GDAŃSK UNIVERSITY OF TECHNOLOGY

The author of the doctoral dissertation: **Milena Marycz, M.Sc. Eng.**
Scientific discipline: **Chemical sciences**

DOCTORAL DISSERTATION

Title of doctoral dissertation: **The use of fungi in biofiltration to remove hydrophobic volatile organic compounds**

Title of doctoral dissertation (in Polish): **Wykorzystanie grzybów w procesie biofiltracji do usuwania lotnych związków organicznych o charakterze hydrofobowym**

First supervisor *signature*	Second supervisor *signature*
PhD. DSc. Eng. Jacek Gębicki, Assoc. Prof.	PhD. DSc. Eng. Anna Brillowska-Dąbrowska, Assoc. Prof.

Gdańsk, May 2023

POLITECHNIKA GDAŃSKA

STATEMENT

The author of the doctoral dissertation: **Milena Marycz, M.Sc. Eng.**

I, the undersigned, declare that I am aware that in accordance with the provisions of Art. 27 (1) and (2) of the Act of 4th February 1994 on Copyright and Related Rights (Journal of Laws of 2021, item 1062), the university may use my doctoral dissertation entitled:
The use of fungi in biofiltration to remove hydrophobic volatile organic compounds
for scientific or didactic purposes.[1]

Gdańsk, 18.05.2023

Milena Marycz
Elektronicznie podpisany przez Milena Marycz
Data: 2023.05.18 02:08:40 +01'00'

.....
signature of the PhD student

Aware of criminal liability for violations of the Act of 4th February 1994 on Copyright and Related Rights and disciplinary actions set out in the Law on Higher Education and Science (Journal of Laws 2021, item 478), as well as civil liability, I declare, that the submitted doctoral dissertation is my own work.

I declare, that the submitted PhD dissertation is my own work performed under and in cooperation with the first supervision of **PhD. DSc. Eng. Jacek Gębicki, Assoc. Prof.**, the second supervision of **PhD. DSc. Eng. Anna Brillowska-Dąbrowska, Assoc. Prof.**

This submitted doctoral dissertation has never before been the basis of an official procedure associated with the awarding of a PhD degree.

All the information contained in the above thesis which is derived from written and electronic sources is documented in a list of relevant literature in accordance with Art. 34 of the Copyright and Related Rights Act.

I confirm that this doctoral dissertation is identical to the attached electronic version.

Gdańsk, 18.05.2023

Milena Marycz
Elektronicznie podpisany przez Milena Marycz
Data: 2023.05.18 02:09:03 +01'00'

.....
signature of the PhD student

I, the undersigned, agree/~~do not agree~~* to include an electronic version of the above doctoral dissertation in the open, institutional, digital repository of Gdańsk University of Technology.

Gdańsk, 18.05.2023

Milena Marycz
Elektronicznie podpisany przez Milena Marycz
Data: 2023.05.18 02:09:20 +01'00'

.....
signature of the PhD student

**delete where appropriate*

[1] Art 27. 1. Educational institutions and entities referred to in art. 7 sec. 1 points 1, 2 and 4–8 of the Act of 20 July 2018 – Law on Higher Education and Science, may use the disseminated works in the original and in translation for the purposes of illustrating the content provided for didactic purposes or in order to conduct research activities, and to reproduce for this purpose disseminated minor works or fragments of larger works.

2. If the works are made available to the public in such a way that everyone can have access to them at the place and time selected by them, as referred to in para. 1, is allowed only for a limited group of people learning, teaching or conducting research, identified by the entities listed in paragraph 1.

GDAŃSK UNIVERSITY OF TECHNOLOGY

DESCRIPTION OF DOCTORAL DISSERTATION

The Author of the doctoral dissertation: Milena Marycz, M.Sc. Eng.

Title of doctoral dissertation: The use of fungi in biofiltration to remove hydrophobic volatile organic compounds

Title of doctoral dissertation in Polish: Wykorzystanie grzybów w procesie biofiltracji do usuwania lotnych związków organicznych o charakterze hydrofobowym

Language of doctoral dissertation: English

First supervisor: PhD. DSc. Eng. Jacek Gębicki, Assoc. Prof.

Second supervisor*: PhD. DSc. Eng. Anna Brillowska-Dąbrowska, Assoc. Prof.

Date of doctoral defense:

Keywords of doctoral dissertation in Polish: biofiltracja, biofiltracja ze złożem zrasznym, grzyby, *Candida subhashii*, LZO, związki hydrofobowe, cytometria przepływowa

Keywords of doctoral dissertation in English: biofiltration, biotrickling filtration, fungi, *Candida subhashii*, VOCs, hydrophobic compounds, flow cytometry

Summary of doctoral dissertation in Polish: Rosnące oczekiwania społeczne dotyczące jakości powietrza oraz zaostrzające się przepisy środowiskowe dotyczące zanieczyszczenia atmosfery spowodowały konieczność minimalizacji i oczyszczania emisji lotnych związków organicznych (LZO). Obecnie duże wyzwanie technologiczne stanowi usuwanie hydrofobowych LZO z powietrza, tak by było zgodne z zasadami zielonej inżynierii. Jednym z rozwiązań jest wykorzystanie grzybów w biofiltracji. Celem niniejszej pracy było opracowanie systemu dezodoryzacji powietrza z hydrofobowych LZO w biofiltrach ze złożem zasiedlanym różnymi gatunkami mikroorganizmów. Rozprawa doktorska oparta została na sześciu artykułach opublikowanych w recenzowanych czasopismach naukowych. Na podstawie przeglądu literatury oraz przeprowadzonych badań własnych (i) wyizolowano z torfu *Candida subhashii*, który nie był wcześniej stosowany w biofiltracji i potwierdzono jego skuteczność asymilacji węgla z wybranych hydrofobowych LZO na porównywalnym poziomie do najczęściej wykorzystywanym do tego celu *Fusarium solani*, (ii) potwierdzono, że jedną z najefektywniejszych metod biologicznych oczyszczania powietrza z hydrofobowych LZO jest grzybowa biofiltracja ze złożem zraszanym, (iii) opracowano metodę immobilizacji różnych gatunków grzybów na powierzchni materiałów wypełniających biofiltry oraz metodę badania różnorodności i żywotności grzybów w procesie biofiltracji.

GDAŃSK UNIVERSITY OF TECHNOLOGY

Summary of doctoral dissertation in English: The growing importance placed on air quality by environmental regulations and public opinion necessitate the minimization and removal of volatile organic compound emissions (VOCs), including odours. The removal of hydrophobic VOCs from the air by biological methods remains a major technical challenge (despite its importance in the shift to green engineering). A potential solution to this challenge is the use of fungi in biofiltration. The aim of this PhD dissertation was to develop a hydrophobic VOC air deodorization system from biofilters inhabited by various species of microorganisms. The content of the dissertation was based on six articles published in peer-reviewed scientific journals. Based on a literature review and the author's own research, (i) *Candida subhashii* isolate, which had not been used in biofiltration before, was isolated from peat, and its effectiveness in carbon assimilation from selected hydrophobic VOCs was confirmed at a level comparable to *Fusarium solani*, the fungus most often used for this purpose. (ii) It was confirmed that fungal biotrickling filtration is among the most effective biological methods of removing hydrophobic VOCs during air purification. (iii) Methods of both immobilizing fungi species on the surface of biofilter packing materials, and testing the diversity and viability of fungi in the biofiltration process were developed.

**delete where appropriate*

***applies to doctoral dissertations written in other languages, than Polish or English*

Dedication

In memory of a best friend
and companion… Codzia

Acknowledgements

I would like to thank the following for their help in completing this thesis:

My Mum who always put my education first and who always supported me.

Professor Anna Brillowska-Dąbrowska and Professor Jacek Gębicki for believing in my ideas, for facilitating the financing of the research, for developing my interest in biofiltration, for their pragmatism in supervising my research and for them constant help. These two scientists are the perfect duo of supervisors, because each of them is wonderful on its own.

Professor Raúl Muñoz Torre, who dedicated his time, resources and guidance through valued opinions and advice in the completion of my research.

Professor Maciej Bagiński, who opened the door to the great scientific world, enabling me to cooperate with some of the best research groups.

Professor Agata Kot-Wasik, who always supported me in an infinite number of ways.

Urszula Kampowska, who always gave me good advice and never left me without help.

My colleagues in the Department of Process Engineering and Chemical Technology at the Gdansk University of Technology and the Department of Chemical Engineering and Environmental Technology at the University of Valladolid, who facilitated me, encouraged me, were bored by me, put up with me, for all the years together.

The project “INTERPHD2” No. POWR.03.02.00-IP.08-00-DOC/16, the project “Integrated Programme of Development of Gdańsk University of Technology” No. POWR.03.05.00-00-Z044/17 and the PROM programme - International scholarship exchange of PhD candidates and academic staff, who provided the funding for my research.

Nomenclature

BF	conventional biofilter
BTF	biotrickling filter
C_{in}	inlet gas concentration
CO_2	carbon dioxide gas
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
EBRT	empty bed residence time
EC	elimination capacity
EPS	extracellular polymeric substance
O_2	oxygen gas
PCR	polymerase chain reaction
pH	power of hydrogen
RE	removal efficiency
SSCP	single strand conformation polymorphism
TCE	trichloroethylene
TGGE	temperature gradient gel electrophoresis
VOC	volatile organic compound

Table of contents

1. Introduction

1.1. The problem of odour nuisance and biological methods of air deodorization

The problem of odour nuisance caused by emissions of volatile organic compounds (VOCs) from various sources is currently a serious social and environmental problem all over the world. Pollution caused by the emission of odour-producing substances has a negative impact on quality of life, creates a sense of discomfort, and may negatively affect human health [1]. Currently, odour nuisance compounds are a real and very common problem due to the fact that their emissions come from many sectors of human activity, including municipal waste landfills, sewage treatment plants, refineries, livestock farms, pulp and fat industry plants, as well as other chemical industries [2–5].

Multiple exposure to air pollution from both outdoor and indoor sources (which is the largest environmental health threat globally) causes 6 million premature deaths [6–8]. The European Environment Agency estimates that in Europe alone (excluding Turkey) air pollution is responsible for 400,000 premature deaths per year [9]. Heart and lung diseases, strokes and lung cancer are now believed to be the most common causes of premature death related to air pollution [10]. These figures are expected to increase due to the long-lasting priority of the economic and energy approach in all branches of human activity, both the increase in economic activity and the construction of energy-efficient buildings [11]. Human exposure to indoor air, and thus to the pollutants contained in it, has increased since the 1970s energy crisis, which led to a change in the concept of designing and constructing residential and utility buildings to increase their energy efficiency used livestock [12,13]. Far from being a simple irritation, this seriously threatens human health, as long-term exposure to pollutants can cause chronic diseases, even low concentrations can lead to serious health effects [8].

The main air pollutants which cause odour nuisances include, among others, volatile organic compounds (VOCs) (e.g. esters, acids, aldehydes, ketones, alcohols) and certain inorganic compounds (mainly hydrogen sulphide and ammonia) [14,15]. Ammonia and hydrogen sulphide are two of the main inorganic odour compounds. Most organic odours are produced by the anaerobic decomposition of nitrogen- or sulphur- containing compounds [16]. The odour quality of the air is determined by the type, the hedonic quality (pleasantness-unpleasantness felt) and the intensity of the smell (depending on the concentration of the odour substance) [17,18]. Other pollutants present in the air, most often odourless, include various types of chemicals, physical agents, particulate matter (both of natural origin and associated with human activity), as well as biological pollutants (mainly pollen, fungi, bacteria, viruses and spores) [19,20]. The main source of odorants (hydrogen sulphide, ammonia, nitric oxide, as well as aldehydes, amines, aromatic hydrocarbons, organic acids and sulphur compounds), both in Poland and around the world, are animal husbandry and slaughterhouses, the utilization of animal by-products, the production of feed and compost, the use of natural fertilizers and municipal sewage sludge in agriculture, sewage treatment plants, chemical and petrochemical industry, the production of furniture boards and the operation of equipment and technological processes [21–28].

The problem of odour nuisance is gaining importance in Poland due to the growing social awareness as well as more restrictive legal regulations currently being developed and introduced in the future [29]. Presently, there are no legal regulations on counteracting odour nuisance. Neither the “Kodeks przeciwdziałania uciążliwości zapachowej” ("Code of counteracting odour nuisance"), which only serves as informative and educational material in the form of technical guidelines, nor “Lista substancji i związków chemicznych, które są przyczyną uciążliwości zapachowej” ("List of substances and chemical compounds that cause odour nuisance") have any legal force. “Lista substancji i związków chemicznych, które są przyczyną uciążliwości zapachowej” was created by Ministry of Climate and Environment on the basis of analyses of substances and chemical compounds considered as nuisances, or potential odour nuisances, generated in Poland. Along with the growing requirements regarding the storage, disposal and management of waste in Poland, an announcement of 3 March 2022 was issued on the publication of the consolidated text of the Act on Waste (Dz.U. 2022 poz. 699). The consolidated text of the Act on Waste of 14 December 2012 (Dz. U. z 2021 r. poz. 779) was published in the attachment to this announcement, taking into account the changes introduced in 2021. One of the main objectives of the introduced regulations is the protection of human life and health and the environment. In particular, waste management must not cause noise or smell nuisances, or threaten water, air, soil, plants, animals or people. The Act also defines actions to protect the environment, human life and health by preventing and reducing waste generation and the negative impact of waste production and management, as well as by reducing the total impact of resource use and improving its efficiency, in order to transition to a circular economy. The aforementioned document also defines the conditions for applying the hierarchy of waste management methods. Firstly, waste must be prepared for reuse or recycled. Recycling also refers to organic recycling, consisting of aerobic processing, including composting or anaerobic processing of waste. Under controlled conditions undergoes biological decomposition with the participation of microorganisms, resulting in organic matter or methane being produced. However, waste whose recovery is not justified for technological, ecological or economic reasons should be neutralized. In 2017, the Chief Inspectorate for Environmental Protection (Główny Inspektorat Ochrony Środowiska) was authorized to prepare and carry out the legislative process of the commonly named “Anti-odour Act” by the Minister of the Environment. On 1 June 2021, the legislative process of the Anti-odour Act began, after the process of submitting it to inter-ministerial arrangements, social consultations and public opinions was completed. In 2020, while the new act was being drafted an expert opinion "Bezpieczne odległości od zabudowań dla przedsięwzięć, których funkcjonowanie wiąże się z ryzykiem powstawania uciążliwości zapachowej" ("Safe distances from buildings for projects whose operation is associated with the risk of odour nuisance") was developed, which enables the creation of temporary solutions to the odour problem. Currently without the above-mentioned regulations and expert opinions, officials in Poland have limited recourse to counteract odour nuisance. One possibility is to impose measures to reduce the negative impact on the environment, including an obligation to perform an ecological inspection, as well as to deodorize the facility that generates unpleasant odours. A fact worth noting is that the European Union leaves the Member States the freedom to act on issues related to counteracting odour nuisance. Taking into account the above-mentioned circumstances, it is now necessary

to develop new methods of neutralizing odours. These methods should be both highly efficient, compliant with the principles of green engineering, and relatively low-cost, and thus more accessible.

Despite the widespread implementation of modern air pollutant neutralization technologies, numerous VOCs are still emitted into the atmosphere. These compounds can be removed by chemical, physical and biological processes. Currently, the most commonly applied methods of deodorization are biological methods, combustion, absorption and adsorption methods, as well as pollutant masking (Figure 1) [30,31].

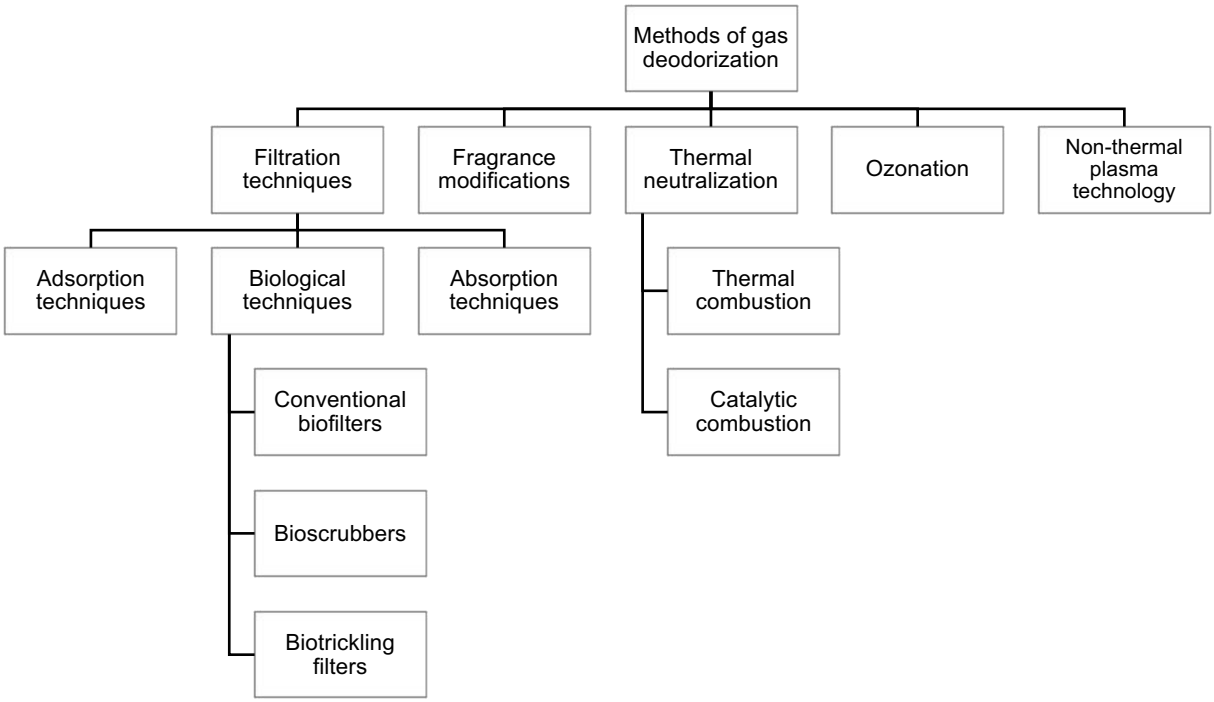


Figure 1. Basic techniques for reducing the emission of odorous compounds.

Three methods of deodorization have been distinguished: removal of pollutants that give off an unpleasant odour, conversion of nuisance odours into odourless or more pleasant ones, and the introduction of masking or neutralizing agents to reduce the intensity or mask the odour [32]. Fragrance modifications do not remove odours, they only mask them, which in many cases does not protect people exposed to their toxic effects. Nevertheless, odour modifications are commonly used to counter the odour nuisance caused by various types of VOCs [33,34]. Implementation is possible in many ways, e.g. the use of another fragrance substance that will soften or completely eliminate the original odour. The reaction between the molecules of the nuisance odour gas and the olfactory receptors of humans can be modified, which leads to a reduction in the sense of smell discomfort [35,36]. Another solution is to mix the malodorous substance with another odorant, which makes the smell of the mixture less invasive [37,38]. However, this method does not remove the source of odours, it only masks them. Therefore, modifications of the odour are used in cases where the odour nuisance occurs seasonally, e.g. due to the need to use antiodorants, which can also be a source of odour nuisance [39].

The first technique for reducing odour emissions is incineration. Combustion as a thermal oxidation process is an effective technique for removing odour compounds. The most commonly used methods are thermal

and catalytic combustion [40]. All these processes require strictly controlled conditions. This technique works on a simple premise, use of the high temperatures to destroy odour compounds in a combustion chamber [41]. This method can be used as an intermediate step in the overall process. Frequently the products of such combustion are much easier to remove in the next steps of air purification [42].

Thermal combustion is a process of oxidizing combustible gases, consisting of heating these gases in a furnace in a mixture with air or oxygen to a high temperature (650-800°C) [43,44]. This method is widely used because most organic compounds can be oxidized and can be used even at very high concentrations. Unfortunately, this solution involves continuous measurements of oxygen, nitrogen oxides, carbon dioxide and carbon monoxide in the exhaust gases [40]. Thermal combustion generates high costs directly related to fuel consumption and often requires pre-treatment of the gases, including removal of water vapour from the gas and removal of solid or liquid contaminants. The main disadvantages of thermal combustion are the occurrence of secondary gas pollutants and economic considerations. If the process parameters are not respected, additional odours and soot are generated, which is inconsistent with the principles of green engineering [33,45].

The second incineration method is catalytic combustion. Compared to thermal combustion, the oxidation of gases takes place on the surface of the catalyst, the use of which allows us to obtain the same degree of combustion at a lower temperature (350-400°C), and thus with lower fuel consumption [46]. This method does not require precise knowledge of the composition and amount of the substance because it allows the removal of compounds even in very high concentrations, and can be used for a large group of odorants (a contraindication to the purification of gases containing fluorine and chlorine compounds) [47,48]. Catalytic combustion is characterized by high deodorization efficiency and allows the removal of slightly dusty and humid gases [49]. The difficulty in carrying out this process lies in the need to ensure a constant concentration of pollutants, frequent inspections and the use of additional purification. Catalytic combustion adversely affects the environment through the formation of sulphur dioxide and hydrogen chloride and the constant exposure of the catalyst to erosion, as well as the frequent blocking of its active surface by dust, which leads to the need for its frequent replacement [50,51].

Non-thermal plasma technology is a method of oxidizing odor pollutants based on the decomposition of gaseous pollutants using plasma at ambient temperature and atmospheric pressure [52]. Plasma, called the fourth state of aggregation, due to its properties different from the solid, liquid and gaseous phases, is formed at temperatures where the average kinetic energies of particles exceed the value of the ionization potential [53]. Plasma is generated between two concentric electrodes placed axially along a gas pipeline (or inside ventilation ducts) through which polluted gas flows. In low-temperature plasma reactors, electricity is not used to heat the gas, which remains "cold". Instead, high-energy electrons and UV light cause the ionization and breakdown of gas molecules, which leads to the formation of chemically active elements. The decomposition of pollutants in the gas stream is the result of the breakdown of particles by direct collisions of electrons and chemical reactions initiated by active elements. Particles of the odorous gas are oxidized by ozone molecules. This method is especially recommended as a method of removing air

pollutants present in very small quantities [54–56]. The efficiency of the plasma process depends to the greatest extent on the power supply system (high power demand is required) [57]. The major advantage of this method is its low cost, as well as the fact that it does not mask the source of malodorous gases, but instead removes them. However, the ozone produced is toxic, unstable (need to be produced directly at the dosing site), has a strong odour, and is corrosive. The ozonation reaction is often characterized by a low rate of oxidation, which depends on the type of chemical compound and the temperature used [58].

Different basic method of reducing the emission of odours compounds is ozonation. Generated ozone oxidizes gaseous pollutants, which results in removing them. The main advantage of this method is low cost. However, effectiveness of this method is not satisfying, because only some pollutants and fumes can be removed. Additionally, it is not recommended by the American Lung Association because it generates unhealthy ozone and degradation products [59,60].

Another widely used class of methods of removing odours are filtration techniques, which include adsorption and absorption techniques, iron catalytic filters and increasingly applied biological techniques [61]. Adsorption techniques rely on the separation of pollutant components from one phase accompanied by their accumulation or concentration at the surface of another. The adsorbents used must be characterized by a large specific surface, examples of which are activated carbon, zeolites and aluminum oxide. The use of high-quality adsorbents allows for high adsorption efficiency, as well as for their more effective regeneration after use. However, as the saturation of the deposit increases, the effectiveness of odour removal decreases proportionally. Adsorption techniques are used with varying degrees of efficiency to remove compounds with high concentrations due to the rapid saturation of the sorbent [31,45,51]. Therefore, the main disadvantage of this solution is the potential risk of pollutant reemission due to the fact that the adsorbent might be too specific or its rapid saturation, as the pollutants are not destructed [59].

Absorption techniques involve dissolving gaseous pollutants in liquid solvents. This process consists of a mass exchange between a soluble gas and a liquid solvent, usually water or water mixed with an admixture of various reagents. Air deodorization using absorption methods is usually recommended when large volume flows are introduced into the atmosphere and the gas is heavily polluted. Most of the described processes using absorption techniques that effectively remove odours are characterized by a multi-stage technological process [45,62]. Most often two- or three-stages processes are used. In the first stage, acidic sorption solutions are used for removal of alkaline impurities. In subsequent stages sodium hydroxide and sodium hypochlorite solutions maybe be used for removal of sulphur compounds [63]. The risk of emissions of organic chlorine compounds is negated by using ozone as an oxidizing agent [64]. The advantage of using absorption techniques is the low investment and operating cost as well as the ease of designing and running the process. Effective removal of water-insoluble compounds is possible in the absorber only with the use of dedicated chemical reagents [31].

Another group of methods belonging to the group of filtration techniques are iron catalytic filters. These filters are made of perforated tanks at the gas inlet and outlet, inside which are corroded steel rods. In this type of filter, hydrogen sulphide, which is an undesirable component of the gas stream flowing through the

tank, reacts with iron oxide. The main environmental problem generated by this method is the formation of a dilute sulphuric acid solution as a by-product. This method is only used to improve the effectiveness of other techniques to eliminate odorogenic substances, it is never used alone [65–67].

On the other hand, biological methods of air deodorization, which belong to filtration techniques, are an ecologically safe and relatively means of cheap odour neutralization [68,69]. Compared to physicochemical methods used to remove gaseous pollutants, these methods are characterized by lower energy demand and do not require either extreme operating conditions or the use of hazardous chemical reagents [3,15]. After starting and stabilizing the process, biological methods become practically maintenance-free. Among all air purification methods they are the most compatible with the principles of green engineering. Biofiltration is effective for waste gases with a concentration lower than 3 g m^{-3} VOCs [70,71]. The biofiltration process involves passing gases through a layer of porous material, which is a habitat for microorganisms. Contaminants diffuse from the gas phase to the biofilm, which is formed on the surface of the bed elements. The compounds adsorbed on the surface or absorbed in the biofilm undergo biodegradation, and the air that is purified and devoid of odorous compounds leaves the biofilter. Dissolved pollutants are decomposed by microorganisms, resulting in simple reaction products such as carbon dioxide (CO_2), water and biomass according to the Equation 1:

$$VOC + O_2 \rightarrow CO_2 + water + biomass + side\ products \quad (1)$$

Therefore, the basic purpose of the biofilter is to bring contaminants (volatile and gaseous substances or aerosols) contained in the air stream into contact with microorganisms under strictly defined conditions [72].

The optimal design of the apparatus and careful selection of process parameters, as well as the appropriate selection of packing materials used in biofiltration, ensure reliability and low investment costs for the entire process [3]. The construction of the column should be strong enough to support the column filling. Most often, fibreglass or various types of glass-fibre-reinforced plastics are used to build the column. Other materials used include polymers, metals protected with anti-corrosion coatings, and even concrete [73]. The packaging material should enable the proper development and growth of microorganisms during the process, and thus maintain the appropriate concentration of biomass in the bed [74,75]. These conditions are affected by bed porosity, low flow resistance and specific surface area. The large specific surface area enables the colonization of many microorganism cells, and also provides places protected against the adverse effects of shear forces associated with the flow of water [76]. The activity of the packing materials towards the removed impurities should also be determined [77,78]. The development of microorganisms takes place on the surface of the filtration column filling, mainly in macropores. The filling should act as a substrate for the development of the biological membrane and enable its complete colonization by microorganisms. Depending on the biofiltration method used, the process uses either microorganisms that naturally colonize the filling material of natural origin (peat, tree bark, cones, straw, wood chips and others), or uses microorganisms which are deliberately inoculated on an inert substrate (polyurethane foam, Palla rings, Białecki rings and others) [3,79,80]. Three technological solutions are most often used in biofiltration

- treatment in conventional biofilters, bioscrubbers and biotrickling filters. In conventional biofiltration, materials naturally colonized by consortia of microorganisms are most frequently used, activated sludge is commonly used in bioscrubbers, while in biotrickling filtration, selected microorganisms capable of decomposing VOCs on inert materials are most often immobilized [81].

Choosing the best air purification method is both complicated and difficult as it depends on many factors [82]. Table 1 summarizes and compares the basic techniques of reducing the emission of odorous compounds. In making this selection, consideration should be given to the chemical nature and characteristics of the gas stream, the amount of emissions produced and total pollutant content, pollutant concentrations, the desired level of gas deodorization and the inlet concentration [83–85]. Low waste (used packing materials can often be reused or recycled) and relatively low unit costs of biofiltration (much lower than in other methods of air purification from pollutants) determine the attractiveness of using this method. VOCs are often present in waste gases in low concentrations and are readily biodegradable. Biofiltration is one of the most dynamically developing technique, especially in the deodorization of gases with temperatures close to ambient temperatures. However, despite the numerous advantages of biofiltration over other methods of air purification, and its increasing implementation and modernization, the purification of gases from hydrophobic VOCs is still a major technological challenge due to their poor solubility in water [86–88]. The low mass transfer of hydrophobic VOCs from the gas phase to the liquid phase and biofilm reduces the supply of substrates to microorganisms, thus limiting the physical contact between the removed compounds and the microorganisms [88]. This phenomenon significantly reduces bioavailability, and thus limits biodegradation. In biological methods, the effectiveness of removing hydrophobic VOCs is significantly influenced not only by the physicochemical properties of the removed compounds, including to a large extent water solubility, but also by their molecular structure and Henry's law constant [60,89,90]. Therefore, in order to improve the removal efficiency of hydrophobic VOCs in biofilters, scientists aim to increase their bioavailability in both biofilm and liquid phase, using various strategies to improve filtration methods: the use of fungal biocatalysts, the addition of a surfactant, two-phase biofilters, the addition of hydrophilic compounds and biofiltration with preliminary treatment [91–98]. Drawing attention to this still existing problem became an inspiration to work on the development of a new proposal for the process of effective air deodorization with hydrophobic VOCs.

Table 1. Basic techniques of reducing the emission of odorous compounds [52,60,99–102].

Method	Parameters	Advantages	Disadvantages
Adsorption techniques	Cin: <10 [g m-3] Gas flow: 5-50000 [m3 h-1] Temp.: <55 [°C] Used solutions: zeolites, activated carbons, aluminium (II) oxide	- low operating costs - ability to recover adsorbents - high efficiency (in the case of a high-grade of activated carbon)	- high investment costs - strong sensitivity to high air humidity and elevated temperature - risk of pollutant emissions - possibility of irreversible contamination of the sorbent - very narrow range of applications (solvent processes, paint shops and chemical industry)
Absorption techniques	C_{in}: 8-50 [g m^{-3}] Gas flow: 100-60000 [m^3 h^{-1}] Temp.: room temperature Used solutions: washing gas with contaminated water	- simple construction of the installation - high efficiency of deodorization	- high operating costs (the need to use expensive chemically resistant and durable construction materials) - not suitable for low concentrations - nuisance of generated sewage - reagents used pose a health risk - risk of environmental pollution due to accidental leakage of reagents
Thermal combustion	C_{in}: 2-90 [g m^{-3}] Gas flow: >10000 [m^3 h^{-1}] Temp.: 371 [°C] Used solutions: thermal oxidation	- high efficiency	- high investment costs - high operating costs - incomplete mineralization - not eco-friendly, release of secondary pollutants
Catalytic combustion	C_{in}: 2-90 [g m^{-3}] Gas flow: >10000 [m^3 h^{-1}] Temp.: 149 [°C] Used solutions: thermal catalysts	- high efficiency - energy efficient	- not eco-friendly, due to the formation of by-products - catalyst deactivation and disposal required
Nonthermal plasma technology	C_{in}: - Gas flow: - Temp.: - Used solutions: reactive radicals and ions react with gaseous pollutants	- low operating costs - low-temperature treatment - plasma generation in ambient air - for all gaseous pollutants - no waste and environmentally friendly - short treatment time	- high investment costs - formation of excess ozone - large number of samples - adaptation mechanisms - determination of effective dose
Ozonation	C_{in}: - Gas flow: - Temp.: - Used solutions: strong oxidizing agent	- low investment costs - low operating costs - in order to increase effectiveness of the process, must be used in conjunction with other methods, e.g. the use of catalysts	- generates unhealthy ozone and degradation products - low rate of oxidation - ability to remove only some vapours and gaseous pollutants

		- microorganisms are removed at the same time - ease of cleaning the installation	
Fragrance modifications	C_{in}: only low concentrations Gas flow: - Temp.: - Used solutions: adding of a volatile substance that changes the nature of the odour or the perceived intensity of the odour	- low investment costs - maintenance-free or easy manual operation - effective use of environmentally friendly masking compounds - very short response time, about a few seconds	- only possible to use this method in the case of odorants with a non-toxic effect and relatively low concentrations - possibility of reducing the immunity of people in the environment - strongly dependent on the state of the atmosphere at a given place and time, primarily: air temperature and humidity, wind speed, direction and gusts - highly efficient ventilation system required
Biological techniques	C_{in}: >3 [g m^{-3}] Gas flow: 200-1500 [m^3 h^{-1}] Temp.: - Used solutions: air flows through a packed bed colonized by microorganisms that are capable of biodegrading pollutants	- low operating costs - ability to remove a wide range of contaminants - follows principles of green engineering - possibility of immediate and flexible change of the biofilter's operating parameters - mostly maintenance-free operation Depending on the type of contamination: - possibility of using various filtration materials and strains of microorganisms - possibility of reusing used filter material, mainly artificial packing materials	- investment costs vary depending on the assumptions and requirements of the project - pollutants to be removed must be biodegradable - gas must not be contaminated with substances toxic to microorganisms, e.g. heavy metal compounds - difficulties in removing hydrophobic compounds - required to control biological parameters - temperature during the pollutant removal process must be within a range that does not affect the deterioration of the activity of microorganisms inhabiting the biofilter column (this condition is met by regulating the purified gases by preliminary flue gas treatment, e.g. by pre-spraying the gases with water, which lowers their temperature and ensures leaching of toxic substances) - biofilters require a period of acclimatization of microorganisms for the biodegradation of pollutants (the required time depends on the species of microorganisms used, ranging from a minimum of one week to even several months) - possibility of generating secondary pollutants

1.2. Biological methods of air deodorization

The use of biological methods for air deodorization has been known for over 70 years [103]. As mentioned before, the biofiltration process can be carried out in two main types of apparatus, i.e. in a conventional biofilter (BF) or in a biotrickling filter (BTF) (Figure 2 a, b). The third group of devices used for biofiltration are bioscrubbers, which are characterized by low efficiency in removing hydrophobic VOCs from the air compared to BFs and BTFs [104] (Figure 2 c). Due to the high proportion of the aqueous phase, this treatment method is suitable for gaseous pollutants with high water solubility. Comparison of biological methods is shown in Table 2. The bioscrubber is a system consisting of two separate reactors, which are the absorber and the aerated activated sludge chamber. The sorbent is most often activated sludge. Gas purification occurs in two stages - absorption of pollutants by a liquid sorbent, and biodegradation in a bioreactor (which can be an activated sludge chamber). The microorganisms involved in the gas purification process are suspended in the mobile liquid phase. The purification of the sorbent (its self-regeneration) occurs as a result of the action of microorganisms in the activated sludge chamber [69,105].

In BFs, the polluted gas is moistened in a separate chamber and then passed through a column filled with materials of mostly natural origin (Figure 2a). Frequently used natural packaging materials are naturally inhabited by various species of microorganisms that form a biofilm layer on the surface of the filling. Contaminants present in the purified gas stream, after passing into the biofilm, are biodegraded to water, carbon dioxide, and converted into biomass [106,107]. Common problems encountered in the operation of BFs are their susceptibility to drying out of the bed, difficulty in controlling the process parameters (e.g. maintaining the required pH value), decrease in efficiency for highly concentrated gas streams, and limited durability of the filling used [108,109]. During the degradation of VOCs in biofiltration, metabolic products of the microorganisms involved in the biofiltration process may be formed, which may constitute secondary pollutants. On the other hand, the advantages of this solution are low investment and operating costs, easy operation and maintenance, and the possibility of cleaning gas streams polluted with odours belonging to various types of chemical groups [3,110].

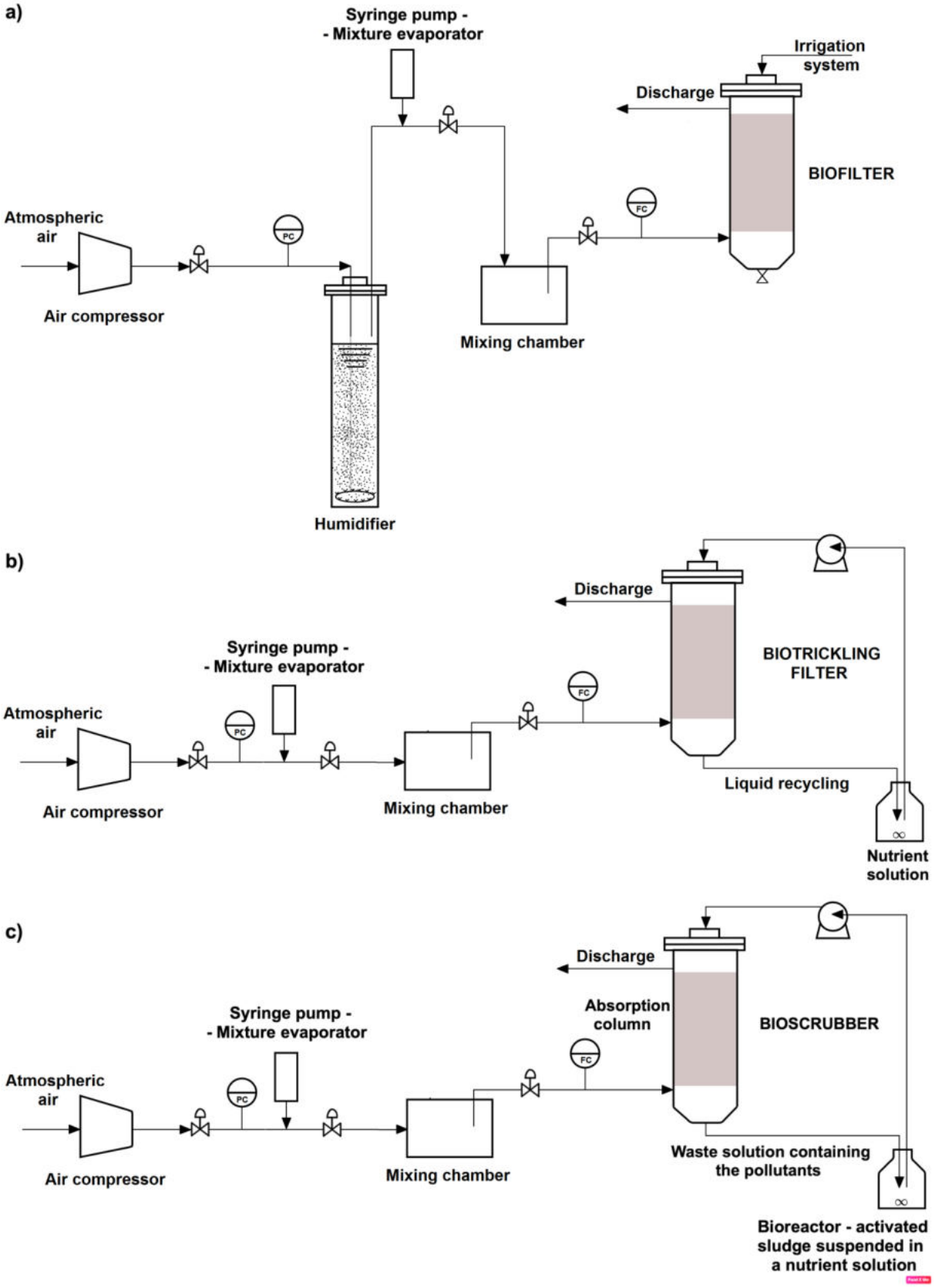


Figure 2. Schematic representation of the experimental set-ups of (a) conventional biofiltration, (b) biotrickling filtration, (c) bioscrubbing.

In a BTF, the absorption and decomposition of pollutants take place in a column through whose filling a liquid enriched with nutrients for microorganisms is trickled (Figure 2b). The liquid phase, which is a medium for microorganisms, is continuously recirculated through the biofilter column, which is filled with a bed without the ability to retain water. The packing material does not contain any nutrients; therefore, all nutrients must be contained in the medium [111,112]. A common procedure used in this type of biofiltration is the immobilization of the inert bed with an isolated strain of known enzymatic activity [107,113,114]. The advantages of biotrickling filtration are the stability of the process, the ability to adjust the pH and temperature of the spray liquid, low flow resistance and small space requirements. However, this type of biofiltration has the following disadvantages: complicated construction, risk of generating secondary pollutants (similar to other biological methods of air deodorization), and an accumulation of excess biomass [14,77,115].

Both conventional biofiltration and biotrickling filtration are based on biofiltration, which is a complex process of removing pollutants in the gas and liquid phase, during which various physical and biological processes take place (Figure 3) [116]. The process of decomposing pollutants is called biodegradation. Odorous pollutants contained in the flowing air are adsorbed on the surface of the mobile liquid phase covering the biofilm produced by microorganisms colonizing the filling of the biofilter column. Adsorbed odour particles penetrate through the liquid phase and then diffuse through the biofilm towards microorganisms. Microorganisms causes decomposition of odour pollution particles, which results in the production of water, carbon dioxide and biomass.

Table 2. Basic biological techniques for reducing the emission of odorous compounds [29,60,100].

Method	Applied solutions	Advantages	Disadvantages
Conventional biofiltration	- single reactors - continuous working time - immobilized biomass - often use natural organic materials, naturally inhabited by microorganisms	- low pressure drops - low investment costs (among all biological methods, they require the smallest initial investment, then operating costs are minimized) - low operating costs - simplicity of commissioning and operation - able to remove hydrophobic VOCs that are difficult to dissolve in water - generates the smallest amount of waste compared to other biological methods of air purification - most often it is not necessary to immobilize microorganisms on an inert material, because materials naturally colonized by microorganisms are often used	- the biofilter bed has a limited lifetime - strict control of working conditions is required - need to maintain constant working conditions of the bioreactor (constant temperature and pH, concentration change in a narrow range) - possibility of problems with deposit clogging - possibility of generating secondary pollutants - in the case of external (soil) biofilters: - large size, large area required, difficult space constraints - poor control of working conditions
Biotrickling filtration	- single reactors - immobilized biomass - most common setup is an inert packing material on which selected species of microorganisms are immobilized - the porosity of the packing material should allow good air flow - mobile liquid phase	- low space requirements - low operating costs - good distribution of nutrients - low flow resistance - high volume throughput - ability to adjust the temperature and pH of the trickled liquid - stability of the process - able to treat acidic VOC degradation products - able to remove hydrophobic VOCs that are difficult to dissolve in water - possible for direct contact of microorganisms (e.g. fungal hyphae) with the removed compound	- investment costs vary depending on the assumptions and requirements of the project - complicated construction - requires additional effort to start the process to immobilize selected microorganisms on the inert packing material - possible leaching of microorganisms from the column packing - possible excessive accumulation of biomass in the filter bed - secondary waste stream is created

Bioscrubbing	- two reactors - the filling of the bioscrubber is sprinkled with liquid containing activated sludge - the mobile liquid phase - suspended biomass - most often the filling is made of neutral materials whose structure is conducive to the development of microorganisms - sometimes bed elements are not only biofilm carriers, but also adsorbents of gaseous pollutants, e.g. activated carbon	- relatively small pressure drop - relatively smaller space requirements (compared to large-area external (soil) biofilters) - high solubility of oxidation products; - better control of operating parameters and work stability - able to cope with severe fluctuations and high flow rates	- investment costs vary depending on the assumptions and requirements of the project - high operating costs - higher energy consumption than biofilter - the need to ensure energy supplies - possible complications in commissioning, operation and maintenance may be encountered - additional air supply may be required - production of liquid waste - sometimes it is necessary to remove excessive sediment - effectiveness of odour removal depends on weather conditions (in the case of external bioscrubbers) - only removal of water-soluble compounds possible

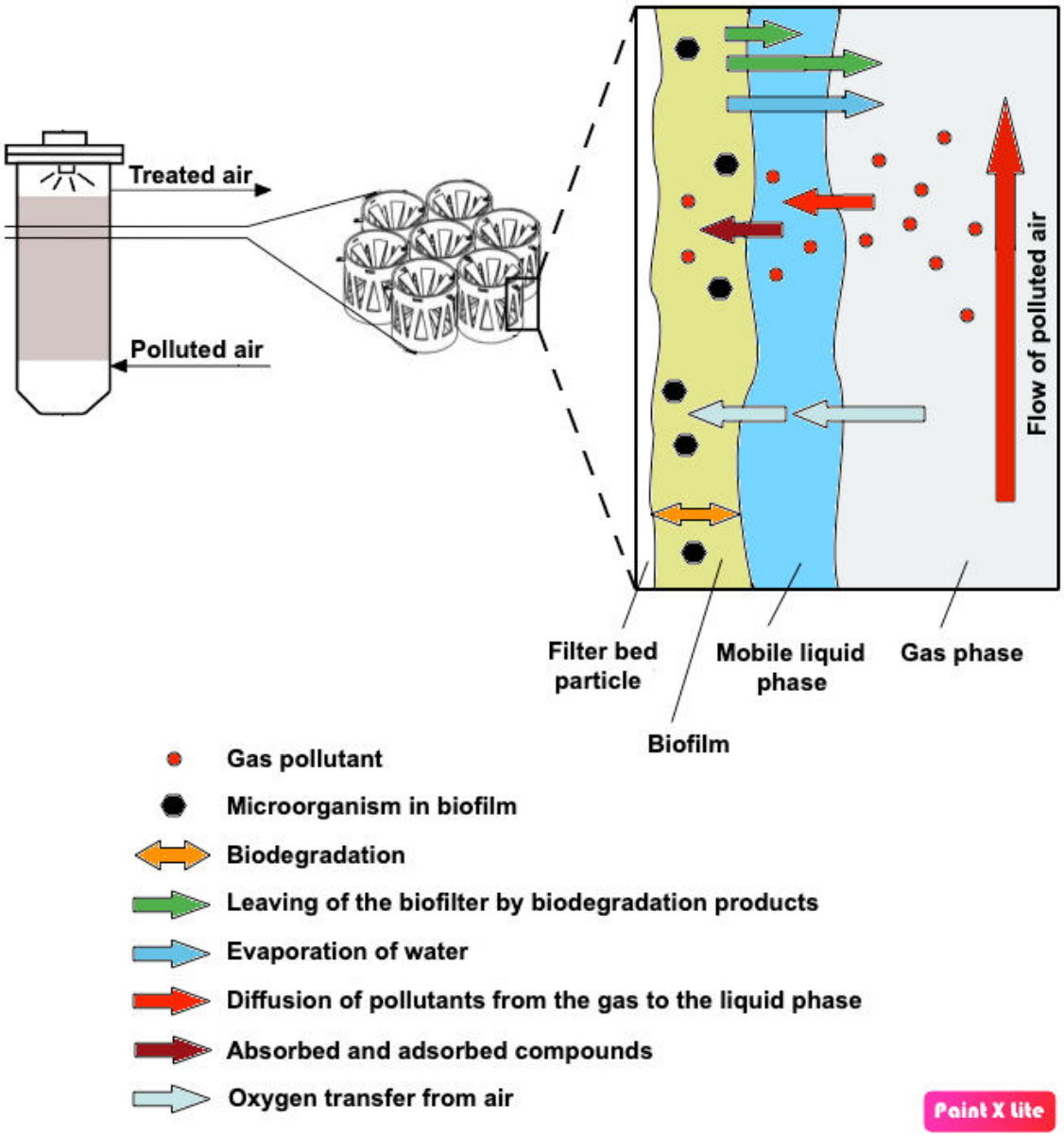


Figure 3. General mechanism of removing gas pollutants in bioreactors.

The biodegradation process takes place in two stages. The first stage is biosorption, which involves organic compounds being retained on the cell surface. On this surface, the penetration of molecules takes place in both directions - odour particles penetrate into the cell, and waste metabolic products exit in the opposite direction. The phenomenon of penetration of intracellular and extracellular contents within the cell enables the uninterrupted continuation of the process of absorbing new pollutant particles. The second stage of biodegradation is mineralization, which consists of the use of energy and biogenic elements for the enzymatic decomposition of VOCs by microorganisms. The decomposition of VOC molecules usually takes place inside the cell, accompanied by the excretion of simple mineral products. In contrast, macromolecular compounds are hydrolyzed outside the cell [86,117].

Usually, the dominant factor in biological treatment, regardless of the technical solutions, is the activity of the microorganisms used [118]. In biological methods, bacteria are most often used, but fungi are also always present in the biofilter and their participation in the purification process depends on the process

conditions [5,119]. In recent years there has been a growing interest in understanding the metabolism of fungi and bacteria involved in the biofiltration process. Due to the large variety of species of microorganisms involved in biofiltration, there is a large variance in the metabolic pathways employed by them [107,120]. To date, the metabolic pathways of bacteria have been extensively studied, while the metabolism of fungi capable of degrading VOCs remains poorly understood. The study and understanding of the metabolism of microorganisms is enabled by molecular techniques, including the next generation sequencing, detection of single strand conformation polymorphism (SSCP), as well as the modified SSCP - DGGE (denaturing gradient gel electrophoresis) method, or the TGGE (temperature gradient gel electrophoresis) method [121–124].

1.3. The use of fungi in biofiltration

When designing biofiltration processes, regardless of technical solutions, one of the most important factors affecting the effectiveness of air deodorization is the type of microorganisms used, and thus their metabolism in relation to the specific odorous VOCs removed [77,116]. The selection of a culture of microorganisms for biofiltration is usually made on the basis of the composition of the purified air and the analysis of the ability of microorganisms to degrade pollutants present in the gas stream [4,5]. In some cases, a single species of microorganism is enough to remove some VOCs from the air, while other times the use of a consortium of microorganisms is required to effectively deodorize the air of pollutants [32,125,126]. The removed VOCs are used by these microorganisms as a source of elements necessary for their lifecycle, mainly carbon, and/or a source of energy [106].

Over the past few years, extensive research has been conducted into the versatility of microorganisms used in biofiltration [5,19,76,97,127–131]. The most commonly used microorganisms in biofiltration are bacteria and fungi. However, to this day, bacteria are used in biofiltration much more often than fungi [109,130]. Particularly high efficiency of bacterial processes has been noted in the case of air deodorization from hydrophilic VOCs [32,132]. Over the past several years, fungi have been garnering more and more interest in this field, especially when it comes to the biodegradation of VOCs of a hydrophobic nature [88,97,133–135]. It is worth mentioning that the terms "bacterial biofilters" or "fungal biofilters" are not always correct in biofiltration. In open biofilters, it is not possible to maintain aseptic conditions, and thus the input composition of microorganisms inhabiting the biofilter bed. In the biofilter during the process, primary microbiota are replaced in various proportions by secondary microbiota, both by fungi and bacteria. On the other hand, under favorable conditions, it is possible to maintain the dominance of microorganisms inhabiting the filling of the biofilter throughout entire duration of the process. This phenomenon is influenced not only by the competitive ability of the microorganisms themselves, but also by the physical and chemical conditions of the process [76].

Bacteria are effectively used in biofiltration to remove hydrogen sulphide, nitrogen compounds and some VOCs [136–138]. The most commonly used bacteria in biofiltration are microorganisms belonging to the following species: *Pseudomonas, Rhodococcus, Burkholderia* and *Bacillus* [132,139–144]. Bacteria,

compared to fungi, are characterized by higher biochemical activity and faster growth [125]. However, hydrophobic VOCs are poorly removed by bacteria colonizing the inert or organic fillings of the biofilter column. The most likely reason is frequent drying in BFs and acidification of the bed filling the column [145,146]. In order to overcome these operational problems, a technique has been developed for the colonization of inert packing materials by fungi [145,147]. Compared to bacteria, fungi are more resistant to dry and acidic conditions, which are the two main factors that most often reduce the efficiency of biofiltration. This is particularly important when biofiltration is carried out on an industrial scale and is in a transitional state, which is often associated with changes in the pH value, rapid changes in the degree of hydration or changes in loads caused by biofilter downtime, which usually have disastrous effects on the bacteria inhabiting the bed biofilter [148,149]. Fungi are also less sensitive to the toxic effects of pollutants and have a greater ability to survive during a shortage of elements necessary for proper functioning than bacteria [150]. In addition, fungi have specific hydrophobic proteins in their cell wall, called hydrophobins, which increase the biodegradation of hydrophobic VOCs directly from the gas phase [145,151–154]. Many species of fungi, mainly yeasts and filamentous fungi, can produce biosurfactants to degrade hydrophobic VOCs and use them as a carbon source [155]. Biosurfactants are amphiphilic molecules containing two parts: hydrophilic (head) and hydrophobic (tail) [98]. These molecules are synthesized as metabolic products, often by-products, using various substrates such as VOCs, carbohydrates, hydrocarbons or fats [156]. Biosurfactants, which enable the emulsification, dispersion and dissolution of insoluble substances, are produced by microorganisms as a mechanism to deal with interfacial challenges [157]. Both biosurfactants (molecules naturally produced by microorganisms during the process) and surfactants (compounds artificially added to the biofiltration process) increase the bioavailability of the hydrophobic VOCs to be removed by reducing the surface tension of the biofilm and interfacial tension at liquid-liquid or liquid-gas interfaces [88]. In 2019, Miller et al. concluded that both surfactants and biosurfactants can be sorbed on cells and affect the hydrophobicity of the outer layers, which directly affects the hydrophobic affinity of microbial cells for poorly soluble VOCs [92]. A variety of fungus able to produce biosurfactants can be found in [156,158–161]. However, cases of negative or neutral impact of surfactants on the process of removing odours from the air have also been reported. Therefore, when designing a biofiltration process, it is necessary to consider whether or not to add a surfactant. In the case of a decision to add these types of compounds, they should be properly selected, as they show high selectivity of action, and by increasing the rate of biodegradation of a given pollutant, they can also block the development of microorganisms responsible for the biofiltration process [162].

Two types of fungi, yeasts and moulds, are mainly used in biofiltration (Figure 4) [146,163,164]. Moulds are distinguished from other microorganisms by having a substrate mycelium that grows into the substrate and aerial mycelium, which is formed by loosely collected hyphae (Figure 4 b) [165]. As already mentioned, the removal of hydrophobic VOCs is currently one of the greatest technological challenges in biofiltration due to their low solubility in the liquid phase. On the other hand, moulds, having an extensive aerial mycelium, have an extensive mass exchange surface from the gas phase to the biofilm. The whole process is also facilitated by the fact that the cell walls of fungal hyphae are hydrophobic, which greatly facilitates

the penetration of hydrophobic pollutants into the fungal biofilm [151,153]. Vergara-Fernández et al. (2006) observed that the solubility of hydrophobic compounds is usually higher in the presence of fungal biomass and their metabolites [151]. One of the first studies focusing on the quantitative determination of the importance of aerial mycelium in the process of n-pentane deodorization in a biofilter inoculated with the fungus species *Fusarium solani*, showed that the aerial mycelium biomass alone accounted for 25.9(±3)% of the total mass of biomass and was responsible for the removal of 71.6(±4)% n-pentane [166]. In addition, the growth of the aerial mycelia of fungi can significantly increase the mass transfer of hydrophobic VOCs to the biofilm phase from the gas phase [97]. To summaries, moulds with aerial mycelium are able to effectively adsorb and biodegrade hydrophobic VOCs due to direct contact with the gas phase.

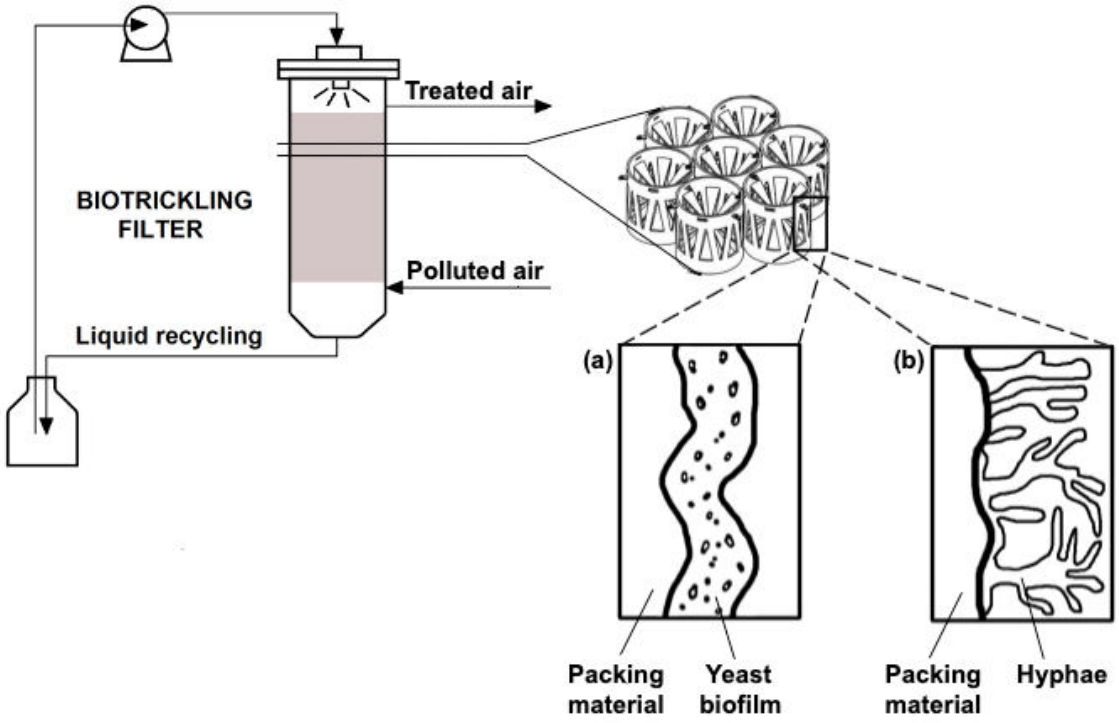


Figure 4. Representation of fungal growth in biotrickling filter. (a) yeast biofilm, (b) mould fungi growth.

Most examples of the use of fungi in biofiltration involve moulds, due to their filamentous mycelium formation. However, the possibility of inoculating biofilter beds with isolated yeast strains is being investigated more frequently, due to the latest optimistic literature reports on this subject [151,164,167,168]. Yeast forms a thin layer (biofilm) on the surface of the inhabited packing material, which performs significant functions during the biofilter process (Figure 4 a). During the biofiltration process, pollutants from the air are absorbed on the surface of the biofilm, while during biotrickling filtration, VOCs are first dissolved in the trickling liquid medium flowing through the biofilm column. Only in the next stage will waste particles penetrate directly into the biofilm. The extracellular polymeric substance (EPS) is responsible for the coherence of the biofilm, and is also its main component (50-80% by weight of the entire biofilm) [169]. The EPS has both hydrophilic and hydrophobic properties, which facilitate the adhesion of both hydrophilic and hydrophobic gas molecules, despite the low solubility of the latter in water [170]. Späth et al. (1998) showed during sorption experiments with benzene, toluene and

xylene for sewage sludge that the extracellular matrix of EPS is a hydrogel and therefore has a hydrophilic character, but also contains hydrophobic regions [171]. The results in which the removed compounds were accumulated in the EPS matrix are presented. Thus, biofilms can react dynamically to sorption processes, while the exact location of hydrophobic VOC sorption in the extracellular matrix is not yet known [172].

In conclusion, there is experimental evidence that the removal efficiency of hydrophobic compounds can be improved by using fungal biofilters. However, literature information on fungal biofiltration of most hydrophobic VOCs is not sufficient to implement this technology in industrial practice. There is a need to conduct such research that will allow us to gain detailed information and experience. Therefore, further research is still needed to optimize this solution and eliminate potential disadvantages associated with these types of new solutions. Further research into the exact mechanisms involved in fungal biofiltration is also needed.

2. Aim of the research work

The development of new process solutions for the removal of hydrophobic VOCs represents a great technological challenge and requires a multifaceted approach, as indicated in the previous chapter of this dissertation. It includes two main aspects: the equipment and process part and the microbiological part (including selection of the appropriate species of microorganisms).

The aim of this PhD dissertation was to develop a hydrophobic VOC air deodorization system from biofilters inhabited by various species of microorganisms. Information collected on the basis of literature reviews in two review publications (P1 and P2) resulted in a decision to develop a fungal BTF. The use of a fungal BTF allows for greater control of process variables compared to bacterial biofilters and enables the removal of gaseous pollutants at higher VOC load rates than in BF. The efficacy of the developed solutions was checked using model organism *Candida subhashii* in BF and BTF to remove a mixture of hydrophobic VOCs on a laboratory scale.

The following tasks were planned to gain the aim:

T1 - Selection of the best biological method of air deodorization and comparison of microorganisms used in biofiltration in terms of their ability to remove hydrophobic compounds based on a literature review.

T2 - Identification of a fungus isolate, previously not used in biofiltration, which will effectively remove hydrophobic VOCs from the air.

- Isolation of pure fungal cultures from peat used in the biotrickling filtration process;
- Selection of fungal isolates capable of using hydrophobic VOCs as a carbon source;
- Species identification of fungi capable of decomposing selected VOCs (selection of a fungus isolate – *C. subhashii*, not used in biotrickling filtration so far, capable of removing selected hydrophobic VOCs from the air).

T3 - Development of a quick and low-cost method of fungal cell immobilization on internal packing material and development of a simple and low-cost method for monitoring the condition of the fungi used during the biofiltration process.

- Development of a new method for the immobilization of yeast and mould on various types of artificial packing materials used in BTF;
- Selection of the material most effectively inhabited by selected fungal isolates;
- Designing and developing methods for evaluating the settlement of fungi and their viability on the support material.

T4 - Elaboration of method allowing for assessment of the fungi immobilization and monitoring their condition during the biofiltration process.

T5 - Checking the ability of the selected fungus to use a mixture of hydrophobic VOCs from the gas phase as a carbon source in BTF.

- Comparison of the efficiency of this process in BT and BTF.

T6 - Comparison of selected fungus with the species most effectively used in biofiltration (according to prior literature) in their ability to use a mixture of hydrophobic VOCs from the gas phase as a carbon source.

3. Description of the research

The implementation of the research objectives has been presented in the scientific publications that are the basis of this doctoral dissertation. The dissertation is based on two review publications (1-2) and four publications (3-6) in which the results and conclusions of the research were presented:

P1 - **Gospodarek, M**., Rybarczyk, P., Szulczyński, B., & Gębicki, J. (2019). Comparative evaluation of selected biological methods for the removal of hydrophilic and hydrophobic odorous VOCs from air. *Processes*, 7(4), 187. doi.org/10.3390/pr7040187 (IF=3.352)

P2 - **Marycz, M.**, Brillowska-Dąbrowska, A., Muñoz R., Gębicki, J. (2022). A state of the art review on the use of fungi in biofiltration to remove volatile hydrophobic pollutants. *Reviews in Environmental Science and Bio/Technology*, 21(1), 225-246. doi.org/10.1007/s11157-021-09608-7 (IF=14.284)

P3 - **Marycz, M.**, Brillowska-Dąbrowska, A., Gębicki, J. (2020). Evaluation of immobilization of selected peat-isolated yeast strains of the species *Candida albicans* and *Candida subhashii* on the surface of artificial support materials used for biotrickling filtration. *Processes*, *8*(7), 801. doi.org/10.3390/pr8070801 (IF=3.352)

P4 - Rybarczyk, P., **Marycz, M.**, Szulczyński, B., Brillowska-Dąbrowska, A., Rybarczyk, A., Gębicki, J. (2021). Removal of cyclohexane and ethanol from air in biotrickling filters inoculated with *Candida albicans* and *Candida subhashii*. *Archives of Environmental Protection*, 47(1), 16-34. doi.org/10.24425/aep.2021.136445 (IF=1.872)

P5 - **Marycz, M.**, Rodríguez, Y., Gębicki, J., Muñoz, R. (2022). Systematic comparison of a biotrickling filter and a conventional filter for the removal of a mixture of hydrophobic VOCs by *Candida subhashii*. *Chemosphere*, 135608. doi.org/10.1016/j.chemosphere.2022.135608 (IF=8.943)

P6 - **Marycz, M.**, Brillowska-Dąbrowska, A., Cantera, S., Gębicki, J., & Muñoz, R. (2023). Fungal co-culture improves the biodegradation of hydrophobic VOCs gas mixtures in conventional biofilters and biotrickling filters. *Chemosphere*, *313*, 137609. doi.org/10.1016/j.chemosphere.2022.137609 (IF=8.943)

3.1. Publication 1: Comparative evaluation of selected biological methods for the removal of hydrophilic and hydrophobic odorous VOCs from air

The first review publication in the series, which is the basis of this doctoral dissertation, is devoted to the comparison of selected biological methods of removing various air pollutants, both hydrophilic and hydrophobic VOCs, and common inorganic odour compounds. The strategy of comparing BF, BTF and bioscrubbers in terms of their suitability for effective odour removal was based on the criteria and their sub-criteria. The following aspects of biofiltration were analysed: efficiency (removal efficiency (RE) and empty bed residence time (EBRT)), costs (investment and operation), technical aspects and problems (periodic replacement of packing elements, periodic liquid regeneration, system complexity and ease of process control) and impact on the environment (dimensions of apparatus and waste streams). A pairwise comparison model was used to compare these methods in terms of their suitability for effective VOC removal. This model was used to assess the effectiveness of selected biological processes in terms of the aforementioned criteria and sub-criteria. In addition, for the same purpose, a decision tree was prepared to verify the input and output data of the pairwise comparison model in various ways.

Taking into account both the economic, technical and environmental aspects, the obtained test results showed that BTFs and BFs are best suited for purifying air of hydrophobic VOCs. However, both BFs and BTFs still encounter problems during reduction of hydrophobic VOCs. Indeed, due to the low water solubility of hydrophobic VOCs, these pollutants are poorly absorbed in recirculating aqueous solution or in bacterial biofilms. In this context, fungal colonization of inert packing materials in BTFs and BFs were chosen for further experimental study after consideration was taken of all relevant criteria, with particular weighting given to the economic and processing constrains of this research project, as well as in purpose overcome the mentioned operational problems.

processes

MDPI

Article

Comparative Evaluation of Selected Biological Methods for the Removal of Hydrophilic and Hydrophobic Odorous VOCs from Air

Milena Gospodarek *, Piotr Rybarczyk, Bartosz Szulczyński and Jacek Gębicki

Department of Process Engineering and Chemical Technology, Faculty of Chemistry, Gdańsk University of Technology, Narutowicza 11/12 Street, 80-233 Gdańsk, Poland; piotr.rybarczyk@pg.edu.pl (P.R.); bartosz.szulczynski@pg.edu.pl (B.S.); jacek.gebicki@pg.edu.pl (J.G.)

* Correspondence: milena.gospodarek@pg.edu.pl; Tel.: +48-58-347-20-58

Received: 8 March 2019; Accepted: 28 March 2019; Published: 1 April 2019

Abstract: Due to increasingly stringent legal regulations as well as increasing social awareness, the removal of odorous volatile organic compounds (VOCs) from air is gaining importance. This paper presents the strategy to compare selected biological methods intended for the removal of different air pollutants, especially of odorous character. Biofiltration, biotrickling filtration and bioscrubbing technologies are evaluated in terms of their suitability for the effective removal of either hydrophilic or hydrophobic VOCs as well as typical inorganic odorous compounds. A pairwise comparison model was used to assess the performance of selected biological processes of air treatment. Process efficiency, economic, technical and environmental aspects of the treatment methods are taken into consideration. The results of the calculations reveal that biotrickling filtration is the most efficient method for the removal of hydrophilic VOCs while biofilters enable the most efficient removal of hydrophobic VOCs. Additionally, a simple approach for preliminary method selection based on a decision tree is proposed. The presented evaluation strategies may be especially helpful when considering the treatment strategy for air polluted with various types of odorous compounds.

Keywords: air deodorization; comparison; biofiltration; volatile organic compound; decision tree

1. Introduction

1.1. Air Deodorization by Biological Methods

Pollution caused by VOCs and other air pollutants, especially odorous compounds, including organic and inorganic compounds, including nitrogen-containing compounds (NH_3, amines) and sulfur-containing compounds (H_2S, mercaptans, sulfides), have adverse effects on both humans and the environment [1–3]. Odorants have been proven to pose toxic effects on human health as well as to negatively influence the quality of life. Thus, a lot of attention has been devoted to the emission control of VOCs and other odorous pollutants in recent years [4,5].

Odorous compounds are emitted from many sectors of human activity, including wastewater treatment plants, communal waste landfills, agriculture and plenty of industrial facilities e.g., crude oil refineries, pulp and paper mills and various chemical industries. Additionally, the need for indoor air treatment is gaining interest [6–11]. Such emissions are controlled by various deodorization techniques. The following methods are most often applied: thermal oxidation, absorption (in water or with chemical reaction), adsorption, masking and biological techniques. The selection of the most appropriate methods is case-specific and depends on the properties of a gas stream, concentration of pollutants, emission source and the desired level of gas deodorization [1,5,12–15].

Among the aforementioned gas deodorization methods, the group of biological methods seems to be superior, especially with the perspective of environment, economy and sustainable development. Biological methods are characterized by low operating costs, low secondary pollution and very high purification efficiency when treating large volumes of gases containing low and medium concentrations of pollutants [16–18]. The process of biological gas treatment is most commonly referred to as "biofiltration". The most common apparatus intended for air biofiltration include conventional biofilters (BF), bioscrubbers (BS) and biotrickling filters (BTF) [19–21]. Beside the differences in the apparatus design, the mechanism of the air treatment process is similar.

The process of biofiltration is based on the degradation of gas contaminants as a result of biological activity of microorganisms inhabiting the porous packing of the biofilter. The microbes are especially present in so-called biofilm developing over the surface of packing elements. The mechanism of the process consists in the diffusion of pollutants from the gas phase to the biofilm surrounded by a liquid phase. This liquid phase may either be supplied as a trickling liquid (biotrickling filter) or may result from former gas humidification (conventional biofilter). Thus, pollutants from the gas phase are either adsorbed on or absorbed by the biofilm and then undergo biodegradation. The stream of treated (cleaned) air leaves the biofilter together with formation of CO_2, H_2O and biomass as biodegradation products [5,22,23] (Figure 1).

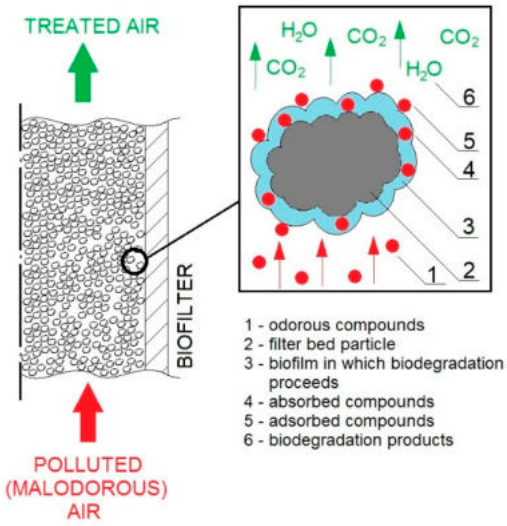


Figure 1. General mechanism of biofiltration.

A conventional biofilter is usually packed with a bed made of organic materials (wood chips, cones, peat) that are naturally colonized by microorganisms capable of degrading various air pollutant impurities. The contaminated gas is humidified in a separate chamber prior to entering the biofilter [24,25].

In the case of BTF, the filter packing is made of inert natural or synthetic materials (ceramic elements, polyurethane foam, lava rock). Such a packing requires inoculation of microbes prior the process start-up and the role of the packing is mainly to give a physical support for the biofilm development. BTF uses a trickling liquid, usually enriched with additional nutrients for microorganisms. Such a configuration enables the pollutants' adsorption/absorption and biodegradation in one apparatus [19,26].

Biological air treatment in bioscrubbers consists in two main processes: absorption of gas contaminants in the liquid phase and biodegradation of these pollutants with the use of additional bioreactors, enabling the liquid regeneration, aeration and circulation [1].

Typical processes of biological air treatment are designed for the removal of water-soluble compounds. The efficiency and the rate of biofiltration of hydrophilic compounds is mainly dependent on the rate of their biodegradation in the biofilm. However, when hydrophobic gas pollutants are considered, the biofiltration efficiency depends drastically on the transfer rate of the components from the gas phase to the liquid, usually aqueous phase [27–29]. This is why biofiltration of hydrophobic

compounds precedes with much lower efficiency than hydrophilic compounds and currently the improvement of the efficiency of hydrophobic compounds biofiltration is a challenge in the design of the biological treatment processes [16,30].

Depending on the air pollutant type and concentrations, one of above discussed biological treatment methods may be chosen. Biological methods of air treatment have been increasingly investigated since the beginning of the 1990s. Firstly, the research concentrated mainly on the effect of basic parameters on the process performance [5,22,31–33]. Furthermore, more in-depth research on the biological aspects have been developing, including the biotechnological assays for the composition of microbial composition of the biofilm. Currently, the research is focused on the improvement of the removal of hydrophobic air pollutants, biofiltration of which is usually limited by low mass transfer rate from the gas to the liquid (biofilm) phase. Parallel to typical experimental investigations, also in the semi-pilot and pilot scales, modeling of these processes is investigated and developed [34–37]. Examples of the latest research include the application of fungi for biofiltration [38], modeling of serial biofiltration unit [39], upgrading of biogas in biofilters [40] or biomass overgrowth control strategies [41]. Research in the field of biotrickling filtration is devoted to e.g., small-scale applications for indoor air treatment [42], application of new strands of microbes [43] or process scaling-up [44]. Examples of current research on bioscrubbers are the emission control of NH_3 from agricultural applications [45] or treatment of air polluted with H2S and others [46–48].

The selection of the most suitable treatment method is a function of several factors, especially when mixtures of hydrophilic or hydrophobic compounds are considered. In this perspective, a broad set of data or mathematical tools aiding the decision-making may be of importance. In the literature, several papers devoted to comparison of various deodorization methods [12,49–51] may be found. However, these papers mainly present the experimental results and economic analyses or compares different processes/process conditions, leaving the reader with general ideas about the processes discussed. Therefore, the development of a comparative tool for the selection of the treatment method is of both scientific and practical importance.

1.2. Assessment of Biological Methods of Air Treatment

In this paper, two approaches of comparative analysis or selection criteria for the treatment of air polluted with various volatile compounds are investigated. An evaluation methodology based on the procedure described by Oliva et al. [15] is proposed in this paper. The adopted method is derived from a pairwise comparison model, using numerical judgments from an absolute scale of numbers. This method was initially proposed by Henri Lebesgue and it enables the comparison of the examined objects, with the aid of the analysis of their properties and selection of the appropriate scale [52]. It is based on comparing elements, in order to receive their assessment, based on a preference. This method also allows to choose which of the analyzed elements are characterized by a larger number of the selected quantitative properties. Pairwise comparison is often a crucial step in multi-criteria decision analysis [53]. This method was chosen due to the fact that it allows for a fair division and balance of the final value into individual components [54,55]. Similar comparisons are made in other fields of science, e.g., assessment of fuels [56], voting system [57–59], psychology [60,61], artificial intelligence system [62] and others [63–65]. They key element is to provide a tool for making an objective comparison taking into account the division into various aspects of a given field.

Additionally, a decision tree procedure for the selection of a treatment technology is proposed. Decision tree is a graphical method of supporting the decision-making process, which can be used for different types of modeled variable, i.e., continuous or discrete. The goal is to create a model that predicts the value of a target variable based on several input variables [66,67]. The main task of the decision tree method is to generate mutually exclusive regions in which there are as many samples as possible classified into one group. These regions are created by successive divisions of the training set, using binary logical rules [68]. The learning process is carried out to obtain the most homogeneous group of sample sets. As an algorithm output, decision trees can provide two types of information:

the description of which group the examined object is located in or the probability of belonging to a given group [69].

1.3. Aim of Investigations

The aim of this paper is to provide a comprehensive assessment of the selected biological methods for the removal of various volatile compounds from air i.e., biofiltration, biotrickilng filtration and biscrubbing with the use of a pairwise comparison model as well as decision tree procedure.

This paper presents three interesting elements from the novelty viewpoint. Firstly, the paper revises the results of selected recent research on the application of biological methods for selected air pollutants, thus it may serve as a source of experimental results. Secondly, the authors adopted a comparison procedure for evaluating the holistic effects of performance, costs and technical aspects of treating air with a given method. Thirdly, a simple tool for preliminary selection of the method is proposed. Such an approach is hardly met in the literature and presents useful way of comparing processes.

2. Materials and Methods

2.1. Data Collection

For the purpose of calculations for a pairwise model as well as the development of a decision tree, literature data was used. For literature search, Science Direct, PubMed and MDPI databases were applied. Articles from last 10 years were selected with the priority of choosing, however older articles were used as well (depending e.g., on the target chemical compound in a given treatment method). Selection of literature data was applied according to the target compound so as to collect data suitable for comparison purposes. Data applied in calculations were taken directly from the literature without normalization procedures.

2.2. Comparative Analysis

A comparative analysis of biological methods in the perspective of the removal of hydrophilic, hydrophobic or inorganic odorous gases is presented with the use of a pairwise comparison model [52]. The numerical procedure used is based on the quantification of a set of parameters previously classified in clusters. It is used to select the best biological process of air purification from impurities. The results obtained on the basis of the semi-quantitative ranking of selected parameters pointed to the advantages and disadvantages of the processes studied. For the purpose of comparison, the focus is on the process performance, technical, economic as well as environmental aspects. The applied method consists in assigning specific values to all highlighted alternatives. Calculations are realized in four main stages i.e., selection of the main criteria determining the deodorization process (C_1–C_4) as well as selection of sub-criteria affecting the main criteria ($C_{1.1}$–$C_{4.2}$); assigning weights to each criterion and sub-criterion ($\boldsymbol{w_i}$ and $\boldsymbol{w_{ij}}$); assigning indicators to sub-criteria (values in the range between 0 and 1); calculation of the results of all alternatives.

The results of all alternatives were obtained using the following equations [28]:

$$R_i = w_{i,j1} \cdot r_{i,j1} + w_{i,j2} \cdot r_{i,j2} + \ldots + w_{i,jn} \cdot r_{i,jn} \; ; \; i = 1, \ldots, n; j = 1, \ldots, n \tag{1}$$

$$R = w_{i1} \cdot R_{i1} + w_{i2} \cdot R_{i2} + \ldots + w_{in} \cdot R_{in} \; ; \; i = 1, \ldots, n; j = 1, \ldots, n \tag{2}$$

where:

$w_{i,j}$ - weight of a given sub-criterion $C_{i,j}$,
$r_{i,j}$ - result of an alternative to the sub-criterion $C_{i,j}$,
R_i - result of an alternative to the criterion C_i,
w_i - weight of given criterion $C_{i,j}$,

R - overall result of the alternative,
n - number of analyzed criteria.

2.3. Decision Tree for the Preliminary Selection of the Deodorization Method

In the second part of this paper, a decision tree procedure was applied. The decision tree has been built based on data from 57 biological processes of removing various chemical compounds from the air i.e., conventional biofiltration, biotrickling filters and bioscrubbers. The data set is presented in Table 5. The following process parameters were selected as input variables: inlet concentration, Henry's constant and empty bed residence time (EBRT). As the result of the decision tree, the most optimal air purification method may be indicated. All calculations were performed in RStudio 1.1.463 [70] using the 'rpart' library [71].

The main advantages of using decision trees are their non-parametric character as well as the automatic identification of the most significant variables by the algorithm and the elimination of statistically insignificant variables. Moreover, the mathematical transformation (e.g., logarithm) of one or more explanatory variables does not change the structure of the tree which is changed only by threshold values. Among the disadvantages should be indicated: a slight modification of the training set (e.g., removal of several observations) can radically change the structure of the tree. In addition, in one step the tree can divide the space only in relation to one variable (in other words: the dividing lines are always perpendicular to the divided axis in the variable space).

2.4. Calculation of Process Performance Parameters

Removal efficiency and empty bed residence time were selected as parameters presenting process performance. These parameters indicate both the degree of the removal of odorous compounds from air as well as gives the information about the rate of the process course based on the air flow rate and the capacity of a bioreactor. Values of RE and EBRT were either taken directly from literature data or calculated according to Formulae (3) and (4):

$$\mathrm{RE} = \left(\frac{\mathrm{C_{in}} - \mathrm{C_{out}}}{\mathrm{C_{in}}}\right) \cdot 100\% \quad (3)$$

$$\mathrm{EBRT} = \frac{\mathrm{V}}{\mathrm{Q}} \quad (4)$$

where:

$\mathrm{C_{in}}$ - inlet concentration of target compound,
$\mathrm{C_{out}}$ - outlet concentration of target compound,
V - volume of the filter bed,
Q - inlet gas flow rate.

3. Results and Discussion

3.1. Selection of Comparison Main Criteria

In order to characterize biological methods of air deodorization, four main criteria were selected: efficiency/process performance, costs, technical aspects and problems as well as the environmental impact. The aforementioned criteria have been chosen in the perspective of possibly complete evaluation of each analyzed treatment method. The selection criteria are presented in Figure 2.

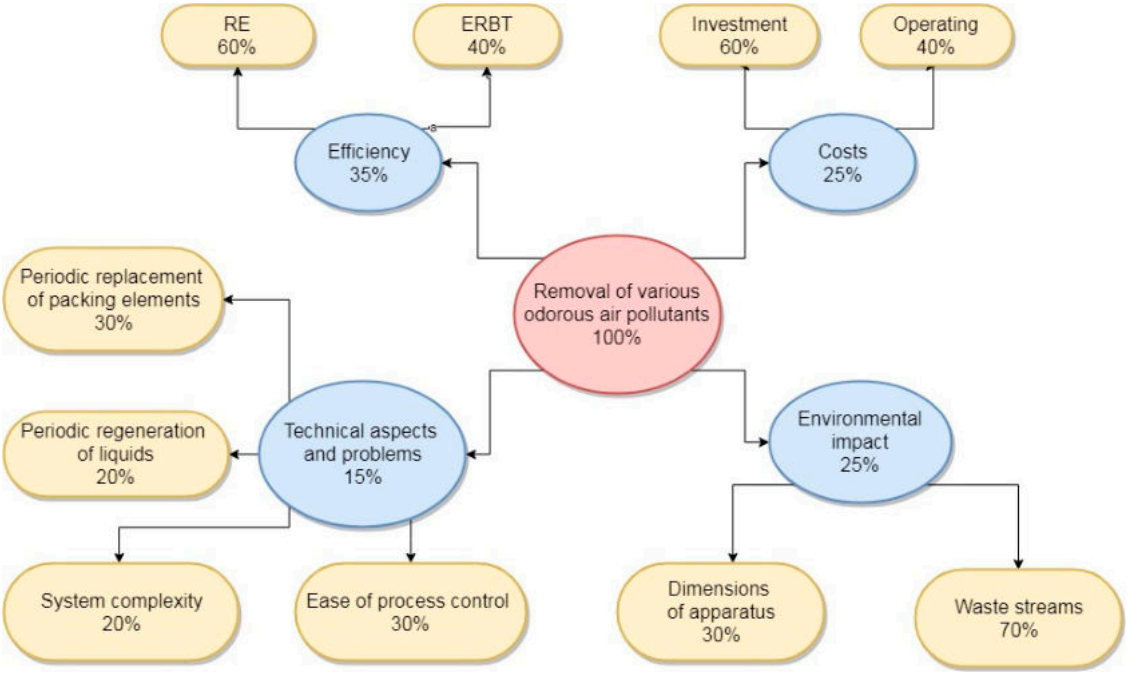


Figure 2. Hierarchy of selected criteria and sub-criteria.

Each of the selected criteria has been divided into second-order criteria. As part of the performance criterion, two sub-criteria were distinguished: removal efficiency (RE) and empty bed residence time (EBRT) [1,4,5,72]. Values of RE inform about the degree of air purification and enable to differentiate among the process performance of analyzed methods. Comparison of the values of empty bed residence time allows for the assessment of the rate of the assessed treatment processes. The criterion related to the costs include investment and operating costs as sub-criteria [12,19,73,74]. These costs are crucial for the process realization, especially when industrial-scale applications are considered.

The authors of this manuscript have proposed two more criteria related to the deodorization strategy i.e., technical problems arising during the process [1,5,19,20,31] as well as the environmental impact of the technology [12,73–75]. Among the technical aspects and possible technical problems, which are thought to greatly affect the long-term process operation, following sub-criteria have been distinguished: periodic replacement of packing elements, the need for periodic regeneration of the liquid, the complexity of the system, as well as the ease of process control and the ability to adapt the system to changes in the charge of current regulation. These sub-criteria have been proposed based on a literature review.

The following sub-criteria have been proposed when the environmental impact of the deodorization technique is considered: dimensions of the apparatus and waste streams generated during the treatment process. Depending on the flow rate of treated air streams, the dimensions of the apparatus may greatly differ (laboratory to industrial scale) [75]. In this perspective, conventional biofilters, especially the open-type, occupy plenty of land area, resulting in a high foot-print. Sizes of biotrickling filters are much smaller. On the other hand, the operation of bioscrubbers generates high volumes of liquid containing compounds absorbed from air and these liquid streams must be further processed [1,5]. This is why the sub-criteria including apparatus size and waste streams generation have been proposed.

3.2. Assigning of Weights to Criteria and Sub-Criteria

The choice of criteria and sub-criteria was made on the basis of a literature review. A literature review was prepared using peer-reviewed journals, other professional literature [72] as well as conference materials. When choosing and proposing the values of the criteria, the authors took

advantage of the process experience of the team [13,21,25,76]. Having the above set of data, the authors attributed the criteria and sub-criteria to the weights, reflecting the importance of the aspect to the entire process of biological purification of air from pollutants. The values of weights are given in Table 1.

Table 1. Weight of the criteria and sub-criteria and ranges of sub-criteria indicators.

Criterion	w_i (%)	Sub-Criterion	$w_{i,j}$ (%)	Range of the Indicators (ID)
C_1 (Efficiency)	35	$C_{1.1}$ (RE)	60	0 . . . 1 (low . . . high)
		$C_{1.2}$ (EBRT)	40	1 . . . 0 (low . . . high)
C_2 (Costs)	25	$C_{2.1}$ (Investment costs)	60	1 . . . 0 (low . . . high)
		$C_{2.2}$ (Operating costs)	40	1 . . . 0 (low . . . high)
C_3 (Technical aspects and problems)	15	$C_{3.1}$ (Periodic replacement of packing elements)	30	1 . . . 0 (slow . . . fast)
		$C_{3.2}$ (Periodic regeneration of liquids)	20	1 . . . 0 (rarely . . . often)
		$C_{3.3}$ (System complexity)	20	1 . . . 0 (low . . . high)
		$C_{3.4}$ (Ease of process control)	30	0 . . . 1 (complex . . . simple)
C_4 (Environmental impact)	25	$C_{4.1}$ (Dimensions of apparatus)	30	1 . . . 0 (small . . . big)
		$C_{4.2}$ (Waste streams)	70	1 . . . 0 (yes . . . no)

3.3. Assigning of Indicators

Based on the literature data presented in Table 2; in Table 3, indexes were assigned to indicators taking into account the "bigger is better" principle, i.e., values were assigned from the range from 0 to 1, where the value of 1 is the most favorable considering the whole group of analyzed methods, as given in Table 2. Each criterion and sub-criterion has been transformed into an indicator (values assigned to sub-criteria $C_{1.1}$–$C_{4.2}$).

The differences in the hydrophilic character of the compounds were estimated using Henry's law constant. The greater the Henry's law constant, the greater the volatility and the lower the solubility of a compound, which is valid for dilute solutions and non-reacting gases at near ambient pressure and temperature. The Henry's law constant (H_c) can be expressed as the dimensionless ratio between the aqueous-phase concentration C_a of a species and its gas-phase concentration C_g [77]:

$$H_C = \frac{C_g}{C_a} \tag{5}$$

Taking into account Equation (4), values of dimensionless Henry's constant in Table 2 were calculated using Formula (5):

$$H_C = \frac{RF}{H} \tag{6}$$

where:

RF - recalculation factor equal to 4.03395×10^{-4}, taken from [77],
H - Henry's constant given as ($mol \cdot m^{-3} \cdot Pa^{-1}$) in Table 2.

Table 2. Literature data for determining the values of sub-criteria 1.1 and 1.2.

			Conventional Biofilter				Biotrickling Filter				Bioscrubber			
Compounds	**H ($mol \cdot m^{-3} \cdot Pa^{-1}$)**	**H_c (-)**	**C_{in} ($mg \cdot m^{-3}$)**	**RE (%)**	**EBRT (s)**	**Reference**	**C_{in} ($mg \cdot m^{-3}$)**	**RE (%)**	**EBRT (s)**	**Reference**	**C_{in} ($mg \cdot m^{-3}$)**	**RE (%)**	**EBRT (s)**	**Reference**
Hydrophilic	-	-	-	99	-	[73]	-	99	-	[73]	-	99	-	[73]
butanol	1.2 [77]	3.4×10^{-4}	900–2600	>73	60	[78]	400–1200	15–99	60–124	[79]	-	98–100	48	[80]
aniline	1.1 [5]	3.7×10^{-4}	-	-	-	-	300	<99	42–166	[81]	-	-	-	-
isopropanol	1.3 [77]	3.1×10^{-4}	1000–8000	81	94.2	[82]	20–65 ($g\ m^{-3} \cdot h^{-1}$)	<95	14–160	[83]	200–500	99	-	[84]
ethanol	9.0 [77]	4.5×10^{-5}	3700	63–85	101	[85]	470	~80	66	[86]	-	80–99	-	[87]
methanol	2.0 [77]	2.0×10^{-4}	-	>95	25	[88]	300–37,000	65	20–65	[89]	50–100	69–81	600	[90]
			0.79–3.3	93.33	38	[91]					-	75	2.5	[92]
Hydrophobic	-	-	-	75	-	[73]	-	50	-	[73]	-	50	-	[73]
hexane	6.1×10^{-3} [77]	6.6×10^{-2}	500–11,000	79	60	[93]	600	57–91	8–30	[94]	6200	70	420	[92]
			200–10,000	59–76	60	[95]								
methane	1.4×10^{-5} [77]	2.9×10^{-1}	4581–4908	43	257	[96]	0–500	~40	240	[97]	-	5–25	1.6	[98]
ethylene	5.9×10^{-5} [77]	6.8	331	100	2160	[99]	8–100 ($g\ m^{-3} \cdot h^{-1}$)	70–95	30	[27,100]	-	-	-	-
α-pinene	$2.9 \cdot \times 10^{-4}$ [77]	1.4	100–450	90	42	[101]	-	-	14–60	[102]	-	-	-	-
			4227	47–67	78	[103]								
styrene	2.7×10^{-3} [77]	1.5×10^{-1}	0.1–0.9	90	9–18	[104]	800–3300	95	60–120	[105]	-	-	-	-
			0.85	97	1845	[106]	55–312	90	15–30	[107]	-	-	-	-
			2.3	90	137–825	[108]	2–1128	99	400	[109]				
toluene	1.5×10^{-3} [77]	2.7×10^{-1}	1.9	>80	21.6	[111]	2200	<99	16.2	[112]	3300	89	-	[110]
			6	<98	70	[113]	1000	60	57	[114]				
Inorganic														
ammonia	5.5×10^{-1} [77]	7.3×10^{-4}	14–350	92–100	17	[115]	9.6	82	1.2	[116]	14	99	142	[117]
							20–100	99	960	[118]				
H2S	1.0×10^{-3} [77]	4.0×10^{-1}	7–3750	100	23–200	[119]	300–650	65–100	53-79	[120]	14–140	98	12–32	[47]

Table 3. Literature data for determining the values of sub-criteria 2.1–4.2 (investment and operating costs are presented for a flowrate 50,000 m^3 h^{-1}).

	Criteria and Sub-Criteria	Units	BF	BTF	BS	Reference
2			Costs			
			Investment			
2.1		(€ per $m^3 \cdot h^{-1}$)	6	11	4	[73]
		(€ per $m^3 \cdot h^{-1}$)	5	10	4	[12]
			Operating			
2.2		(€ per $m^3 \cdot h^{-1}$)	2	1.2	3.6	[74]
		(€$\cdot 10^{-4} \cdot m^{-3}$)	0.2	0.1	0.28	[73]
3			Technical aspects and problems			
			Periodic replacement of element			
3.1	Packing material	(years)	2	10	10	[73]
	Annual/material-reagents	(kg $m^3 \cdot h$)	4	0.1	0.1	[73]
	Packing material	(%)	47	44	4	[74]
			Periodic regeneration of liquids			
3.2	Annual water consumption	(L$\cdot m^{-3} \cdot h \cdot 10^2$)	2.4	6.3	3.3	[73]
	Water that can be replaced with secondary effluent	(-)	Possible	Possible	Impossible	[73]
			Complexity of the system			
3.3	Basic elements of the apparatus	(-)	-Humidification chamber -Packed bioreactor	-Packed bioreactor -Liquid container -Pump	-Absorption column -Pump -Absorbent tank	[5]
	number of basic elements of the apparatus	(-)	2	3	3	(-)
			Ease of process control			
3.4	impact on the control process	V	Low	High	Medium	(-)
	Customization at work	(-)	Impossible	High	Medium	(-)
4			Environmental impact			
			Dimensions of the apparatus			
4.1	The size of the apparatus	[$m^2 \cdot m^{-3} \cdot h \cdot 10^2$]	1.75	0.25	0.1	[73]
	Surface area	(-)	High	Low	Low	[87]
			Waste streams			
4.2	Use of filling	(-)	Possible	Impossible	Impossible	[73]
	The possibility of replacing water with sewage	(-)	Possible	Possible	Impossible	[12]
	Volume of liquid	vol<<VOL	-	vol	VOL	[12]
	-	-	Very low	Medium	High	[-]

The values of indicators (Figure 3) are proposed and derived from data presented in Table 2; Table 3 as well as additional literature data [1,19,20,31].

The values of indicators for investment and operating costs are presented in Figure 3a. The obtained results indicate that BSs are characterized by the lowest investment costs (ID = 1), while the highest investment costs are found for BTFs (ID = 0.3). Such a result may be justified by the number of basic elements of apparatus included in the system (BTF contains the highest number of elements, i.e., biofilter chamber, packed bed, circulating pumps, liquid containers, trickling system).

Interestingly, the lowest operating costs are associated with BTF operation (ID = 1), while the highest are attributed to bioscrubbers. The operating costs of BFs (ID = 0.66) are medium in the compared group of methods. It mainly results from the periodic maintenance requirements, as described more precisely by the technical-aspects-and-problems sub-criterion. The BS process is associated with the highest operating costs (ID = 0.33).

Figure 3b shows the values of indicators for periodic replacement of packing elements, periodic regeneration of liquid, complexity of the system and possibility of process control. The literature indicates that the packing material in BTF (ID = 1) should be replaced the least frequently. It is mainly because of the fact that inert, ceramic or synthetic materials are applied as packing materials and their durability is much higher than for natural packing materials, used in BFs [1,5]. By contrast, the results indicate that BF requires the most frequent replacement of the packed bed (ID = 0.2) because BFs are usually packed with natural organic packing materials. The replacement of liquid, due to apparatus construction, does not typically apply for BF (ID = 1). Interestingly, in terms of BS and BTF it is possible to replace water with so called secondary wastewater [73]. Periodic liquid replacement is the biggest problem in the case of BS (ID = 0.3), despite the fact that water consumption compared to the other two processes is at an average level. This results from the inability to use "wastewater" for secondary

use. BTF and BS are characterized by the greatest complexity of the system (ID = 0.8). They have three or more basic elements of the apparatus (e.g., bioreactors, packing elements, pumps, trickling system, absorption column etc.). BF, on the other hand, is a relatively simple system (ID = 1), having only two basic elements in its construction (humidification chamber and biofilter itself).

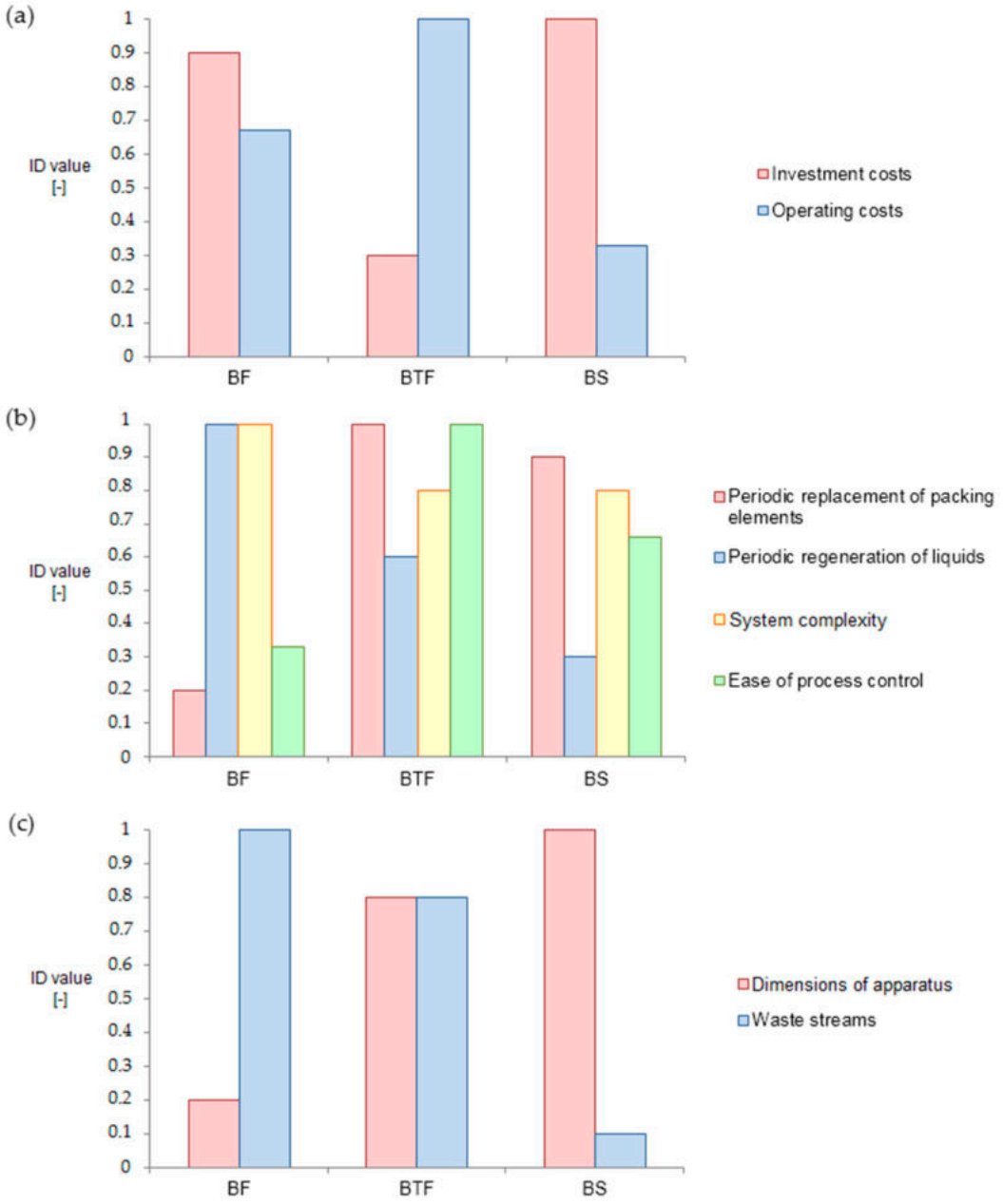


Figure 3. Diagrams presenting indicator values for each sub-criterion: (**a**) diagram of criterion 2. (**b**) diagram of criterion 3. (**c**) diagram of criterion 4.

The results regarding the possibility of a system control indicate that BTFs are characterized with ID = 1. This is because this treatment method enables the application of the most complex, effective and quickly responding control system (e.g., control and regulation of trickling liquid frequency, pH, flow rate or composition) [5,31]. The control of process realized in BS is much lower (ID = 0.67) and the lowest control possibility is for BF (ID = 0.33), indicating high inertness of the process and little regulation possibility in the case of conventional biofilters.

The size of the apparatus (both occupied surface area and capacity) is the largest for BF and this is why the ID calculated for BF is equal to 0.2 (ID = 0.2) (Figure 3c). BS has the smallest area occupied by the apparatus (ID = 1), however the volume of liquid used for the BS process is high. The amount of waste produced is the smallest in the case of BF (ID = 1). Additionally, further reuse of packing material (for anther purposes e.g., land fertilizers) as well as the replacement of water by secondary water [12] is possible. The least favorable method in terms of generation of waste streams is BS (ID = 0.1), indicating the need of further processing of generated wastewater for the recovery of absorbed compounds as well as down-stream water purification.

3.4. Results of a Pairwise Comparison

Table 4 was prepared in order to summarize the results collected during a pairwise evaluation procedure. The results contained therein q compare the cost-effectiveness of each analyzed method, in general, as well as the given criterion. Table 4 presents the aggregated results for the criterion (C1–C4) and overall result of the alternative (depicted as Summary) to highlight the weaknesses and strengths of the processes studied. The values given in Table 4 were calculated on the basis of Formulae (1) and (2).

The summary results for each investigated treatment method indicate that BTF is the best method for removing hydrophilic compounds. In the case of hydrophobic compounds, BTs are the most convenient, with BTFs presenting very similar efficiency. The removal of inorganic compounds are characterized by the same tendency. In the case of the three methods analyzed, it is least profitable to use BS. These results are supported by the literature data [1,12,19,20,30,73].

Table 4. Scores of the alternatives for each criterion.

Process	Hydrophilic Compounds			Hydrophobic Compounds			Inorganic Compounds		
	BF	**BTF**	**BS**	**BF**	**BTF**	**BS**	**BF**	**BTF**	**BS**
C1	0.76	0.93	0.59	0.90	0.88	0.44	0.71	0.67	0.65
C2	0.80	0.58	0.73	0.80	0.58	0.73	0.80	0.58	0.73
C3	0.56	0.88	0.69	0.56	0.88	0.69	0.56	0.88	0.69
C4	0.76	0.80	0.37	0.76	0.80	0.37	0.76	0.80	0.37
Summary	**0.74**	**0.77**	**0.60**	**0.78**	**0.76**	**0.56**	**0.72**	**0.71**	**0.61**

The distribution of the results of individual variants from various criteria is given in Table 4. The results show that BF is a superior technique, among others compared, for the removal of hydrophobic and inorganic compounds from air. BTF proved to be the most beneficial method for removing hydrophilic compounds from air. However, it is of worth to note that very similar results are obtained for BF and BTF for all investigated types of target compounds. The most convenient process in the perspective of exploitation and operating costs is BF, while the least-economic seems to be BTF. BTF and BS presented as the most favorable processes in terms of technical aspects and possibilities of problem elimination. Processes realized in BF and BTF are also the most environmentally-friendly.

Analyzing the obtained results for the removal of hydrophobic compounds, BF is found to be the most efficient. However, BTF attains similar performance as BF in terms of the final summary result.

In terms of efficiency of H2S and ammonia removal, BF and BTF performance is better than BS. Both methods i.e., BF and BTF are characterized by the lowest negative influence on the environment.

Figure 4 presents the percentage distribution of the results of individual variants from various criteria, based on data from Table 4. The analysis of Figure 4 allows to identify the adequacy of various methods for the removal of odorous compounds.

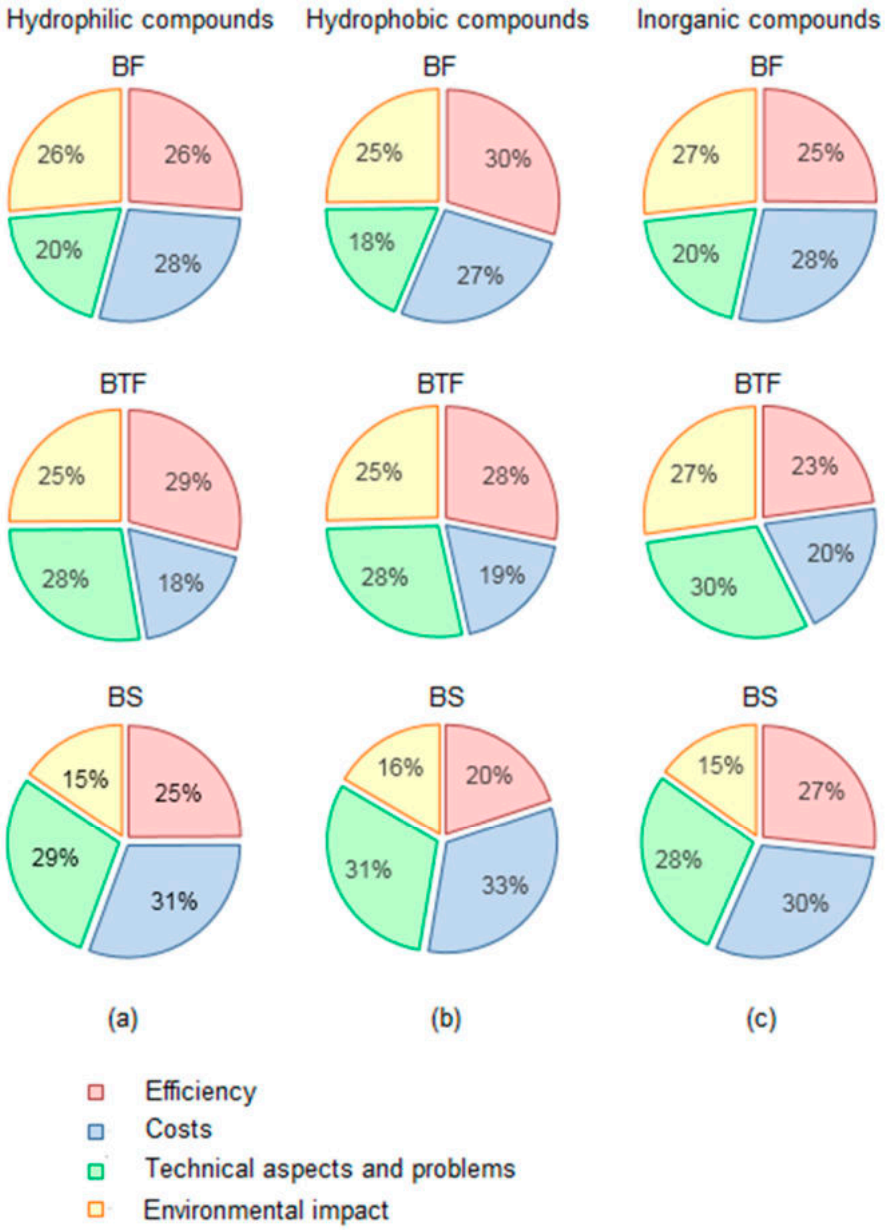


Figure 4. Distribution of alternative results related to different criteria for: (**a**) hydrophilic compounds, (**b**) hydrophobic compounds and (**c**) inorganic compounds.

The results showed that for hydrophilic compounds (Figure 4a), the highest removal efficiency is obtained using BF. BS proved to be the most beneficial due to the costs involved. Similar conclusions for BS concerned technical aspects as well as possible problems faced during the system operation. BF and BTF methods are the most environment-friendly methods. In this respect, BS differs significantly from the other two methods, due to the large production of sewage and the inability of replacing the absorbent by a secondary wastewater.

Analyzing the results for methods of hydrophobic compounds removal from air (Figure 4b), it can be stated that the greatest advantage of the BF method is high removal efficiency and environmental friendliness. The use of the BS method is reasonable when attention is paid to the costs incurred and the technical aspects together with rather low possibility of exploitation problems. In the case of BTF, the results reveal a comparable and even distribution of results for all criteria.

The results obtained for the methods in terms of the removal of inorganic compounds (Figure 4c) revealed that BTF is the optimal method when environmental issues and technical aspects with possible

exploitation problems are considered. On the other hand, BS is outstanding in terms of efficiency and costs of treatment. Due to the fact that BF is characterized by a balanced distribution of results for all analyzed criteria, it may be regarded as a suitable method of purifying air from H_2S and NH_3. Additionally, BF seems to be the best choice when none of the criteria is favored, and only optimal profitability is sought in every respect.

A similar approach of a pairwise comparison was taken up by Oliva et al. [15]. However, the comparison was focused on the advanced oxidation processes, but also included biofiltration methods. In recent years, comparative evaluation of biological methods of air treatment have been proposed by other researchers [12,73,74]. Similar to the results of a pairwise comparison presented in this paper, conventional biofilters seem to be the best choice. Comparing the obtained results with the outcomes of this paper it can be stated that bioscrubbers are the least-favored method of removing compounds of various types from the air.

3.5. Result of Decision Tree for the Preliminary Selection of the Deodorization Method

A decision tree for preliminary selection of an air deodorization method is presented in Figure 5. Table 5 presents the results of investigations used previously for the pairwise comparison procedure as well as additional data collected with the purpose of the tree development. The probability of belonging to a given group of processes is shown at the bottom of Figure 5. The presented decision tree shows, based on the input data used, that only two parameters are important when choosing the proper method i.e., the inlet concentration of a target compound and the hydrophobicity of the compound, represented by Henry's law constant.

Table 5. Data set used for the decision tree development.

Process	Compound	H ($mol \cdot m^{-3} \cdot Pa^{-1}$)	C_{in} ($mg \cdot m^{-3}$)	EBRT (s)	References
BF	butanol	1.2	2600	60	[78]
BF	isopropanol	1.3	8000	94.1	[82]
BF	ethanol	9	3700	101	[85]
BF	methanol	2	3.3	38	[91]
BF	hexane	0.0061	10,000	60	[95]
BF	methane	0.000014	4908	257	[96]
BF	ethylene	0.000059	331	2160	[99]
BF	α-pinene	0.00029	450	42	[101]
BF	styrene	0.0027	0.85	1845	[106]
BF	toluene	0.0015	1.9	21.6	[111]
BF	ammonia	0.59	350	17	[115]
BF	hydrogen sulfide	0.001	3750	200	[119]
BF	hexane	0.0061	700	30	[121]
BF	ethanol	9	3700	150	[85]
BF	phenol	0.025	1000	54	[122]
BF	dichloromethane	0.0036	175	60	[123]
BF	methylamine	0.35	136	220	[124]
BF	dimethyl sulfide	0.0056	400	27	[125]
BF	triethylamine	0.066	3000	60	[126]
BF	toluene	0.0015	2800	516	[127]
BF	styrene	0.0027	250	81	[128]
BS	butanol	1.2	1000	48	[80]
BS	isopropanol	1.3	500	60	[84]
BS	methanol	2	100	600	[90]
BS	hexane	0.0061	6200	420	[92]
BS	toluene	0.0015	3300	89	[110]
BS	ammonia	0.59	14	142	[117]
BS	hydrogen sulfide	0.001	140	32	[47]
BS	trichloroethylene	0.0011	300	931	[129]
BS	acetone	0.27	118	195	[130]

Table 5. *Cont.*

Process	Compound	H ($mol \cdot m^{-3} \cdot Pa^{-1}$)	C_{in} ($mg \cdot m^{-3}$)	EBRT (s)	References
BS	1,2-dichloroethane	0.0089	2400	300	[131]
BS	ethyl acetate	0.059	500	84	[132]
BTF	butanol	1.2	1200	124	[79]
BTF	aniline	1.1	300	166	[81]
BTF	isopropanol	1.3	65	160	[83]
BTF	ethanol	9	470	66	[86]
BTF	methanol	2	300	65	[89]
BTF	hexane	0.0061	600	30	[94]
BTF	methane	0.000014	500	240	[97]
BTF	ethylene	0.000059	100	30	[27,100]
BTF	styrene	0.0027	3300	120	[105]
BTF	toluene	0.0015	1128	400	[109]
BTF	ammonia	0.59	100	960	[118]
BTF	hydrogen sulfide	0.001	650	79	[120]
BTF	methyl mercaptan	0.0038	25	50	[28]
BTF	dimethyl sulfide	0.0056	25	123	[120]
BTF	nitrobenzene	0.64	300	24	[133]
BTF	aniline	52	60	42	[81]
BTF	trichloroethylene	0.0011	300	21	[134]
BTF	chlorobenzene	0.0027	1700	60	[135]
BTF	toluene	0.0015	1000	60	[114]
BTF	methyl acrylate	0.049	5000	400	[136]
BTF	methyl acrylate	0.049	5000	200	[136]
BTF	acetone	0.27	8000	137	[137]
BTF	styrene	0.0027	1000	90	[105]
BTF	formaldehyde	3.2	100	80	[138]
BTF	isopropanol	1.3	1000	140	[139]

The decision tree learning algorithm is a non-arbitrary algorithm. A tree was "learned" by splitting the training set into subsets based on an attribute value test. This process was repeated on each derived subset in a recursive manner called recursive partitioning. The recursion is completed when the subset at a node has all the same values of the target variable, or when splitting no longer adds value to the predictions.

In the decision tree model development, only process performance was included, while cost analysis was excluded. Considering such an approach, for high inlet concentrations (higher than 2500 $mg \cdot m^{-3}$), the best treatment option is to use the conventional biofilter. Traditional biofilters based on natural packing materials, for both hydrophilic and hydrophobic compounds, tend to be applied when relatively high inlet concentrations are used [78,82,93,95,96,119]. The use of large EBRT values is necessary for high inlet concentrations, which consequently significantly increases the dimensions of the apparatus. Organic packing materials are cheaper than synthetic ones. The use of a trickling or absorption liquid (BTF and BS) at high concentrations generates the necessity of its frequent replacement (hydrophobic compounds very quickly achieve the saturation state of the liquid), the use of surfactants or increase in the dimensions of the apparatus. The results of applied algorithms show that for the inlet concentrations lower than 2500 $mg \cdot m^{-3}$ and for hydrophilic compounds, biotrickling filtration seems to be the best treatment method. Similar results are obtained for hydrophobic compounds for the inlet concentration range between 475 and 2500 $mg \cdot m^{-3}$. If concentration is lower than 475 $mg \cdot m^{-3}$, the better choice will be the application of a conventional biofilter. Bioscrubbers may be used for compounds characterized by Henry's law constant between 0.0075 and 0.62 $mol \cdot m^{-3} \cdot Pa^{-1}$ and for inlet concentrations lower than 2500 $mg \cdot m^{-3}$. In this group of compounds, the use of a conventional biofilter should also be considered (due to the low differences in probability of belonging to a given group: 50% and 38% for the bioscrubber and conventional biofilter, respectively).

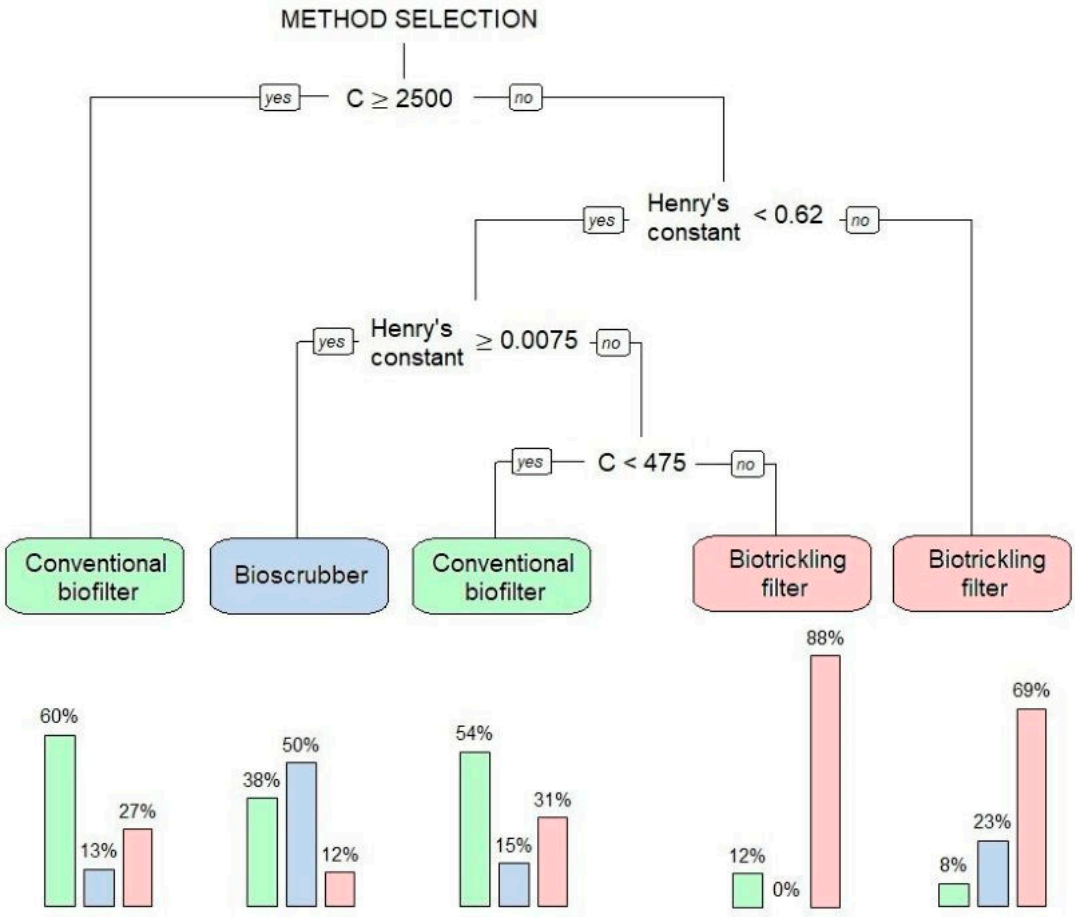


Figure 5. Developed decision tree for the selection of the most suitable biological treatment method (C—inlet concentration, $mg \cdot m^{-3}$; Henry's constant, $mol \cdot m^{-3} \cdot Pa^{-1}$).

3.6. Practical Applications and Future Research Perspectives

The presented comparative analysis together with a proposed decision tree model seem to be useful when selecting a treatment procedure for air polluted with odorous compounds. Current development of the legislation regarding the odorous quality of air implies the increased interest in sustainable and efficient deodorization methods, thus the use of biological methods with applications in industrial, agricultural as well as indoor air treatment applications will in particular be increasing in the nearby future. Additionally, currently observed development of these methods, especially when biotrickling filtration is considered, suggests highly probable possibility of eliminating most of the related problems e.g., the effective removal of hydrophobic air pollutants or efficient long-term operation of biological systems. The results of comparative assessment of investigated deodorization methods presented in this paper may, therefore, aid the decision-making process when considering the most efficient biological method of air deodorization.

This paper presents a prototype of a decision model, which after expansion, based on a larger set of input data, will allow for a quick selection of the appropriate method of purification of air polluted with specific compounds. Such an extension of the procedure proposed in this paper is planned by the authors. The future model will take into account the inlet concentrations, geometry as well as the dimensions of apparatus and all important process parameters, including the gas flow rate but also the packed bed material as well as microbial species, especially those selected for the efficient removal of specific compounds from air. In this perspective, the authors believe that a future model will aid and simplify the selection of the treatment method, especially for industrial applications (e.g., pulp and paper, chemical of pharmaceutical) providing that all required input data are available.

4. Conclusions

The results of the comparative evaluation indicate that conventional biofilters and biotrickling filters exhibit similar and good performance of treatment of hydrophobic compounds. Biotrickling filters are superior in terms of the removal of hydrophilic compounds while bioscrubbers present moderate or low performance when compared to BF and BTF. The decision rules obtained from the decision tree method suggest that the most important parameters in the method selection are: inlet concentration and Henry's constant. Based on the literature data presented in this study, the decision tree output suggests using conventional biofilters for the treatment of relatively highly concentrated streams (concentration above 2500 $mg \cdot m^{-3}$). For streams with concentrations of odorous compounds lower than depicted, biotrickling filtration is a more suitable method than biofiltration or bioscrubbing. This manuscript reveals the first iteration of the problem related to the selection of the treatment method for hydrophilic and hydrophobic odorous compounds using a proposed decision algorithm. Further expansion of this algorithm is planned in the future and it will be based on more complex input data, including a packing material type or microbial species with the perspective of facilitating the method selection process.

Author Contributions: M.G., P.R., B.S. and J.G. wrote a paper. M.G. revised the literature data, prepared a method of comparison and performed the calculations. P.R. revised the literature for the introduction and discussed the results. B.S. developed a decision tree model. J.G. supervised the preparation of the manuscript, consulted with discussions, and prepared the final conclusions.

Funding: This research received no external funding.

Acknowledgments: The authors are grateful for the support by the project "INTERPHD2" No. POWR.03.02.00-IP.08-00-DOC/16.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Barbusinski, K.; Kalemba, K.; Kasperczyk, D.; Urbaniec, K.; Kozik, V. Biological methods for odor treatment—A review. *J. Clean. Prod.* **2017**, *152*, 223–241. [CrossRef]
2. Qian, H.; Cheng, Y.; Yang, C.; Wu, S.; Zeng, G.; Xi, J. Performance and biofilm characteristics of biotrickling filters for ethylbenzene removal in the presence of saponins. *Environ. Sci. Pollut. Res.* **2018**, *25*, 30021–30030. [CrossRef]
3. Wu, H.; Yan, H.; Quan, Y.; Zhao, H.; Jiang, N.; Yin, C. Recent progress and perspectives in biotrickling filters for VOCs and odorous gases treatment. *J. Environ. Manag.* **2018**, *222*, 409–419. [CrossRef] [PubMed]
4. Brancher, M.; Griffiths, K.D.; Franco, D.; de Melo Lisboa, H. A review of odour impact criteria in selected countries around the world. *Chemosphere* **2017**, *168*, 1531–1570. [CrossRef]
5. Rybarczyk, P.; Szulczyński, B.; Gębicki, J.; Hupka, J. Treatment of malodorous air in biotrickling filters: A review. *Biochem. Eng. J.* **2019**, *141*, 146–162. [CrossRef]
6. Darlington, A.B.; Dat, J.F.; Dixon, M.A. The Biofiltration of Indoor Air: Air Flux and Temperature Influences the Removal of Toluene, Ethylbenzene, and Xylene. *Environ. Sci. Technol.* **2001**, *35*, 240–246. [CrossRef] [PubMed]
7. Darlington, A.; Chan, M.; Malloch, D.; Pilger, C.; Dixon, M.A. The Biofiltration of Indoor Air: Implications for Air Quality. *Indoor Air* **2000**, *10*, 39–46. [CrossRef]
8. Torpy, F.; Clements, N.; Pollinger, M.; Dengel, A.; Mulvihill, I.; He, C.; Irga, P. Testing the single-pass VOC removal efficiency of an active green wall using methyl ethyl ketone (MEK). *Air Qual. Atmos. Health* **2018**, *11*, 163–170. [CrossRef]
9. Ondarts, M.; Hort, C.; Platel, V.; Sochard, S. Indoor Air Purification by Compost Packed Biofilter. *Int. J. Chem. React. Eng.* **2010**, *8*. [CrossRef]
10. Pratt, C.; Walcroft, A.S.; Tate, K.R.; Ross, D.J.; Roy, R.; Reid, M.H.; Veiga, P.W. Biofiltration of methane emissions from a dairy farm effluent pond. *Agric. Ecosyst. Environ.* **2012**, *152*, 33–39. [CrossRef]
11. Dumont, E.; Hamon, L.; Lagadec, S.; Landrain, P.; Landrain, B.; Andrès, Y. NH_3 biofiltration of piggery air. *J. Environ. Manag.* **2014**, *140*, 26–32. [CrossRef]

12. Miller, U.; Sówka, I.; Grzelka, A.; Pawnuk, M. Application of biological deodorization methods in the aspect of sustainable development. In Proceedings of the SHS Web Conference, Gdańsk-Nynäshamn, Poland, 18–20 September 2018; Volume 57.
13. Szulczyński, B.; Wasilewski, T.; Wojnowski, W.; Majchrzak, T.; Dymerski, T.; Namieśnik, J.; Gębicki, J. Different Ways to Apply a Measurement Instrument of E-Nose Type to Evaluate Ambient Air Quality with Respect to Odour Nuisance in a Vicinity of Municipal Processing Plants. *Sensors* **2017**, *17*, 2671. [CrossRef] [PubMed]
14. Khan, F.I.; Ghoshal, A.K. Removal of Volatile Organic Compounds from polluted air. *J. Loss Prev. Process Ind.* **2000**, *13*, 527–545. [CrossRef]
15. Oliva, G.; Zarra, T.; Naddeo, V.; Munoz, R.; Lebrero, R.; Angeles, R.; Belgiorno, V.; Ángeles, R.; Belgiorno, V. Comparative Analysis of AOPs and Biological Processes for the Control of VOCs Industrial Emissions. In Proceedings of the Chemical Engineering Transactions, Milan, Italy, 9–12 September 2018; Volume 68, pp. 451–456.
16. Schiavon, M.; Ragazzi, M.; Rada, E.C.; Torretta, V. Air pollution control through biotrickling filters: A review considering operational aspects and expected performance. *Crit. Rev. Biotechnol.* **2016**, *36*, 1143–1155. [CrossRef] [PubMed]
17. Yu, J.; Liu, W.; Cheng, Z.; Jiang, Y.; Cai, W.; Chen, J. Dichloromethane removal and microbial variations in a combination of UV pretreatment and biotrickling filtration. *J. Hazard. Mater.* **2014**, *268*, 14–22.
18. Abraham, S.; Joslyn, S.; Suffet, I.H. (Mel) Treatment of odor by a seashell biofilter at a wastewater treatment plant. *J. Air Waste Manag. Assoc.* **2015**, *65*, 1217–1228. [CrossRef] [PubMed]
19. Mudliar, S.; Giri, B.; Padoley, K.; Satpute, D.; Dixit, R.; Bhatt, P.; Pandey, R.; Juwarkar, A.; Vaidya, A. Bioreactors for treatment of VOCs and odours—A review. *J. Environ. Manag.* **2010**, *91*, 1039–1054. [CrossRef]
20. Vikrant, K.; Kim, K.H.; Szulejko, J.E.; Pandey, S.K.; Singh, R.S.; Giri, B.S.; Brown, R.J.C.; Lee, S.H. Bio-filters for the treatment of VOCs and odors—A review. *Asian J. Atmos. Environ.* **2017**, *11*, 139–152. [CrossRef]
21. Szulczyński, B.; Gębicki, J.; Namieśnik, J. Monitoring and efficiency assessment of biofilter air deodorization using electronic nose prototype. *Chem. Pap.* **2018**, *72*, 527–532. [CrossRef]
22. McNevin, D.; Barford, J. Biofiltration as an odour abatement strategy. *Biochem. Eng. J.* **2000**, *5*, 231–242. [CrossRef]
23. Chmiel, K.; Jastrzębski, A.B.; Palica, M. Biofiltracja lotnych związków organicznych. *Przem. Chem.* **2005**, *6*, 442–445.
24. Ferdowsi, M.; Avalos Ramirez, A.; Jones, J.P.; Heitz, M. Elimination of mass transfer and kinetic limited organic pollutants in biofilters: A review. *Int. Biodeterior. Biodegrad.* **2017**, *119*, 336–348. [CrossRef]
25. Szulczyński, B.; Rybarczyk, P.; Gębicki, J. Monitoring of n-butanol vapors biofiltration process using an electronic nose combined with calibration models. *Monatshefte für Chem. Chem. Mon.* **2018**, *149*, 1693–1699. [CrossRef] [PubMed]
26. Viotti, P.; Schiavon, M.; Gavasci, R.; Capodaglio, A.G. Removal of benzene and toluene from a refinery waste air stream by water sorption and biotrickling filtration. *Interdiscip. J. Appl. Sci.* **2015**, *10*, 720–727.
27. Zdeb, M.; Lebiocka, M. Microbial removal of selected volatile organic compounds from the model landfill gas. *Ecol. Chem. Eng. S* **2016**, *23*, 215–228. [CrossRef]
28. Lebrero, R.; Rodríguez, E.; Estrada, J.M.; García-Encina, P.A.; Muñoz, R. Odor abatement in biotrickling filters: Effect of the EBRT on methyl mercaptan and hydrophobic VOCs removal. *Bioresour. Technol.* **2012**, *109*, 38–45. [CrossRef]
29. Yang, C.; Qian, H.; Li, X.; Cheng, Y.; He, H.; Zeng, G.; Xi, J. Simultaneous Removal of Multicomponent VOCs in Biofilters. *Trends Biotechnol.* **2018**, *36*, 673–685. [CrossRef]
30. Cheng, Y.; He, H.; Yang, C.; Zeng, G.; Li, X.; Chen, H.; Yu, G. Challenges and solutions for biofiltration of hydrophobic volatile organic compounds. *Biotechnol. Adv.* **2016**, *34*, 1091–1102. [CrossRef] [PubMed]
31. Iranpour, R.; Cox, H.H.J.; Deshusses, M.A.; Schroeder, E.D. Literature review of air pollution control biofilters and biotrickling filters for odor and volatile organic compound removal. *Environ. Prog.* **2005**, *24*, 254–267. [CrossRef]
32. Lu, C.; Lin, M.-R.; Chu, C. Temperature Effects of Trickle-Bed Biofilter for Treating BTEX Vapors. *J. Environ. Eng.* **1999**, *125*, 775–779. [CrossRef]

33. Mirpuri, R.; Jones, W.; Bryers, J.D. Toluene degradation kinetics for planktonic and biofilm-grown cells ofPseudomonas putida 54G. *Biotechnol. Bioeng.* **1997**, *53*, 535–546. [CrossRef]
34. Vaiškūnaitė, R.; Zagorskis, A. Modelling of a biofiltration process of volatile organic compound mixtures in a biofilter. *Biotechnol. Biotechnol. Equip.* **2016**, *30*, 1217–1222. [CrossRef]
35. Gąszczak, A.; Bartelmus, G.; Burghardt, A.; Rotkegel, A.; Sarzyński, R. Experiments and modelling of a biotrickling filter (BTF) for removal of styrene from airstreams. *J. Chem. Technol. Biotechnol.* **2018**, *93*, 2659–2670. [CrossRef]
36. Ahmed, W.; Shareefdeen, Z.M.; Jabbar, N.A. Dynamic modeling and analysis of biotrickling filters in continuous operation for H2S removal. *Clean Technol. Environ. Policy* **2014**, *16*, 1757–1765. [CrossRef]
37. Almenglo, F.; Ramírez, M.; Gómez, J.M.; Cantero, D.; Gamisans, X.; Dorado, A.D. Modeling and control strategies for anoxic biotrickling filtration in biogas purification. *J. Chem. Technol. Biotechnol.* **2016**, *91*, 1782–1793. [CrossRef]
38. Vergara-Fernández, A.; Revah, S.; Moreno-Casas, P.; Scott, F. Biofiltration of volatile organic compounds using fungi and its conceptual and mathematical modeling. *Biotechnol. Adv.* **2018**, *36*, 1079–1093. [CrossRef]
39. Yoon, H.; Song, M.J.; Kim, D.D.; Sabba, F.; Yoon, S. A Serial Biofiltration System for Effective Removal of Low-Concentration Nitrous Oxide in Oxic Gas Streams: Mathematical Modeling of Reactor Performance and Experimental Validation. *Environ. Sci. Technol.* **2019**, *53*, 2063–2074. [CrossRef]
40. Toledo-Cervantes, A.; Lebrero, R.; Cavinato, C.; Muñoz, R. Biogas upgrading using algal-bacterial processes. *Microalgae Based Biofuels Bioprod.* **2017**, 283–304. [CrossRef]
41. Han, M.-F.; Wang, C.; Liu, H. Comparison of physical technologies for biomass control in biofilters treating gaseous toluene. *J. Air Waste Manag. Assoc.* **2018**, *68*, 1118–1125. [CrossRef]
42. López de León, L.R.; Deaton, K.E.; Deshusses, M.A. Miniaturized Biotrickling Filters and Capillary Microbioreactors for Process Intensification of VOC Treatment with Intended Application to Indoor Air. *Environ. Sci. Technol.* **2019**, *53*, 1518–1526. [CrossRef]
43. Aguirre, A.; Bernal, P.; Maureira, D.; Ramos, N.; Vásquez, J.; Urrutia, H.; Gentina, J.C.; Aroca, G. Biofiltration of trimethylamine in biotrickling filter inoculated with Aminobacter aminovorans. *Electron. J. Biotechnol.* **2018**, *33*, 63–67. [CrossRef]
44. San-Valero, P.; Gabaldón, C.; Álvarez-Hornos, F.J.; Izquierdo, M.; Martínez-Soria, V. Removal of acetone from air emissions by biotrickling filters: Providing solutions from laboratory to full-scale. *J. Environ. Sci. Health Part A* **2018**, 1–8. [CrossRef]
45. Dumont, É. Impact of the treatment of NH_3 emissions from pig farms on greenhouse gas emissions. Quantitative assessment from the literature data. *New Biotechnol.* **2018**, *46*, 31–37. [CrossRef] [PubMed]
46. Wysocka, I.; Gębicki, J.; Namieśnik, J. Technologies for deodorization of malodorous gases. *Environ. Sci. Pollut. Res.* **2019**, 1–26. [CrossRef] [PubMed]
47. Potivichayanon, S.; Pokethitiyook, P.; Kruatrachue, M. Hydrogen sulfide removal by a novel fixed-film bioscrubber system. *Process Biochem.* **2006**, *41*, 708–715. [CrossRef]
48. Barbusiński, K.; Kalemba, K. Use of biological methods for removal of H2S from biogas in wastewater treatment plants—A review. *Archit. Civ. Eng. Environ.* **2016**, *9*, 103–112. [CrossRef]
49. Malakar, S.; Saha, P.D.; Baskaran, D.; Rajamanickam, R. Comparative study of biofiltration process for treatment of VOCs emission from petroleum refinery wastewater—A review. *Environ. Technol. Innov.* **2017**, *8*, 441–461. [CrossRef]
50. Taghipour, H.; Shahmansoury, M.R.; Bina, B.; Movahdian, H. Comparison of the biological NH_3 removal characteristics of a three stage biofilter with a one stage biofilter. *Int. J. Environ. Sci. Technol.* **2006**, *3*, 417–424. [CrossRef]
51. Gao, L.; Keener, T.C.; Zhuang, L.; Siddiqui, K.F. A technical and economic comparison of biofiltration and wet chemical oxidation (scrubbing) for odor control at wastewater treatment plants. *Environ. Eng. Policy* **2001**, *2*, 203–212. [CrossRef]
52. Saaty, T.L. Relative Measurement and Its Generalization in Decision Making Why Pairwise Comparisons are Central in Mathematics for the Measurement of Intangible Factors The Analytic Hierarchy/Network Process. *RACSAM-Revista de la Real Academia de Ciencias Exactas Fisicas y Naturales Serie A Matematicas* **2008**, *102*, 251–318. [CrossRef]

53. Brunelli, M.; Canal, L.; Fedrizzi, M. Inconsistency indices for pairwise comparison matrices: A numerical study. *Ann. Oper. Res.* **2013**, *211*, 493–509. [CrossRef]
54. Chang, D.Y. Applications of the extent analysis method on fuzzy AHP. *Eur. J. Oper. Res.* **1996**, *95*, 649–655. [CrossRef]
55. Kabir, G.; Sadiq, R.; Tesfamariam, S. A review of multi-criteria decision-making methods for infrastructure management. *Struct. Infrastruct. Eng.* **2014**, *10*, 1176–1210. [CrossRef]
56. Soltani, A.; Sadiq, R.; Hewage, K. Selecting sustainable waste-to-energy technologies for municipal solid waste treatment: A game theory approach for group decision-making. *J. Clean. Prod.* **2016**, *113*, 388–399. [CrossRef]
57. Palmer, E.J.; Devitt, P.G.; De Young, N.J.; Morris, D. Assessment of an electronic voting system within the tutorial setting: A randomised controlled trial [ISRCTN54535861]. *BMC Med. Educ.* **2005**, *5*, 24. [CrossRef]
58. Laukkanen, S.; Kangas, A.; Kangas, J. Applying voting theory in natural resource management: A case of multiple-criteria group decision support. *J. Environ. Manag.* **2002**, *64*, 127–137. [CrossRef]
59. Nurmi, H. Voting Procedures: A Summary Analysis. *Br. J. Political Sci.* **1983**, *13*, 181–208. [CrossRef]
60. Oishi, S.; Hahn, J.; Schimmack, U.; Radhakrishan, P.; Dzokoto, V.; Ahadi, S. The measurement of values across cultures: A pairwise comparison approach. *J. Res. Pers.* **2005**, *39*, 299–305. [CrossRef]
61. Martignon, L.; Hoffrage, U. Fast, frugal, and fit: Simple heuristics for paired comparison. *Theory Decis.* **2002**, *52*, 29–71. [CrossRef]
62. Chan, F.T.S.; Jiang, B.; Tang, N.K.H. The development of intelligent decision support tools to aid the design of flexible manufacturing systems. *Int. J. Prod. Econ.* **2000**, *65*, 73–84. [CrossRef]
63. Kok, M.; Lootsma, F.A. Pairwise-comparison methods in multiple objective programming, with applications in a long-term energy-planning model. *Eur. J. Oper. Res.* **1985**, *22*, 44–55. [CrossRef]
64. Kaya, T.; Kahraman, C. Multicriteria decision making in energy planning using a modified fuzzy TOPSIS methodology. *Expert Syst. Appl.* **2011**, *38*, 6577–6585. [CrossRef]
65. Colombo, J.; Richman, W.A.; Shaddy, D.J.; Follmer Greenhoot, A.; Maikranz, J.M. Heart Rate-Defined Phases of Attention, Look Duration, and Infant Performance in the Paired-Comparison Paradigm. *Child Dev.* **2001**, *72*, 1605–1616. [CrossRef]
66. Quinlan, J.R. Induction of decision trees. *Mach. Learn.* **1986**, *1*, 81–106. [CrossRef]
67. Breiman, L.; Friedman, J.H.; Olshen, R.A.; Stone, C.J. *Classification and Regression Trees*; Chapman & Hall/CRC: New York, NY, USA, 1984.
68. Hastie, T.; Tibshirani, R.; Friedman, J. *The Element of Statistical Learning*; Springer: New York, NY, USA, 2001.
69. Chang, L.-Y.; Wang, H.-W. Analysis of traffic injury severity: An application of non-parametric classification tree techniques. *Accid. Anal. Prev.* **2006**, *38*, 1019–1027. [CrossRef] [PubMed]
70. RStudio Team. RStudio Integrated Development Environment for R. Available online: https://www.rstudio.com/ (accessed on 25 February 2019).
71. Therneau, T.; Atkinson, B. *Rpart: Recursive Partitioning and Regression Trees*; Comprehensive R Archive Network (CRAN): Rochester, MN, USA, 2018.
72. Joanna, K.; Mazur-Chrzanowska, B.; Wyszyński, B. *Odory*; Wydawnictwo Naukowe PWN: Warszawa, Poland, 2012.
73. Estrada, J.M.; Bart Kraakman, N.J.R.; Muñoz, R.; Lebrero, R. A Comparative Analysis of Odour Treatment Technologies in Wastewater Treatment Plants. *Environ. Sci. Technol.* **2011**, *45*, 1100–1106. [CrossRef] [PubMed]
74. Estrada, J.M.; Kraakman, B.; Lebrero, R.; Muñoz, R. A sensitivity analysis of process design parameters, commodity prices and robustness on the economics of odour abatement technologies. *Biotechnol. Adv.* **2012**, *30*, 1354–1363. [CrossRef] [PubMed]
75. Webb, D.S. Best Design Practices for Odour Management for Organics Processing Facilities. In Proceedings of the A&WMA Waste Conference 2014, Long Beach, CA, USA, 24–27 June 2014.
76. Rybarczyk, P.; Szulczyński, B.; Gębicki, J. Investigations on the Removal of Hydrophobic Odorous Volatile Organic Compounds by Biotrickling Filtration Monitored with Electronic Nose. *Chem. Eng. Trans.* **2018**, *68*. [CrossRef]
77. Sander, R. Compilation of Henry's law constants (version 4.0) for water as solvent. *Atmos. Chem. Phys.* **2015**, *15*, 4399–4981. [CrossRef]

78. Eshraghi, M.; Parnian, P.; Zamir, S.M.; Halladj, R. Biofiltration of n-butanol vapor at different operating temperatures: Experimental study and mathematical modeling. *Int. Biodeterior. Biodegrad.* **2017**, *119*, 361–367. [CrossRef]
79. Schmidt, T.; Anderson, W.A.; Schmidt, T.; Anderson, W.A. Biotrickling Filtration of Air Contaminated with 1-Butanol. *Environments* **2017**, *4*, 57. [CrossRef]
80. Wübker, S.M.; Friedrich, C.G. Reduction of biomass in a bioscrubber for waste gas treatment by limited supply of phosphate and potassium ions. *Appl. Microbiol. Biotechnol.* **1996**, *46*, 475–480. [CrossRef]
81. Li, G.; Wan, S.; An, T. Efficient bio-deodorization of aniline vapor in a biotrickling filter: Metabolic mineralization and bacterial community analysis. *Chemosphere* **2012**, *87*, 253–258. [CrossRef] [PubMed]
82. Krailas, S.; Tongta, S.; Meeyoo, V. Macrokinetic determination of isopropanol removal using a downward flow biofilter. *Songklanakarin J. Sci. Technol.* **2004**, *26*, 55–64.
83. San-Valero, P.; Penya-Roja, J.M.; Sempere, F.; Gabaldón, C. Biotrickling filtration of isopropanol under intermittent loading conditions. *Bioprocess Biosyst. Eng.* **2013**, *36*, 975–984. [CrossRef] [PubMed]
84. Whaley, M.B.; Monroig, P.; Villarubia, E. Allergan-isopropyl alcohol emissions removal using a unique biological system. *Environ. Prog.* **1998**, *17*, 256–260. [CrossRef]
85. Auria, R.; Aycaguer, A.-C.; Devinny, J.S. Influence of Water Content on Degradation Rates for Ethanol in Biofiltration. *J. Air Waste Manag. Assoc.* **1998**, *48*, 65–70. [CrossRef]
86. Morotti, K.; Ramirez, A.A.; Jones, J.P.; Heitz, M. Analysis and comparison of biotreatment of air polluted with ethanol using biofiltration and biotrickling filtration. *Environ. Technol.* **2011**, *32*, 1967–1973. [CrossRef]
87. Singh, A.; Shareefdeen, Z.; Ward, O.P. Bioscrubber Technology. In *Biotechnology for Odor and Air Pollution Control*; Springer: Berlin/Heidelberg, Germany, 2005; pp. 169–193.
88. Allen, L.; Ellis, S. Laboratory Evaluation of Trickling Biofiltration for Treatment of Kraft Mill Noncombustion Air Emissions. In Proceedings of the TAPPI 2000 Environmental Confrence Processdins, Denver, CO, USA, 21–24 May 2000; pp. 293–309.
89. Avalos Ramirez, A.; Peter Jones, J.; Heitz, M. Control of methanol vapours in a biotrickling filter: Performance analysis and experimental determination of partition coefficient. *Bioresour. Technol.* **2009**, *100*, 1573–1581. [CrossRef]
90. DeHollander, G.R.; Overcamp, T.J.; Grady, C., Jr.; Grady, C. Performance of a Suspended-Growth Bioscrubber for the Control of Methanol. *J. Air Waste Manag. Assoc.* **1998**, *48*, 872–876. [CrossRef]
91. Prekumar, R.; Krishnamohan, N. Removal of Methanol from Waste Gas Using Biofiltration. *J. Appl. Sci. Res.* **2010**, *6*, 1898–1907.
92. Lalanne, F.; Malhautier, L.; Roux, J.-C.; Fanlo, J.-L. Absorption of a mixture of volatile organic compounds (VOCs) in aqueous solutions of soluble cutting oil. *Bioresour. Technol.* **2008**, *99*, 1699–1707. [CrossRef] [PubMed]
93. Arriaga, S.; Revah, S. Removal of n-hexane by Fusarium solani with a gas-phase biofilter. *J. Ind. Microbiol. Biotechnol.* **2005**, *32*, 548–553. [CrossRef] [PubMed]
94. Tu, Y.; Yang, C.; Cheng, Y.; Zeng, G.; Lu, L.; Wang, L. Effect of saponins on n-hexane removal in biotrickling filters. *Bioresour. Technol.* **2015**, *175*, 231–238. [CrossRef] [PubMed]
95. Arriaga, S.; Revah, S. Improving hexane removal by enhancing fungal development in a microbial consortium biofilter. *Biotechnol. Bioeng.* **2005**, *90*, 107–115. [CrossRef]
96. Nikiema, J.; Bibeau, L.; Lavoie, J.; Brzezinski, R.; Vigneux, J.; Heitz, M. Biofiltration of methane: An experimental study. *Chem. Eng. J.* **2005**, *113*, 111–117. [CrossRef]
97. Lebrero, R.; Hernández, L.; Pérez, R.; Estrada, J.M.; Muñoz, R. Two-liquid phase partitioning biotrickling filters for methane abatement: Exploring the potential of hydrophobic methanotrophs. *J. Environ. Manag.* **2015**, *151*, 124–131. [CrossRef] [PubMed]
98. Liu, F.; Fiencke, C.; Guo, J.; Rieth, R.; Dong, R.; Pfeiffer, E.-M. Performance evaluation and optimization of field-scale bioscrubbers for intensive pig house exhaust air treatment in northern Germany. *Sci. Total Environ.* **2017**, *579*, 694–701. [CrossRef]
99. Kim, J.-O. Degradation of benzene and ethylene in biofilters. *Process Biochem.* **2003**, *39*, 447–453. [CrossRef]
100. Barzgar, S.; Hettiaratchi, J.P.; Pearse, L.; Kumar, S. Inhibitory effects of acidic pH and confounding effects of moisture content on methane biofiltration. *Bioresour. Technol.* **2017**, *245*, 633–640. [CrossRef]
101. van Groenestijn, J.W.; Liu, J.X. Removal of alpha-pinene from gases using biofilters containing fungi. *Atmos. Environ.* **2002**, *36*, 5501–5508. [CrossRef]

102. Montes, M.; Daugulis, A.J.; Veiga, M.C.; Kennes, C. Removal of α-pinene from waste gases in biotrickling filters with the addition of silicone oil or polymers. *J. Biotechnol.* **2010**, *150*, 221. [CrossRef]
103. Miller, M.J.; Allen, D.G. Modelling transport and degradation of hydrophobic pollutants in biofilter biofilms. *Chem. Eng. J.* **2005**, *113*, 197–204. [CrossRef]
104. Kraakman, N.J.R.; van Groenestijn, J.W.; Koers, B.; Heslinga, D.C. Mededelingen Faculteit Lanbouwkundige en Toegepaste Biologische Wetenschapper. *Univ. Gent* **1997**, *62*, 1535–1541.
105. Zamir, S.M.; Babatabar, S.; Shojaosadati, S.A. Styrene vapor biodegradation in single- and two-liquid phase biotrickling filters using Ralstonia eutropha. *Chem. Eng. J.* **2015**, *268*, 21–27. [CrossRef]
106. Ramay, M.J.; Porter, R.; Johnson, S.; Dasgupta, A.; Robbins, J. Pilot biofilter demonstration for styrene, MEK (methyl ethyl ketone), and methylene chloride emissions. In Proceedings of the 4th International In Situ On-Site Bioremediation Symposium, New Orleans, LA, USA, 28 April–1 May 1997; Volume 5, pp. 225–230.
107. San-Valero, P.; Dorado, A.D.; Martínez-Soria, V.; Gabaldón, C. Biotrickling filter modeling for styrene abatement. Part 1: Model development, calibration and validation on an industrial scale. *Chemosphere* **2018**, *191*, 1066–1074. [CrossRef]
108. Rene, E.R.; Murthy, D.V.S.; Swaminathan, T. Performance evaluation of a compost biofilter treating toluene vapours. *Process Biochem.* **2005**, *40*, 2771–2779. [CrossRef]
109. Kim, D.; Cai, Z.; Sorial, G.A. Behavior of trickle-bed air biofilter for toluene removal: Effect of non-use periods. *Environ. Prog.* **2005**, *24*, 155–161. [CrossRef]
110. Díaz, L.F.; Muñoz, R.; Bordel, S.; Villaverde, S. Toluene biodegradation by Pseudomonas putida F1: Targeting culture stability in long-term operation. *Biodegradation* **2008**, *19*, 197–208. [CrossRef] [PubMed]
111. Delhoménie, M.-C.; Bibeau, L.; Gendron, J.; Brzezinski, R.; Heitz, M. A study of clogging in a biofilter treating toluene vapors. *Chem. Eng. J.* **2003**, *94*, 211–222. [CrossRef]
112. Cox, H.H.J.; Deshusses, M.A. Co-treatment of H2S and toluene in a biotrickling filter. *Chem. Eng. J.* **2002**, *87*, 101–110. [CrossRef]
113. García-Peña, E.I.; Hernández, S.; Favela-Torres, E.; Auria, R.; Revah, S. Toluene biofiltration by the fungus *Scedosporium apiospermum* TB1. *Biotechnol. Bioeng.* **2001**, *76*, 61–69. [CrossRef]
114. Cox, H.H.J.; Deshusses, M.A. Biomass control in waste air biotrickling filters by protozoan predation. *Biotechnol. Bioeng.* **1999**, *62*, 216–224. [CrossRef]
115. Liang, Y.; Quan, X.; Chen, J.; Chung, J.S.; Sung, J.Y.; Chen, S.; Xue, D.; Zhao, Y. Long-term results of ammonia removal and transformation by biofiltration. *J. Hazard. Mater.* **2000**, *80*, 259–269. [CrossRef]
116. Melse, R.W.; Ploegaert, J.P.M.; Ogink, N.W.M. Biotrickling filter for the treatment of exhaust air from a pig rearing building: Ammonia removal performance and its fluctuations. *Biosyst. Eng.* **2012**, *113*, 242–252. [CrossRef]
117. Nisola, G.M.; Cho, E.; Orata, J.D.; Redillas, M.C.F.R.; Farnazo, D.M.C.; Tuuguu, E.; Chung, W.J. NH_3 gas absorption and bio-oxidation in a single bioscrubber system. *Process Biochem.* **2009**, *44*, 161–167. [CrossRef]
118. Jun, Y.; Xu, W. Ammonia biofiltration and community analysis of ammonia-oxidizing bacteria in biofilters. *Bioresour. Technol.* **2009**, *100*, 3869–3876. [CrossRef] [PubMed]
119. Yang, Y.; Allen, E.R. Biofiltration Control of Hydrogen Sulfide 1. Design and Operational Parameters. *Air Waste* **1994**, *44*, 863–868. [CrossRef]
120. Chen, Y.; Wang, X.; He, S.; Zhu, S.; Shen, S. The performance of a two-layer biotrickling filter filled with new mixed packing materials for the removal of H2S from air. *J. Environ. Manag.* **2016**, *165*, 11–16. [CrossRef]
121. Morgenroth, E.; Schroeder, E.D.; Chang, D.P.Y.; Scow, K.M. Nutrient Limitation in a Compost Biofilter Degrading Hexane. *J. Air Waste Manag. Assoc.* **1996**, *46*, 300–308.
122. Zilli, M.; Converti, A.; Lodi, A.; Borghi, M.D.; Ferraiolo, G. Phenol removal from waste gases with a biological filter byPseudomonas putida. *Biotechnol. Bioeng.* **1993**, *41*, 693–699. [CrossRef] [PubMed]
123. Ergas, S.J.; Kinney, K.; Fuller, M.E.; Scow, K.M. Characterization of compost biofiltration system degrading dichloromethane. *Biotechnol. Bioeng.* **1994**, *44*, 1048–1054. [CrossRef] [PubMed]
124. Chou, M.-S.; Shiu, W.-Z.; Chou, S. Bioconversion of Methylamine in Biofilters. *J. Air Waste Manag. Assoc.* **1997**, *47*, 58–65. [CrossRef]
125. Smet, E.; Van Langenhove, H.; Verstraete, W. Isobutyraldehyde as a competitor of the dimethyl sulfide degrading activity in biofilters. *Biodegradation* **1997**, *8*, 53–59. [CrossRef]

126. Tang, H.-M.; Hwang, S.-J.; Hwang, S.-C. Waste Gas Treatment in Biofilters. *J. Air Waste Manag. Assoc.* **1996**, *46*, 349–354.
127. Shareefdeen, Z.; Baltzis, B.C. Biofiltration of toluene vapor under steady-state and transient conditions: Theory and experimental results. *Chem. Eng. Sci.* **1994**, *49*, 4347–4360. [CrossRef]
128. Arnold, M.; Reittu, A.; von Wright, A.; Martikainen, P.J.; Suihko, M.-L. Bacterial degradation of styrene in waste gases using a peat filter. *Appl. Microbiol. Biotechnol.* **1997**, *48*, 738–744. [CrossRef] [PubMed]
129. Hecht, V.; Brebbermann, D.; Bremer, P.; Deckwer, W.-D. Cometabolic degradation of trichloroethylene in a bubble column bioscrubber. *Biotechnol. Bioeng.* **1995**, *47*, 461–469. [CrossRef]
130. Hammervold, R.E.; Overcamp, T.J.; Grady, C., Jr.; Smets, B.F.; Hammervold Enotech, R.E.; Thomas Overcamp, C.J.; Grady, C. Sorptive Slurry Bioscrubber for the Control of Acetone. *J. Air Waste Manag. Assoc.* **2000**, *50*, 954–960. [CrossRef]
131. Koutinas, M.; Martin, J.; Peeva, L.G.; Mantalaris, A.; Livingston, A.G. An Oil-Absorber−Bioscrubber System To Stabilize Biotreatment of Pollutants Present in Waste Gas. Fluctuating Loads of 1,2-Dichloroethane. *Environ. Sci. Technol.* **2006**, *40*, 595–602. [CrossRef] [PubMed]
132. Koutinas, M.; Peeva, L.G.; Livingston, A.G. An attempt to compare the performance of bioscrubbers and biotrickling filters for degradation of ethyl acetate in gas streams. *J. Chem. Technol. Biotechnol.* **2005**, *80*, 1252–1260. [CrossRef]
133. Oh, Y.-S.; Bartha, R. Removal of nitrobenzene vapors by a trickling air biofilter. *J. Ind. Microbiol. Biotechnol.* **1997**, *18*, 293–296. [CrossRef]
134. Quan, Y.; Wu, H.; Yin, Z.; Fang, Y.; Yin, C. Effect of static magnetic field on trichloroethylene removal in a biotrickling filter. *Bioresour. Technol.* **2017**, *239*, 7–16. [CrossRef] [PubMed]
135. Zilli, M.; Nicolella, C. Removal of monochlorobenzene from air in a trickling biofilter at high loading rates. *J. Chem. Technol. Biotechnol.* **2012**, *87*, 1141–1149. [CrossRef]
136. Wu, H.; Yin, Z.; Quan, Y.; Fang, Y.; Yin, C. Removal of methyl acrylate by ceramic-packed biotrickling filter and their response to bacterial community. *Bioresour. Technol.* **2016**, *209*, 237–245. [CrossRef]
137. Rene, E.R.; Špačková, R.; Veiga, M.C.; Kennes, C. Biofiltration of mixtures of gas-phase styrene and acetone with the fungus Sporothrix variecibatus. *J. Hazard. Mater.* **2010**, *184*, 204–214. [CrossRef] [PubMed]
138. Prado, Ó.J.; Veiga, M.C.; Kennes, C. Removal of formaldehyde, methanol, dimethylether and carbon monoxide from waste gases of synthetic resin-producing industries. *Chemosphere* **2008**, *70*, 1357–1365. [CrossRef] [PubMed]
139. Pérez, M.C.; Álvarez-Hornos, F.J.; San-Valero, P.; Marzal, P.; Gabaldón, C. Microbial community analysis in biotrickling filters treating isopropanol air emissions. *Environ. Technol.* **2013**, *34*, 2789–2798. [CrossRef] [PubMed]

3.2. Publication 2: A state of the art review on the use of fungi in biofiltration to remove volatile hydrophobic pollutants

This review focused on the bioavailability of hydrophobic VOCs in fungal biofilters, and investigated fungal properties and other factors affecting both bioavailability and biodegradation, and strategies to improve bioavailability using selected fungal species. This led to increased knowledge of, and better design of hydrophobic VOC biofiltration experiments.

Recently, fungi have gained increased popularity based on their ability to biodegrade hydrophobic odour compounds. This article presents an overview of the latest research (primarily focusing on the last decade) of the biocatalytic activity of fungi and the assessment of their practical application in biofiltration systems for hydrophobic VOC removal.

Despite improvements in technologies for removing air pollutants, purifying air of hydrophobic VOCs remains challenging due to the low water solubility of these contaminants. This publication compared fungi and bacteria used in biotrickling filtration, and found that selected species of fungi are more effective than bacteria at filtering hydrophobic VOCs in biofilters. The use of fungal BTFs has the potential advantage of allowing us to more finely control process conditions, such as biofilm thickness and pH, enabling gas pollution abatement to be used at higher VOC loading rates than with BFs. Additionally, the article highlights the potential of fungi to remove hydrophobic VOCs that have not been used in biofiltration so far.

Rev Environ Sci Biotechnol (2022) 21:225–246
https://doi.org/10.1007/s11157-021-09608-7

REVIEW PAPER

A state of the art review on the use of fungi in biofiltration to remove volatile hydrophobic pollutants

Milena Marycz · Anna Brillowska-Dąbrowska · Raúl Muñoz · Jacek Gębicki

Received: 25 August 2021 / Accepted: 29 December 2021 / Published online: 22 January 2022

Abstract The physical/chemical abatement of gas pollutants creates many technical problems, is costly and entails significant environmental impacts. Biological purification of off-gases is a cheap and ecologically safe way of neutralization of gas pollutants. Despite the recent advances, the main technological challenge nowadays is the purification of volatile organic compounds (VOCs) of hydrophobic character due to their low solubility in water. Among all known biological methods of air purification, the most cost-effective biodegradation of hydrophobic VOCs is conducted by biotrickling filters. In this context, fungi have gained an increasing interest in this field based on their ability to biodegrade hydrophobic VOCs. In addition, biotrickling filtration using fungi can support a superior hydrophobic VOC abatement when compared to the bacterial biofilters. This paper aims at reviewing the latest research results concerning biocatalytic activity of fungi and evaluating the possibilities of their practical application in biofiltration systems to remove hydrophobic VOCs.

Keywords Biofiltration · Biotrickling filtration · Fungi · VOCs · Hydrophobic compounds · EPS

M. Marycz (✉) · J. Gębicki
Department of Process Engineering and Chemical Technology, Faculty of Chemistry, Gdansk University of Technology, 11/12 G. Narutowicza Street, 80-233 Gdansk, Poland
e-mail: milena.marycz@pg.edu.pl

M. Marycz · R. Muñoz
Institute of Sustainable Processes, Universidad de Valladolid, Dr Mergelina s/n, 47011 Valladolid, Spain

A. Brillowska-Dąbrowska
Department of Molecular Biotechnology and Microbiology, Faculty of Chemistry, Gdansk University of Technology, 11/12 G. Narutowicza Street, 80-233 Gdansk, Poland

1 Introduction

Anthropogenic activity introduces different gas pollutants into the environment, the removal of which entails many technical problems and often generates high costs. Recently, social awareness has resulted in more emphasis on ecological aspects in all human activities. Hence, the selection of the optimal method for air pollutant removal should not be based exclusively on financial aspects but also, and more importantly, on the environmental friendliness following green engineering principles. Despite the fact that the techniques of mitigation of gases generating odour nuisance are relatively well-known, the sustainable removal of hydrophobic volatile organic compounds (VOCs) has not been fully tackled. Thus, biological processes for exhaust gas purification have been extensively investigated in the last few decades. Compared to the other off-gas treatment techniques

such as chemical absorption, adsorption and incineration, biotechnologies are characterized by their low operation costs, practically unattended operation, low emission of the secondary pollutants and high purification efficiency upon treatment of high volumes of gas with low or medium concentration of VOCs (Jianming et al. 2014; Abraham et al. 2015; Schiavon et al. 2016; Gospodarek et al. 2019b).

The first biological filter was employed in 1893 in England for sewage treatment. Originally, biofilters were constructed using rock or slag as a filtration medium (Metcalf et al. 1979; Chaudhary et al. 2003). In a publication from 1923, H. Bach presented for the first time the concept of controlling odor (hydrogen sulphide) emissions from composting plants and wastewater treatment plants by means of a soil bed (Leson and Winer 1991). Since 1950, biofiltration has been also used for the filtration of off-gases. In the early 1950s, the first successful applications and patents for biofilters were filed in the United States and Germany (Pomeroy 1957). The developed systems were highly effective, unfortunately only for a short time. They were characterized by a simple structure, consisting only of open spaces filled with soil, under which perforated pipes that distributed air were placed. Due to their structure, these systems required a lot of space due to the low specific activity of the soil. Their major disadvantages were susceptibility to cracking, acidification, drying out and uneven air distribution, which resulted from low air permeability through soil layers. Thus, microorganisms have been employed for the removal of VOCs from air for almost 70 years. Similarly, a detailed investigation was conducted on removal of hydrogen sulphide, sulphur dioxide and thiols from air using microorganisms (Gumerman and Carlson 1966; Carlson et al. 1970; Bremner and Banwart 1976; Baltensperger et al. 2008). One of the first applications of fungi in biofiltration was presented in 1982 in the United Kingdom (Wheatley et al. 1982) in order to improve the economy of odour abatement during sewage treatment. In this case study, the filter bed packing of the pilot installation was colonized with filamentous fungi of *Fusarium* and *Geotrichum* species. These organisms developed and multiplied strongly on the highly acidic medium, which effectively outcompeted other microorganisms. The plant was used to treat/process the sewage from a production of dairy products. The process was conducted at pH 4–5 with a high biological oxygen demand. In 1977 in Germany, Bohn and Bohn, designed the first soil biofilter for the removal of organic waste gases (Bohn and Bohn 1986). In 1987, they discovered that sorption was not responsible for odor removal by biofiltration, but biodegradation (Detchanamurthy and Gostomski 2012).

S. Ottengrafa in the 1980s extended the use of biofiltration to the treatment of VOCs and hazardous air pollutants (Ottengraf and Oever 1983). Compost was used to effectively remove moderate concentrations of VOCs from the air. Extending biofiltration research has resulted in the development of two new bioreactor configurations for air pollution control, biotrickling filter and bioscrubbers. In biotrickling filter, a nutrient-laden aqueous solution is constantly recirculated through an inert packing material colonized by microorganisms, treating the gas pollutant present in the gas emission pumped co or countercurrently. On the other hand, bioscrubbers are composed of an absorption unit, where gas pollutant absorption occur, coupled with a suspended growth bioreactor for the biodegradation of the absorbed VOCs. In conventional biofilters, natural packing materials are most often used, e.g. peat, compost, wood chips. This type of biofilter often uses unidentified microorganisms that naturally inhabit a given packed bed. Most of these microorganisms are autotrophs. Sometimes the packing material is additionally inoculated with heterotrophic organisms, whose task is to increase the efficiency of the organic gas pollutant degradation process. The stream of gas to be purified is passed through the bed, in which the removal of pollutants takes place. In biotrickling filters, inert beds naturally laking microorganisms and nutrients, are used as packing materials (e.g. polyurethane foam, Pall rings, Rashig rings) (Marycz et al. 2020). Therefore, packed bed material inoculation with selected microorganisms is needed in biotrickling filters. The nutrients, along with the water phase, are continuously recirculated through the biofilter bed. Finally, bioscrubbers are composed of two main units: an absorber and an aerated bioreactor. The first step of the process takes place in the absorber, which is the absorption of pollutants by the recirculating sorbent. The second step takes place in the bioreactor column, where biodegradation of the compounds removed from the air takes place. The microorganisms used in the process are typically activated sludge, so they are

suspended in the liquid phase flowing through the device. This method of biofiltration is especially dedicated to the removal of hydrophilic gas pollutants, due to the fact that the water phase governs pollutant removal in bioscrubbers. The development of biotrickling filters and bioscrubbers allowed greater control of process variables such as pH and biofilm thickness, and subsequently gas pollutant abatament can be operated at higher VOC loading rates than with conventional biofilters.

In 1995, Weber and Hartmans (1996) concluded that fungi could play a significant role in the degradation of gas pollutants in biotrickling filter (BTF), which had been usually attributed to bacteria so far. They inoculated two BTFs for toluene removal with different inocula and observed dominant growth of fungi in one BTF over bacteria in the other BTF, although operation conditions were the same for both. Under nutrient limiting conditions, the fungal BTF revealed significantly higher ability to remove toluene (27 g of carbon per m^3 per hour versus 13 g of carbon per m^3 per h in the bacterial BTF). Figure 1 shows a schematic diagram where the main milestones of the development of biofiltration are depicted, including the use of fungi for biofiltration of hydrophobic VOCs (Yadav et al. 1995).

The elimination of gas pollutants in BTFs is the result of a complex combination of various biological and physico-chemical phenomena (Fig. 2). The process of air purification by biological methods consists of the application of microorganisms, most frequently bacteria and fungi, to decompose the VOC to non-toxic or less toxic compounds. While polluted air is passed through the filter bed packing typically upwards, the nutrient medium is recirculated in the column to provide moisture and mineral nutrients for the growth of pollutants-decomposing microorganisms (Cox and Deshusses 1998). During biofiltration, the pollutants contained in air adsorb on a surface of thin biolayer (biofilm), while during biotrickling filtration the gas pollutant must dissolved first in a trickling aqueous solution flowing over this biofilm. Then, the pollutants diffuse into the biofilm, which covers the column's packing (Figure B). Dissolved pollutants present inside the biofilm are decomposed by microorganisms, which results in the formation of

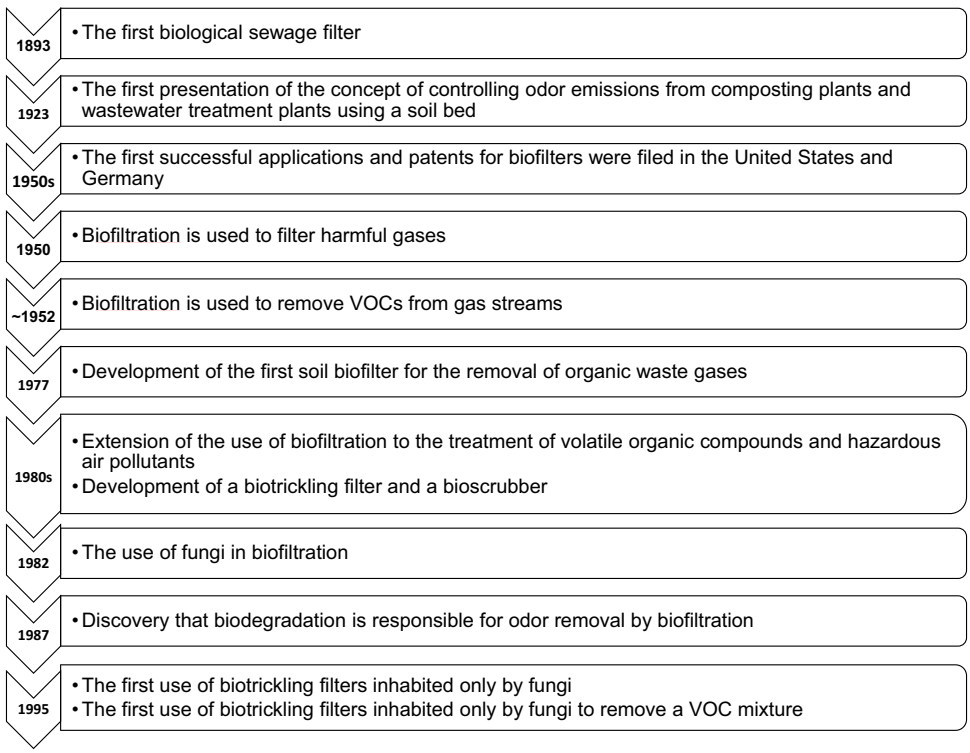


Fig. 1 Main milestones in the development of fungal biofiltration

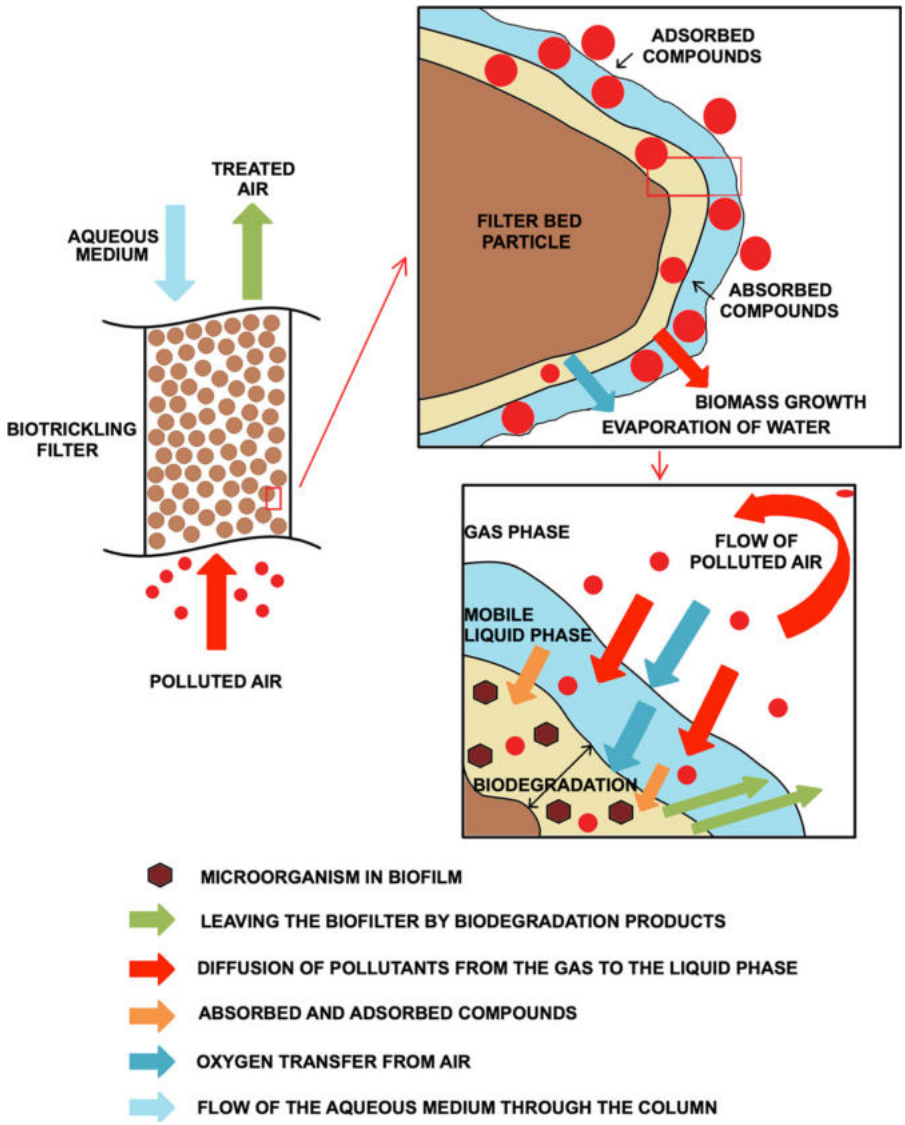


Fig. 2 General mechanism of gas pollutant removal in biotrickling filtration

simple reaction products such as CO_2, water and biomass following the Eq. (1):

$$\mathrm{VOC} + \mathrm{O_2} \rightarrow \mathrm{H_2O} + \mathrm{CO_2} + \text{biomass} + \text{side products} \quad (1)$$

Biofilm—one of the form of microorganims' growth promote an increase in biofiltration effectiveness up to a point when the amount of biomass is large enough to trigger clogging, which impairs the biofiltration process by creating preferential pathways and anaerobic conditions (Devinny and Ramesh 2005). An advantage of biofilters and BTF is the fact that pollutants are not only transferred to the water phase or the biofilm, but converted into biomass, a compounds less harmful and odorous than their parent pollutants (Revah et al. 2011). Biological processes for off-gas treatment are usually conducted at room temperature and under atmospheric pressure. A drawback of bioprocesses, relatively easy to cope with in BTF, is the sensitivity of microorganisms to non-optimal conditions of temperature, pH or humidity (Kośmider et al. 2012).

The process of air purification consists not only of transferring pollutants from the gas to another phase

(aqueous phase or biofilm), but also of removing them: i.e. transformed into less toxic, non-toxic and odorless compounds, converted into biomass or mineralized. The process of decomposition of gas pollutants by microorganisms can take place in two stages: biosorption followed by mineralization, co-metabolic biodegradation or partial biotransformation to intermediates. The first one, so-called biosorption, involves trapping the gas pollutants on the surface of microorganisms' cells, where a bidirectional exchange occurs: pollutants molecules diffuse into the cells, while enzymes and metabolites travel in the opposite direction (Fig. 3). The second stage, mineralization of the gas pollutants, takes place inside the cells. VOCs are decomposed by microbial enzymes, which results in production of simple molecules. The products of this reaction undergo oxidation after diffusion inside the microorganisms' cells (Lalanne et al. 2008; Yang et al. 2018).

Adsorption onto the surface of the packing material is often negligible in BTFs due to the inert nature of the bed. Biotrickling filters employ inert beds, which are free of microorganisms and nutrients, for instance polyurethane foam, Pall rings, Rashig rings, expanded clay, ceramic saddles, tri-pack, alginate balls, volcanic rock and perlite (Marycz et al. 2020). In this way, it is possible to inoculate them with selected microorganisms. Nutrients together with water are continuously recirculated through the BTF bed packing, which often has no water retention capabilities. The trickling fluid flowing down the bioreactor is in contact with the biofilm and provides the conditions for control of microbial growth, such as pH, mineral nutrients concentration, conductivity, temperature, etc. Inside the biofilm, pollutant biodegradation occurs due to the catalytic action of bacteria and/or fungi developing in a complex ecosystem. The BTF can be also occupied by predators, for instance protozoa and other higher organisms. Kinetics of pollutants elimination in the biofilm are driven by environmental conditions. BTFs operate in a continuous mode supplying the microorganisms with necessary mineral elements for microbial growth, such as nitrogen, phosphorus, potassium and trace elements. Predators, for example protozoa, nematodes and higher organisms, observed in BTF play an important role in the recycling of key nutrients. Pollutants biodegradation can be accompanied by formation of the final products such as chlorides or sulphates and/or partially oxidized metabolites (i.e. carboxylic acids), which can inhibit biomass growth. The periodic renewal of the trickling solution allows mitigating this product-based inhibition while replenishing essential nutrients. Typically, less than 10% of the carbon present in the pollutants removed is transferred to the trickling liquid and eliminated in this way (Cox et al. 1998).

In recent years, many efforts have been devoted to optimize methods for the abatement of hydrophobic VOCs using microorganisms. Biofiltration is typically cost-effective in the case of exhaust gases with low VOCs concentration (< 3 g/m^3) (van Groenestijn and Hesselink 1993). However, conventional biofilters based on compost encounter problems for the elimination of hydrophobic VOCs such as aromatic compounds, alkenes and alkanes. Due to their low solubility in water, these compounds are hardly absorbed by the bacterial biofilms. BTFs inhabited by fungi on an inert material are used to overcome these problems (Cox 1995; Groenestijn et al. 1995). Fungi are more resistant to acidic and dry conditions than bacteria, which is a useful feature upon biofilters maintenance. The hypotheses supporting why fungi exhibit a relatively better performance during the abatement of hydrophobic VOCs are: (i) the aerial mycelium of fungi, which is in direct contact with the gas phase, can absorb hydrophobic VOCs much faster than the flat surfaces of the bacterial biofilm. The aerial mycelium supports high surface area of the biofilm in gas phase, which results in more effective trapping of the hydrophobic VOCs (van Groenestijn et al. 2001): (ii) the extracellular polymeric substance (EPS) can participate in the degradation and sorption of the hydrophobic VOCs (Avalos Ramirez et al. 2012; Han et al. 2020); and finally (iii) the release of surface active substances synthesized by fungi (Ron and Rosenberg 2001b). These biosurfactants can decrease the surface tension and thus facilitate transport of the hydrophobic VOCs to biologically active surfaces.

The topic of fungi applied in biofiltration in recent years has been extensively described by scientists. To date, many aspects of biofiltration involving fungi have been researched and described. However, the reviews published so far indicate new, and therefore unresolved, challenges that remain to be resolved and therefore inspired the authors to write this review. The publication of van Groenestijn and co-workers from 2001, as one of the first, indicated the superiority of the

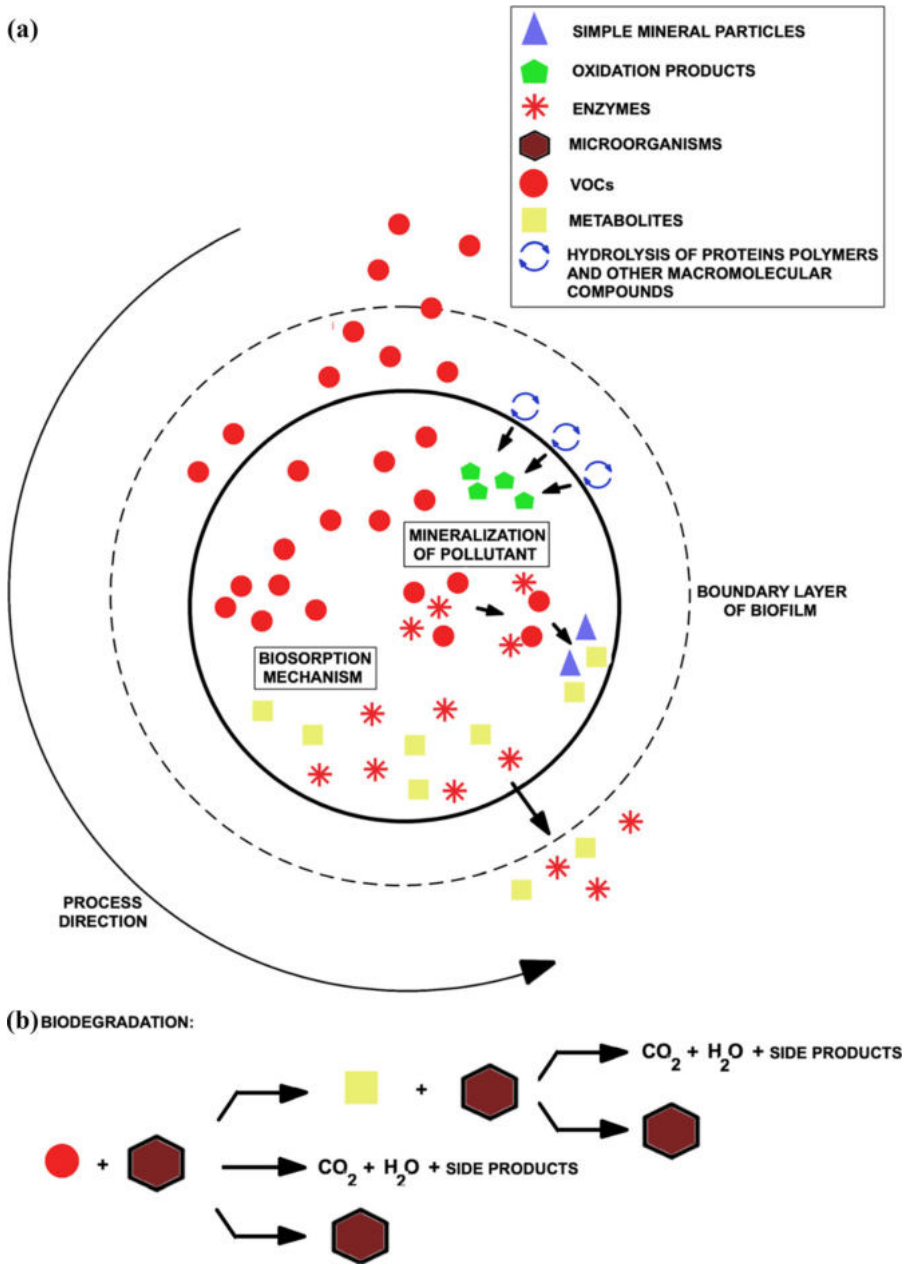


Fig. 3 Phenomena involved in the operation of biofilters during pollutant mineralization. **a** Physiochemical mechanisms in biosorption and mineralization of pollutants. **b** Biodegradation processes during VOC removal

use of fungal over bacterial biofilters for the removal of hydrophobic VOCs (van Groenestijn et al. 2001). The research described in this review identified the important working conditions of fungal biofilters. The review by Kennes and Veiga in 2004 pointed out the dynamic development in the creation of new types of bioreactors, new carrier materials and more efficient biocatalysts (Kennes and Veiga 2004). At that time, the search for efficient fungal biocatalysts, mainly for VOC biofiltration, was presented as a novelty. The publication described novel isolated fungal strains capable of degrading mainly alkylbenzenes. This publication inspired the authors of this review to collect and systematize knowledge about fungal

species capable of removing hydrophobic VOCs that have been studied in biotrickling filters in the last 10 years. The review paper of Vergara-Fernández et al. (2018), using conceptual and mathematical models, proved that the abatement efficiency of bacterial biofilters is lower for compounds that are poorly soluble in water compared to fungal biofilters. This review indicated that despite the fact that the main problem in biofilters is the description of the phenomenon of mass and momentum transfer between gas, liquid and biofilm, in order to advance design and optimization of biofilters, it is necessary to understand in detail the relationship between the rate of biodegradation and knowledge of the internal growth of microorganisms in the columns, on various types of packing materials. In a 2018 review by Prenafet-Boldú and co-workers, the the role of melanised hydrocarbonoclastic fungi in biofiltration and biosafety have been discussed was discussed extensively (Prenafeta-Boldú et al. 2018). At that time, there was also no revision of the fungal activity on the removal of VOCs, especially of the hydrophobic nature of their modeling. This review included a detailed description of the phenomena occurring during the biofiltration process (mass, heat and momentum transport) as well as the growth and biodegradation kinetics of bacteria and fungi.

The aim of this paper is the presentation and discussion of the investigation results from the last 10 years on the removal of the hydrophobic VOCs using fungi and their consortia in biofiltration systems. A comparative analysis of fungi and bacteria for the removal of hydrophobic VOCs in biofiltration systems will be carried out. Additionally, the biodegradation potential of fungi not previously exploited in biofiltration will be discussed in term of hydrophobic VOCs removal in biofiltration systems.

2 Comparison of fungi and bacteria in biotrickling filtration

One of the most important parameters in the design of biotrickling filters is the selection of the suitable microorganisms capable of biodegrading the target pollutants. In order to provide the highest biodegradation efficiency, proper operational parameters must be adjusted in every process. The tuning of the parameters influencing on microorganisms growth can result not only in an increase in the VOC biodegradation activity but also cause an increase in microbial growth, which will eventually trigger the process of pollutant removal (under non mass transfer limiting conditions). The microorganisms forming the consortium present in the BTF packing must possess suitable metabolic properties and be capable of cooperation with all microorganisms present in the consortium.

Microorganisms require many macro and micro nutrients to grow and support metabolic activity. Biomass growth and pollutant biodegradation in a bioreactor depend on the number and concentration of nutrients available. These elements are either naturally present in filter bed packings or added to the bed packings when using synthetic or inert materials. Microorganisms are composed of 4 basic elements: carbon, hydrogen, nitrogen and oxygen. In this context, a typical composition of the fungal cells can be described as $C_4H_7N_{0.6}O_2$, whereas for bacteria the stoichiometric formula is $C_5H_{8.3}NO_{1.35}$ (Shareefdeen et al. 2005).

Microorganisms also require trace elements (Mg, Mn, K, Ca, P, S and Fe) for proper development and operation of enzymes and osmotic equilibrium. Oxygen supply to the microorganisms can be troublesome when treating high loads of highly or moderately soluble VOC or in BTF with thick biofilm layers (Shareefdeen et al. 2005). Regardless of the bioreactor configuration, enzymatic activity of bacteria is often regarded as the dominant factor during biological air purification. However, fungal activity can also play a key role depending on the operational conditions prevailing in the bioreactor. In BTFs operated under non sterile conditions, the packed bed is always inhabited by both bacteria and fungi. Microbial population structure is influenced by the competition among VOC degrading strains, process conditions, technical solutions as well as by microbial contamination since sterile conditions are very difficult to achieve in full scale air purification facilities. Therefore, the composition of microorganisms changes with time and very often without a significant impact on the macroscopic VOC abatement performance. So far, most of the papers published put an emphasis on bacterial population structure in BTFs. Nevertheless, regardless of the conditions in the bioreactor, BTF packing becomes inhabited by other strains that do not belong to the primary microbiota—these could be

fungi as well as bacteria feeding on metabolites or cell debris. Thus, the primary microbiota can be substituted with the secondary microbiota. In this context, Maestre et al. (2007) described the change from the dominant bacteria into fungi inside the bed packing in a biofilter treating toluene inoculated only with bacteria (Maestre et al. 2007). The reason underlying this shift in the dominant microbial community was the increased acidification in the biofilter. Interestingly, no decrease in pollutant removal efficiency was observed. On the contrary, a significant improvement in toluene removal was recorded. Due to the fact that fungi are able to survive in much more extreme environmental conditions than bacteria, it is much easier for them to maintain dominant status in the bed packing under long term operation.

Most examples of aromatic hydrocarbons degradation by fungi found in literature are based on a co-metabolism consisting of independent cooperation with the other organisms. One microorganisms typically breaks down the target pollutant into an intermediate metabolite, which becomes available for a second organism that benefits from its partial degradation (Chang et al. 1993). However, there are many studies where fungi were able to use the target organic pollutant as the sole source of carbon and energy. Rybarczyk et al. (2021) reported an efficient removal of cyclohexane and ethanol (95–99%) from air in a BTF inoculated only with the fungal species *Candida albicans* and *Candida subhashi*. When comparing the advantages and disadvantages of bacteria and fungi for the removal of gas pollutants in BTFs, scientists have typically focused only on the values of VOC removal efficiency. However, a complete comparative analysis must also take into account the elimination capacity, microbial acclimation time and VOC loading rate and process robustness.

Fungi are eucaryotic organisms that substantially differ in structure and metabolic processes from bacteria, which are the representatives of the procaryotic domain. Table 1 presents a general comparison of morphological and phenotype features of fungi and bacteria (Griffin 1985; Pietarinen et al. 2008).

Today, approximately 70 thousand species of fungi have been described in literature, but it is estimated that their number is much higher in nature (Ławrynowicz 2002). Saprobionts (sporophytes) are an ecological group of fungi, the most frequently presented in papers dealing with gas biofiltration

Table 1 Comparison of morphological and phenotype features of fungi and bacteria

Feature	Fungi	Bacteria
Size	ca. 10 μm (on average one order of magnitude larger than bacteria)	ca. 1 μm
Composition of the cell wall	Made of polysaccharides, chitin and other substances	Made of peptidoglycan
Osmophilic nutrition	Yes (they secrete enzymes decomposing organic substances and then absorb decomposed nutrient via osmosis)	No (large majority)

(Thormann and Rice 2007; Gospodarek et al. 2019a). They play a very important role in nature by decomposing organic substrates. Saprobionts convert complex organic substances into simple inorganic compounds and products of their own metabolism, playing a key role on the cycles of the elements in nature. Their specific structure and course of metabolic processes make fungi successful candidates for the removal of VOCs. As above highlighted, the main advantages of fungi over bacteria include:

- much higher resistance to environmental factors such as temperature, pH and humidity (Kennes and Veiga 2004).
- ability to survive upon shortage of nutrients. Indeed, when the concentration of chemical substances/nutrients is too low, blocking of enzymes synthesis is not observed.
- lower sensitivity to the toxic impact of pollutants.
- some fungi do not need additional time for adaptation, which is typically necessary to start the synthesis of the degradation enzymes in bacteria (Carrera 2010).

In the next sections of the publication (Sects. 2 and 2.1), the authors use the term "biofiltration" to refer to conventional biofiltration and biotrickling filtration processes. This generalization aims at presenting the phenomena which, regardless of the process in which they were described, also take place in both types of the aforementioned biofiltration. In addition, an indisputable advantage of the fungi used in biofilters is their much higher resistance to drying and acidification compared to other organisms (Cox 1995; van Groenestijn et al. 2001). Under mesophilic conditions

(15–40 °C), bacteria grow at pH of 5–9, whereas fungi can grow at pH 2–7. The growth as well as the biocatalytic activity of most microorganisms decreases substantially beyond pH 4–8. It is worth noting that almost all microorganisms present in BTFs do not tolerate pH variations higher than 2–3 units (Shareefdeen et al. 2005), whereas microbial activity drops significantly under dry environmental conditions.

Another advantage of fungi in comparison to bacteria is the fact that they possess an aerial mycelium, which significantly increases the surface area of the biofilm in the gas phase, resulting in more effective capture of hydrophobic VOCs (van Groenestijn et al. 2001). All filamentous fungi exhibit branched hypha or numerous hyphae concentrated in one place. Two types of mycelium can be distinguished—substrate (submerged) and aerial (surface) (Fig. 4). The former penetrates the substrate in order to absorbs water and nutrients, whereas the latter develops on the substrate's surface and is used for respiration and reproduction (Bowman and Free 2006; Ruiz-Herrera 2016). In addition, hydrophobins play a key role in the process of growth and development of the filamentous fungi. These proteins are produced by fungi and participate in the formation of surface structures and hyphae, and their attachment to different types of hydrophobic surfaces. Hydrophobins fulfil these functions as they are produced by fungi on the hydrophobic-hydrophilic surfaces. In BTFs, such surface is established at a liquid-purified gas interface. Based on these properties, fungi can be used in biofilters and biotrickling filters for highly efficient removal of pollutants directly from the gas phase, which allows overcoming VOC mass transfer resistance in the aqueous phase (Wösten et al. 1999; Wösten 2001) and confirms fungi as perfect candidates for removal of the hydrophobic VOCs.

The hydrophobicity of fungal surface can increase proportionally with an increase in the presence of hydrophobic substrates. This phenomenon explains the high efficiency of fungi removing hydrophobic VOCs in biofilters (Vergara-Fernández et al. 2006). However, high pressure drops are recorded when using filamentous fungi, which ultimately entails operational problems such as clogging and channelling of bed packings in biofilters. A solution to these problems can be application of saprophytes during fungal biofiltration. Thus, the addition of higher organisms to biofilters or BTFs prevents from rapid increase in pressure drop and reduces energy consumption for gas circulation. Woertz and co-workers proved that saprophytes in biofilters were relatively easy to maintain during the biofiltration process and could be successfully used to control fungal biomass overgrowth (Woertz et al. 2003). The reduction in the supplementation of some nutrients, such as phosphate and potassium ions or nitrogen, can also limit biomass growth in biofiltration systems (Wübker and Friedrich 1996). Many fungi species also exhibit high potential

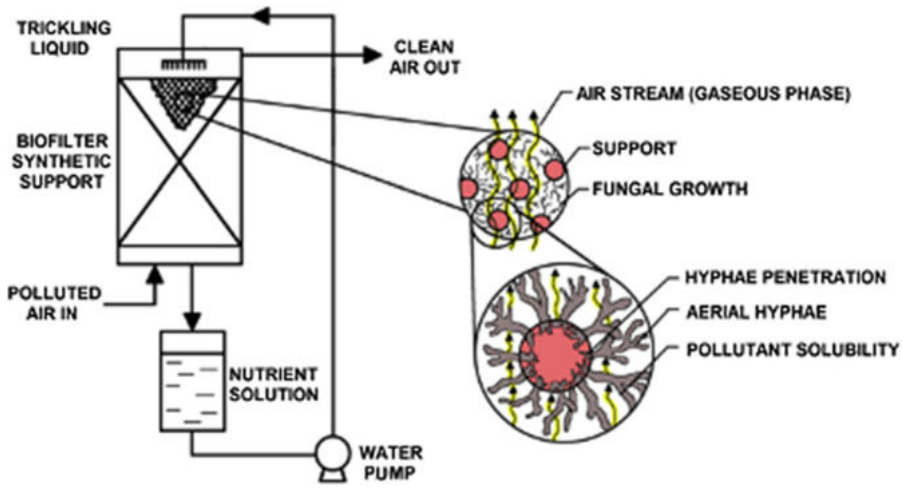


Fig. 4 Representation of fungal growth in a biotrickling filter

of surviving during "pollutant surges" and recover full functionality even after few days of starvation (Jin et al. 2007; Rene et al. 2012). Particular attention should be paid to the robustness of VOC purifying biofiltration units on an industrial scale, where transient conditions are relatively common as a result of process shutdown and restart, sudden changes in the VOC loadings. Substrate starvation, which occurs when the bioreactor is deprived of energy and carbon source, is one of the most typical process perturbations. The time of recovery of the biocatalytic activity is influenced by the type of VOC, fasting period, bioreactor configuration and operational parameters.

2.1 Fungal biofilm in biofiltration systems and EPS production

Both bacteria and fungi are able to produce a biofilm with similar functions. However, the publication focuses on a detailed description of only the fungal biofilm due to the topic discussed in the publication. Formation of a biofilm's structure is a multi-stage process that depends on the properties and structure of the material of the bed packing and on the properties of the microorganisms inhabiting the bioreactor. Figure 5 illustrates the four main stages of biofilm formation. In the first stage, suspended fungal cells attach to the surface of the column's bed packing. Initially, cells bind onto the substrate via reversible and non-specific interactions (including hydrophobic van der Waals interactions, electrostatic interaction, gravitational forces) (Flemming and Wingender 2010). The extracellular structures of mould fungi, substrate (submerged) mycelia, play an important role in this stage. In the second stage of biofilm formation, a specific reaction takes place between the adhering fungi and the substrate. In this context, the adhesins produced by *Candida* species have been thoroughly examined and described (Sundstrom 2002; Rapoport et al. 2011). Strong adhesion of the microbial cells to the substrate for a long time creates irreversible connection, which at this stage can be broken only in a mechanical way. The degree of adhesion depends on the physico-chemical properties of the packing, trickling liquid flow rate in BTFs, flow rate of polluted air and the concentration and species of the fungi colonizing the BTF. At this stage of the process, fungal cells produce an extracellular matrix based on extracellular polymeric substance (EPS). Adhesion of cells to the substrate and EPS formation are followed

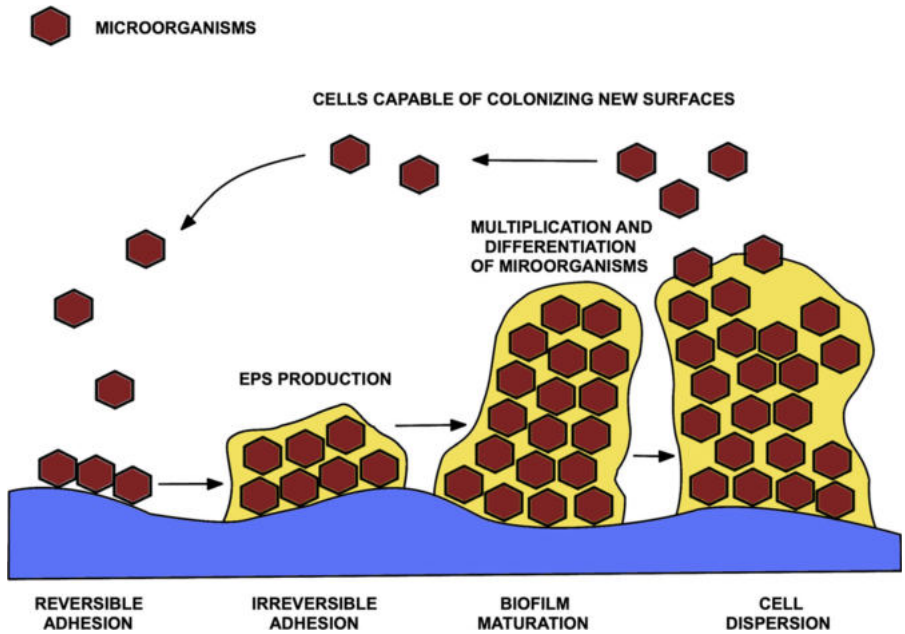


Fig. 5 Stages of biofilm formation in a biofiltration systems

by the third stage of biofilm formation, which involves multiplication and differentiation of the fungal cells.

The factors impacting the extent and rate of biofilm growth include:

- availability of the elements indispensable for life and growth of fungi.
- content of nutrients in substrate and trickling liquid.
- oxygen availability.
- trickling liquid flow rate.
- flow rate of supplied polluted air.
- VOC concentration.
- pH,
- ambient temperature,
- solubility of VOCs in water.

During the last stage of biofilm formation, fungal cells detach from the biofilm structure and via gravitational forces and bulk transport with the trickling liquid, they expand over new surfaces of the BTF bed packing to create a new biofilm. Mature biofilms are a compact, three-dimensional structure composed of a few up to several layers of the fungal cells of the same or different species embedded in EPS. EPS is the main constituent of the biofilm (it corresponds to 90% of the biomass in a mature biofilm, the remaining being fungal cells) responsible for adhesion to the surface of the bed packing and for cohesion inside the film. EPS also forms a scaffolding for a three-dimensional biofilm structure. The main components of EPS and their functions are shown in Table 2. The components of the matrix stabilize the biofilm structure and participate in the formation and maturing of the biofilm, being also a source of nutrients and water. EPS can be also a source of substrate during periods of starvation. The components of the matrix can also protect fungal cells against physical factors, mainly UV radiation.

The knowledge of the structure and functional properties of the biofilm is central to the understanding of its role. Despite the fact that carbohydrates and proteins are considered the main EPS components, biochemical properties of these compounds remain unclear due to their complex structure and unique combinations, and to the fact that each organism can produce different EPS. Moreover, elucidating biofilm's composition can help explaining the structure–function relations, which can facilitate the design of new strategies to maximize VOC removal in BTFs. This can be the basis of the selection of other species of microorganisms within the consortium, which will work more effectively than pure strains.

EPS are located on or around the surface of the fungal cell and they are considered glycocalyx or slime, which facilitates and accelerates adhesion of fungi to the substrate. EPS contains mainly fungal secretions from the cell's surface, cellular lysates and hydrolysates and organic substances adsorbed from the environment. EPS is a complex mixture of biomolecules (proteins, polysaccharides, nucleic acids, lipids and other macromolecules), which are released by the microorganisms and maintain microbial aggregates together. Proteins and exopolysaccharides are the key components of the macromolecules, which represents 40–95% of EPS dry matter. EPS was referred to as "a home of biofilm cells" by Flemming et al. (2007), (Flemming et al. 2007), which can be attributed to its three-dimensional (3D) polymer network (comprising over 90% of biofilms). In practice, EPS participates in the transition from reversible to irreversible adhesion of single cells (inhabitation of inert material inside the biofiltration systems) in immobilized but dynamic microbial environment in BTF. This facilitates the formation of compact, three-dimensional polymer networks, which connects and temporarily immobilizes biofilm's cells. EPS can take part in the degradation and sorption of organic and inorganic compounds and as a barrier to protect cells from hostile environments.

Extracellular polysaccharides, proteins and DNA are strongly hydrated molecules of hydrophilic character, the remaining EPS being composed of hydrophobic molecules. The hydrophobic character of EPS is associated to the acetylene and methyl groups, and lipids combined with polysaccharides, present in the matrix (Neu et al. 1992). Biosurfactants produced by microorganisms in BTFs play an important role during VOC treatment, influencing the surface tension of the trickling solution and thus facilitating gas exchange between the gas and liquid phases. Biosurfactants are surface active substances synthesized by living cells, including yeast (Ron and

Table 2 Main components of EPS and their functions (Singh et al. 2006; Flemming and Wingender 2010; Lewandowski and Boltz 2011)

Main component of EPS	Function
Water	Water constitutes 97% of the entire EPS biomass—protects biofilm from drying
Proteins	Enzymatic activity allowing decomposition of exogenous macromolecules to monomers constituting nutrients for microorganisms. Degradation of EPS structure leads to biofilm or cell dettachment
Proteins and polysaccharides	Adhesion (cohesion) of biofilm
	Intercellular communication
	Maintenance of mechanical stability
	Adhesion of free cells to biotic and abiotic surfaces
	Aggregation of cells and density increase
	Water retention allowing maintenance of aqueous microenvironment, providing cells with tolerance to drying
	Formation of protective barrier allowing tolerance
	To antimicrobial factors
	Sorption of organic compounds enabling
	Accumulation of nutrients and xenobiotics sorption
Lipids and glycoproteins	Potential source of carbon, nitrogen and phosphorous compounds utilized by microorganisms in the biofilm as nutrients
Extracellular DNA (eDNA)	Architectural role and conditions proper distribution of microorganisms in biolayer (Smalyukh et al. 2008; Gloag et al. 2013)

Table 3 Examples of surface active compounds produced by fungi

Fungi	Biosurfactant	References
Candida antarctica	Mannosylerythritol lipids	(Rodrigues et al. 2006)
Candida batistae & *Candida bombicola*	Sophorolipids	(Bhardwaj et al. 2013; De et al. 2015)
Torulopsis bombicola	Sophorolipids	(Siñeriz et al. 2001; De et al. 2015)
Candida petrophilum	Peptidolipid	(Siñeriz et al. 2001; De et al. 2015)
Ustilago sp.	Cellobioselipids	(Siñeriz et al. 2001)
Torulopsis sp.	Sophorolipids	(Ito and Inoue 1982; Ron and Rosenberg 2001a)

Rosenberg 2001b), that decrease surface tension, undergo biodegradation and they are generally non-toxic (Neu 1996). Table 3 compiles the main surfactants synthesized by fungal species. In this context, the synthesis of surfactants such as glycolipids and phospholipids is boosted when microorganisms use hydrocarbons as a source of carbon. It was hypothesized that these chemicals are synthesized in order to emulsify the hydrocarbon substrate and to facilitate their transport to the cells (Flemming and Wingender 2010).

3 Application of fungi to biofiltration of hydrophobic compounds

The low aqueous solubility of hydrophobic VOCs is one of the main limitations of biological methods, which entails the need for large gas residence times and bioreactor volumes. A low VOC solubility in water influences gas–liquid or gas-biofilm pollutant transfer, thus significantly limiting the bioavailability of hydrophobic compounds or the possibility of their leaching. In this context, surface active compounds,

which are characterized by their ability to change free interphase energy, can decrease the surface tension of the aqueous phase/biofilm and improve the gas–liquid/biofilm mass transfer (Miller et al. 2019). Similarly, the addition of a hydrophilic compound to the BTF can increase the efficiency of hydrophobic VOC removal. This can result in stimulated fungal growth and increased carbon demand of the microbial species inhabiting the biofilm (Cheng et al. 2020), leading to co-metabolism of the hydrophilic compounds in the presence of hydrophobic compounds. However, despite the fact that the synergistic effects of simultaneous removal of hydrophilic and hydrophobic VOCs in biofiltration processes are known (Zhang et al. 2006; Yang et al. 2018), the molecular mechanisms of this improvement have not been identified yet. However, the investigations focused on the mechanism of VOC trapping in the mycelium and in the fungal structure did confirm that fungi could uptake hydrophobic VOCs directly from the gas phase (Krailas et al. 2000). Prenafeta-Boldú and co-workers have published a series of publications in which they demonstrated that black yeast (*Capnodiales* and *Chaetothyriales*) can be successfully used for air biofiltration (Prenafeta-Boldú et al. 2008, 2012, 2019; Mayer et al. 2021). These fungi showed a high ability to eliminate VOCs, even those of a hydrophobic nature (e.g. toluene, p-xylene) (Prenafeta-Boldú et al. 2012).

Table 4 presents a review compiling studies of hydrophobic VOCs removal in the biotrickling filtration processes published over the last 10 years. The model fungi employed in these studies were capable of biodegrading aliphatic and aromatic pollutants such as α-pinene, styrene, alkyl benzenes and BTEX (Cox et al. 1996, 1997; Braun-Lüllemann et al. 1997; Shareefdeen et al. 2005).

The overview of the studies using fungi for gas purification of hydrophobic VOCs in biotrickling filtration processes presented in Table 4 shows that the vast majority of studies are focused on the removal of single compounds. The hydrophobic VOCs most frequently removed in BTF inhabited by fungi include toluene, styrene, α–pinene and TCE. The most frequently used trickling liquid is mineral salt medium, often enriched with organic nutrients necessary for the proper functioning and growth of the microorganisms used. EBRT values ranged from several seconds to several minutes (from 15 to 405 s). The different EBRT values resulted, among others, from the variety of dimensions of biofiltration systems, and thus the scale of tests and flowrates of purified gas. Gas chromatography with a flame ionization detector was most often used to evaluate the VOC removal efficiency of the processes. On the other hand, the most popular packing material used in BTFs is polyurethane foam (Moe and Irvine 2000), which is characterised by a high porosity, proper size of the pores to be inhabited by the fungal cells, low density and low pressure drop (Moe and Irvine 2000). Perlite is the second most frequently used packing material in BTFs. Perlite is a naturally occurring amorphous volcanic glass with high thermal and mechanical stability. It is widely used in BTFs due to its non-toxicity, resistance to organic compounds and ease to support the immobilization of microorganisms.

The popular fungal species used in biofiltration studies to abate hydrophobic VOCs were mould (e.g. *Fusarium* sp.) and yeast (e.g. *Candida* sp., *Cladophialophora* sp.). The most efficient fungal species used for the purification of toluene were *Exophiala lecanii-corni* (RE 95%) (Woertz et al. 2001) and *Cladophialophora* sp. (RE 99%) (Woertz et al. 2002). *Sporothrix variecibatus* was the most common species in styrene biodegradation studies, supporting a RE of 95% at EBRTs of 19–77 s (Rene et al. 2010a). In addition, the removal of styrene in the presence of acetone (hydrophilic compound) increased, achieving a RE of 97.5% at an EBRT of 360 s (Rene et al. 2010b). α-pinene removal was carried out with *Ophiostoma* sp. (RE 95%, EBRT 143 s) (Jin et al. 2006), while TCE abatement in the presence of methanol (hydrophilic compound) has been conducted with a consortium of *Fusarium verticillioides* and *Fusarium solani* (max. RE 87.1%, EBRT 9 s) (Quan et al. 2018). Hexane has been effectively removed using *Cladophialophora* sp. (RE 99%, EBRT 60 s) (Arriaga and Revah 2009) and *Candida subhashii* was successfully used to remove cyclohexane in the presence of ethanol (hydrophilic compound) (RE 98.9%, EBRT 60 s) (Rybarczyk et al. 2021).

4 Untested fungi with potential to be used for VOC abatement

Table 5 displays fungal species that have not been used in gas phase bioreactors for hydrophobic VOC abatement based on the comprehensive literature search

Table 4 Examples of fungi treating hydrophobic VOCs in biotrickling filtration processes

No	Predominate strain	Contaminant	Packing material	RE (%)	EBRT (s)	EC (g m^{-3} h^{-1})	C_{in} (g m^{-3})	References
1	*Paecilomyces variotii*	Toluene	Vermiculite (particle size 2.4–5.0 mm)	5.8 ± 4.7%	60.0	–	1.0 ± 0.3	(Estrada et al. 2013)
2	*Phanerochaete chrysosporium*	Toluene	Steel pall rings and pumice grains	> 80.0% in one-liquid phase BTF for inlet toluene concentrations < 2.5 g m^{-3}, and REs > 70.0% were obtained for concentrations < 18 g m^{-3} in two-liquid phase BTF	60.0	810.0	0.5–4.4 and 0.5–24.7 for one-liquid phase (OLP-BTF) and two-liquid phase BTF (TLP-BTF), respectively	(Yousefinejad et al. 2019)
3	*Fusarium oxysporum, Paramicrosporidium saccamoebae*	Toluene	Ceramic particles	92.5%	77.0	98.1	0.2 – 0.3	(Zhang et al. 2019)
4	*Cladophialophora* sp. strain CBS 110,553 (ATCC MYA-2335)—non-virulent	Toluene	Perlite granules (PEG) and polyurethane foam cubes (PUC)	25–100%	CSTR* (n):—for PEG: 33–86 (48 to 12 s);– for PUC: 9–16	5–30	0.02–0.2	(Prenafeta-Boldú et al. 2008)
5	*Candida palmioleophila strain MA-M11*	Styrene	Ceramic Raschig rings	80.0%	136.0, 90.0, 68.0, 45.0, 34.0	126.0	0.3–2.7	(Li et al. 2019)
6	*Sporothrix variecibatus*	Styrene	Lava rock into silicone oil	> 90.0%	91.2 (days 1–55), 40.2 (days 55–83), and 20.1 (days 84–110)	172.8 (without silicone oil) and 670.0 (with silicone oil)	0.6–17.5	(Rene et al. 2011)
7	*Sporothrix variecibatus*	Styrene	Ceramic monolith	95.0%	77.0–19.0	67.4	0.1 and 2.5	(Rene et al. 2010a)
8	*Sporothrix variecibatus*	Styrene–acetone mixture	Perlite	97.5% for styrene and 75.6% for acetone	As low as 17.1	360.0	Styrene 0.0–6.3; acetone 0.0–8.9	(Rene et al. 2010b)

Table 4 continued

No	Predominate strain	Contaminant	Packing material	RE (%)	EBRT (s)	EC (g m^{-3} h^{-1})	C_{in} (g m^{-3})	References
9	*Ophiostoma* sp.	α-pinene	Polypropylene Pall rings of a nominal height of 15 mm was mixed with perlite	89.0%	65.0	31.0	–	(Jin et al. 2007)
10	*Candida boidinii, Ophiostoma stenoceras*	α-pinene (mixtures of methanol, α-pinene and H_2S)	Polyurethane foam	Methanol 100%; α-pinene 67.0%; $H_2S > 99.0\%$	38.0–26.0	Methanol 302.0; α-pinene 175.0; H_2S 191.0	Methanol 0.1–3.3, α-pinene 0.1–2.7, H_2S 0.0–1.4	(López et al. 2013)
11	*Ophiostoma* sp.	α-pinene	Lava rock	95.0%	26.0; 38.0; 72.0	143.0	2.5	(Jin et al. 2006)
12	*Gibberella moniliformis (Fusarium verticillioides)* and *Fusarium solani*	Trichloroethane (TCE) with methanol	Diatomaceous earth pellets	81.4–87.1%	For methanol and TCE were 72.0 and 9.0, respectively	*LR: loading rate* TCE 3.2–12.9; methanol 103.7–414.8	–	(Chheda and Sorial 2017)
13	*Ascomycota strain*	TCE	Wood chips	52.9%; 39.4%	405.0	4.9–3.6	–	(Quan et al. 2018)
14	*Candida subhashii*	Cyclohexane with ethanol	Polyurethane foam	Cyclohexane 98.9%; ethanol 99.5%	60.0	Cyclohexane 89.0; ethanol 36.7;	Cyclohexane 45.0 [g m^{-3} h^{-1}]–90.0 [g m^{-3} h^{-1}]; ethanol 36.9	(Rybarczyk et al. 2021)
15	*Candida albicans*	Cyclohexane with ethanol	Polyurethane foam	Cyclohexane 75.8%; ethanol 94.9%	60.0	Cyclohexane 67.5; ethanol 35.0	Cyclohexane 45.0 [g m^{-3} h^{-1}], 90.0 [g m-3 h^{-1}]; ethanol 36.9	(Rybarczyk et al. 2021)
16	*Gibberella moniliformis (Fusarium verticillioides)*	n-hexane	Perlite	84.0% (pH = 4); 64.0% (pH = 7)	120.0	47.7 (pH = 4); 36.3 (pH = 7)	–	(Zehraoui et al. 2014)

Table 4 continued

No	Predominate strain	Contaminant	Packing material	RE (%)	EBRT (s)	EC (g m^{-3} h^{-1})	C_{in} (g m^{-3})	References
17	*Paecilomyces variotii*	Hexanol	Vermiculite (particle size 2.4–5.0 mm)	43.4 ± 6.7%	60.0	–	0.17	(Estrada et al. 2013)
18	*Aspergillus candidus, Penicillium frequentans*	Xylene	1-cm^3 foam cubes	67.6%	–	2.0	–	(Li and Liu 2006)
19	*Fusarium sp*	Dimethyl disulfide (DMDS)	Polyurethane foam	~ 100%	40.0	86.0	–	(Arellano-García et al. 2018)
20	*Cladosporium sphaerospermum*	BTEX (mixed)	Blue mussel shells (Mytilus edulis shells, about 10 cm in length)	76.4%	90.0	–	–	(Raboni et al. 2017)
21	*Paecilomyces variotii*	Propanal	Vermiculite (particle size 2.4–5.0 mm)	72.8 ± 6.6%	60.0	–	0.7 ± 0.2	(Estrada et al. 2013)
22	*Paecilomyces variotii*	Methyl isobutyl ketone (MIBK)	Vermiculite (particle size 2.4–5.0 mm)	15.0 ± 5.3%	60.0	–	1.0 ± 0.3	(Estrada et al. 2013)
23	*Cladosporium sphaerospermum*	Methyl propyl ketone, MEK, toluene, and n-butyl acetate	Polyurethane foam	98.0%	15.0	92.0	0.1, 0.1, 0.2, and 0.0, respectively	(Qi et al. 2005)

**CSTR* completely stirred tank reactors

Table 5 Examples of fungi that degrade xenobiotics

Xenobiotics	Division/class	Species	References
Chlorinated organic compounds, e.g. TCE	Basidiomycota/ Agaricomycetes	*Trametes versicolor*	(Marco-Urrea et al. 2006)
Aliphatic hydrocarbons	Ascomycota/ Sordariomycetes	*Trichoderma asperellum*	(Husaini et al. 2008)
	Ascomycota/Eurotiomycetes	*Penicillium* sp.	(Husaini et al. 2008)
		Aspergillus sp.	(Husaini et al. 2008)
Low molecular weight aromatic hydrocarbons	Basidiomycota/ Agaricomycetes	*Phanerochaete chrysosporium*	(Jorio et al. 2009)
	Ascomycota/Eurotiomycetes	*Paecilomyces variotii*	(García-Peña et al. 2001)
	Ascomycota/ Sordariomycetes	*Scedosporium apiospermum*	(García-Peña et al. 2001)
Aromatic nitro compounds	Ascomycota/ Saccharomycetes	*Yarrowia lipolytica*	(Ziganshin et al. 2007)
	Basidiomycota/ Agaricomycetes	*Gymnopilus luteofolius*	(Anasonye et al. 2015)
		Kuehneromyces mutabilis	(Anasonye et al. 2015)
		Phanerochaete velutina	(Anasonye et al. 2015)
Hydrocarbon mixtures, e.g. crude oil, engine oil	Ascomycota/ Saccharomycetes	*Yarrowia lipolytica*	(Ferreira et al. 2009)
	Ascomycota/Eurotiomycetes	*Aspergillus* sp.	(Thenmozhi et al. 2013)
	Mucoromycota/Mucorales	*Rhizopus* sp.	(Thenmozhi et al. 2013)

herein conducted. The main fungal candidate for gas biofiltration are mainly white rot fungi with ability to decompose xenobiotics. In fact, many fungal enzymes participating in the decomposition of lignin, including lignin and manganese peroxidase as well as laccase, exhibit a low substrate specificity towards multiple organic compounds of different structure (Cullen and Kersten 1992; Reddy and Mathew 2001). These fungi have been successfully applied in the degradation of toxic, persistent and hardly biodegradable pollutants, which cannot be decomposed by other microorganisms, and of compounds with limited solubility in water (Table 5). For instance, the fungi *Trametes versicolor* was able to limit significantly the toxicity of chlorinated organic compounds. White rot fungi and some yeast strains can biodegrade highly toxic nitroaromatic compounds under aerobic conditions in a multi-stage process consisting of the reduction of nitro groups and/or aromatic rings. These facts support the potential of the genus *Trametes, Trichoderma*, *Penicillium*, *Aspergillus*, *Phanerochaete, Paecilomyces, Scedosporium*, *Yarrowia*, *Gymnopilus, Kuehneromyces* and *Rhizopus* to biodegrade hydrophobic VOC in biofilters and BTFs.

5 Summary and future prospects

Despite bacterial biofiltration units are commercially available for air purification, their effectiveness rapidly drops as a result of the low pH, low humidity and nutrient limitations under long term operation. Fungal biofilters and BTFs are considered as more resistant to low humidity and pH and more effective for the removal of hydrophobic VOCs. The most recent literature in the field indicates that many species of fungi, including mold fungi (e.g. *Fusarium* sp.), yeast (e.g. *Candida* sp.) and *Cladophialophora* sp., are able to remove the most common hydrophobic VOCs emitted from industry (α-pinene, styrene, toluene, n-hexane, cyclohexane, TCE) with efficiencies above 90% under optimal design and operational conditions. Fungi can absorb hydrophobic VOCs faster than bacterial biofilm and exhibit unique properties that

justify further investigations to fully exploit the potential of these microorganisms.

Compared to conventional fungal biofilters and BTFs, where columns are colonized with one selected species of fungus, an interesting alternative is to use a consortium of different species of fungi to remove hydrophobic VOCs mixtures. In the future, it is necessary to focus on the search for synergistic consortia of fungi, so that individual species of fungi mutually increase their effectiveness in removing hydrophobic VOCs due to cross-feeding interactions or to the excretion of surfactants to the trickling solutions. In this context, understanding the mechanisms inside the fungal cells used in biofiltration systems to remove hydrophobic VOCs and elucidating the biocatalytic properties of fungi and the role of EPS in relation to hydrophobic VOCs will facilitate the selection of the most effective species of fungi, and thus allow the optimization of the biofiltration process. The prospect of using mutagenesis to increase their ability to remove hydrophobic compounds will provide great opportunities for the development of high performance fungal species.

Finally, the use of fungi for the biofiltration of hydrophobic VOCs is in line with the principles of green engineering, which aims at developing processes in a manner conducive to reducing pollution, promoting sustainable development and minimizing the risk to human health and the environment. One of the most promising applications of fungal biofiltration for the principles of green technology is the development of microbial fuel cell technology. This platform is a source of power production, which can be an interesting alternative to the methods used so far, due to the ability of microorganisms to generate electricity while removing pollutants in bioelectrochemical gas-phase reactors.

Acknowledgements The authors are grateful for the support by the project "Integrated Programme of Development of Gdansk University of Technology" No. POWR.03.05.00-00-Z044/17. The support from the regional government of Castilla y León and the EU-FEDER programme (CLU 2017-09 and UIC 315) is also acknowledged.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Abraham S, Joslyn S, Suffet IH (2015) Treatment of odor by a seashell biofilter at a wastewater treatment plant. J Air Waste Manag Assoc 65:1217–1228. https://doi.org/10.1080/10962247.2015.1075918

Anasonye F, Winquist E, Räsänen M et al (2015) Bioremediation of TNT contaminated soil with fungi under laboratory and pilot scale conditions. Int Biodeterior Biodegrad 105:7–12

Arellano-García L, Le Borgne S, Revah S (2018) Simultaneous treatment of dimethyl disulfide and hydrogen sulfide in an alkaline biotrickling filter. Chemosphere 191:809–816. https://doi.org/10.1016/j.chemosphere.2017.10.096

Arriaga S, Revah S (2009) Mathematical modeling and simulation of hexane degradation in fungal and bacterial biofilters: effective diffusivity and partition aspects. Can J Civ Eng 36:1919–1925. https://doi.org/10.1139/L09-090 (**This article is one of a selection of papers published in this Special Issue on Biological Air Treatment**)

Avalos Ramirez A, García-Aguilar BP, Jones JP, Heitz M (2012) Improvement of methane biofiltration by the addition of non-ionic surfactants to biofilters packed with inert materials. Process Biochem 47:76–82. https://doi.org/10.1016/j.procbio.2011.10.007

Baltensperger D, Barbarick K, Roberts C et al (2008) Sulfur: a missing link between soils, crops, and nutrition, vol 50. Agronomy Monograph. Madison

Bhardwaj G, Singh Cameotra S, Kumar Chopra H (2013) Biosurfactants from Fungi: a review petroleum & environmental biotechnology biosurfactants from fungi: a review. J Pet Environ Biotechnol 4:2157–7463. https://doi.org/10.4172/2157-7463.1000160

Bohn H, Bohn R (1986) Gas scrubbing by bio-washers and biofilters. Pollut Eng 18:34–35

Bowman SM, Free SJ (2006) The structure and synthesis of the fungal cell wall. BioEssays 28:799–808. https://doi.org/10.1002/bies.20441

Braun-Lüllemann A, Majcherczyk A, Hüttermann A (1997) Degradation of styrene by white-rot fungi. Appl Microbiol Biotechnol 47:150–155. https://doi.org/10.1007/s002530050904

Bremner JM, Banwart WL (1976) Sorption of sulfur gases by soils. Soil Biol Biochem 8:79–83

Carlson DA, Leiser CP, Gumerman R (1970) The soil filter: A treatment process for removal of odorous gases. Fed Water Pollut Contr Assoc Rep WP 00883–03

Carrera LV (2010) Two ex situ fungal technologies to treat contaminated soil. In: Academic dissertation, University of Helsinki, Faculty of Agriculture and Foresty, Helsinki

Chang M-K, Voice TC, Criddle CS (1993) Kinetics of competitive inhibition and cometabolism in the biodegradation of benzene, toluene, and p-xylene by two Pseudomonas isolates. Biotechnol Bioeng 41:1057–1065. https://doi.org/10.1002/bit.260411108

Chaudhary DS, Vigneswaran S, Ngo HH et al (2003) Biofilter in water and wastewater treatment. Korean J Chem Eng 20:1054–1065. https://doi.org/10.1007/BF02706936

Cheng Y, Li X, Liu H et al (2020) Effect of presence of hydrophilic volatile organic compounds on removal of hydrophobic n-hexane in biotrickling filters. Chemosphere 252:126490. https://doi.org/10.1016/j.chemosphere.2020.126490

Chheda D, Sorial GA (2017) Evaluation of co-metabolic removal of trichloroethylene in a biotrickling filter under acidic conditions. J Environ Sci (china) 57:54–61. https://doi.org/10.1016/j.jes.2016.12.008

Cox H (1995) Styrene Removal from Waster Gas by the Fungus Exophiala Jeanselmei in a Biofilter. Rijksuniversiteit Groningen, the Netherlands

Cox H, Nguyen T, Deshusses M (1998) Elimination of toluene vapors in biotrickling filters: performance and carbon balances. In: Proceedings of the annual meeting & exhibition of the air & waste management association, pp 14–18

Cox HH, Deshusses MA (1998) Biological waste air treatment in biotrickling filters. Curr Opin Biotechnol 9:256–262. https://doi.org/10.1016/S0958-1669(98)80056-6

Cox HHJ, Magielsen FJ, Doddema HJ, Harder W (1996) Influence of the water content and water activity on styrene degradation by Exophiala jeanselmei in biofilters. Appl Microbiol Biotechnol 45:851–856. https://doi.org/10.1007/s002530050773

Cox HHJ, Moerman RE, van Baalen S et al (1997) Performance of a styrene-degrading biofilter containing the yeast Exophiala jeanselmei. Biotechnol Bioeng 53:259–266. https://doi.org/10.1002/(SICI)1097-0290(19970205)53:3%3c259::AID-BIT3%3e3.0.CO;2-H

Cullen D, Kersten P (1992) Applied molecular genetics of filamentous fungi (Fungal enzymes for lignocellulose degradation). Springer

De S, Malik S, Ghosh A et al (2015) A review on natural surfactants. RSC Adv 5:65757–65767. https://doi.org/10.1039/c5ra11101c

Detchanamurthy S, Gostomski PA (2012) Biofiltration for treating VOCs: an overview. Rev Environ Sci Biotechnol 11(3):231–241. https://doi.org/10.1007/s11157-012-9288-5

Devinny JS, Ramesh J (2005) A phenomenological review of biofilter models. Chem Eng J 113:187–196

Estrada JM, Hernández S, Muñoz R, Revah S (2013) A comparative study of fungal and bacterial biofiltration treating a VOC mixture. J Hazard Mater 250–251:190–197. https://doi.org/10.1016/j.jhazmat.2013.01.064

Ferreira T, Azevedo D, Coelho MA (2009) The crude oil degrading potential of Yarrowia lipolytica. New Biotechnol 25:S80–S81

Flemming HC, Neu TR, Wozniak DJ (2007) The EPS matrix: the "House of Biofilm Cells." J Bacteriol 189:7945–7947

Flemming HC, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623–633

García-Peña EI, Hernández S, Favela-Torres E et al (2001) Toluene biofiltration by the fungus *Scedosporium apiospermum* TB1. Biotechnol Bioeng 76:61–69. https://doi.org/10.1002/bit.1026

Gloag ES, Turnbull L, Huang A et al (2013) Self-organization of bacterial biofilms is facilitated by extracellular DNA. Proc Natl Acad Sci USA 110:11541–11546. https://doi.org/10.1073/pnas.1218898110

Gospodarek M, Rybarczyk P, Brillowska-Dąbrowska A, Gębicki J (2019a) The use of various species of fungi in biofiltration of air contaminated with odorous volatile organic compounds. In: E3S web of conferences, vol 100, p 00021. https://doi.org/10.1051/e3sconf/201910000021

Gospodarek M, Rybarczyk P, Szulczyński B, Gębicki J (2019b) Comparative evaluation of selected biological methods for the removal of hydrophilic and hydrophobic odorous VOCs from air. Processes 7:187. https://doi.org/10.3390/pr7040187

Griffin DM (1985) A comparison of the roles of bacteria and fungi. Bacteria in Nature. Springer, pp 221–255

Groenestijn J van, Harkes M, Cox H, Doddema H (1995) Ceramic materials in biofiltration. In: Proceedings USC-TRG conference on biofiltration. Los Angeles, USA

Gumerman R, Carlson DA (1966) Hydrogen sulfide and methyl mercaptan removal with soil columns. In: 21st industrial waste conference, Purdue University. West Lafayette, IN, pp 172–191

Han MF, Hu XR, Wang YC et al (2020) Comparison of separated and combined photodegradation and biofiltration technology for the treatment of volatile organic compounds: a critical review. Crit Rev Environ Sci Technol. https://doi.org/10.1080/10643389.2020.1854566

Husaini A, Roslan H, Hii K et al (2008) Biodegradation of aliphatic hydrocarbon by indigenous fungi isolated from used motor oil contaminated sites. World J Microbiol Biotechnol 24:2789–2797

Ito S, Inoue S (1982) Sophorolipids from torulopsis bombicola: possible relation to alkane uptake. Appl Environ Microbiol 43:1278–1283. https://doi.org/10.1128/aem.43.6.1278-1283.1982

Jianming Y, Wei L, Zhuowei C et al (2014) Dichloromethane removal and microbial variations in a combination of UV pretreatment and biotrickling filtration. J Hazard Mater 268:14–22. https://doi.org/10.1016/j.jhazmat.2013.12.068

Jin Y, Guo L, Veiga MC, Kennes C (2007) Fungal biofiltration of α-pinene: effects of temperature, relative humidity, and transient loads. Biotechnol Bioeng 96:433–443. https://doi.org/10.1002/bit.21123

Jin Y, Veiga MC, Kennes C (2006) Performance optimization of the fungal biodegradation of α-pinene in gas-phase biofilter. Process Biochem 41:1722–1728. https://doi.org/10.1016/j.procbio.2006.03.020

Jorio H, Jin Y, Elmrini H et al (2009) Treatment of VOCs in biofilters inoculated with fungi and microbial consortium.

Environ Technol 30:477–485. https://doi.org/10.1080/09593330902778849
Kennes C, Veiga MC (2004) Fungal biocatalysts in the biofiltration of VOC-polluted air. J Biotechnol 113:305–319
Kośmider J, Mazur-Chrzanowska B, Wyszyński B (2012) Odory. Wydawnictwo Naukowe PWN
Krailas S, Pham QT, Amal R et al (2000) Effect of inlet mass loading, water and total bacteria count on methanol elimination using upward flow and downward flow biofilters. J Chem Technol Biotechnol 75:299–305. https://doi.org/10.1002/(SICI)1097-4660(200004)75:4%3c299::AID-JCTB210%3e3.0.CO;2-P
Lalanne F, Malhautier L, Roux J-C, Fanlo J-L (2008) Absorption of a mixture of volatile organic compounds (VOCs) in aqueous solutions of soluble cutting oil. Biores Technol 99:1699–1707. https://doi.org/10.1016/J.BIORTECH.2007.04.006
Ławrynowicz M (2002) Krolestwo grzybow na przelomie tysiacleci. Wiadomości Botaniczne 46:19–25
Leson G, Winer AM (1991) Biofiltration: an innovative air pollution control technology for VOC emissions. J Air Waste Manag Assoc 41:1045–1054. https://doi.org/10.1080/10473289.1991.10466898
Lewandowski Z, Boltz JP (2011) Biofilms in water and wastewater treatment. Treatise on water science. Elsevier, pp 529–570
Li K, Zhou J, Wang L et al (2019) The styrene purification performance of biotrickling filter with toluene-styrene acclimatization under acidic conditions. J Air Waste Manag Assoc 69:944–955. https://doi.org/10.1080/10962247.2019.1604450
Li L, Liu JX (2006) Removal of xylene from off-gas using a bioreactor containing bacteria and fungi. Int Biodeterior Biodegrad 58:60–64. https://doi.org/10.1016/J.IBIOD.2006.07.002
López ME, Rene ER, Malhautier L et al (2013) One-stage biotrickling filter for the removal of a mixture of volatile pollutants from air: performance and microbial community analysis. Bioresour Technol 138:245–252. https://doi.org/10.1016/J.BIORTECH.2013.03.136
Maestre JP, Gamisans X, Gabriel D, Lafuente J (2007) Fungal biofilters for toluene biofiltration: evaluation of the performance with four packing materials under different operating conditions. Chemosphere 67:684–692. https://doi.org/10.1016/J.CHEMOSPHERE.2006.11.004
Marco-Urrea E, Gabarrell X, Sarrà M, Caminal G, Vicent T, Reddy CA (2006) Novel aerobic perchloroethylene degradation by the white-rot fungus Trametes versicolor. Environ Sci Technol 40(24):7796
Marycz M, Brillowska-Dąbrowska A, Gębicki J (2020) Evaluation of immobilization of selected peat-isolated yeast strains of the species Candida albicans and Candida subhashii on the surface of artificial support materials used for biotrickling filtration. Processes 8:801. https://doi.org/10.3390/pr8070801
Mayer VE, de Hoog S, Cristescu SM et al (2021) Volatile organic compounds in the azteca/cecropia ant-plant symbiosis and the role of black fungi. J Fungi 7:836. https://doi.org/10.3390/JOF7100836
Metcalf L, Harrison PE, Tchobanoglous G (1979) Wastewater Engineering: treatment, disposal, and reuse. McGraw-Hill, New York
Miller U, Sówka I, Skrętowicz M (2019) Zastosowanie surfaktantów w biotechnologii środowiska. In: EKO-DOK 11th conference on interdisciplinary problems in environmental protection and engineering. Polanica-Zdrój (Poland)
Moe WM, Irvine RL (2000) Polyurethane foam medium for biofiltration. I: characterization. J Environ Eng 126:815–825. https://doi.org/10.1061/(asce)0733-9372(2000)126:9(815)
Neu TR (1996) Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. Microbiol Rev 60:151–166
Neu TR, Dengler T, Jann B, Poralla K (1992) Structural studies of an emulsion-stabilizing exopolysaccharide produced by an adhesive, hydrophobic Rhodococcus strain. J Gen Microbiol 138:2531–2537. https://doi.org/10.1099/00221287-138-12-2531
Ottengraf SPP, van den Oever AHC (1983) Kinetics of organic compound removal from waste gases with a biological filter. Biotechnol Bioeng 25:3089–3102. https://doi.org/10.1002/BIT.260251222
Pietarinen VM, Rintala H, Hyvärinen A et al (2008) Quantitative PCR analysis of fungi and bacteria in building materials and comparison to culture-based analysis. J Environ Monit 10:655–663. https://doi.org/10.1039/b716138g
Pomeroy RD (1957) De-odoring of gas streams by the use of micro-biological growths. U.S. Patent No. 2,793,096. U.S. Patent and Trademark Office, Washington, DC
Prenafeta-Boldú FX, de Hoog GS, Summerbell RC (2018) Fungal Communities in Hydrocarbon Degradation. Microbial communities utilizing hydrocarbons and lipids: Members, metagenomics and ecophysiology, handbook of hydrocarbon and lipid microbiology. Springer, Cham
Prenafeta-Boldú FX, Guivernau M, Gallastegui G et al (2012) Fungal/bacterial interactions during the biodegradation of TEX hydrocarbons (toluene, ethylbenzene and p-xylene) in gas biofilters operated under xerophilic conditions. FEMS Microbiol Ecol 80:722–734. https://doi.org/10.1111/J.1574-6941.2012.01344.X
Prenafeta-Boldú FX, Illa J, van Groenestijn JW, Flotats X (2008) Influence of synthetic packing materials on the gas dispersion and biodegradation kinetics in fungal air biofilters. Appl Microbiol Biotechnol 79(2):319–327. https://doi.org/10.1007/S00253-008-1433-2
Prenafeta-Boldú FX, Roca N, Villatoro C et al (2019) Prospective application of melanized fungi for the biofiltration of indoor air in closed bioregenerative systems. J Hazard Mater 361:1–9. https://doi.org/10.1016/J.JHAZMAT.2018.08.059
Qi B, Moe WM, Kinney KA (2005) Treatment of paint spray booth off-gases in a fungal biofilter. J Environ Eng 131:180–189. https://doi.org/10.1061/(ASCE)0733-9372(2005)131:2(180)
Quan Y, Wu H, Guo C et al (2018) Enhancement of TCE removal by a static magnetic field in a fungal biotrickling filter. Bioresour Technol 259:365–372. https://doi.org/10.1016/J.BIORTECH.2018.03.031
Raboni M, Torretta V, Viotti P (2017) Treatment of airborne BTEX by a two-stage biotrickling filter and biofilter,

exploiting selected bacterial and fungal consortia. Int J Environ Sci Technol 14:19–28. https://doi.org/10.1007/s13762-016-1127-8

Rapoport A, Borovikova D, Kokina A et al (2011) Immobilisation of yeast cells on the surface of hydroxyapatite ceramics. Process Biochem 46:665–670. https://doi.org/10.1016/j.procbio.2010.11.009

Reddy CA, Mathew Z (2001) Bioremediation potential of white rot fungi. In: British mycological society symposium series, vol. 23. pp 52–78

Rene ER, López ME, Veiga MC, Kennes C (2010a) Performance of a fungal monolith bioreactor for the removal of styrene from polluted air. Biores Technol 101:2608–2615. https://doi.org/10.1016/J.BIORTECH.2009.10.060

Rene ER, Mohammad BT, Veiga MC, Kennes C (2012) Biodegradation of BTEX in a fungal biofilter: influence of operational parameters, effect of shock-loads and substrate stratification. Bioresour Technol 116:204–213. https://doi.org/10.1016/J.BIORTECH.2011.12.006

Rene ER, Montes M, Veiga MC, Kennes C (2011) Styrene removal from polluted air in one and two-liquid phase biotrickling filter: steady and transient-state performance and pressure drop control. Bioresour Technol 102:6791–6800. https://doi.org/10.1016/J.BIORTECH.2011.04.010

Rene ER, Špačková R, Veiga MC, Kennes C (2010b) Biofiltration of mixtures of gas-phase styrene and acetone with the fungus Sporothrix variecibatus. J Hazard Mater 184:204–214. https://doi.org/10.1016/J.JHAZMAT.2010.08.024

Revah S, Vergara-Fernández A, Hernández S (2011) Fungal biofiltration for the elimination of gaseous pollutants from air. Mycofactories. Bentham Science Publishers Ltds, pp 109–120

Rodrigues L, Banat IM, Teixeira J, Oliveira R (2006) Biosurfactants: potential applications in medicine. J Antimicrob Chemother 57:609–618. https://doi.org/10.1093/jac/dkl024

Ron EZ, Rosenberg E (2001a) Natural roles of biosurfactants. Environ Microbiol 3:229–236

Ron EZ, Rosenberg E (2001b) Natural roles of biosurfactants. Minirev Environ Microbiol 3:229–236. https://doi.org/10.1046/j.1462-2920.2001.00190.x

Ruiz-Herrera J (2016) Fungal cell wall: structure, synthesis, and assembly. CRC Press

Rybarczyk P, Marycz M, Szulczyński B et al (2021) Removal of cyclohexane and ethanol from air in biotrickling filters inoculated with Candida albicans and Candida subhashii. Arch Environ Prot. https://doi.org/10.24425/aep.2021.136445

Schiavon M, Ragazzi M, Rada EC, Torretta V (2016) Air pollution control through biotrickling filters: a review considering operational aspects and expected performance. Crit Rev Biotechnol 36:1143–1155. https://doi.org/10.3109/07388551.2015.1100586

Shareefdeen Z, Herner B, Singh A (2005) Biotechnology for air pollution control. Springer, Berlin

Siñeriz F, Hommel RK, Kleber H-P (2001) Production of biosurfactants. Encyclopedia of life support systems. Eolls Publishers, Oxford

Singh R, Paul D, Jain RK (2006) Biofilms: implications in bioremediation. Trends Microbiol 14:389–397

Smalyukh II, Butler J, Shrout JD et al (2008) Elasticity-mediated nematiclike bacterial organization in model extracellular DNA matrix. Phys Rev E Stat Nonlinear Soft Matter Phys 78:030701. https://doi.org/10.1103/PhysRevE.78.030701

Sundstrom P (2002) Adhesion in Candida spp. Cell Microbiol 4:461–469. https://doi.org/10.1046/j.1462-5822.2002.00206.x

Thenmozhi R, Arumugam K, Nagasathya A et al (2013) Studies on Mycoremediation of used engine oil contaminated soil samples. Pelagia Res Libr Adv Appl Sci Res 4:110–118

Thormann MN, Rice AV (2007) Fungi from peatlands. Fungal Divers 24:241–299

van Groenestijn JW, Hesselink PGM (1993) Biotechniques for air pollution control. Biodegradation 4:283–301. https://doi.org/10.1007/BF00695975

van Groenestijn JW, van Heiningen WNM, Kraakman NJR (2001) Biofilters based on the action of fungi. Water Sci Technol 44:227–232

Vergara-Fernández A, Revah S, Moreno-Casas P, Scott F (2018) Biofiltration of volatile organic compounds using fungi and its conceptual and mathematical modeling. Biotechnol Adv 36:1079–1093. https://doi.org/10.1016/j.biotechadv.2018.03.008

Vergara-Fernández A, van Haaren B, Revah S (2006) Phase partition of gaseous hexane and surface hydrophobicity of Fusarium solani when grown in liquid and solid media with hexanol and hexane. Biotechnol Lett 28:2011–2017. https://doi.org/10.1007/s10529-006-9186-4

Weber FJ, Hartmans S (1996) Prevention of clogging in a biological trickle-bed reactor removing toluene from contaminated air. Biotechnol Bioeng 50:91–97. https://doi.org/10.1002/(SICI)1097-0290(19960405)50:1%3c91::AID-BIT10%3e3.0.CO;2-A

Wheatley AD, Mitra RI, Hawkes HA (1982) Protein recovery from dairy industry wastes with aerobic biofiltration. J Chem Technol Biotechnol 32:203–212. https://doi.org/10.1002/jctb.5030320125

Woertz JR, Kinney KA, Kraakman NJR et al (2003) Mite growth on fungus under various environmental conditions and its potential application to biofilters. Exp Appl Acarol 27(4):265–276

Woertz JR, Kinney KA, McIntosh NDP, Szaniszlo PJ (2001) Removal of toluene in a vapor-phase bioreactor containing a strain of the dimorphic black yeastExophiala lecaniicorni. Biotechnol Bioeng 75:550–558. https://doi.org/10.1002/bit.10066

van Woertz W, van Heiningen M, Ee J, van Heiningen W, van Eekert M et al (2002) Dynamic bioreactor operation: effects of packing material and mite predation on toluene removal from off-gas. Appl Microbiol Biotechnol 58:690–694. https://doi.org/10.1007/s00253-002-0944-5

Wösten HAB (2001) Hydrophobins: multipurpose proteins. Annu Rev Microbiol 55:625–646. https://doi.org/10.1146/annurev.micro.55.1.625

Wösten HAB, van Wetter M-A, Lugones LG et al (1999) How a fungus escapes the water to grow into the air. Curr Biol 9:85–88. https://doi.org/10.1016/S0960-9822(99)80019-0

Wübker SM, Friedrich CG (1996) Reduction of biomass in a bioscrubber for waste gas treatment by limited supply of phosphate and potassium ions. Appl Microbiol Biotechnol 46:475–480

Yadav JS, Wallace RE, Reddy CA (1995) Mineralization of mono- and dichlorobenzenes and simultaneous degradation of chloro- and methyl-substituted benzenes by the white rot fungus Phanerochaete chrysosporium. Appl Environ Microbiol 61:677–680

Yang C, Qian H, Li X et al (2018) Simultaneous removal of multicomponent VOCs in biofilters. Trends Biotechnol 36:673–685. https://doi.org/10.1016/J.TIBTECH.2018.02.004

Yousefinejad A, Zamir SM, Nosrati M (2019) Fungal elimination of toluene vapor in one- and two-liquid phase biotrickling filters: effects of inlet concentration, operating temperature, and peroxidase enzyme activity. J Environ Manag 251:109554. https://doi.org/10.1016/J.JENVMAN.2019.109554

Zehraoui A, Wendell D, Sorial GA (2014) Treatment of hydrophobic VOCs in trickling bed air biofilter: emphasis on long-term effect of initial alternate use of hydrophilic VOCs and microbial species evolution. J Air Waste Manag Assoc 64:1102–1111. https://doi.org/10.1080/10962247.2014.925522

Zhang Y, Liss SN, Allen DG (2006) The effects of methanol on the biofiltration of dimethyl sulfide in inorganic biofilters. Biotechnol Bioeng 95:734–743. https://doi.org/10.1002/bit.21033

Zhang Y, Liu J, Qin Y et al (2019) Performance and microbial community evolution of toluene degradation using a fungi-based bio-trickling filter. J Hazard Mater 365:642–649. https://doi.org/10.1016/J.JHAZMAT.2018.11.062

Ziganshin AM, Gerlach R, Borch T et al (2007) Production of eight different hydride complexes and nitrite release from 2,4,6-Trinitrotoluene by Yarrowia lipolytica. Appl Environ Microbiol 73:7898–7905. https://doi.org/10.1128/AEM.01296-07

3.3. Publication 3: Evaluation of immobilization of selected peat-isolated yeast strains of the species *Candida albicans* and *Candida subhashii* on the surface of artificial support materials used for biotrickling filtration

The first part of the experimental research was aimed at identifying environmental fungal isolates from peat capable of degrading hydrophobic VOCs. In order to familiarize with the methodology of conducting biofiltration processes, the research began with the removal of n-butanol in BTF [173]. Beginning of research on a hydrophilic compound was motivated by the fact that this compound is characterized by high biodegradability, so the process of its removal from the air is a problem-free and efficient process, and thus allows for easy verification of the correctness of the process. Conducting this experiment made it possible to collect a sample from a working biofilter in order to isolate the strains of microorganisms inhabiting it. Using peat from a working biofilter column, rather than fresh peat, increased the chance of isolating organisms that were able to use VOCs as a carbon source. The experiment was carried out in a two-section BTF filled with a commercially purchased mixture of peat and perlite, naturally inhabited by various species of microorganisms. From the 3rd day of the process to the end of the process, stabilization of the RE value in the range of 85-92% was achieved. The sample was taken on the last day of the process. Subsequently, the fungal strains were cultured on Sabouraud medium, and a short method developed by Brillowska-Dąbrowska et al. was used to isolate the DNA of the strains [174]. Primers ITS1 and ITS4 were used to obtain polymerase chain reaction (PCR) products. One isolate of *C. subhashii* and five isolates of *C. albicans* were identified in the tested peat sample. For further stages of research, isolates were selected that were characterized by the highest efficiency of carbon assimilation from selected VOCs, both hydrophobic and hydrophilic. Subsequently, new methods were proposed to immobilize fungi on various types of artificial support materials used for BTF (Bialecki rings (25x25), Pall rings, polyurethane foam, Bialecki rings (50x50)). An innovative method was also developed that allowed for safe detachment of fungal cells from the surface of the packing material, so as not to damage them. The assessment of fungal immobilization and their viability on the support material was carried out using the following methods: optical microscopy, flow cytometry and tests using propidium iodide and annexin V. The viability of fungi isolated from the surface of support materials was determined at the level of 95%. The article also compares materials that can be used as BTF packing materials, using flow cytometry and optical microscopy, in terms of the effectiveness of their immobilization by selected species of fungi. On the basis of the proposed comparative tests and the analyses carried out, polyurethane foam was selected as the best support material in the BTF for immobilization by fungal species. This study also showed that the proposed methods of immobilization of fungal species as well as methods of its assessment, apart from their relatively low price, are effective, quick and simple. The proposed methods, together with the obtained results, could be useful in improving biofiltration processes and providing comprehensive knowledge about the types of microorganisms inhabiting the biofilter bed. This would allow to increase its efficiency by isolating a specific strain of microorganisms and inoculating them on the aseptic packing materials.

The publication presents experiments leading to the implementation of task T2 *Identification of a fungus isolate, previously not used in biofiltration, which will effectively remove hydrophobic VOCs from the air* and T3 *Development of a quick and low-cost method of fungal cell immobilization on internal packing material and development of a simple and low-cost method for monitoring the condition of the fungi used during the biofiltration process*.

The novelties presented in it are:

- Species identification isolate, capable of removing selected hydrophobic VOCs from the air, not yet used in BTF, as *C. subhashii.*
- Development of a proprietary, cheap and simple method of fungi immobilization on various types of packaging materials most commonly used in BTF (Bialecki rings (25x25), Pall rings, polyurethane foam, Bialecki rings (50x50)).
- Proposal of full method for the assessment of fungal colonization and their viability on the packing material in BTF using the following methods: optical microscopy, flow cytometry and tests using propidium iodide and annexin V.

processes

MDPI

Article

Evaluation of Immobilization of Selected Peat-Isolated Yeast Strains of the Species *Candida albicans* and *Candida subhashii* on the Surface of Artificial Support Materials Used for Biotrickling Filtration

Milena Marycz [1,*], Anna Brillowska-Dąbrowska [2] and Jacek Gębicki [1]

[1] Department of Process Engineering and Chemical Technology, Faculty of Chemistry, Gdansk University of Technology, 11/12 G. Narutowicza Street, 80-233 Gdansk, Poland; jacek.gebicki@pg.edu.pl
[2] Department of Molecular Biotechnology and Microbiology, Faculty of Chemistry, Gdansk University of Technology, 11/12 G. Narutowicza Street, 80-233 Gdansk, Poland; annbrill@pg.edu.pl
* Correspondence: milena.marycz@pg.edu.pl; Tel.: +48-58-347-2058

Received: 15 May 2020; Accepted: 3 July 2020; Published: 8 July 2020

Abstract: The paper describes the process of *n*-butanol abatement by unicellular fungi, able to deplete n-butanol content in gas, by using *n*-butanol as source of carbon. Isolated and identified fungi species *Candida albicans* and *Candida subhashii* were subjected to a viability process via assimilation of carbon from hydrophilic and hydrophobic compounds. The isolates, which exhibited the ability to assimilate carbon, were immobilized on four different types of artificial support materials used for biotrickling filtration. Application of optical microscopy, flow cytometry and the tests employing propidium iodide and annexin V revealed viability of the fungi isolated on support materials' surfaces at the average level of 95%. The proposed method of immobilization and its evaluation appeared to be effective, cheap and fast. Based on performed comparative analyses, it was shown that polyurethane foam and Bialecki rings (25 × 25) could be attractive support materials in biotrickling filtration.

Keywords: biotrickling filtration; fungi; polyurethane foam; Pall rings; Bialecki rings; *n*-butanol; cyclohexane; flow cytometry

1. Introduction

One of the most common techniques of biological gas treatment is biofiltration. The process of biofiltration consists in passing a polluted stream of gas through a filter bed, inhabited by microorganisms (bacteria, fungi), belonging to different species. The impurities diffuse from the gas phase to the biofilm forming on the surface of elements of the packed bed. The compounds adsorbed on the surface of elements or absorbed in the biofilm undergo biodegradation, and the air free of odorous compounds leaves the biofilter. The most popular apparatus for biofiltration includes conventional biofilters, biotrickling filters and bioscrubbers [1–3].

Microorganisms and type of packing are crucial elements for biological air purification processes. The selection of microorganisms colonizing the biofilter located on the packing is pivotal for the effective degradation of specific groups of chemical compounds. Biofilters can be populated with a single strain of microorganisms or their consortia [3].

A literature review of available techniques for removal of odorous volatile organic compounds (VOCs) from air revealed that biotrickling filtration was a highly effective method for the removal of contaminants from gaseous streams [1]. The most important element of the biofiltration apparatus

is a column packing that is colonized by the microorganisms responsible for VOC removal, the compounds of odor nuisance. Usually, ambiguous term like fungi or microbial consortium is used, without clear indication of the species present in a biofilter bed [4–9]. At present, especially bacterial strains used in biofiltration are a subject of extensive studies [10,11]. In the case of fungi, especially those used in conventional biofiltration are under investigation [12]. Table 1 presents typical fungi and types of packing material employed in the conventional biofilters and biotrickling filters. Considering the technical and operational differences between conventional biofiltration and biotrickling filtration [2,13], the beds of these biofilters differ in the types of fungal strains present in a packing material. Depending on the mode of biofiltration, the process makes use of the microorganisms either naturally inhabiting the packing material (e.g., bark, leaves or peat in conventional biofilters, straw, wood shavings, compost, cones) [3,14–17] or those intentionally inoculated on the filter bed elements (e.g., inoculation of polyurethane foam or ceramic materials for biotrickling filter, alginate beads, ceramic saddles, Pall rings, Raschig rings, tri-Pack) [18–21]. Accordingly, assuring of high efficiency of malodorous compounds removal requires not only selection of suitable microorganisms but also fitting of such biofilter packing, which is easily colonized by selected microorganisms and allows their growth during the process allows maintenance of proper biomass concentration on the filter bed. Cultivation of the microorganisms occurs both in interstitial spaces as well as in micropores, the size of which allows penetration of the cells of microorganisms forming so-called sites protected from the impact of adverse shearing forces due to water flow. The biofilter's packing material can play the role of not only a support material for biological film development but also an adsorbent, an ion exchanger, a nutrient medium and a substance buffering the biochemical reaction environment [22]. Apart from the effectiveness of microorganism colonization, the price should also be taken into account while selecting the biofilter's packing. Hence, the following features of the packing became a target: low price, effective colonization with the microorganisms and stimulation of growth of the microorganisms capable of malodorous compounds removal. Such material should be characterized by a significant specific surface area but it should not influence on biofilm formation [23–25].

Regarding the fungi which are to colonize the biofilm substrate, a big surface area of thread-like structure of a fungi's hyphae allows easy assimilation of carbon from organic compounds present in a surrounding gas phase [26]. Wu et al. revealed that fungi could be as effective as bacteria in the removal of organic contaminants from air [27] and some research indicate that fungi can assimilate carbon with higher efficiency as compared to bacteria [28,29]. The information about VOCs assimilation by the fungi isolated from pine needles can be found in literature [30]. As opposed to bacteria, fungi are more resistant to low humidity and high acidity. It is especially beneficial for the removal of hydrophobic compounds [31]. Moreover, fungi can be applied in acidic gas streams with high concentration of organic contaminants [32]. Abovementioned properties of fungi make them an interesting subject for further investigation.

In microbiological processes it is necessary to provide carbon, nitrogen and phosphorus for microorganisms. The last two can be easily accessed through ammonium salts, nitrates or phosphates. The problem of carbon assimilation is far more complex. Despite the fact that there is about 30 times more carbon in the environment as compared to nitrogen, the multiplicity of its chemical connections causes that carbon containing compounds can affect microorganisms in different ways e.g., enhance as well as inhibit their growth. The form of carbon most suitable for assimilation are carbohydrates, preferably glucose. However, in the absence of carbohydrates, microorganisms can obtain carbon from other sources e.g., volatile organic compounds present in air. To improve the ability of microorganism to decompose VOCs in biotrickling filtration processes, it is desirable to provide the surface of biofilm i.e., packing elements covered with biofilm as densely as possible, to increase the contact area between the biofilm and gas phase. In practice, it is realized by immobilization of the microorganisms on a support material. A literature review revealed a variety of different supports e.g., expanded clay aggregate [33], tuff [34], activated carbon [35], perlite [36], alginate beads [37], polyurethane foam [38], ceramic saddles [39], Pall rings [40–42], and Raschig rings [43].

Table 1. Examples of fungi employed for gas purification in conventional biofiltration and biotrickling filtration processes.

Predominate Species	Contaminant	Packing Material	Reference
Conventional Biofilter—microorganisms naturally inhabiting the packing material			
Paecilomyces variotii	toluene	ceramic rings	[44]
Phanerochaete chrysosporium	butanol	straw	[45]
Trametes versicolor	aniline	peat	
Pichia pastoris	methanol	perlite	[46]
Cladosporium sphaerospermum	xylene	wood shavings	[47]
Ophiostoma sp.	α-pinene	volcanic rock	[48]
Aspergillus niger	*n*-hexane	kermesite	[49]
Candida utilis	ethanol	sugarcane bagasse	[50]
Scedosporium apiospermum	toluene	GAC:vermiculite (15:85)	[51]
		perlite	[52]
Paecilomyces variotii	toluene	perlite	
Sporothrix veriecibatus	styrene	perlite	[53]
Exophiala jeanselmei	styrene	perlite	[54]
Exophiala sp.	BTEX	perlite	[55]
Fusarium solani	*n*-hexane	perlite	[56]
Biotrickling Filter—microorganisms intentionally inoculated on the filter bed elements			
Sporothrix variecibatus	styrene	ceramic monolith	[57]
Fusarium solani	*n*-hexane	perlite	[58]
Candida palmileophila strain MA-M11	styrene	ceramic Raschig rings	[59]
Cladosporium sphaerospermum	methyl propyl ketone, MEK, toluene, and *n*-butyl acetate	polyurethane foam	[60–63]
Candida boidinii, *Ophiostoma stenoceras*	α-pinene	polyurethane foam	[64]

GAC—Granular Activated Carbon, BTEX—Benzene, Toluene, Ethylbenzene and Xylenes, MEK—Methyl Ethyl Ketone.

Flow cytometry can be used to check the effectiveness of fungi colonization in a quantitative as well as qualitative manner [65]. Flow cytometry is a rapid and sensitive technique with important applications in biology and medicine [66,67]. This technique allows analysis of the microorganisms involved in technological processes as well as characterization of the starter cultures aimed at precise determination of their species composition. Monitoring of film damage can provide fast identification of microorganism failure or their death. Flow cytometry has already been utilized for *Candida albicans* [68,69], however, up to our best knowledge, it has not been implemented for investigation of fungi viability during biofiltration processes.

The paper presents the investigation on isolation of fungi from peat as a natural type of packing used in the classic biofilters. The study concerns immobilization of selected fungi (*Candida albicans* and *Candida subhashii)* on the surface of typical support materials of the biotrickling filters i.e., polyurethane foam, Pall rings and Bialecki rings. Effectiveness of their colonization was measured and compared using flow cytometry and optical microscopy. Moreover, the investigations aimed at determination of viability and ability to assimilate carbon from such compounds as *n*-butanol and cyclohexane, representing hydrophilic and hydrophobic compounds removed by the biotrickling filters, were carried out for the aforementioned fungal isolates. The obtained information can be employed for proper design of the process and maintenance of the optimum operation conditions. Additionally, correctly selected support material with respect to fungi types determines achievement of assumed deodorization effectiveness in the biotrickling filters.

2. Materials and Methods

2.1. Chemicals and Reagents

N-butanol was purchased from POCH (Gliwice, Poland) and was used in the biofiltration experiments as a carbon source for the microorganisms. The composition of the used trickling medium is as follows: $Na_2HPO_4 \cdot 2H_2O$ (15.2 g/dm^{-3}), KH_2PO_4 (3 g/dm^{-3}), NaCl (0.5 g/dm^{-3}) and NH_4Cl (1 g/dm^{-3}). All materials were purchased from POCH (Gliwice, Poland). Cyclohexane was purchased from Merck (Darmstadt, Germany).

The primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) [70] were purchased from Genomed (Warsaw, Poland). The High GC PCR mix was purchased from A&A Biotechnology, Gdynia, Poland.

2.2. Support Materials

Characteristics of the materials employed as a surface for immobilization of selected fungi is presented in Table 2. The shape and general appearance of utilized types of support materials are illustrated in Figure 1.

Table 2. Characteristics of investigated materials used for immobilization of fungi [71].

Type of Material Characteristics	Polyurethane Foam	Bialecki Rings (25 × 25)	Pall Rings (50 × 50)	Bialecki Rings (50 × 50)
material	polyurethane	polypropylene	polypropylene admixed with chalk	polypropylene
color/transparency	blue color, low transparency	milky color, high transparency	milky color, medium transparency	dark green color, no transparency
surface	600 m^2/m^3	245 m^2/m^3 (in bulk); 0.0047 m^2/piece (in bulk)	110 m^2/m^3 (in bulk); 0.017 m^2/piece (in bulk)	155 m^2/m^3 (in bulk); 0.024 m^2/piece (in bulk)
number	6369 pieces/m^3 (arranged)	52000 pieces/m^3 (in bulk)	6500 pieces/ m^3 (in bulk); 8980 pieces/m^3 (arranged)	6500 pieces/ m^3 (in bulk); 8980 pieces/m^3 (arranged)

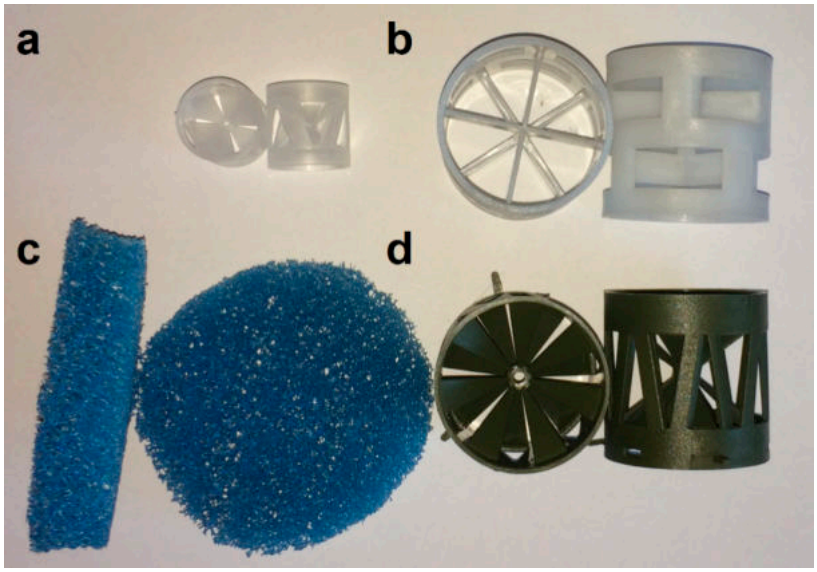


Figure 1. Selected examined support materials. (**a**) Bialecki rings (25 × 25), (**b**) Pall rings, (**c**) polyurethane foams, (**d**) Bialecki rings (50 × 50).

2.3. Isolation of Fungi Species Present in Peat and Their Species Identification

For the experiment, the peat samples were taken from a biofilter column and cultivated in Sabouraud agar, which is selective for fungal growth. The isolation and preservation of pure culture was carried out as follows: the peat specimens were collected at day 20 of the process from a biofilter removing *n*-butanol. This approach allowed easy selection of the strains being able to survive in the *n*-butanol environment (hydrophilic compound). Then, several (up to 20) streak plates were prepared to obtain pure colonies. Next, the pure isolates were kept frozen at −80 °C (glycerol/water 1/3). After obtaining the isolates, the identification by means of PCR (Polymerase Chain Reaction) and ITS sequencing (Genomed, Warszawa, Poland) was performed. In order to isolate DNA, a short method developed by Brillowska-Dąbrowska [72,73] was used. The PCR mixture was prepared for each of the isolates as indicated in Table 3. The conditions of PCR are described in [3].

Table 3. Composition of the reaction mixture for the PCR (Polymerase Chain Reaction) reaction.

Reagent	Volume (µL)
Water	6.0
2xPCR Mix Plus High GC	10.0
Starter ITS1 (10 mM)	1.0
Starter ITS4 (10 mM)	1.0
Matrix	2.0
Final volume	20.0

2.4. Selection of Fungal Isolates Able to Utilize Hydrophilic and Hydrophobic Compounds as a Carbon Source

After identification (by ITS region sequencing), the isolates were taken for the measurement of capability of *n*-butanol, cyclohexane and their mixture (50/50) biodegradation. Determination of their ability to assimilate carbon for tested compounds was performed as follows: the incubation of all samples was carried out at 24 °C for 16 h and the optical density was measured at 595 nm wavelength [74–76]. This wavelength is applied for optical density measurement of fungi cultures. *n*-butanol, cyclohexane and selected 2-component mixtures (50/50 (v/v)) at the concentrations of 10%, 1% and 0.1%, were added to the cultures at 24, 48, 72 and 96 h. Observation of increasing optical density (OD) value over time for specific concentration was a sign of an increase in tested isolates, and thus the ability to assimilate carbon from the tested compounds.

2.5. Immobilization of Selected Fungal Isolates on the Support Materials (Polyurethane Foam, Pall Rings and 2 Types of Bialecki Rings)

The examined support materials were polyurethane foam (Ultramare, Warsaw, Poland) cut into approximately 100 mm diameter and 20 mm high discs, Pall rings (50 × 50) and 2 types of Bialecki rings (25 × 25 and 50 × 50) (LCS, Cracow, Poland). The immobilization of fungi on the support particles was carried out in 500 ml sterile beakers containing single type of the substrate and 100 mL growth Sabouraud medium with 10% (v/v) inoculum, under orbital agitation at 100 rpm and at 24 °C (Figure 1a,b). After 24 h, 50% of the volume of medium was replaced with the same volume of the minimal medium: $Na_2HPO_4 \cdot 2H_2O$ (15.2 g/dm^{-3}), KH_2PO_4 (3 g/dm^{-3}), NaCl (0.5 g/dm^{-3}) and NH_4Cl (1 g/dm^{-3}) (Figure 1c). The beakers with appropriate contents were shaken throughout the whole immobilization. The next day, all medium was poured out, replacing it with the minimal medium (Figure 1d). The procedure was repeated after one day with the difference that the medium spilled was replaced with the Sabouraud medium (Figure 1e). After the same time interval the support materials were completely colonized (Figure 1f). The samples were routinely taken for analysis (optical density measurement). After 5 days from the beginning, the polyurethane foam material was completely colonized.

Due to lower porosity and the specific surface of Pall rings and 2 types of Bialecki rings as compared to polyurethane foam, and therefore greater difficulty in immobilizing the fungus on the

surface of the support material, the process has been extended over time. In order to immobilize fungi on these materials, all the above mentioned stages must be carried out, except that all incubation times of the cultures should be extended from 24 to 48 h. Therefore, after 9 days from the beginning, the supporting materials were completely colonized. The process of fungi immobilization on the aforementioned support materials is schematically presented in Figure 2.

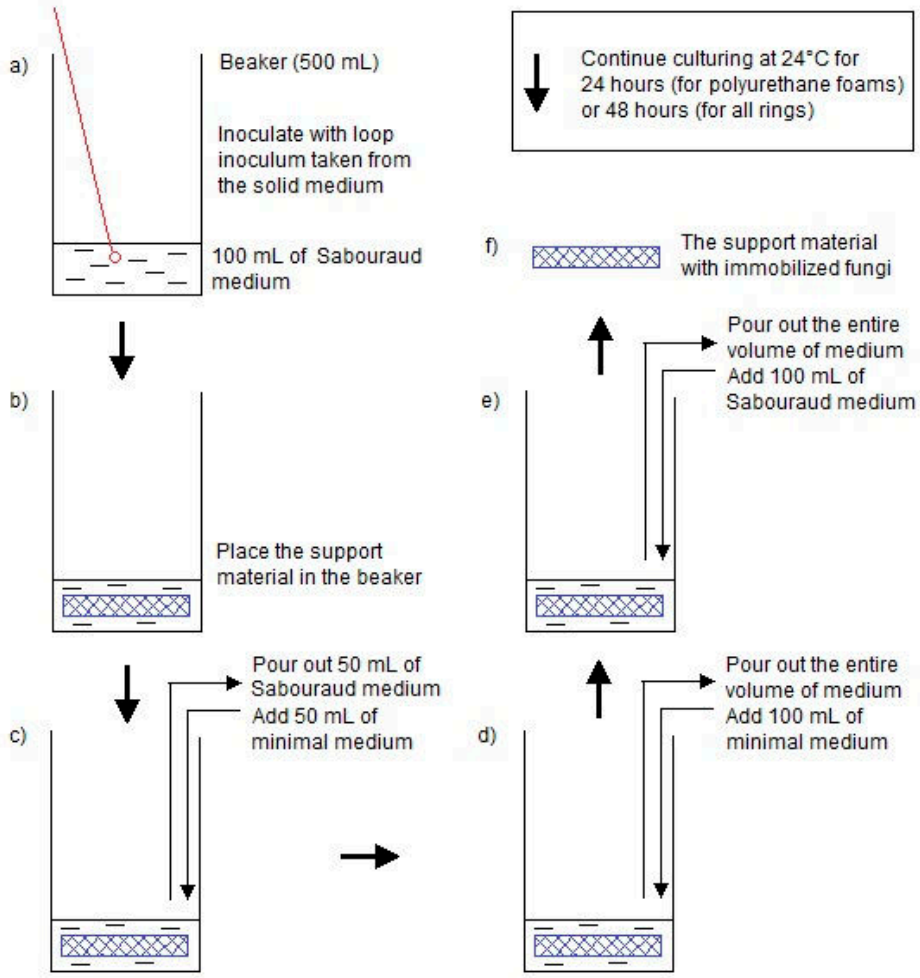


Figure 2. Scheme (**a**)–(**f**) showing immobilization of selected fungi on the support materials.

2.6. Microscopic Observations

Viability staining technique was used for evaluation of vitality of investigated fungi. Pictures of immobilized fungi were made using transmitted light, employing an optical microscope with a 10× long working distance lens (LAB 40 Series Optical Microscope, Katowice, Poland).

2.7. Preparation of Fungal Cells to Cytometric Analysis

A fragment of the investigated support material was placed in 40 mL of physiological salt solution buffered with phosphate (pH 7.6, 0.01 M) and then shaken in an ultrasonic washer (100 W) for 15 s. Next, the vessel was placed in ice for 15 s. In order to remove contaminants, cell suspension was filtered through a nylon with 400-mesh. The residue was washed twice with PBS and suspended in 1 ml of PBS after centrifugation at 6000 rpm and at 4 °C (Eppendorf Centrifuge 5415 R, Hamburg, Germany) for 10 min. The number of cells in the examined sample was determined using a flow cytometer. The volume corresponding to 1 million of fungal cells was utilized in further investigations.

2.7.1. Cytometric Analysis of Distribution in the Life Cycle of Fungi after Immobilization

Cytometric analysis of distribution in the life cycle of cells is based on detection of increased permeability of cell membrane for propidium iodide (PI), which binds with DNA in a stoichiometric way. This phenomenon and the knowledge that the amount of bound propidium iodide is proportional to cell DNA make it possible to determine the cell population in a given phase of the life cycle. PI is also capable of binding with RNA, which can lead to erroneous results. Hence, RNase A is added to the staining solution.

Then, 300 µL of PBS (Phosphate Buffered Saline), 300 µL of sodium deoxycholate (25 mM) (Sigma Aldrich, Darmstadt, Germany) and 0.3 µL of PI (2 mg/mL) (Sigma Aldrich, Darmstadt, Germany) were added to the pellet. The final concentration of PI in the tubes was 1 mg/ml. Staining was carried out for 30 min at 24 °C in the dark. Measure the fluorescence intensity emitted by DNA-bound propidium iodide using a flow cytometer (Merck Millipore guava easyCyte 8, Darmstadt, Germany). The excitation wavelength for propidium iodide is 488 nm, emissions are 617 nm. Each flow cytometric susceptibility test analyzed 10,000 events or yeast cells. The threshold between permeable and impermeable membranes was determined based on publication [77]. In order to obtain reliable results, the number of cells from 3 tested elements of each type were measured, settled in the same way, under the same conditions. The technical replicates were not included in the study.

2.7.2. Microbial Populations Viability State after Immobilization (Annexin V Test)

Annexin V test is aimed at investigation of the changes in asymmetry and integrity of cytoplasmic membrane of cells. A method consisting in double staining with annexin V marked with fluorescein and propidium iodide, which allows identification of the cells subjected to apoptosis or necrosis, was utilized in order to detect changes of cytoplasmic membrane after immobilization of fungal cells on the surface of selected materials.

100 µL of annexin V binding buffer (BD Pharmingen, San Diego, CA, USA), 0.5 µL of FITC Annexin V (Annexin V fluorescein conjugate; Introgen by Thermo Fisher, Eugene, OR, USA) and 0.25 µL of 7-aminoactinomycin D (Sigma, Dorset, UK) were added to the pellet. Staining was carried out for 15 min at 24 °C in the dark. These two dye conjugates can emit red and green fluorescence excited by 488 nm wavelength and can be detected by a fluorescence detector of the flow cytometry. In order to obtain reliable results the number of cells from 3 tested elements of each type were measured, settled in the same way, under the same conditions. The technical replicates were not included in the study.

3. Results and Discussion

3.1. Measurement of Viability and Carbon Assimilation from Hydrophilic and Hydrophobic Compounds

Fungi were isolated from the samples of peat collected from the biofilter intended for removal of *n*-butanol vapors from air. Five isolates were identified as *Candida albicans* and one as *Candida subhashii*. Next, evaluation of the microorganisms able to assimilate carbon from selected volatile organic compounds (*n*-butanol (B), cyclohexane (C) and their mixture of 50/50) was performed. For the liquid culture of the test isolates after 24, 48, 72 and 96 h, the test compound solutions and their mixture at the concentrations of 10%, 1% and 0.1% were successively added. Every 24 h after the addition of the test compound solution, the optical density of the culture was measured at 595 nm wavelength.

The obtained optical density values (Figure 3) showed that *C. albicans* MG2, *C. albicans* MG4, *C. albicans* MG5 and *C. subhashii* MG6 in concentrations of tested compounds grew with time (an increase in optical density value). *C. albicans* MG2 has higher OD value when fungi grow in the media "B 0.1%", "C + B 10%", "C + B 0.1%" and the OD is increased by ~130%, 130% and 97%, respectively. In addition, *C. albicans* MG3 grows better in "C 1%" supplemented media than *C. albicans* MG2. 50% OD increase and can use "C + B 0.1%" as a source of carbon, however in lower yield than *C. albicans* MG2 (70% OD increase). Remarkably, *C. albicans* MG4 is able to assimilate carbon from the medium in broad range "C + B 10%" at the same extent as *C. albicans* MG2 (~130% OD increase). On the other hand,

C. albicans MG5 is able to grow in the media "B 0.1%", "C + B 1%", "C + B 0.1%" owing to the following OD: ~190, ~100 and ~60%. Finally, *C. subhashii* MG6 is able to use cyclohexane and *n*-butanol from the media complemented as "B 10%", "B 0.1%" and "C + B 0.1%", the OD increase is ~200, ~140 and ~70%, respectively. *C. subhashii* MG6 is able to grow only in "B 10%" medium, other isolates require at least mixture of cyclohexane and *n*-butanol to show higher OD after 72 h of incubation. On the other hand, *C. albicans* MG1 and *C. albicans* MG3 did not increase their optical density, so they cannot be used as a source of carbon in tested compound. The studies have shown that the strains isolated from peat, i.e., the isolates of *Candida albicans* and *Candida subhashii*, have a promising future regarding the use of volatile organic compounds as a source of carbon (Figure 3).

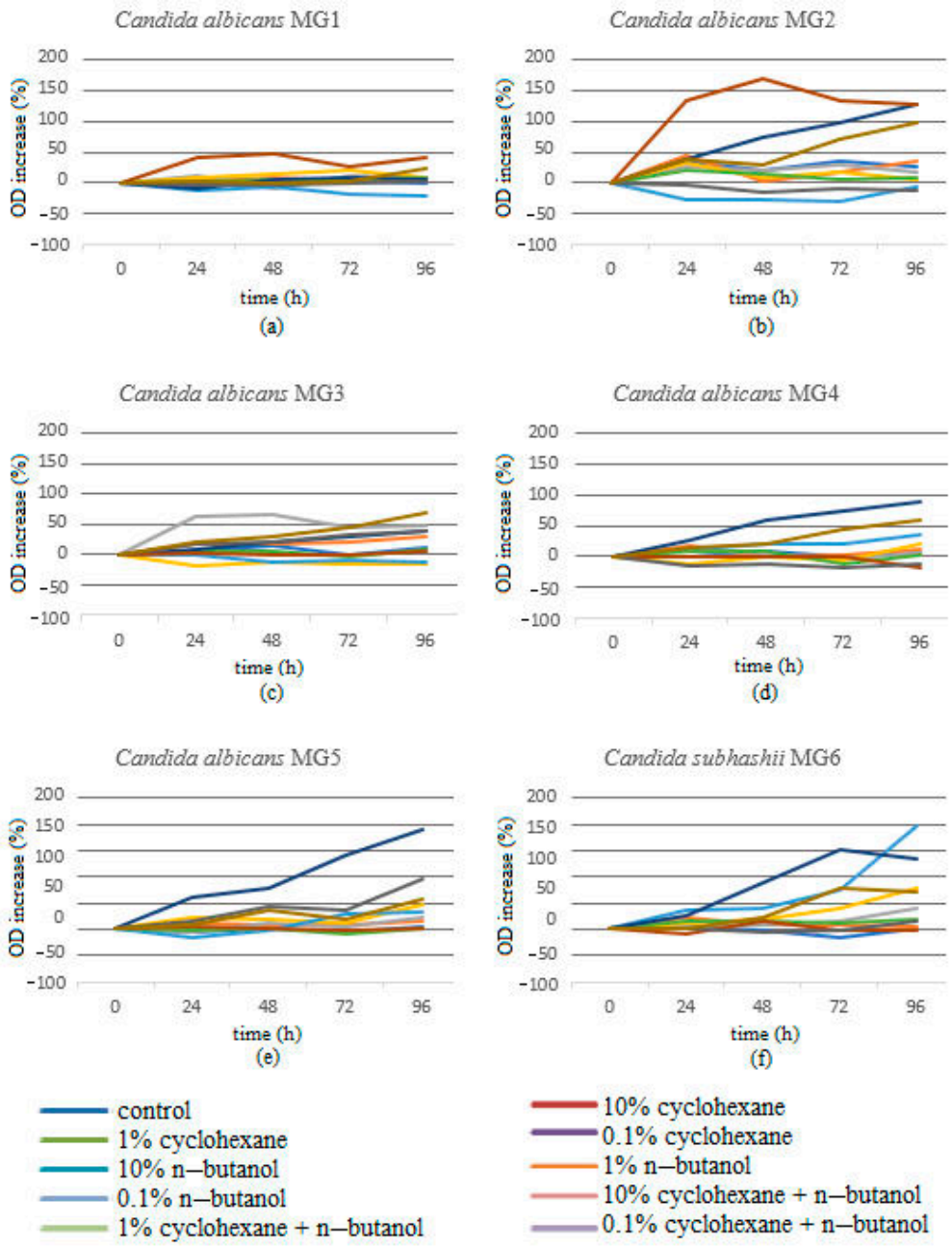


Figure 3. The growth curves of examined strains cultured in the presence of cyclohexane, *n*-butanol and their selected mixtures. (**a**) *Candida albicans* MG1. (**b**) *Candida albicans* MG2. (**c**) *Candida albicans* MG3. (**d**) *Candida albicans* MG4. (**e**) *Candida albicans* MG5. (**f**) *Candida subhashii* MG6. OD—optical density.

Based on the results presented in Figure 3, 2 isolates (*C. albicans* MG2 and *C. subhashii* MG6) exhibiting the highest rate of carbon assimilation from the tested compounds were selected. They were utilized in the further stage of investigation for immobilization on the surface of selected materials employed in biotrickling filtration.

3.2. Evaluation of Immobilization of Fungal Isolates on Support Materials Using Microscopic and Cytometric Measurements

Microscopic observations of the cells of *Candida albicans* and *Candida subhashii* on polyurethane foam reveal that after assumed incubation time selected fungi species are able to form biofilms of relatively comparable thickness on the surface of the foam (Figure 4). The optical microscope image confirmed colonization of the microorganisms on the foam's surface manifested by a presence of numerous stained fungal cells (Figure 4). The colonization was also confirmed by the investigation carried out with a flow cytometer. Obtained information (Table 4) shows that the highest colonization effectiveness (from a quantitative standpoint) on the polyurethane foam occurs for *C. subhashii* 67 ± 0.4 million cells, whereas for *C. albicans* it amounts to 53.5 ± 0.6 million cells.

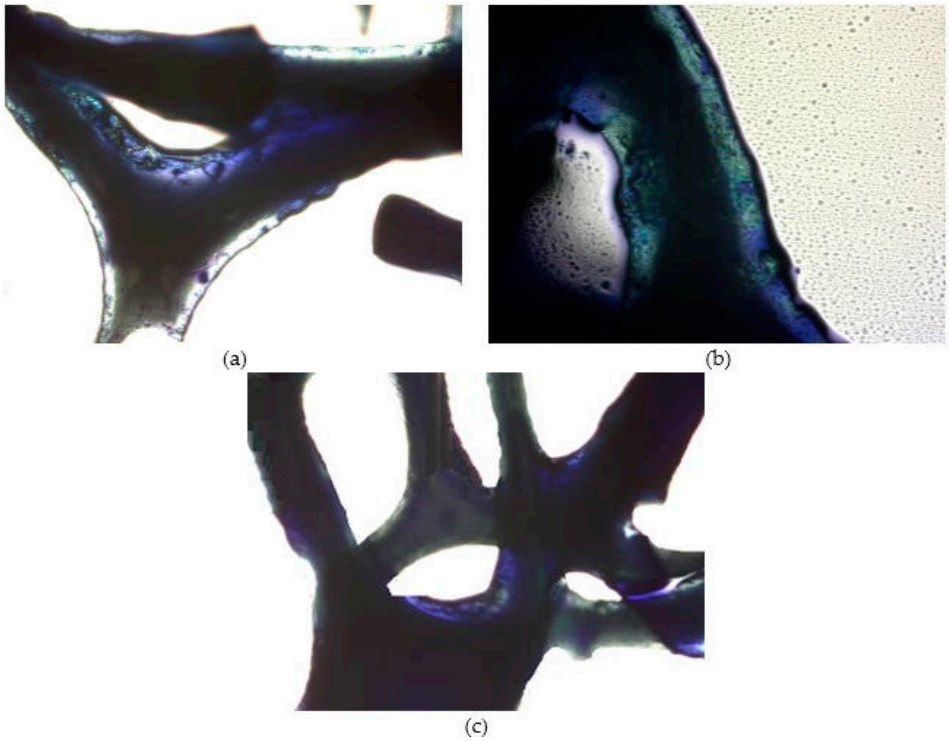


Figure 4. Optical microscope biofilm images. (**a**) The optical microscope images (10×) of a biofilm produced by *C. albicans* on polyurethane foam. (**b**) The optical microscope images (10×) of a biofilm produced by *C. subhashii* on polyurethane foam. (**c**) Control image: clean polyurethane foam.

The microscope images presenting the effect of immobilization of two different fungal isolates on the surface of Bialecki rings (25 × 25) illustrate 2 various ways of the same surface colonization by different fungal isolates (Figure 5). The isolate of *C. albicans* colonizes the surface in form of the clusters. The *C. subhashii* isolate colonized of the surface in a non-uniform way, small clusters of fungal cells were present. The fact that fungi did not occupy the entire available surface is a convenient phenomenon because it enables population growth during running of the processes, in which they will be utilized. According to the microscopic observation and the analyses of fungal cells count using the cytometer (Table 4), the most effective colonization (from a quantitative standpoint) on the Bialecki rings' surface occurs for the isolate *C. subhashii* (1.21 million per 1 cm^2). The smaller numerous colonization was found for *C. albicans* (1.06 million per 1 cm^2).

Table 4. Cytometric count of selected fungal cells after detaching them from the support materials.

	Polyurethane Foam		**Bialecki Ring (25 × 25)**		**Pall Ring (50 × 50)**		**Bialecki Ring (50 × 50)**	
	C. albicans	***C. subhashii***	***C. albicans***	***C. subhashii***	***C. albicans***	***C. subhashii***	***C. albicans***	***C. subhashii***
Number of cells from one element of the support material	$(53.5 \pm 0.6) \times 10^6$	$(67.0 \pm 0.4) \times 10^6$	$(49.9 \pm 0.5) \times 10^6$	$(56.8 \pm 0.4) \times 10^6$	$(77.2 \pm 0.6) \times 10^6$	$(72.2 \pm 0.3) \times 10^6$	$(69.6 \pm 0.7) \times 10^6$	$(82.4 \pm 0.5) \times 10^6$
Number of cells from one element of the support material per 1 cm^2	ca. 0.55×10^6	ca. 0.69×10^6	ca. 1.06×10^6	ca. 1.21×10^6	ca. 0.45×10^6	ca. 0.42×10^6	ca. 0.2×910^6	ca. 0.34×10^6
Volume of one element of the support material	157 cm^3		12.27 cm^3		98.13 cm^3		98.13 cm^3	

± standard deviation, ca.—circa.

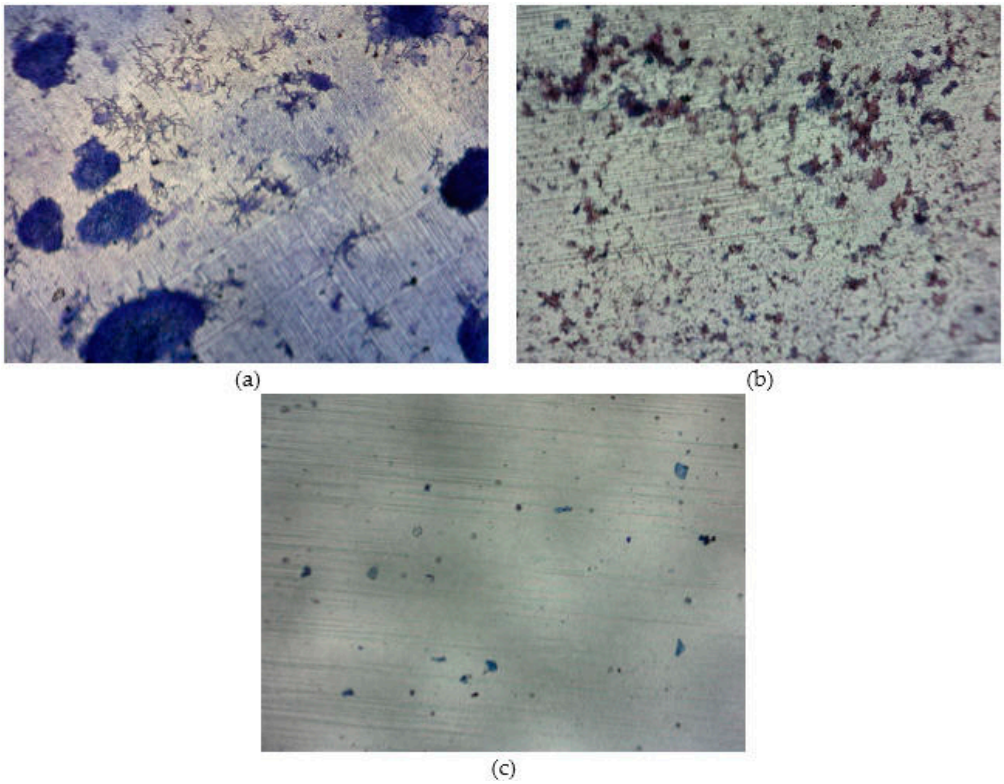


Figure 5. Optical microscope biofilm images. (**a**) The optical microscope image (10×) of a biofilm produced by *C. albicans* on Bialecki ring (25 × 25). (**b**) The optical microscope image (10×) of a biofilm produced by *C. subhashii* on Bialecki ring (25 × 25). (**c**) Control image: clean Bialecki ring (25 × 25).

Analyzing the microscope images showing the effect of colonization of Pall rings with fungi, which have different surface than previously examined Bialecki rings (Table 2). *C. albicans* and the isolate of *C. subhashii* were immobilized on Pall rings' surface (Figure 6) in a similar manner, however different than in the case of Bialecki rings (25 × 25). The cells covered the surface uniformly, locally the cell clusters are also visible. Comparing these two isolates, in the case of *C. subhashii* the average size of the cell clusters is bigger than for of *C. albicans* which translates into the occupied surface by them. Looking at the results of the fungal cell count using the cytometer (Table 4), the biggest number of cells immobilized on the Pall ring occurs for *C. albicans* (0.45 million per 1 cm^2), then for *C. subhashii* (0.42 million per 1 cm^2).

Due to the fact that the third type of the investigated rings—Bialecki rings (50 × 50)—were manufactured from dark green, completely opaque polypropylene, it was not possible to perform observations with the optical microscope. Nevertheless, these rings are made of the same material (the only difference is in color and transparency) (Table 2) and by the same producer as previously analyzed Bialecki rings (25 × 25). Thus, surface of these rings is colonized in a similar way, so the investigation (comparison) should concern only structure of the entire ring and efficiency of its colonization. Looking at the results of fungal cells count using the cytometer (Table 4), the biggest number of cells immobilized on Pall ring occurs for *C. subhashii* (0.34 million per 1 cm^2), then for *C. albicans* (0.29 million per 1 cm^2).

Comparing the obtained results regarding application of particular support materials in the biotrickling filter, it can be noticed that the best operational parameters are exhibited by Bialecki ring (25 × 25). The biggest number of fungal cells (per 1 cm^2) are colonized on its surface as well as 1 m^3 of this support material also contains the biggest number of fungal cells. Another carrier material characterized by the largest colonization of fungi on the surface is occupied by polyurethane foam. Additionally, it is characterized by the biggest specific surface area, which provides excellent contact of the gas under purification with the biofilm surface on the support material. It should be noted

that counting of microorganisms on polyurethane foam requires an exceptional method as the results obtained with application of standard procedure are not realistic. This is due to the fact that the method used to detach fungal cells from the surface does not allow to detach cells from the inside of the foam disk without damaging them. The structure of foam is the reason of retaining the cells inside the disk—both those colonizing and already detached. To perform the examination of the cells presence inside the cells, the disc was cut into 1–2 mm slices stained with methylene blue. Microscopic observation of stained cells allowed for examination the level of their detaching. Such examination exhibited that the number of cells indicated by cytometry is false in case of polyurethane foam discs. Polyurethane foam offers maintenance of constant microbiological activity of biofilm and uniform distribution of biofilm surface over the entire cross-section. It seems that these two types of carrier materials can be successfully used for the immobilization of fungi and used for biotrickling filtration.

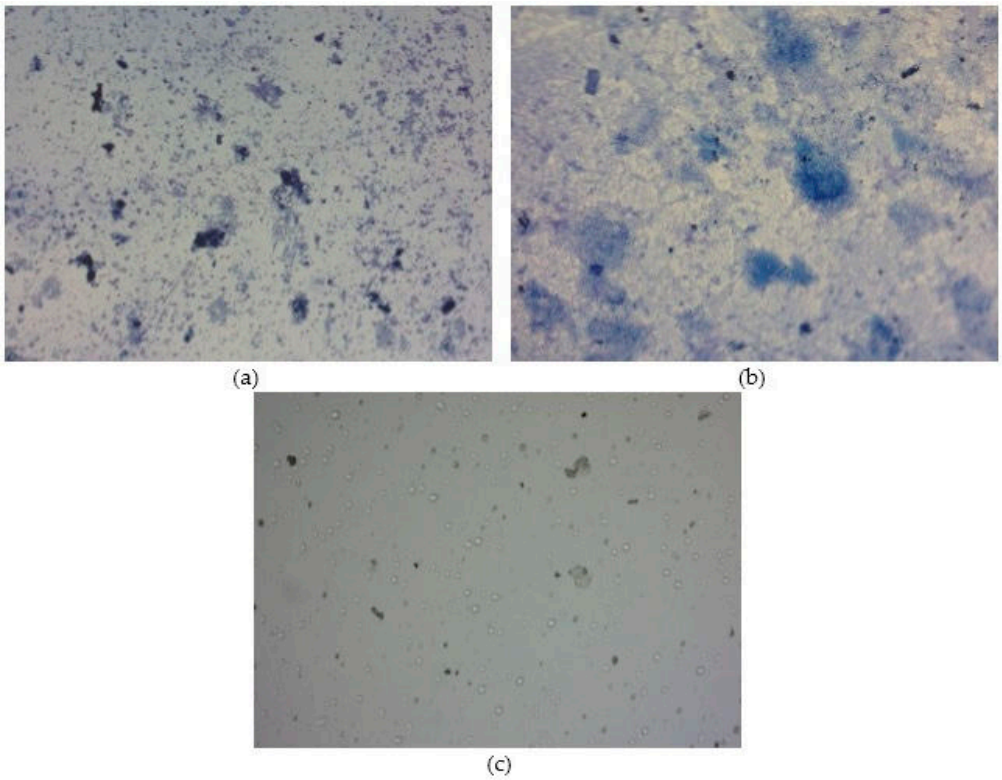


Figure 6. Optical microscope biofilm images. (**a**) The optical microscope image (10×) of a biofilm produced by *C. albicans* on Pall ring. (**b**) The optical microscope image (10×) of a biofilm produced by *C. subhashii* on Pall ring (**c**) Control image: clean Pall ring.

3.3. Measurement of Viability of Fungal Isolates Immobilized on Support Material Surface

Membrane permeability after immobilization on the support materials was measured by flow cytometry using propidium iodide, a nucleic acid-binding fluorochrome largely excluded by the intact cell membrane. The results of these studies show that the flow cytometry provides a rapid and sensitive in vitro method for antifungal susceptibility testing of *Candida albicans* and *Candida subhashii*. After staining of all selected fungal isolates with PI, it was determined that viability remained at a very high level (above 95%). Constant percentages of fungi with permeable membranes were observed and they were on average 4.5–5% for selected fungal isolates from polyurethane foams, 1–3% for Bialecki rings (25 × 25), 1.5–2% Bialecki rings (50 × 50) and 1.25–3% for Pall rings (Figure 7).

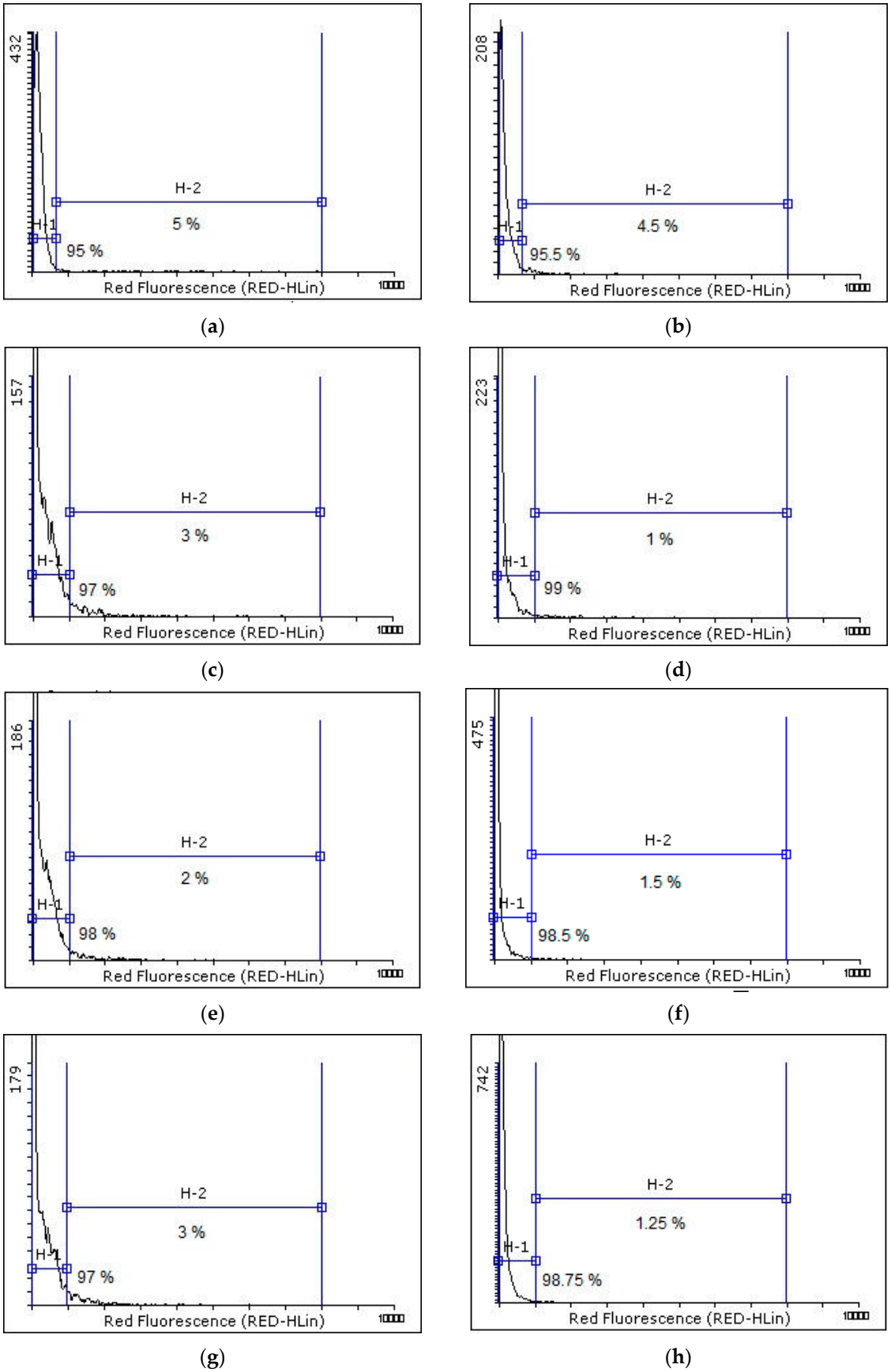
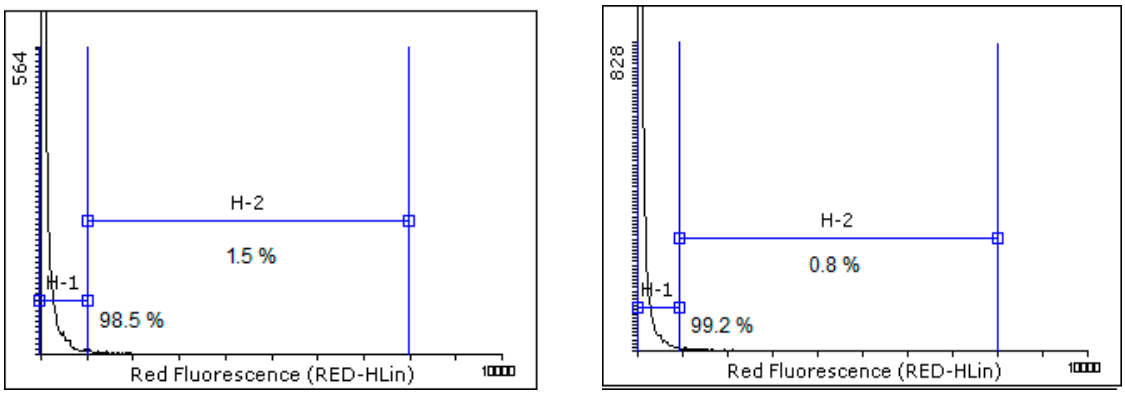


Figure 7. *Cont.*



(**i**) (**j**)

Figure 7. Analysis of viability determined by flow cytometry for PI-stained selected fungal isolates. (**a**) *C. albicans* from polyurethane foam. (**b**) *C. subhashii* from polyurethane foam. (**c**) *C. albicans* from Bialecki ring (25 × 25). (**d**) *C. subhashii* from Bialecki ring (25 × 25). (**e**) *C. albicans* from Pall ring. (**f**) *C. subhashii* from Pall ring. (**g**) *C. albicans* from Bialecki ring (50 × 50). (**h**) *C. subhashii* from Bialecki ring (50 × 50). The regions H-2 indicated by the bars represent the percentage of fungi with permeable membranes, whereas H-1 are living cells. (**i**) *C. albicans*—control with cells not immobilized. (**j**) *C. subhashii*—control with cells not immobilized.

Immobilization processes can change the microbial population's viability state on the support materials, which includes intact cells, apoptosis-like decayed cells, necrotic cells, and mechanically damaged cells. In this study, the Annexin V binding assay is used to detect microbial population's viability state by the flow cytometry [78–80].

Simultaneous supply of propidium iodide and annexin to the cells allows differentiation between intact cells, necrotic cells as well as early and late apoptosis cells. Annexin V is a protein, which possesses high affinity to phosphatidylserine and thus makes it possible to detect apoptosis-like decayed cells. Propidium iodide binds to cell DNA; it must be emphasized that at low concentrations it can penetrate only through damaged cell membranes, which lost their integrity. The results presented in Figure 8a–h can be interpreted in the following way: intact cells, which are not penetrated by propidium iodide and not connected with annexin V, are located in the lower left quarter. One of the features characteristic for apoptosis-like decayed cells is phosphatidylserine translocation to the external layer of a lipid membrane. In normal living cells this lipid is present only in the internal layer of the membrane. The upper left quarter is occupied by necrotic cells absorbing propidium iodide, which possess phosphatidylserine on the cytosol side of the membrane, so they do not bind annexin V. The lower right quarter corresponds to early apoptosis cells binding annexin V, the plasmalemma of which is tight and impermeable for iodide. In the upper right quarter there are medium and late apoptosis cells with permeable membrane, which bind annexin and absorb propidium iodide.

Analyzing the changes in structure of the cytoplasmic membrane of all investigated fungi strains on all tested support materials, it can be noticed that in every case the majority of cells remained alive (Figure 8). It is also clear that population distribution in the figures does not differ significantly within a single isolate. Relatively highest decay occurs for the cells, which were immobilized on Bialecki rings 50 × 50. Annexin V test confirmed the results obtained in the experiment on analysis of viability determined by the flow cytometry for PI-stained, which show that the cell immobilization process as well as in principle the dangerous process of cell detachment from the support material's surface are not serious issues here (Figure 8). Cell detachment from the support material imposes a risk of cell membrane damage, which can result even in cell decay. This phenomenon is especially adverse in this type of studies because membrane permeability was employed as a measure of fungal cells viability. Hence, if the results of cytometric investigations had revealed high cell mortality, it would not have been possible to identify the cause of their low viability without additional examinations. It could

result from both imperfect method of cell immobilization on the support material as well as from improper cell detachment. Favorably, both cytometric analyses revealed high viability of all isolates on all support materials, so they can be recommended for future investigations on viability of fungal cells immobilized on the materials utilized in the biotrickling filters. Analyzing Figure 9, regularity can be found; the bigger diameter of the pellet, the bigger number of damaged cells. It results from the fact that for bigger pellets it is more difficult to detach cells from its entire surface. The investigations presented in this paper confirm high effectiveness of the method of colonization of polyurethane and polypropylene materials by fungal (yeast) cells, which, besides ascomycetes, are the fungi species most frequently applied to removal of malodorous compounds from air. Proposed method of cell detachment from the support materials' surface combined with cytometric investigations constitutes a full methodology allowing control over efficiency of colonization of particular support material. The authors are sure that the proposed methodology can be also successfully implemented to control viability of microorganisms during biotrickling filtration processes.

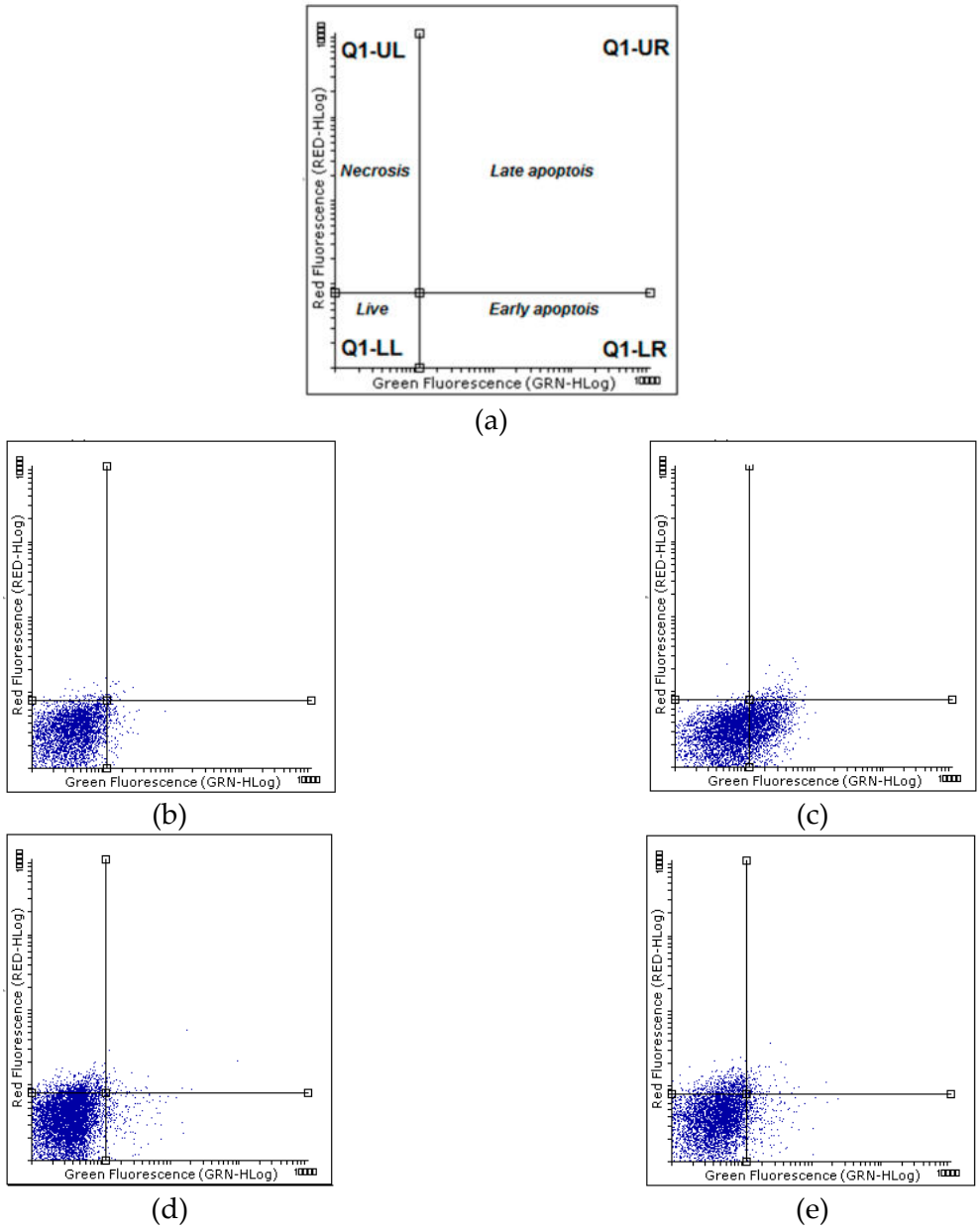


Figure 8. *Cont.*

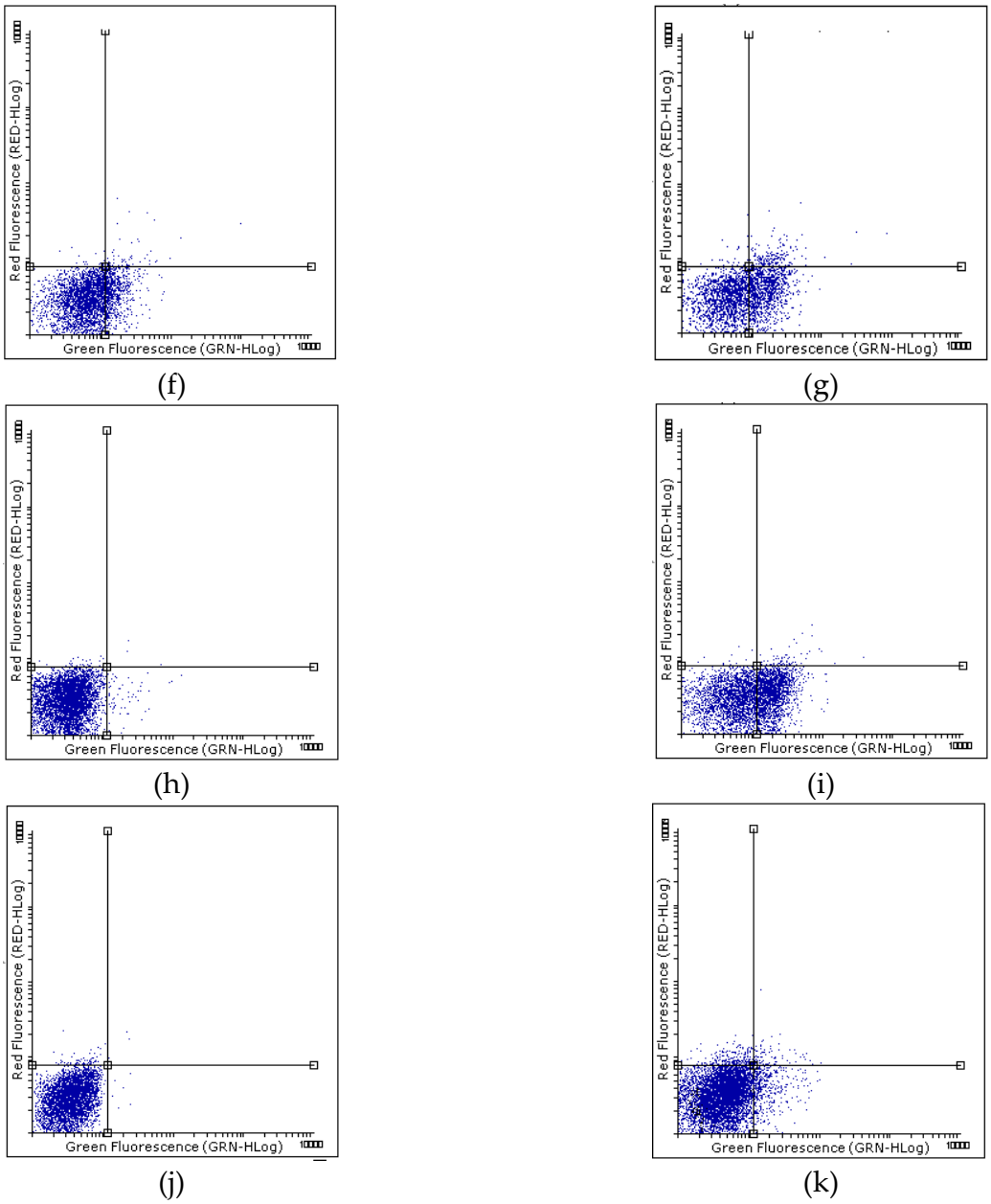


Figure 8. Flow cytometry cytograms of cell viability (intact cells (Q1-LL), early apoptosis cells (Q1-LR), late apoptosis cells (Q1-UR), necrotic cells (Q1-UL)) (**a**) and (**b**) *C. albicans* from polyurethane foam. (**c**) *C. subhashii* from polyurethane foam. (**d**) *C. albicans* from Bialecki ring (25 × 25). (**e**) *C. subhashii* from Bialecki ring (25 × 25). (**f**) *C. albicans* from Pall ring. (**g**) *C. subhashii* from Pall ring. (**h**) *C. albicans* from Bialecki ring (50 × 50). (**i**) *C. subhashii* from Bialecki ring (50 × 50). (**j**) *C. albicans*—control with cells not immobilized. (**k**) *C. subhashii*—control with cells not immobilized.

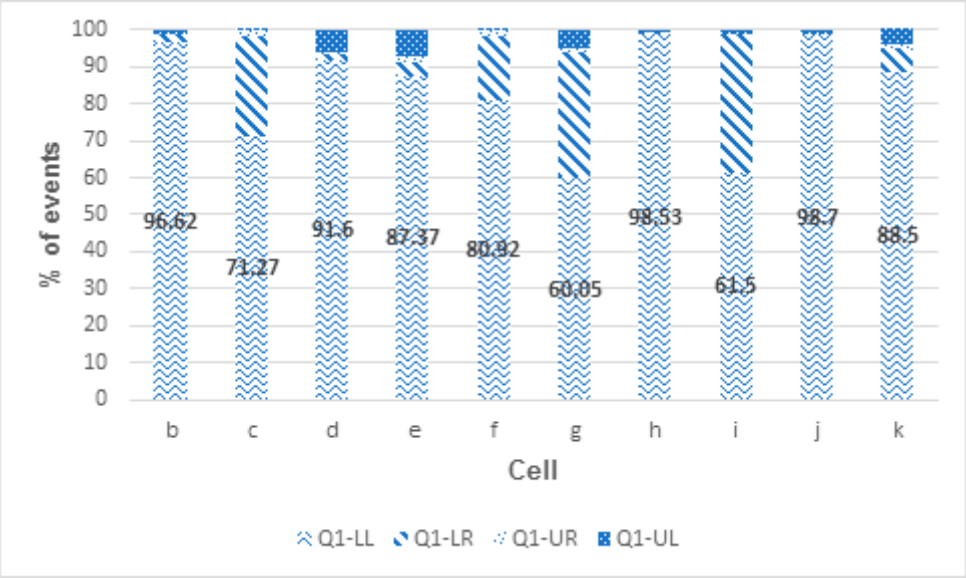


Figure 9. Percentage distribution of cells of selected fungal isolates showing changes in the structure of the cytoplasmic membrane. The diagrams take into account the percentage of cells in the cytograms' quarters (Figure 8): (intact cells (Q1-LL), early apoptosis cells (Q1-LR), late apoptosis cells (Q1-UR), necrotic cells (Q1-UL)). (**b**) *C. albicans* from polyurethane foam. (**c**) *C. subhashii* from polyurethane foam. (**d**) *C. albicans* from Bialecki ring (25 × 25). (**e**) *C. subhashii* from Bialecki ring (25 × 25). (**f**) *C. albicans* from Pall ring. (**g**) *C. subhashii* from Pall ring. (**h**) *C. albicans* from Bialecki ring (50 × 50). (**i**) *C. subhashii* from Bialecki ring (50 × 50). (**j**) *C. albicans*—control with cells not immobilized. (**k**) *C. subhashii*—control with cells not immobilized.

4. Conclusions

Strains of species of unicellular fungi from the peat samples collected from the operating biofilter intended for removal of *n*-butanol vapors from air streams were isolated and recognized. Identified fungi species included: 5 isolates of *C. albicans* (*C. albicans* MG1, *C. albicans* MG2, *C. albicans* MG3, *C. albicans* MG4, *C. albicans* MG5) and *C. subhashii* MG6. Optical density measurements revealed that some fungal isolates were able to assimilate carbon directly from the compounds subjected to removal. The investigations employed *n*-butanol as hydrophilic compound and cyclohexane as hydrophobic one. The purpose of such an approach was to show that fungi inoculated on artificial support material of the biotrickling filter are able to survive and additionally to take part in air purification from malodorous compounds via carbon assimilation from removed compounds. The immobilization process of selected fungal isolates was conducted on four popular artificial support materials utilized in biotrickling filtration, namely polyurethane foam, Bialecki rings (25 × 25) and (50 × 50) as well as Pall rings (50 × 50). Optical and cytometric measurements showed that the immobilization was successful. Propidium iodide and annexin V tests revealed fungal isolates viability at average level of 95%. Moreover, proposed method of cell detachment from the support materials' surface combined with cytometric investigations constitutes a full methodology allowing control over efficiency of colonization of particular support material. Additionally, it can be cheap, fast and highly efficient methodology, which could be a starting point for the biofiltration process aimed at removal of malodorous compounds by selected fungi species. Based on performed comparative analyses, it was found that two types of the support materials, polyurethane foam and Bialecki rings (25 × 25), could be attractive candidates for biotrickling filtration. Polyurethane foam offers maintenance of constant microbiological activity of biofilm and uniform distribution of biofilm surface over the entire cross-section. Small-size Bialecki rings provide unrivalled high colonization of fungal cells per 1 cm^2.

The obtained results could be useful for improving biofiltration processes and provide comprehensive knowledge about the types of microorganisms existing inside the biofilter bed,

allowing to enhance its performance through isolation of a specific microbial strain and artificial inoculation in the biofilter bed.

Author Contributions: Writing—original draft, M.M. and J.G.; Formal analysis, M.M. and J.G.; Investigation, M.M.; Methodology, M.M. and A.B.-D.; Supervision, A.B.-D. and J.G.; Validation, M.M. and J.G.; Visualization, M.M.; Writing—review & editing, M.M., A.B.-D. and J.G. All authors have read and agreed to the published version of the manuscript.

Funding: The authors are grateful for the support by the project "INTERPHD2" No. POWR.03.02.00-IP.08-00-DOC/16.

Acknowledgments: We thank company LCS (Gdansk, Poland) which manufactures plastic components, for providing us with the support materials.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gospodarek, M.; Rybarczyk, P.; Szulczyński, B.; Gębicki, J. Comparative Evaluation of Selected Biological Methods for the Removal of Hydrophilic and Hydrophobic Odorous VOCs from Air. *Processes* **2019**, *7*, 187. [CrossRef]
2. Rybarczyk, P.; Szulczyński, B.; Gębicki, J.; Hupka, J. Treatment of malodorous air in biotrickling filters: A review. *Biochem. Eng. J.* **2019**, *141*, 146–162. [CrossRef]
3. Gospodarek, M.; Rybarczyk, P.; Brillowska-Dąbrowska, A.; Gębicki, J. The use of various species of fungi in biofiltration of air contaminated with odorous volatile organic compounds. *E3S Web Conf.* **2019**, *100*, 00021. [CrossRef]
4. Chheda, D.; Sorial, G.A. Evaluation of co-metabolic removal of trichloroethylene in a biotrickling filter under acidic conditions. *J. Environ. Sci. (China)* **2017**, *57*, 54–61. [CrossRef] [PubMed]
5. Cox, H.H.J.; Sexton, T.; Shareefdeen, Z.M.; Deshusses, M.A. Thermophilic biotrickling filtration of ethanol vapors. *Environ. Sci. Technol.* **2001**, *35*, 2612–2619. [CrossRef]
6. Elmrini, H.; Bredin, N.; Shareefdeen, Z.; Heitz, M. Biofiltration of xylene emissions: Bioreactor response to variations in the pollutant inlet concentration and gas flow rate. *Chem. Eng. J.* **2004**, *100*, 149–158. [CrossRef]
7. Kim, D.; Cai, Z.; Sorial, G.A. Behavior of trickle-bed air biofilter for toluene removal: Effect of non-use periods. *Environ. Prog.* **2005**, *24*, 155–161. [CrossRef]
8. Grove, J.A.; Zhang, H.; Anderson, W.A.; Moo-Young, M. Estimation of Carbon Recovery and Biomass Yield in the Biofiltration of Octane. *Environ. Eng. Sci.* **2009**, *26*, 1497–1502. [CrossRef]
9. Shahi, A.; Rai, B.N.; Singh, R.S. Analysis of Metabolites and Carbon Balance in the Biofilteration of Cumene Using Loofa Sponge as Biofilter Media. *Appl. Biochem. Biotechnol.* **2016**, *180*, 338–348. [CrossRef]
10. Bordoloi, A.; Gostomski, P.A. Carbon recovery and the impact of start-up conditions on the performance of an unsaturated *Pseudomonas putida* biofilm compared with soil under controlled environmental parameters in a differential biofilter. *J. Chem. Technol. Biotechnol.* **2019**, *94*, 600–610. [CrossRef]
11. Jang, J.H.; Hirai, M.; Shoda, M. Styrene degradation by Pseudomonas sp. SR-5 in biofilters with organic and inorganic packing materials. *Appl. Microbiol. Biotechnol.* **2004**, *65*, 349–355. [CrossRef] [PubMed]
12. Kołwzan, B.; Adamiak, W.; Dziubek, A.M. Możliwości Zastosowania Grzybów w Technologiach Oczyszczania i Remediacji Wybranych Elementów Środowiska. *Ochrona Środowiska* **2018**, *40*, 3–20.
13. Oliva, G.; Zarra, T.; Naddeo, V.; Munoz, R.; Lebrero, R.; Angeles, R.; Belgiorno, V.; Ángeles, R.; Belgiorno, V. Comparative Analysis of AOPs and Biological Processes for the Control of VOCs Industrial Emissions. *Chem. Eng. Trans.* **2018**, *68*, 451–456.
14. Saliling, W.J.B.; Westerman, P.W.; Losordo, T.M. Wood chips and wheat straw as alternative biofilter media for denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations. *Aquac. Eng.* **2007**, *37*, 222–233. [CrossRef]
15. Mitin, A.; Nikolajkina, N.; Pushnov, A. Aerodynamic resistance of a biofilter with a packing of pine cones. *J. Environ. Eng. Landsc. Manag.* **2015**, *23*, 138–146. [CrossRef]
16. Rene, E.R.; Murthy, D.V.S.; Swaminathan, T. Performance evaluation of a compost biofilter treating toluene vapours. *Process Biochem.* **2005**, *40*, 2771–2779. [CrossRef]
17. Luo, J.; Lindsey, S. The use of pine bark and natural zeolite as biofilter media to remove animal rendering process odours. *Bioresour. Technol.* **2006**, *97*, 1461–1469. [CrossRef]

18. Tian, S.L.; Zhang, L.H.; Wang, Q.H.; Wang, X.M.; Xie, W.M. Study on hydrogen sulfide removal based on bench-scale experiment by bio-trickling filter. *J. Zhejiang Univ. Sci. A* **2007**, *8*, 1141–1148. [CrossRef]
19. Ruggeri, B.; Sassi, G. Experimental sensitivity analysis of a trickle bed bioreactor for lignin peroxidases production by P. chrysosporium. *Process Biochem.* **2003**, *38*, 1669–1676. [CrossRef]
20. Piché, S.; Larachi, F.; Grandjean, B.P.A. Loading Capacity in Packing Towers—Database, Correlations and Analysis. *Chem. Eng. Technol.* **2001**, *24*, 373–380. [CrossRef]
21. Rybarczyk, P.; Szulczyński, B.; Gospodarek, M.; Gębicki, J. Effects of n-butanol presence, inlet loading, empty bed residence time and starvation periods on the performance of a biotrickling filter removing cyclohexane vapors from air. *Chem. Pap.* **2019**, *74*, 1039–1047. [CrossRef]
22. Górka, A.; Papciak, D.; Zamorska, J.; Antos, D. The influence of biofilm on the effectiveness of ion exchange process. *Ind. Eng. Chem. Res.* **2008**, *47*, 7456–7464. [CrossRef]
23. Papciak, D. Effect Of Nitrification-Filter Packing Material On The Time To Reach Its Operation Capacity. In *Environmental Engineering*; Taylor & Francis Group: London, UK, 2007; pp. 125–132.
24. Papciak, D.; Zamorska, J. Wypełnienie biofiltru, a efektywność procesu nitryfikacji. *Zesz. Nauk. Politech. Rzesz. Oficyna Wydawnicza Politech. Rzesz.* **2003**, *35*, 147–160.
25. Cohen, Y. Biofiltration—The treatment of fluids by microorganisms immobilized into the filter bedding material: A review. *Bioresour. Technol.* **2001**, *77*, 257–274. [CrossRef]
26. Kraakman, N.J.R.; Rocha-Rios, J.; van Loosdrecht, M.C.M. Review of mass transfer aspects for biological gas treatment. *Appl. Microbiol. Biotechnol.* **2011**, *91*, 873–886. [CrossRef]
27. Qian, H.; Cheng, Y.; Yang, C.; Wu, S.; Zeng, G.; Xi, J. Performance and biofilm characteristics of biotrickling filters for ethylbenzene removal in the presence of saponins. *Environ. Sci. Pollut. Res.* **2018**, *25*, 30021–30030. [CrossRef] [PubMed]
28. Giri, B.S.; Kim, K.H.; Pandey, R.A.; Cho, J.; Song, H.; Kim, Y.S. Review of biotreatment techniques for volatile sulfur compounds with an emphasis on dimethyl sulfide. *Process Biochem.* **2014**, *49*, 1543–1554. [CrossRef]
29. Cox, H.H.; Deshusses, M.A. Biological waste air treatment in biotrickling filters. *Curr. Opin. Biotechnol.* **1998**, *9*, 256–262. [CrossRef]
30. Repečkienė, J.; Švedienė, J.; Paškevičius, A.; Tekorienė, R.; Raudonienė, V.; Gudeliūnaitė, E.; Baltrėnas, P.; Misevičius, A. Succession of microorganisms in a plate-type air treatment biofilter during filtration of various volatile compounds. *Environ. Technol.* **2015**, *36*, 881–889. [CrossRef] [PubMed]
31. Prenafeta-Boldú, F.X.; Illa, J.; van Groenestijn, J.W.; Flotats, X. Influence of synthetic packing materials on the gas dispersion and biodegradation kinetics in fungal air biofilters. *Appl. Microbiol. Biotechnol.* **2008**, *79*, 319–327. [CrossRef] [PubMed]
32. Zehraoui, A.; Kapoor, V.; Wendell, D.; Sorial, G.A. Impact of alternate use of methanol on n-hexane biofiltration and microbial community structure diversity. *Biochem. Eng. J.* **2014**, *85*, 110–118. [CrossRef]
33. Gisvold, B.; Ødegaard, H.; Füllesdal, M. Enhanced removal of ammonium by combined nitrification/adsorption in expanded clay aggregate filters. *Water Sci. Technol.* **2000**, *41*, 409–416. [CrossRef]
34. Pol, A.; van Haren, F.J.J.; den Camp, H.J.M.O.; van der Drift, C. Styrene Removal from Waste Gas with a Bacterial Biotrickling Filter. *Biotechnol. Lett.* **1998**, *20*, 407–410. [CrossRef]
35. Kirchner, K.; Schlachter, U.; Rehm, H.-J. Biological purification of exhaust air using fixed bacterial monocultures. *Appl. Microbiol. Biotechnol.* **1989**, *31–31*, 629–632. [CrossRef]
36. Paca, J.; Koutsky, B.; Maryska, M.; Halecky, M. Styrene degradation along the bed height of perlite biofilter. *J. Chem. Technol. Biotechnol.* **2001**, *76*, 873–878. [CrossRef]
37. Chung, Y.-C.; Huang, C.; Tseng, C.-P.; Rushing Pan, J. Biotreatment of H2S- and NH3-containing waste gases by co-immobilized cells biofilter. *Chemosphere* **2000**, *41*, 329–336. [CrossRef]
38. Ramírez, M.; Gómez, J.M.; Aroca, G.; Cantero, D. Removal of hydrogen sulfide by immobilized Thiobacillus thioparus in a biotrickling filter packed with polyurethane foam. *Bioresour. Technol.* **2009**, *100*, 4989–4995. [CrossRef]
39. Li, G.; He, Z.; An, T.; Zeng, X.; Sheng, G.; Fu, J. Comparative study of the elimination of toluene vapours in twin biotrickling filters using two microorganisms Bacillus cereus S1 and S2. *J. Chem. Technol. Biotechnol.* **2008**, *83*, 1019–1026. [CrossRef]
40. Cox, H.H.J.; Deshusses, M.A. Biomass control in waste air biotrickling filters by protozoan predation. *Biotechnol. Bioeng.* **1999**, *62*, 216–224. [CrossRef]

41. Allen, L.; Ellis, S. Laboratory Evaluation of Trickling Biofiltration for Treatment of Kraft Mill Noncombustion Air Emissions. In Proceedings of the TAPPI 2000 Environmental Confrence Process, Denver, CO, USA, 6–10 May 2000; pp. 293–309.
42. Fernández, M.; Ramírez, M.; Pérez, R.M.; Gómez, J.M.; Cantero, D. Hydrogen sulphide removal from biogas by an anoxic biotrickling filter packed with Pall rings. *Chem. Eng. J.* **2013**, *225*, 456–463. [CrossRef]
43. Sharvelle, S.; McLamore, E.; Banks, M.K. Hydrodynamic Characteristics in Biotrickling Filters as Affected by Packing Material and Hydraulic Loading Rate. *J. Environ. Eng.* **2008**, *134*, 346–352. [CrossRef]
44. Aizpuru, A.; Dunat, B.; Christen, P.; Auria, R.; García-Peña, I.; Revah, S. Fungal Biofiltration of Toluene on Ceramic Rings. *J. Environ. Eng.* **2005**, *131*, 396–402. [CrossRef]
45. Adamiak, W.; Szklarczyk, M. Possibilities of using ligninolytic fungi for biological waste gas treatment. *Environ. Prot. Eng.* **2001**, *27*, 45–58.
46. Arriaga, S.; Serrano, M.A.; Barba de la Rosa, A.P. Methanol vapor biofiltration coupled with continuous production of recombinant endochitinase Ech42 by Pichia Pastoris. *Process Biochem.* **2012**, *47*, 2311–2316. [CrossRef]
47. Jorio, H.; Jin, Y.; Elmrini, H.; Nikiema, J.; Brzezinski, R.; Heitz, M. Treatment of VOCs in biofilters inoculated with fungi and microbial consortium. *Environ. Technol.* **2009**, *30*, 477–485. [CrossRef] [PubMed]
48. Jin, Y.; Veiga, M.C.; Kennes, C. Performance optimization of the fungal biodegradation of α-pinene in gas-phase biofilter. *Process Biochem.* **2006**, *41*, 1722–1728. [CrossRef]
49. Vergara-Fernández, A.; Scott, F.; Moreno-Casas, P.; Díaz-Robles, L.; Muñoz, R. Elucidating the key role of the fungal mycelium on the biodegradation of n-pentane as a model hydrophobic VOC. *Chemosphere* **2016**, *157*, 89–96. [CrossRef]
50. Christen, P.; Domenech, F.; Michelena, G.; Auria, R.; Revah, S. Biofiltration of volatile ethanol using sugar cane bagasse inoculated with Candida utilis. *J. Hazard. Mater.* **2002**, *89*, 253–265. [CrossRef]
51. García-Peña, E.I.; Hernández, S.; Favela-Torres, E.; Auria, R.; Revah, S. Toluene biofiltration by the fungus *Scedosporium apiospermum* TB1. *Biotechnol. Bioeng.* **2001**, *76*, 61–69. [CrossRef] [PubMed]
52. Estévez, E.; Veiga, M.C.; Kennes, C. Fungal biodegradation of toluene in gas-phase biofilters. *Proc. Eur. Symp. Environ. Biotechnol.* **2004**, 337–340.
53. Rene, E.R.; Veiga, M.C.; Kennes, C. Biodegradation of gas-phase styrene using the fungus Sporothrix variecibatus: Impact of pollutant load and transient operation. *Chemosphere* **2010**, *79*, 221–227. [CrossRef] [PubMed]
54. Cox, H.H.J.; Moerman, R.E.; van Baalen, S.; van Heiningen, W.N.M.; Doddema, H.J.; Harder, W. Performance of a styrene-degrading biofilter containing the yeastExophiala jeanselmei. *Biotechnol. Bioeng.* **1997**, *53*, 259–266. [CrossRef]
55. Mohammad, B.T.; Rene, E.R.; Veiga, M.C.; Kennes, C. Performance of a thermophilic gas-phase biofilter treating high BTEX loads under steady- and transient-state operation. *Int. Biodeterior. Biodegrad.* **2017**, *119*, 289–298. [CrossRef]
56. Arriaga, S.; Revah, S. Removal of n-hexane by Fusarium solani with a gas-phase biofilter. *J. Ind. Microbiol. Biotechnol.* **2005**, *32*, 548–553. [CrossRef] [PubMed]
57. Rene, E.R.; López, M.E.; Veiga, M.C.; Kennes, C. Performance of a fungal monolith bioreactor for the removal of styrene from polluted air. *Bioresour. Technol.* **2010**, *101*, 2608–2615. [CrossRef] [PubMed]
58. Arriaga, S.; Revah, S. Mathematical modeling and simulation of hexane degradation in fungal and bacterial biofilters: Effective diffusivity and partition aspectsThis article is one of a selection of papers published in this Special Issue on Biological Air Treatment. *Can. J. Civ. Eng.* **2009**, *36*, 1919–1925. [CrossRef]
59. Li, K.; Zhou, J.; Wang, L.; Mao, Z.; Xu, R. The styrene purification performance of biotrickling filter with toluene-styrene acclimatization under acidic conditions. *J. Air Waste Manag. Assoc.* **2019**, *69*, 944–955. [CrossRef]
60. Qi, B.; Moe, W.M.; Kinney, K.A. Treatment of paint spray booth off-gases in a fungal biofilter. *J. Environ. Eng.* **2005**, *131*, 180–189. [CrossRef]
61. Weber, F.J.; Hage, K.C.; de Bont, J.A. Growth of the fungus Cladosporium sphaerospermum with toluene as the sole carbon and energy source. *Appl. Environ. Microbiol.* **1995**, *61*, 3562–3566. [CrossRef]
62. Giraud, F.; Guiraud, P.; Kadri, M.; Blake, G.; Steiman, R. Biodegradation of anthracene and fluoranthene by fungi isolated from an experimental constructed wetland for wastewater treatment. *Water Res.* **2001**, *35*, 4126–4136. [CrossRef]

63. Qi, B.; Moe, W.; Kinney, K. Biodegradation of volatile organic compounds by five fungal species. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 684–689. [PubMed]
64. López, M.E.; Rene, E.R.; Malhautier, L.; Rocher, J.; Bayle, S.; Veiga, M.C.; Kennes, C. One-stage biotrickling filter for the removal of a mixture of volatile pollutants from air: Performance and microbial community analysis. *Bioresour. Technol.* **2013**, *138*, 245–252. [CrossRef] [PubMed]
65. Vermes, I.; Haanen, C.; Steffens-Nakken, H.; Reutellingsperger, C. A novel assay for apoptosis Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *J. Immunol. Methods* **1995**, *184*, 39–51. [CrossRef]
66. Prigione, V.; Lingua, G.; Filipello Marchisio, V. Development and Use of Flow Cytometry for Detection of Airborne Fungi. *Appl. Environ. Microbiol.* **2004**, *70*, 1360–1365. [CrossRef]
67. Chen, J.; Xue, C.; Zhao, Y.; Chen, D.; Wu, M.-H.; Wang, J. Microfluidic Impedance Flow Cytometry Enabling High-Throughput Single-Cell Electrical Property Characterization. *Int. J. Mol. Sci.* **2015**, *16*, 9804–9830. [CrossRef] [PubMed]
68. Green, L.; Petersen, B.; Steimel, L.; Haeber, P.; Current, W. Rapid determination of antifungal activity by flow cytometry. *J. Clin. Microbiol.* **1994**, *32*, 1088–1091. [CrossRef]
69. Ordóñez, J.V.; Wehman, N.M. Amphotericin B susceptibility ofCandida species assessed by rapid flow cytometric membrane potential assay. *Cytometry* **1995**, *22*, 154–157. [CrossRef]
70. White, T.; Bruns, T.; Lee, S. *Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics*; Acad Press: San Diego, CA, USA, 1990; pp. 315–322.
71. LCS–Spółka z o.o. n.d. Available online: http://www.lcs24.pl/pierscienie#header (accessed on 6 May 2020).
72. Brillowska-Dąbrowska, A.; Świerkowska, A.; Lindhardt Saunte, D.M.; Arendrup, M.C. Diagnostic PCR tests for Microsporum audouinii, M. canis and Trichophyton infections. *Med. Mycol.* **2010**, *48*, 486–490. [CrossRef]
73. Brillowska-Dąbrowska, A.; Saunte, D.M.; Arendrup, M.C. Five-hour diagnosis of dermatophyte nail infections with specific detection of Trichophyton rubrum. *J. Clin. Microbiol.* **2007**, *45*, 1200–1204. [CrossRef] [PubMed]
74. Broekaert, W.F.; Terras, F.R.G.; Cammue, B.P.A.; Vanderleyden, J. An automated quantitative assay for fungal growth inhibition. *FEMS Microbiol. Lett.* **1990**, *69*, 55–59. [CrossRef]
75. Nilsson, I.; Möller, A.; Mattiasson, B.; Rubindamayugi, M.S.T.; Welander, U. Decolorization of synthetic and real textile wastewater by the use of white-rot fungi. *Enzyme Microb. Technol.* **2006**, *38*, 94–100. [CrossRef]
76. Martínez-Luis, S.; Rodríguez, R.; Acevedo, L.; González, M.C.; Lira-Rocha, A.; Mata, R. Malbrancheamide, a new calmodulin inhibitor from the fungus Malbranchea aurantiaca. *Tetrahedron* **2006**, *62*, 1817–1822. [CrossRef]
77. Martinez-Rojano, H.; Mancilla-Ramirez, J.; Quiñonez-Diaz, L.; Galindo-Sevilla, N. Activity of hydroxyurea against Leishmania mexicana. *Antimicrob. Agents Chemother.* **2008**, *52*, 3642–3647. [CrossRef] [PubMed]
78. Yang, N.; Wang, C.; Han, M.-F. Gel-encapsulated microorganisms used as a strategy to rapidly recover biofilters after starvation interruption. *J. Environ. Manag.* **2020**, *261*, 110237. [CrossRef]
79. Chen, B.; Yang, Z.; Pan, J.; Ren, Y.; Wu, H.; Wei, C. Functional identification behind gravity-separated sludge in high concentration organic coking wastewater: Microbial aggregation, apoptosis-like decay and community. *Water Res.* **2019**, *150*, 120–128. [CrossRef] [PubMed]
80. Henry, C.M.; Hollville, E.; Martin, S.J. Measuring apoptosis by microscopy and flow cytometry. *Methods* **2013**, *61*, 90–97. [CrossRef]

3.4. Publication 4: Removal of cyclohexane and ethanol from air in biotrickling filters inoculated with *Candida albicans* and *Candida subhashii*

The aim of the work was to apply and evaluate the effectiveness of the above proposed methods as part of a separate process of deodorization of air polluted with cyclohexane and ethanol in BTF. A new method of immobilizing *C. subhashii* and *C. albicans* on an artificial support material (polyurethane foam) was successfully used. In addition, the developed method for effective and safe detachment of fungal cells from the surface of polyurethane foam was used. A cytometric analysis of the distribution in the life cycle of fungi and the state of viability were carried out, both immediately after immobilization and after 100 days of the process. Optical microscopy studies were also carried out. Based on the results of the above-mentioned tests, the formation of a stable biofilm on the surface of the material filling both biofilter columns was observed. The formation of a stable biofilm immediately after immobilization proves the effectiveness of the process of settling the inert material by the fungus cells. The development and growth of the biofilm, and thus the good condition of the fungal cells after 100 days of the process, proves that both species can use the tested compounds as a carbon source, which is tantamount to removing these compounds from the deodorized air.

The publication presents experiments leading to the implementation of task T4 *Elaboration of method allowing for assessment of the fungi immobilization and monitoring their condition during the biofiltration process*.

Archives of Environmental Protection
Vol. 47 no. 1 pp. 26–34

PL ISSN 2083-4772
DOI 10.24425/aep.2021.136445

Removal of cyclohexane and ethanol from air in biotrickling filters inoculated with *Candida albicans* and *Candida subhashii*

Piotr Rybarczyk[1]*, Milena Marycz[1], Bartosz Szulczyński[1], Anna Brillowska-Dąbrowska[2], Agnieszka Rybarczyk[3], Jacek Gębicki[1]

[1]Department of Process Engineering and Chemical Technology, Faculty of Chemistry, Gdańsk University of Technology
[2]Department of Molecular Biotechnology and Microbiology, Faculty of Chemistry, Gdańsk University of Technology
[3]Department of Histology, Faculty of Medicine, Medical University of Gdańsk

*Corresponding author's e-mail: piotr.rybarczyk@pg.edu.pl

Keywords: biofilm, flow cytometry, removal efficiency, ethanol, biofiltration, cyclohexane

Abstract: This paper presents investigations on the removal of cyclohexane and ethanol from air in polyurethane-packed biotrickling filters, inoculated with *Candida albicans* and *Candida subhashii* fungal species. Results on process performance together with flow cytometry analyses of the biofilm formed over packing elements are presented and discussed. The results indicate that the presence of ethanol enhances the removal efficiency of cyclohexane from air. This synergistic effect may be attributed to both co-metabolism of cyclohexane with ethanol as well as increased sorption efficiency of cyclohexane to mineral salt medium in the presence of ethanol. Maximum elimination capacities of 89 g m^{-3} h^{-1} and 36.7 g m^{-3} h^{-1} were noted for cyclohexane and ethanol, respectively, when a mixture of these compounds was treated in a biofilter inoculated with *C. subhashii*. Results of flow cytometry analyses after 100 days of biofiltration revealed that about 91% and 88% of cells in biofilm remained actively dividing, respectively for *C. albicans* and *C. subhashii* species, indicating their good condition and ability to utilize cyclohexane and ethanol as a carbon source.

Introduction

The use of biofiltration processes to purify air from odorous compounds has been practiced for many years. Biofiltration, compared to other deodorization methods, has a number of advantages, such as low process costs, high purification efficiency for large volumes of gases with low odor concentrations, and very low emissions of secondary pollutants (Mudliar et al. 2010). A particularly attractive way of conducting biofiltration is the use of biotrickling filters. This is due to the possibility of quick adjustment of key process parameters (pH, composition and flow rate of the spraying liquid), which results in higher efficiency of air deodorization and longer life-time of such systems, compared to conventional biofilters (Rybarczyk et al. 2019a). In biotrickling filters, gas contaminated with odorous compounds is passed through a bed made of inert elements (e.g. polyurethane foam, ceramic elements), previously inoculated with consortium of microorganisms. The bed is trickled with liquid enriched with mineral salts. The trickling liquid circulates in a closed system, with the possibility of periodic replacement or regeneration. As a result of the growth of microorganisms, the so-called biofilm is formed over the packing elements. It is the biofilm in which compounds are adsorbed and absorbed from the gaseous phase and then undergo biodegradation.

It is well-known that efficient performance of biotrickling filters is greatly dependent on the biofilm formed over the packing elements (Purswani et al. 2011). What is more, microorganisms inhabiting the biofilm differ greatly in terms of both the rate of biofilm formation as well as ability to degrade pollutants (Feng et al. 2019). Usually, bacteria species are used for biofilter inoculation. Interestingly, fungi are known to be able to maintain the microbial activity under shock loads of pollutants, starvation periods or drought episodes (Rybarczyk et al. 2019b; Zhang et al. 2019). Besides, due to filamentous morphology, fungi offer a large surface area for, and thus increase the mass transfer of pollutants from the gas phase to the biofilm (Ferdowsi et al.,2017; Spigno et al. 2003). Because of the above listed features, fungi are of special interest when designing biotrickling filtration processes.

Biofiltration systems are especially efficient when water-soluble compounds are considered. Such compounds easily

break the mass transfer barrier between gaseous and aqueous (i.e. biofilm) phases and the rate of their biodegradation is mainly governed by the rate of biodegradation within the biofilm. Contrary, for poorly water-soluble compounds, i.e. hydrophobic ones, the efficiency of biofiltration depends greatly on the mass transfer rate between the above mentioned phases, and thus the biofiltration efficiency is much lower than for hydrophilic compounds (Cheng et al. 2016; Gospodarek et al. 2019). Several measures may be applied to improve the biofiltration performance with respect to hydrophobic compounds, including the addition of surfactants, especially biosurfactants, application of selected microbial species, including fungi, reactor modification, selection of proper process conditions as well as co-treatment with hydrophilic compounds (Cheng et al. 2020; He et al. 2020; Miller et al. 2019; Miller et al. 2020; Rybarczyk et al. 2020, 2019b; Yang et al. 2018, 2010).

In this paper, the possibility of using selected *Candida* fungi to simultaneously remove from air compounds with extremely different affinity to the aqueous phase was investigated. Hydrophobic cyclohexane and hydrophilic ethanol were used as model compounds. These compounds are found in post-processing gases from, e.g., paint, petroleum and food industries (Avalos Ramirez et al. 2007; Zhanga et al. 2018). Biotrickling filtration of air containing single cyclohexane or ethanol was previously investigated (Avalos Ramirez et al. 2007; Cox et al. 2001; Salamanca et al. 2017). In this paper, a mixture of cyclohexane and ethanol was subjected to biofiltration in two biotrickling filters, inoculated with *Candida albicans* and *Candida subhashii*, respectively. The composition of biofilms formed in two biotrickling filters was tested for purity of inhabiting fungi and compared between the process start-up and steady-state operation conditions using flow cytometry technique. To the best knowledge of authors of this paper, the above given fungi have not been used so far in biotrickling filters for air purification, and the search for new species of microorganisms capable of biodegradation of pollutants, especially of a hydrophobic nature, is an important trend in the environmental research.

Materials and methods

Investigations were performed on biotrickling filters made of plexi-glass columns of the following dimensions: 0.08 m in internal diameter and 0.68 m in height. Biofilters were packed with polyurethane foam discs (pore size PPI 10, Ultramare, Poland; dimensions of a single disc: 0.08 m in diameter, 0.01 m in height) up to the working volume of 2.5 dm^3 each. Biofilters were fed with a gas mixture from the bottom, while the trickling liquid was supplied from the top of a bioreactor, by means of a peristaltic pump. Gaseous mixtures of air with cyclohexane and ethanol (POCH, Poland) were obtained by passing the purified and dried air via a porous sinter through vials containing liquid cyclohexane and ethanol. The gas flow rate was controlled and regulated using a precise mass flow controller (Vögtlin, Switzerland). Gas flow rate of 2.5 dm^3 min^{-1} was used throughout the experiments, resulting in empty bed residence time (EBRT) equal to 1 min. Pressure drop across the packings of biotrickling filters was monitored using MPX5010dp sensors (NXP, the Netherlands) working in the range from 0 to 10 kPa. Maximum noted pressure did not exceed 2 kPa and no biomass overgrowth was observed throughout the experiments.

Gaseous samples containing cyclohexane and ethanol were taken from inlet and outlet gas streams. Samples were collected in Tedlar bags and concentrations of the above given volatile organic compounds were determined using gas chromatography technique using a DB-WAX column (30 m × 0.53 mm × 1 µm; Agilent Technologies, USA) and flame ionization detector (Varian CP-3800, VarianAnalytical Instruments, USA). Nitrogen was used as a carrier gas. The parameters of the analytical program were as follows: oven temperature: 100°C; FID detector temperature: 200°C, carrier gas flow rate: 3 cm^3 min^{-1}; split ratio: 10.

During the start-up period (first 7–10 days of biofiltration process), packing elements of biofilters were trickled with a Buffered Peptone Water medium (Merck, Germany). Then, mineral salt medium (MSM) was introduced. MSM contained the following salts dissolved in 1 dm^3 of distilled water: $Na_2HPO_4 \cdot 2H_2O$ (7.39 g), KH_2PO_4 (3 g), NaCl (0.5 g) and NH_4Cl (1 g) (POCH, Poland). Trickling liquid solutions were autoclaved before introducing to biofilters (Prestige Medical, England) and the MSM solution was exchanged once a week throughout the whole reported time period. Trickling liquid was sprayed over the packing elements with a frequency of 0.5 minutes per each hour, with a volumetric flow rate of 0.2 dm^3 min^{-1}.

Prior to the biofiltration start-up, packing elements made of polyurethane foam discs were inoculated with *Candida albicans* (biotrickling filter "A") and *Candida subhashii* (biotrickling filter "B"). Immobilization of fungi on polyurethane discs was realized using sterile beakers (each 1 dm^3 in volume) in which 600 cm^3 of Sabouraud medium (BTL, Poland) containing 10% (v/v) of selected fungi species inoculums was placed. These beakers were agitated in an orbital shaker (100 rpm, 24°C). After 24 hours of shaking, half of the medium volume was replaced with a fresh MSM solution. After the next 24 hours of shaking, the whole volume of the medium was replaced by a fresh portion of MSM solution. Then, a similar procedure was repeated, but MSM solution was replaced with Sabouraud medium in two steps as described above. The inoculation procedure lasted for 120 hours. Each day of inoculation, samples of media were taken and optical density measurements at wavelength of 595 nm using Thermo Scientific Multiskan FC spectrophotometer (Thermo Fisher Scientific, Finland) were routinely performed.

Two series of experiments were performed. In the first series (I), the performance of two biotrickling filters A and B was studied. Both biotrickling filters were initially fed with cyclohexane only, and at the 38th day of the process, ethanol was introduced into the gas stream. Additionally, flow cytometry analyses aiming at the determination of the general condition of microorganism populations inhabiting packing elements of biofilters were performed.

In the second series of experiments (II), one biotrickling filter inoculated with *C. subhashii* was investigated. Selection of these fungi species out of two investigated was done due to pathogenic characteristics of *C. albicans* as well as due to expected high performance of *C. subhashii* in the biotrickling filtration of hydrophobic volatile organic compounds. In this series, the biofilter was fed with a mixture of cyclohexane and ethanol from the process initiation. This approach was intended in order to study the effect of ethanol addition on the

performance of cyclohexane biofiltration, in comparison to experiments in series I. In series II, the liquid phase (MSM) was also investigated in terms of variations of pH as well as the concentrations of treated compounds during the biofiltration process.

The experimental staining technique with methylene blue (Sigma Aldrich, USA) was used to assess the formation of biofilm, containing the tested fungi, on the polyurethane foam elements. Photos of immobilized fungi were taken using transmitting light optical microscope with a 10× working distance lens (LAB 40 Series Optical Microscope, OPTA-TECH, Poland).

For cytometric analyses, single polyurethane discs were taken from each of the working biofilters. Each disc was placed in a beaker and suspended in 40 cm^3 of 0.01 M phosphate buffered saline solution (PBS, pH = 7.6) and shaken (4 times of 15 s shaking) in an ultrasonic bath (Bandelin Sonorex, Germany). After each shaking step, a beaker with a disk was placed in an water-ice bath for 15 s. The cell suspension was filtered using a 400-mesh nylon net to remove impurities. The precipitates were washed twice with PBS solution and suspended in 35 cm^3 of PBS solution after the centrifugation (6000 rpm, 6 min, 4°C; Eppendorf Centrifuge 5418R, Germany). A cell count was determined using a flow cytometry technique (Merck Millipore Guava easyCyte 8, Germany). A suspension volume containing 1 million of fungi cells was used in further investigations.

For the determination of microbial population condition using flow cytometry, 100 µL of AAB buffer (Annexin V Binding Buffer, BD Biosciences, Pharmingen, USA), 0.5 µL of FITC Annexin V (Annexin V fluorescein conjugate; Life Technologies Limited, Scotland) and 0.25 µL of 7-aminoactinomycin D (Sigma Aldrich, Germany) were added to the pellet. Staining with Annexin V was carried out for 15 minutes at 24°C in the dark. Finally, 100 µL of AAB buffer was added to the finished sample, in order to increase the sample volume. This conjugate can emit red and green fluorescence detected by fluorescence detector of flow cytometer.

For cytometric analyses of cell cycle of fungi, to the pellet were added: 300 µL of PBS, 300 µL of sodium deoxycholate (25 mM) (Sigma Aldrich, Germany) and 0.3 µL of propidium iodide, PI (2 mg cm^{-3}) (Sigma Aldrich, Germany). The final concentration of PI in the tubes was 1 mg/ml. Staining with propidium iodide was carried out for 30 minutes at 24°C in the dark. The measurement of fluorescence emitted by PI binded to DNA was performed using a flow cytometer. For propidium iodide, the wavelengths of excitation and emission are 488 and 617 nm, respectively. 10 000 of cells was analyzed during a single measurement.

The process conditions as well as performance of biotrickling filtration were described and evaluated using inlet loading (IL), empty bed residence time (EBRT), removal efficiency (RE) and elimination capacity (EC), according to the below given formulae:

$$IL = \frac{Q \cdot C_{in}}{V} \quad (1)$$

$$EBRT = \frac{V}{Q} \quad (2)$$

$$RE = \frac{C_{in} - C_{out}}{C_{in}} \quad (3)$$

$$EC = IL \cdot RE \quad (4)$$

where: Q – volumetric gas flow rate (m^3 h^{-1}), V – total volume of a biofilter packing (m^3), C_{in}, C_{out} – inlet and outlet concentrations of cyclohexane or ethanol in the gas stream (ppm v/v), respectively.

Results and discussion

Performance of biotrickling filtration using Candida albicans *and* Candida subhashii

In the first series of experiments, two biotrickling filtration processes were investigated. A mixture of air with cyclohexane and ethanol was treated either in a biofilter inoculated with *Candida albicans* (biofilter A) or *Candida subhashii* (biofilter B). Biotrickling filters were fed in a following manner. Initially (during the biofiltration start-up period), a gas stream contained only cyclohexane. Ethanol was added to the feeding gas stream after 38 days of biofiltration. During the first 10–14 days of biofiltration, the removal of cyclohexane from air is low. The results show that the removal efficiency of cyclohexane reaches the values of about 0.3–0.35 after the first 10–14 days of biofiltration (biofilter A, Fig. 1A). When biofilter B is concerned (Fig. 1B), values of RE fluctuate and do not exceed 0.25 during the first 30–35 days of biofiltration. A low removal efficiency is related to two main aspects: firstly, during the start-up period, the process runs under unsteady--state conditions when a biofilm formation precedes and the physico-chemical equilibria are set for the system. Secondly, the microbial flora inoculated onto the biofilters' packing elements undergoes acclimation to the treated compounds. The stabilization of the RE values, for both biotrickling filters, after about 14–38 days of the process duration indicate that steady--state conditions were attained since an increase of inlet loading at 21st day of biofiltration did not result in a decrease of the process performance.

At 38th day of biotrickling filtration processes, ethanol was added to the gas stream fed to the bioreactors. The addition of hydrophilic ethanol resulted in the enhancement of bioflitration performance, both for biofilters A and B (Figs. 1A and 1B). The removal efficiency of ethanol reaches about 99% after 45–50 days after the biofiltration process start-up. The values of RE, both for ethanol and cyclohexane, are slightly higher for the biotrickling filter A than B.

The increase of a process performance with respect to hydrophobic cyclohexane, upon the addition of hydrophilic ethanol, may be attributed to the promotion effect of the removal of hydrophobic compound. This effect may be a result of the stimulated growth and increased demand for carbon of microbial species inhabiting the biofilm (Cheng et al. 2020), leading to a co-metabolism of cyclohexane in the presence of ethanol. Moreover, the improved removal efficiency of hydrophobic air pollutants in the presence of ethanol may have resulted from improved sorption conditions. Cyclohexane is hardly soluble in water (up to about 55 mg dm^{-3} at 25°C), while it is easily soluble in ethanol (completely miscible)

(Yalkowsky et al. 2016). On the other hand, ethanol is easily absorbed in water, resulting in some content of ethanol in the mineral salt medium solution circulating in the biotrickling filter (discussed later, Fig. 3). Thus, the sorption efficiency of cyclohexane to mineral salt medium may be enhanced in the presence of ethanol. However, beside the fact that synergistic effects for simultaneous removal of hydrophilic and hydrophobic VOCs treated in biofiltration processes are already identified (Yang et al. 2018; Zhang et al. 2006), the actual mechanisms of this improvement are not yet defined.

Fig. 2A presents the performance of a biotrickling filter fed with other mode of feed gas supply: the treated gas stream contained cyclohexane and ethanol just from the process start-up. The following observations may be formulated when the course of a process performance (Fig. 2A) is compared with previously discussed courses (Figs. 1A and 1B). Firstly, the removal efficiencies of ethanol and cyclohexane are higher for both the start-up period (about two first weeks of biofiltration) as well as the steady-state conditions (after about 20 days of biofiltration) when two modes of feed supply are considered. Secondly, the removal performance with respect to cyclohexane is much higher in the second mode (Fig. 2A) than in the previous mode (Fig. 1B). Such observations may be explained by the increased metabolism of cyclohexane in the presence of ethanol, which resulted in promoted development of microbial flora within the biofilm and enhanced biofiltration performance with respect to cyclohexane. The following values of elimination capacity (EC) were reached for the investigated process performance: for cyclohexane, about 67.5 and 89 g m^{-3} h^{-1} (Fig. 1 and Fig. 2A, respectively) and about 35–36.7 g m^{-3} h^{-1} for ethanol. Biotrickling filtration of ethanol was studied by Cox at al. and Avalos Ramirez and co-workers, and the values of EC were reached as high as 220 and 970 g m^{-3} h^{-1}, respectively (Avalos Ramirez et al. 2007; Cox et al. 2001). For cyclohexane, elimination capacity of about 38 g m^{-3} h^{-1} was reached by Salamanca and co-workers for biotrickling filtration on polyurethane foam using *Acivodorax* sp. CHX100 (Salamanca et al. 2017). Please note that the mentioned studies dealt with the removal from air of single VOCs and the results presented in this paper reveal enhanced removal of cyclohexane in the presence of ethanol, allowing for much higher elimination capacity of cyclohexane than previously reported in the literature (up to 89 g m^{-3} h^{-1} in this study compared to about 38 g m^{-3} h^{-1} in reached by Salamanca and co-workers).

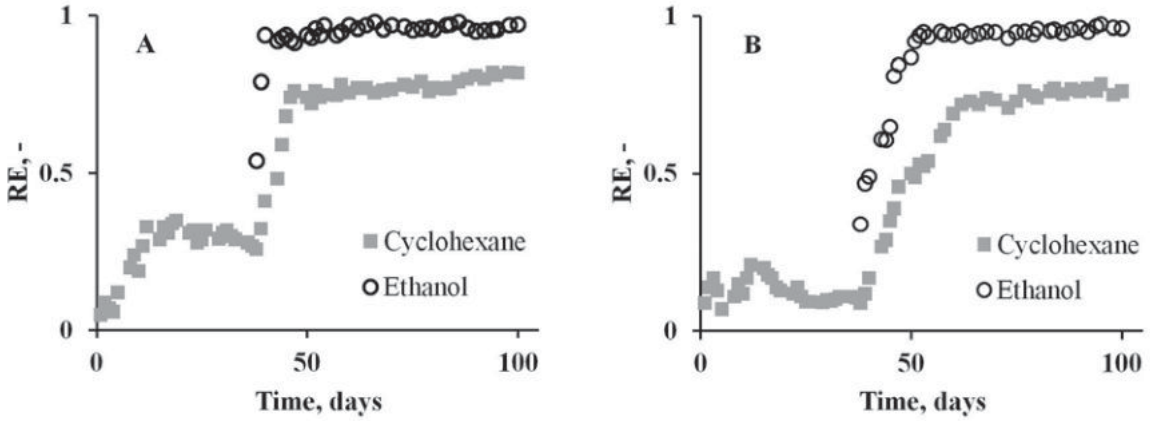


Fig. 1. Performance of a biotrickling filter inoculated with *Candida albicans* (Fig. 1A) and *Candida subhashii* (Fig. 1B). Inlet loading of cyclohexane: 45 g m^{-3} h^{-1} (C_{in} = 200 ppm v/v; days 1–20) and 90 g m^{-3} h^{-1} (C_{in} = 400 ppm v/v; days 21–100); inlet loading of ethanol: 36,9 g m^{-3} h^{-1} (ethanol introduced on 38th day of biofiltration; C_{in} = 400 ppm v/v).

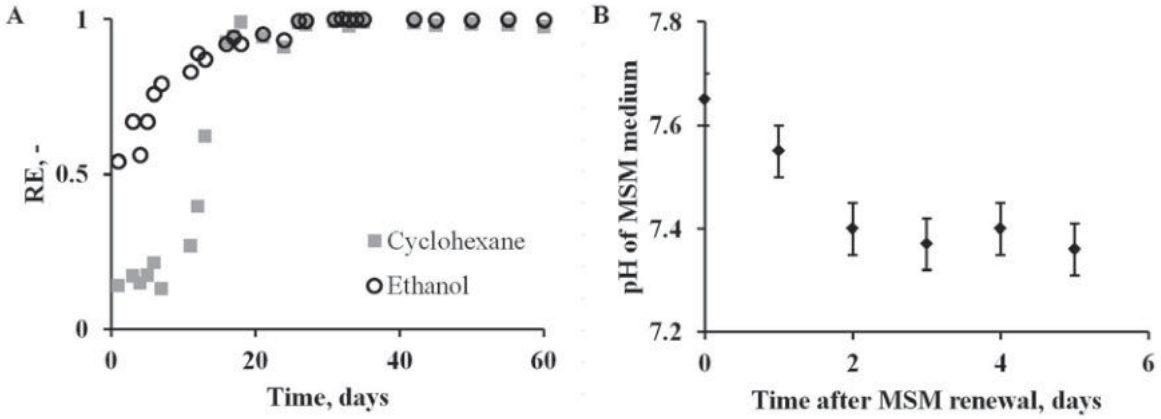


Fig. 2. Performance of a biotrickling filter inoculated with *Candida subhashii* (inlet loading for cyclohexane: 45 g m^{-3} h^{-1} (C_{in} = 200 ppm v/v; days 1–35) and 90 g m^{-3} h^{-1} (C_{in} = 400 ppm v/v; days 36–60); inlet loading for ethanol: 36,9 g m^{-3} h^{-1}; C_{in} = 400 ppm v/v) (Fig. 2A). Effect of biofiltration time on the pH of mineral salt medium (Fig. 2B).

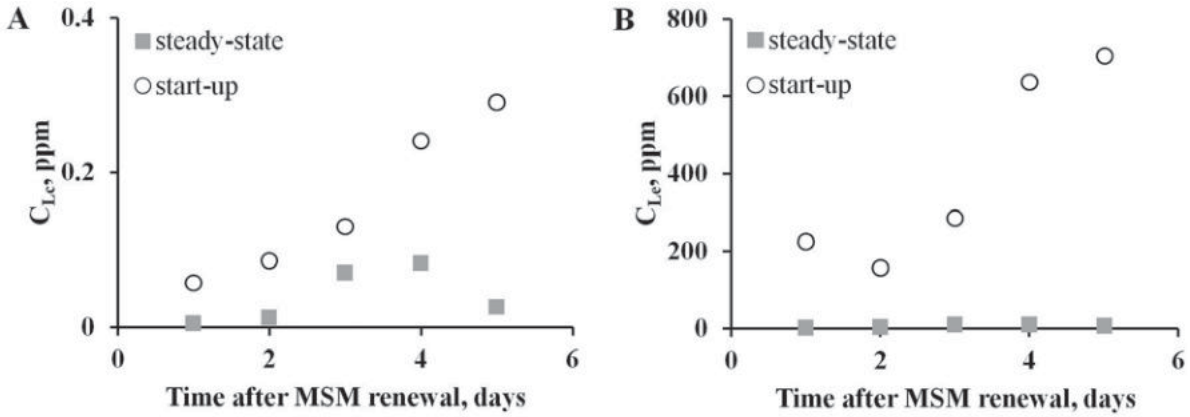


Fig. 3. Effects of biofiltration time on the composition of mineral salt medium with respect to cyclohexane (C_{Lc}, Fig. 3A) and ethanol (C_{Le}, Fig. 3B)

The effects of the gas stream composition on the physico-chemical parameters of the trickling liquid were investigated in order to evaluate possible effects on the process performance. Changes in pH of the trickling liquid as well as variations in the concentrations of treated compound in the liquid phase were evaluated. Fig. 2B shows changes of the pH values of MSM as a function of time after the introduction of a fresh portion of a trickling liquid (exchange of a trickling liquid was performed once a week throughout the whole time of experiments). The results show that only a slight pH drop of the solution was observed, probably due to products of microbial metabolism, typically leading to acidification of aqueous solutions, in the time period between the introduction of new portion of MSM and its exchange.

Interesting results are presented in Figs. 3A and 3B. The concentrations of the treated compounds were investigated in the trickling liquid for time intervals between the solution exchange, both for the start-up period as well as during the operation of biotrickling filters at steady-state conditions. During the start-up period, the concentrations of both cyclohexane and ethanol in the MSM solution increase with time after the solution renewal. It must be noted that due to the low solubility in water, the identified concentrations of cyclohexane are about 3 orders of magnitude lower than for hydrophilic ethanol. The increase of the target compounds concentrations may be explained by their partial absorption in the trickling liquid, which is especially true for ethanol. This is why the ethanol concentration in the MSM solution increases with time after a solution renewal during the start-up period (unsteady-state condition, Fig. 3B). As a result of absorption of ethanol vapors in MSM solution, its concentration in the gas phase decreases, thus a gas stream undergoing the biofiltration process is diluted. Therefore, the decrease of ethanol concentration in the outlet gas stream is attributed not only to the biofiltration process, but also partially to its absorption in MSM solution. Interestingly, the concentrations of ethanol in the MSM solution under steady-state conditions are very low, regardless of the time since the trickling liquid renewal. This observation suggests that a decrease of ethanol concentration in the outlet gas stream is predominantly the result of its biodegradation, i.e., the rate of biofiltration is high enough for its complete biotransformation and removal from the gaseous phase. Similar observations are valid for cyclohexane (Fig. 3A), bearing in mind its very limited solubility in aqueous solutions.

Optical microscopic analyses of fragments of biotrickling filter packing materials

Figures 4a to 4d present optical microscope images of biofilms formed on the structure of polyurethane foam applied as a packing material in the investigated biotrickling filters (Fig. 4e is a control showing polyurethane foam only). The below presented images were taken after the immobilization procedure (just prior to the introduction of inoculated packing elements to biotrickling filters) and after 100 days of biofiltration in order to evaluate the development of the biofilm. Optical microscopy observations reveal that both selected fungi species are able to form a biofilm over the biofilter packing elements and the thickness of developed biofilms is about 4 to 5 times higher than in the start-up period. Based on images presented in Figs. 4a to 4e it was found that the thickness of biofilm observed after the immobilization procedure was about 30 and 60 µm for *C. albicans* and *C. subhashii*, respectively, while the thickness of biofilm measured after 100 days of biofiltration was about 130 and 150 for µm for *C. albicans* and C. *subhashii*, respectively.

Flow cytometry analysis of applied fungi species

Cells of *Candida albicans* and *Candida subhashii*, sampled during the biofiltration start-up and after 100 days of biofiltration process, were subjected to flow cytometry analyses in order to determine the general condition of the fungi species. First series of analyses included a test with Annexin V which resulted in identification of apoptotic and necrotic cells. During the apoptosis, phosphatidylserine is transported from internal to external leaflet of the phospholipid bilayer. Annexin V is a specific reagent with high affinity to phosphatidylserine. In the commercial tests, Annexin V is conjugated with fluorescein. After binding with plasma membrane of apoptotic cells, green fluorescence may be detected. Additionally, propidium iodide is applied in this test, allowing for the differentiation of three main types of cells: alive, early apoptotic as well as necrotic and late apoptotic (Fig. 5a) (Chen et al. 2019; Henry et al. 2013; Vermes et al., 1995).

The results presented in Figs. 5b to 5e and in Table 1 show that the prominent fraction of cells remain alive after 100 days of biofiltration. Within a group of a given fungi species, the distribution of cell populations is similar for samples taken during the start-up period and after 100 days of biofiltration. This observation suggests that the same fungi species are

inhabiting the biotrickling filter packing elements as were those initially inoculated, indicating that no contamination of microbial cultures occurred.

The results presented in Table 1 inform that a decrease in the number of alive cells for both investigated fungi species is about 3%. It seems that the reduced number of alive cells results from a natural physiology of these microorganisms and it is not a result of the negative influence of the conditions induced by the biofiltration process on their viability.

Flow cytometry – evaluation of the cell cycle

Cytometric analysis of the cell cycle allow to determine the state of the cell population, i.e. how many of the cells in the analyzed population are actively dividing (i.e. are alive, denoted by index H-1 on the histogram), how many are at rest or how many die by apoptosis (denoted by index H-2 on the histogram). Membrane perforation, which is one of the stages of cell fixation during preparation for cytometric analysis, allows propidium iodide to enter the cytoplasm and the nucleus and, as a result, bind (intercalate) with double-stranded DNA (Martinez-Rojano et al. 2008; Ramani et al. 1997). The results presented in the attached histograms (Figs. 6a–6d) show that both *C. albicans* and *C. subhashii* remain in a good general condition, i.e., their cells stay alive, when biofilm samples taken during the process start-up and after 100 days of biofiltration are compared. During the start-up period, predominating cells remain in the interphase (H-1 = 96.5% for *C. albicans* and H-2 = 92% for *C. subhashii*), thus indicating that these cells are alive. After 100 days of the process, the number of alive cells slightly decreases at the expense of growth in the number of the dying cell population, indicated by H-2 index on the histograms. However, this is a natural process because the population is aging physiologically during the analyzed time interval and no drastic changes are observed that indicate

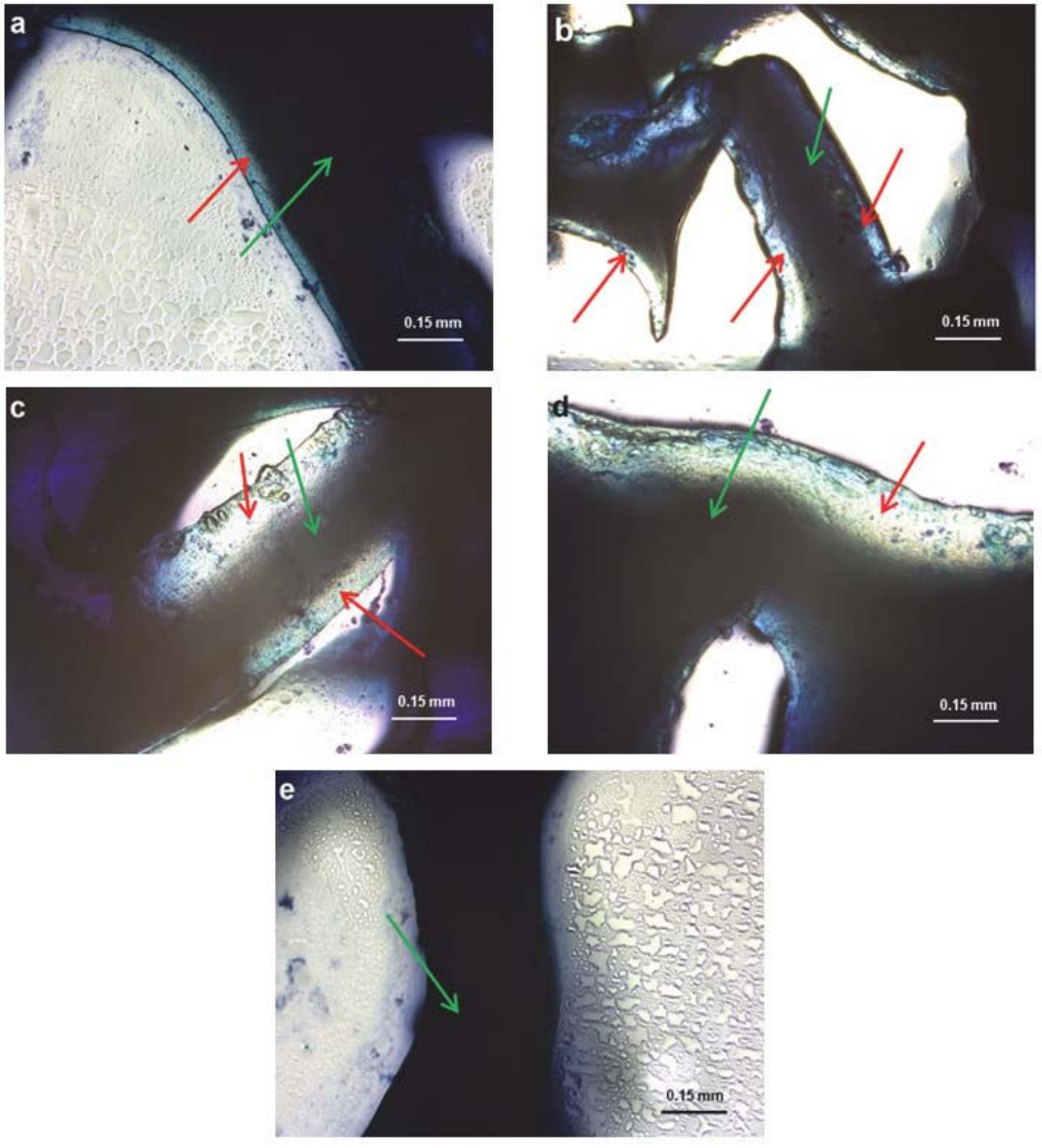


Fig. 4. Optical microscopy images (magnification: 10×; cells were stained with methylene blue): a – image of *C. albicans* biofilm after immobilization; b – image of *C. subshashii* biofilm after immobilization; c – image of *C. albicans* biofilms after 100 days of biofiltration; d – image of *C. subhashii* biofilm after 100 days of biofiltration; e – image of a fragment of polyurethane foam (control sample without a biofilm); red arrow – biofilm; green arrow – polyurethane foam

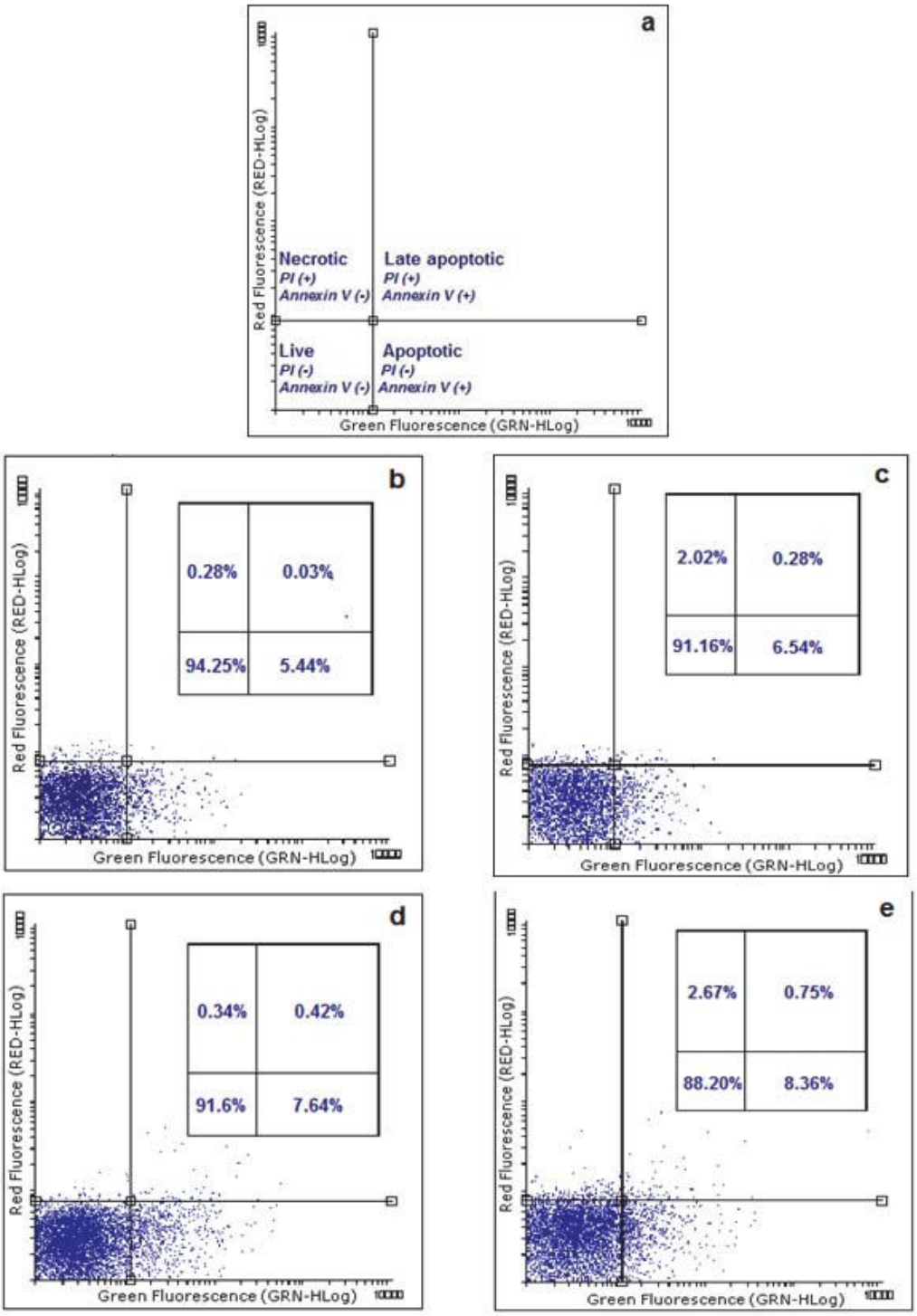


Fig. 5. Flow cytometry cytograms of cell viability: a – general information on how to read the cytogram; b – *C. albicans* after immobilization (start-up of the process); c – *C. subhashii* after immobilization (start-up of the process); d – *C. albicans* after 100 days of bilfiltration; e – *C. subhashii* after 100 days of biofiltration

Table 1. Summary of results obtained from Annexin V test

Species/Type of cell populations	Alive, %	Early apoptotic, %	Necrotic and late apoptotic, %
C. albicans (start-up)	94.25	5.44	0.31
C. albicans (after 100 days of biofiltration)	91.6	7.64	0.76
C. subhashii (start-up)	91.16	6.54	2.30
C. subhashii (after 100 days of biofiltration)	88.20	8.36	3.42

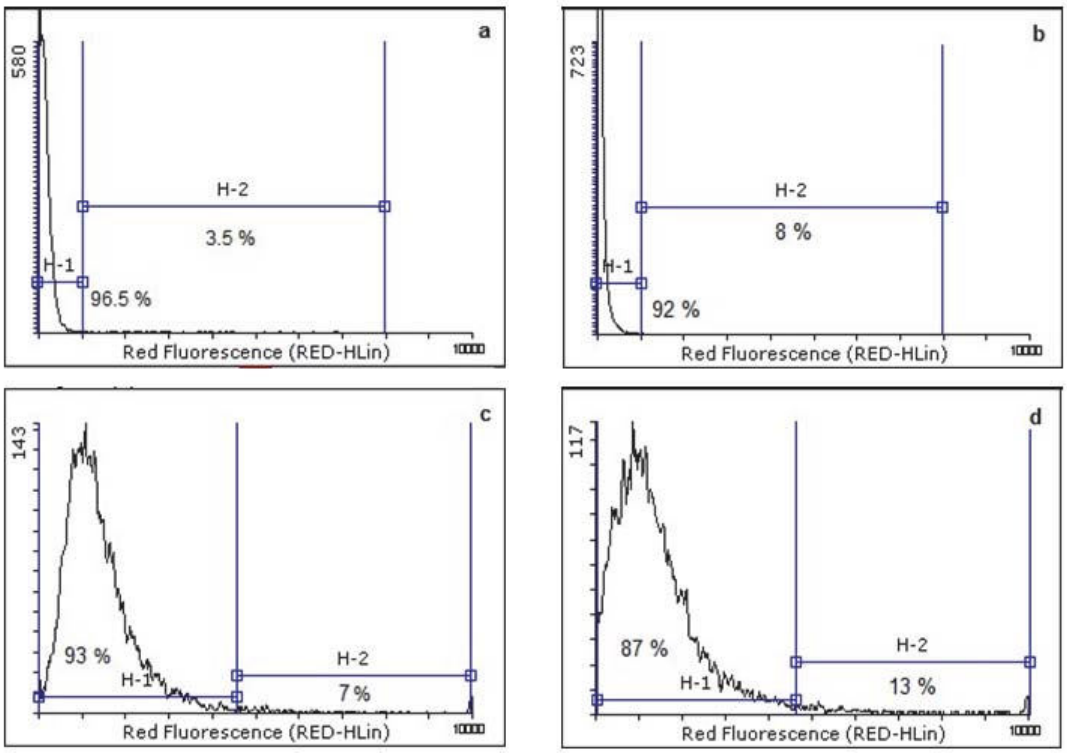


Fig. 6. Flow cytometry histograms of the cell cycle of investigated fungal isolates stained with propidium iodide: a – *C. albicans* after immobilization (start-up of the process); b – *C. subhashii* after immobilization (start-up of the process); c – *C. albicans* after 100 days of biofiltration; d – *C. subhashii* after 100 days of biofiltration

a negative impact of the process conditions on the viability of the species of investigated fungi. The results of flow cytometry analyses support the postulate that the use of *C. albicans* and *C. subhashii* in biofiltration processes can be upheld either as the main component of the inoculum or as additives that improve the process efficiency.

Conclusions

In this paper, *Candida albicans* and *Candida subhashii* species were inoculated to polyurethane-packed biotrickling filters to treat the air stream polluted with cyclohexane and ethanol. The obtained results of flow cytometry analyses indicate that the investigated fungi species may be applied to remove the above mentioned volatile organic compounds from their mixture with air. A stable biofilm has been formed on the packing elements of biotrickling filters, enabling a biofiltration performance with high removal efficiency. A synergistic effect of ethanol on the biofiltration of cyclohexane was identified. It was found that the removal efficiency of cyclohexane is higher when ethanol is introduced from the process start-up comparing to the case when it is introduced to a steady-state operating biotrickling filter, treating cyclohexane solely from the process start-up. Enhancement of biotrickling filtration of cyclohexane may result from both its co-metabolism with ethanol as well as improved sorption properties of trickling liquid for cyclohexane in the presence of ethanol, thus decreasing the mass transfer barrier for hydrophobic VOC. Additionally, investigations on the composition of liquid phase with respect to the treated compounds were performed. The results indicate that negligibly small concentrations of these compounds are found in the liquid phase in the steady-state conditions, when the removal efficiency of cyclohexane and ethanol are about 95–99%. The results of these studies indicate that the mechanism of enhanced removal of hydrophobic volatile organic compounds in the presence of hydrophilic compounds is rather complex and requires more in-depth elucidation, especially when multi-component systems are considered. In such future investigations, attention should be paid to link the gas-phase composition with changes occurring in the biofilm structure and composition as well as in the liquid phase, especially in the perspective of mathematical model formulation for biotrickling filtration of multi-component gas streams and specified single-component inoculum.

Acknowledgements

This research was funded by the Ministry of Science and Higher Education, Poland, through the specific subsidy awarded to Faculty of Chemistry, Gdańsk University of Technology (grant number 33882).

References

Avalos, Ramirez, A., Jones, J.P. & Heitz, M. (2007). Biotrickling filtration of air contaminated with ethanol, *Journal of Chemical Technology and Biotechnology*, 82, pp. 149–157, https://doi.org/10.1002/jctb.1644.

Cheng, Y., He, H., Yang, C., Zeng, G., Li, X., Chen, H. & Yu, G. (2016). Challenges and solutions for biofiltration of hydrophobic volatile organic compounds, *Biotechnology Advances*, 34, 1091–1102, https://doi.org/10.1016/j.biotechadv.2016.06.007.

Cheng, Y., Li, X., Liu, H., Yang, C., Wu, S., Du, C., Nie, L. & Zhong, Y. (2020). Effect of presence of hydrophilic volatile organic compounds on removal of hydrophobic n-hexane in biotrickling filters, *Chemosphere* 252, 126490, https://doi.org/10/1016/j.chemosphere.2020.126490.

Cox, H.H.J., Sexton, T., Shareefdeen, Z.M. & Deshusses, M.A. (2001). Thermophilic Biotrickling Filtration of Ethanol Vapors, *Environmental Science and Technology*, 35, pp. 2612–2619, https://doi.org/10.1021/es001764h.

Ferdowsi, M., Avalos, Ramirez, A., Jones, J.P. & Heitz, M. (2017). Elimination of mass transfer and kinetic limited organic pollutants in biofilters: A review, *International Biodeterioration and Biodegradation*, 119, pp. 336–348, https://doi.org/10.1016/j.ibiod.2016.10.015.

Gospodarek, M., Rybarczyk, P., Szulczyński, B. & Gębicki, J. (2019). Comparative Evaluation of Selected Biological Methods for the Removal of Hydrophilic and Hydrophobic Odorous VOCs from Air, *Processes 7*, 187, https://doi.org/10.3390/pr7040187.

He, S., Ni, Y., Lu, L., Chai, Q., Yu, T., Shen, Z. & Yang, C. (2020). Simultaneous degradation of n-hexane and production of biosurfactants by Pseudomonas sp. strain NEE2 isolated from oil-contaminated soils, *Chemosphere* 242, 125237, https://doi.org/10.1016/j.chemosphere.2019.125237.

Martinez-Rojano, H., Mancilla-Ramirez, J., Quiñonez-Diaz, L. & Galindo-Sevilla, N. (2008). Activity of hydroxyurea against Leishmania mexicana, Antimicrobial Agents Chemotheraphy 52, pp. 3642–3647, https://doi.org/10.1128/aac.00124-08.

Miller, U., Sówka, I. & Adamiak, W. (2019). The effect of betaine on the removal of toluene by biofiltration, SN Applied Sciences 1, https://doi.org/10.1007/s42452-019-0832-6.

Mudliar, S., Giri, B., Padoley, K., Satpute, D., Dixit, R., Bhatt, P., Pandey, R., Juwarkar, A. & Vaidya, A. (2010). Bioreactors for treatment of VOCs and odours – A review, *Journal of Environ*

Purswani, J., Juárez, B., Rodelas, B., Gónzalez-López, J. & Pozo, C. (2011). Biofilm formation and microbial activity in a biofilter system in the presence of MTBE, ETBE and TAME, *Chemosphere* 85, pp. 616–624, https://doi.org/10.1016/j.chemosphere.2011.06.106.

Ramani, R., Ramani, A. & Wong, S.J. (1997). Rapid Flow Cytometric Susceptibility Testing of Candida albicans, *Journal of Clinical Microbiology* 35(9):2320-4, DOI: 10.1128/jcm.35.9.2320-2324.1997.

Rybarczyk, P., Szulczyński, B. & Gębicki, J. (2020). Simultaneous Removal of Hexane and Ethanol from Air in a Biotrickling Filter – Process Performance and Monitoring Using Electronic Nose, *Sustainability* 12, 387, https://doi.org/10.3390/su12010387.

Rybarczyk, P., Szulczyński, B., Gębicki, J. & Hupka, J. (2019a). Treatment of malodorous air in biotrickling filters: A review, *Biochemical Engineering Journal* 141, pp. 146–162, https://doi.org/10.1016/j.bej.2018.10.014.

Rybarczyk, P., Szulczyński, B., Gospodarek, M. & Gębicki, J. (2019b). Effects of n-butanol presence, inlet loading, empty bed residence time and starvation periods on the performance of a biotrickling filter removing cyclohexane vapors from air, *Chemical Papers* 74, pp. 1039–1047, https://doi.org/10.1007/s11696-019-00943-2.

Salamanca, D., Dobslaw, D. & Engesser, K.-H. (2017). Removal of cyclohexane gaseous emissions using a biotrickling filter system, *Chemosphere* 176, pp. 97–107, https://doi.org/10.1016/j.chemosphere.2017.02.078.

Spigno, G., Pagella, C., Fumi, M.D., Molteni, R. & De Faveri, D.M. (2003). VOCs removal from waste gases: Gas-phase bioreactor for the abatement of hexane by Aspergillus niger, *Chemical Engineering Science* 58, pp. 739–746, https://doi.org/10.1016/S0009-2509(02)00603-6.

Yalkowsky, S.H., He, Y. & Jain, P. (2016). Handbook of Aqueous Solubility Data, Handbook of Aqueous Solubility Data. CRC Press, https://doi.org/10.1201/ebk1439802458.

Yang, C., Chen, H., Zeng, G., Yu, G. & Luo, S. (2010). Biomass accumulation and control strategies in gas biofiltration, *Biotechnology Advances* 28, 4, pp. 531–540, https://doi.org/10.1016/j.biotechadv.2010.04.002.

Yang, C., Qian, H., Li, X., Cheng, Y., He, H., Zeng, G. & Xi, J. (2018). Simultaneous Removal of Multicomponent VOCs in Biofilters, *Trends in Biotechnology* 36, 7, pp. 673–685, https://doi.org/10.1016/j.tibtech.2018.02.004.

Zhang, Y., Liss, S.N. & Allen, D.G. (2006). The effects of methanol on the biofiltration of dimethyl sulfide in inorganic biofilters, *Biotechnology and Bioengineering* 95, pp. 734–743, https://doi.org/10.1002/bit.21033.

Zhang, Y., Liu, J., Qin, Y., Yang, Z., Cao, J., Xing, Y. & Li, J. (2019). Performance and microbial community evolution of toluene degradation using a fungi-based bio-trickling filter, *Journal of Hazardous Materials* 365, pp. 642–649, https://doi.org/10.1016/j.jhazmat.2018.11.062.

Zhanga, Y., Denga, W., Qina, Y., Yanga, Z., Liua, J. & Lia, J. (2018) Research on Simultaneous Removal of Cyclohexane and Methyl Acetate in Biotrickling Filters, Proceedings of the 2nd International Conference of Recent Trends in Environmental Science and Engineering, Niagara Falls, Canada, https://doi.org/10.11159/rtese18.107.

Usuwanie z powietrza cykloheksanu i etanolu w biofiltrach strużkowych zasiedlonych grzybami Candida albicans i Candida subhashii

Streszczenie: W pracy przedstawiono badania nad usuwaniem cykloheksanu i etanolu z powietrza w boifiltrach zraszanych, wypełnionych pianką poliuretanową, zasiedloną grzybami z gatunku Candida albicans i Candida subhashii. Przedstawiono i omówiono wyniki dotyczące wydajności procesu (na podstawie pomiarów techniką chromatografii gazowej) wraz z wynikami cytometrii przepływowej dla utworzonego biofilmu. Uzyskano wartości zdolności usuwania, wynoszące około 89 g m^{-3} h^{-1} i 36.7 g m^{-3} h^{-1}, odpowiednio dla cykloheksanu i etanolu, gdy te związki jednocześnie poddawano procesowi biofiltracji w biofiltrze zaszczepionym Candida subhashii. Wyniki wskazują, że obecność etanolu powoduje zwiększenie skuteczności usuwania cykloheksanu z powietrza. Wzrost skuteczności usuwania z powietrza cykloheksanu w obecności etanolu może wynikać z polepszonego metabolizmu cykloheksanu w takich warunkach oraz z ograniczenia bariery dla przenikania masy, wskutek lepszych właściwości sorpcyjnych cieczy zraszającej wobec cykloheksanu w obecności etanolu.

3.5. Publication 5: Systematic comparison of a biotrickling filter and a conventional filter for the removal of a mixture of hydrophobic VOCs by *Candida subhashii*

The research was carried out as part of the first scientific internship in the laboratory Department of Chemical Engineering and Environmental Technology at the University of Valladolid under supervision of Prof Raúl Muñoz Torre. The aim of the study was to compare the efficiency of a BF and a BTF using the *C. subhashii* species previously isolated from peat to remove a mixture of hydrophobic VOCs. This part of the research focused solely on the *C. subhashii* isolate, due to the fact that it was characterized by the best carbon assimilability from selected VOCs, and it was never used in biofiltration. This decision was also influenced by the information that *C. albicans* may be a pathogenic fungus species for immunocompromised people. In rare cases, this species can cause a fungal infection called candidiasis [175,176].

As a model mixture of hydrophobic VOCs, n-hexane, TCE, toluene and α-pinene were selected. Each of the selected compounds are considered significant air pollutants due to their toxicity and cover a wide range of VOCs (respectively aliphatic hydrocarbons, halogenated hydrocarbons, aromatic hydrocarbons and terpenes) emitted by industrial activities. In addition, the components of the composed mixture are characterized by moderate to high hydrophobicity, which is most often the main technical limitation of BTFs and BFs. The composition of the tested mixture did not include hydrophilic compounds due to the fact that their absorption and decomposition by microorganisms in biofilters are often simple.

The results of all the experiments presented in the article, both carried out in BF, BTF and a batch test conducted in serum bottles, confirmed the ability of *C. subhashii* immobilized on polyurethane foam to biodegrade n-hexane, TCE, toluene and α-pinene. BTF showed a higher VOC reduction efficiency than BF. All biodegradation tests described showed a consistent biodegradation pattern: toluene ≈ n-hexane > α-pinene > TCE.

To date, Henry's law constants have been used to determine the role of the hydrophobicity of the tested VOCs in their mass transfer to the liquid phase in biofiltration. On the other hand, the biodegradation patterns obtained as experimental results (toluene ≈ n-hexane > α-pinene > TCE) differed from those theoretically expected, according to Henry's law constants (in order of decreasing solubility: n-hexane > α-pinene > TCE > toluene). Therefore, it was decided for the first time in biofiltration to use Hansen's solubility parameters, which so far have been mainly used in the field of polymers. The obtained values were fully consistent with the obtained biodegradation pattern. The presence of the dripping liquid phase did not alter the degradation patterns observed. This phenomenon suggests that it is possible that the obtained biodegradation pattern is also dependent on the microorganism that inhabits the biofilter, and not only on the type of biofiltration used. The verification of this assumption was carried out in the next stage of research, described in the next article (P6).

The publication presents experiments leading to the implementation of task T5 *Checking the ability of the selected fungus to use a mixture of hydrophobic VOCs from the gas phase as a carbon source in BTF*.

The novelty presented in it is:

- Confirmation of the ability of *C. subhashii* to biodegrade a mixture of hydrophobic VOCs (n-hexane, TCE, toluene and α-pinene, each of which is considered a significant air pollutant), regardless of the gas-phase bioreactor configuration assessed.

Chemosphere 306 (2022) 135608

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Systematic comparison of a biotrickling filter and a conventional filter for the removal of a mixture of hydrophobic VOCs by *Candida subhashii*

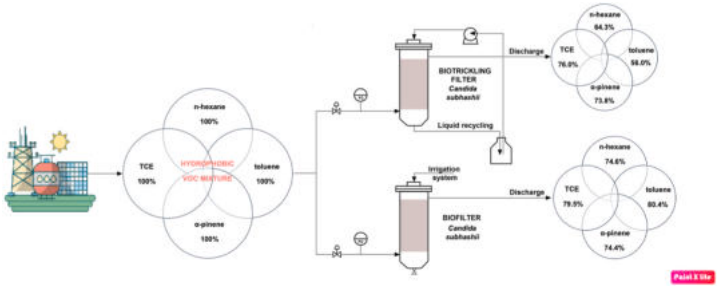
Milena Marycz [a,b], Yadira Rodríguez [a], Jacek Gębicki [b], Raúl Muñoz [a,*]

[a] Institute of Sustainable Processes, Universidad de Valladolid, Dr. Mergelina s/n, 47011, Spain
[b] Department of Process Engineering and Chemical Technology, Faculty of Chemistry, Gdansk University of Technology, 11/12 G. Narutowicza Street, 80-233, Gdansk, Poland

HIGHLIGHTS

- A fungal BF and BTF treating hydrophobic VOCs were systematically compared.
- *subhashii* supported an effective removal of VOCs at short EBRT of 30 s.
- Fungal BTF supported a slightly higher VOC abatement performance than fungal BF.
- The ability of *C. subhashii* to remove VOCs was also confirmed in batch assays.
- A consistent biodegradation pattern was recorded: toluene ≈ n-hexane>α-pinene > TCE.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Dr A ADALBERTO NOYOLA

Keywords:
Biofiltration
Fungi
n-hexane
TCE
Toluene
α-pinene

ABSTRACT

This work systematically compared the potential of a conventional fungal biofilter (BF) and a fungal biotrickling filter (BTF) for the abatement of a mixture of hydrophobic volatile organic compounds (VOCs). *Candida subhashii* was herein used for the first time, to the best of the author's knowledge, to remove *n*-hexane, trichloroethylene, toluene and α-pinene under aerobic conditions. *C. subhashii* immobilized on polyurethane foam supported steady state removal efficiencies of *n*-hexane, trichloroethylene, toluene and α-pinene of 25.4 ± 0.9%, 20.5 ± 1.0%, 19.6 ± 1.5% and 25.6 ± 2.8% in the BF, and 35.7 ± 0.9%, 24.0 ± 1.6%, 44.0 ± 1.7% and 26.2 ± 1.8% in the BTF, respectively, at relatively short gas residence times (30 s). The ability of *C. subhashii* to biodegrade *n*-hexane, TCE, toluene and α-pinene was confirmed in a batch test conducted in serum bottles, where a biodegradation pattern (toluene ≈ *n*-hexane > α-pinene > trichloroethylene) comparable to that recorded in the BF and BTF was recorded.

1. Introduction

The removal of hazardous gas pollutants prior discharge into the environment entails many technical problems and is often costly. When selecting the best technology to remove hazardous gas pollutants, parameters such as a high pollutant removal efficiency, low capital and operational costs, environmentally friendliness and low environmental impacts are typically considered (Kośmider et al., 2012; Revah et al., 2011). In recent years, biological waste gas and odor treatment methods have become increasingly popular (Gospodarek et al., 2019; Szulczyński

* Corresponding author.
E-mail address: mutora@iq.uva.es (R. Muñoz).

https://doi.org/10.1016/j.chemosphere.2022.135608
Received 1 February 2022; Received in revised form 8 June 2022; Accepted 2 July 2022
Available online 7 July 2022

et al., 2019; Abraham et al., 2015). One of the most common biological gas purification techniques is biofiltration, which is a relatively cheap and ecologically safe process based on packed bed systems. Biofiltration consists of circulating the contaminated gas stream through a filter bed inhabited by pollutant degrading microorganisms (Rybarczyk et al., 2019a; Lebrero et al., 2016; Guieysse et al., 2008). Pollutants diffuse from the gas phase to the so-called biofilm formed on the surface of the packing material. The compounds adsorbed on the surface or absorbed into the biofilm undergo biodegradation in the presence of nutrients (N, P, etc) and electron acceptors (typically O_2), and the purified and odorless gas stream leaves the biofiltration unit (Rybarczyk et al., 2019b; Raboni et al., 2017). The biofiltration process can be engineered as a biofilter or biotrickling filter, the latter involving a continuous recirculation of a nutritive aqueous solution that helps controlling the pH, supplying nutrients and improving gas-biofilm pollutant mass transfer (Mudliar et al., 2010; Estrada et al., 2013).

Biofiltration is cost effective for waste gases with a low concentration (<3 g m^{-3}) of volatile organic compounds (VOCs) (van Groenestijn and Hesselink, 1993; ÓJ Veiga and Kennes, 2008). However, conventional biofilters, based on the action of microorganisms typically growing on organic packing materials such as compost, and biotrickling filters constructed with inorganic packing materials, still face problems during the abatement of hydrophobic VOCs such as aromatics, alkenes and alkanes. Indeed, these gas pollutants are poorly absorbed in bacterial biofilms or in the recirculating aqueous solution due to the low aqueous solubility of hydrophobic VOCs. In addition, the operational stability of conventional biofilters is often hampered by acidification and drying of the filter bed. In this context, fungal colonization of inert packing material in biofilters or biotrickling filters has been proposed in order to overcome these operational problems (Cox, 1995; Groenestijn et al., 1995).

Fungi are more resistant to acidic and dry conditions than bacteria, and their cell wall contains hydrophobic proteins called hydrophobins that can enhance the abatement of hydrophobic VOCs (Cox, 1995; Jorio et al., 2009; Marycz et al., 2022). In recent years, the potential of fungi in biofiltration applications has been revisited, since these microorganisms have been shown to be able to biodegrade many complex organic compounds. For example, *Fusarium solani* supported an effective removal of hydrophobic VOCs in biofilters (Arriaga and Revah, 2005; Arriaga et al., 2006; Vergara-Fernández et al., 2016; Rybarczyk et al., 2021). Similarly, *Cladosporium sphaerospermum* was able to remove BTEX, methyl propyl ketone, MEK, toluene and *n*-butyl acetate in a biotrickling filter (Raboni et al., 2017; Qi et al., 2005). Two types of fungi are typically used in biofiltration: molds and yeasts. Mold fungi form a mycelium composed of loosely collected hyphae, the so-called air mycelium. This aerial mycelium of mold fungi, which is in direct contact with the gas phase, can adsorb and biodegrade hydrophobic VOCs. On the other hand, yeasts are single-celled fungi that reproduce by budding. The species *Candida subhashii*, which was pioneering in the removal of mixtures of hydrophobic and hydrophilic VOCs, belongs to the group of yeasts. Indeed, *C. subhashii* was successfully used in a biotrickling filter for the continuous cyclohexane removal with ethanol as a co-substrate (Rybarczyk et al., 2021) based on its effective immobilization on biofilter packing materials (Marycz et al., 2020). Applications of bacterial biofiltration as an alternative for odor abatement has been extensively investigated and reviewed in the past 35 years (Gospodarek et al., 2019; Marycz et al., 2022). However, the potential of yeasts for air biofiltration has been poorly explored in literature and its applicability is still incipient (Marycz et al., 2022). The review recently published by Marycz et al. (2022) (Marycz et al., 2022) embraced the main research performed on fungal and yeast species capable of removing hydrophobic VOCs in BTF in the last 10 years. Additionally, species of fungi and yeasts that have not been used in biofiltration to remove hydrophobic VOCs were proposed and their biodegradation potential was justified. Vergara-Fernández et al. (2018) stated that the removal efficiency of hydrophobic compounds is lower in bacterial biofilters than in fungal biofilters (Vergara-Fernández et al., 2018a). Moreover, Prenafeta-Boldú et al. (2018) (Prenafeta-Boldú et al., 2018) indicated that special attention should be paid to elucidate the phenomena underlying fungal and yeast based biofiltration process such as heat, momentum and mass transport, as well as microbial growth and biodegradation kinetics. Indeed, systematic studies comparing the ability of fungi to abate hydrophobic VOCs in different bioreactor configurations have not been reported to date. Moreover, the performance of *C. subhashii* for the biofiltration of a hydrophobic VOC mixture has never been assessed.

VOCs are emitted in industrial activities (e.g. chemical, printing, petrochemical industries). VOCs are generally classified as aliphatic hydrocarbons (e.g., *n*-hexane), halogenated hydrocarbons (e.g., TCE), aromatic hydrocarbons (e.g., toluene), and terpenes (e.g., α-pinene) (Yang et al., 2009). *n*-hexane, TCE, toluene and α-pinene are representative VOCs from each class and are considered to be relevant air pollutants as a result of their toxicity (Yang et al., 2009; Liu et al., 2007). In addition, these VOCs exhibit a moderate to high hydrophobicity, which is typically considered the main technical limitation of biofilters and biotrickling filters.

The present work aims at systematically comparing a conventional biofilter and a biotrickling filter colonized by *C. subhashii* in terms of their ability to remove a hydrophobic VOC mixture composed of hexane, pinene, trichloroethylene (TCE) and toluene. Additionally, batch biodegradation tests were carried out in order to confirm the metabolic capacity of *C. subhashii* to biodegrade the target mixture of hydrophobic VOCs.

2. Materials and methods

2.1. Microorganisms and inoculum

C. subhashii was used in this work as a biocatalyst (Marycz et al., 2020). Aliquots of 333 mL of sterile liquid Sabouraud medium (BTL, Poland) (containing the carbon and energy source) in 500 mL Erlenmeyer flasks (E-flasks) were inoculated with *C. subhashii* agar using an inoculation loop under sterile conditions. The E-flasks were incubated at 25 °C for 9 days in a rotary shaker (Thermo Fisher Scientific, U.S.) at 200 rpm. Then, 1000 mL of inoculum were centrifuged under sterile conditions at 24 °C for 5 min at 3000 rpm (Sorvall Legend RT Plus Centrifuge, U.S.). After centrifugation, the fungal pellet was re-suspended and washed with 200 mL of minimal nutrient medium (MSM), and centrifuged again under similar conditions. The fungal pellet was re-suspended in 200 mL of MSM and 90-mL cell culture aliquots were used to inoculate the polyurethane foam used as a packing material in the fungal biofilter and biotrickling filter.

2.2. VOCs and minimal nutrient medium

n-hexane (Sigma-Aldrich, South Korea), trichloroethylene (Panreac AppliChem, Spain), toluene (Sigma-Aldrich, USA) and α-pinene (Sigma-Aldrich, USA) were used as model hydrophobic indoor air pollutants. The MSM used for fungal growth in the batch liquid cultures and biofiltration columns was previously reported by Marycz (Marycz et al., 2020). This medium was composed of: Na_2HPO_4 $2H_2O$ (Panreac AppliChem, Spain) (15.2 g L^{-1}), KH_2PO_4 (Panreac AppliChem, Spain) (3 g L^{-1}), NaCl (Panreac AppliChem, Spain) (0.5 g L^{-1}) and NH_4Cl (Sigma-Aldrich, USA) (1 g L^{-1}). After mixing all components, the pH of the medium was ~7. The composition of the Sabouraud mineral salt medium was as follows: casein hydrolyzate (Panreac AppliChem, Spain) (5 g L^{-1}), meat extract (Panreac AppliChem, Spain) (5 g L^{-1}), glucose (Panreac AppliChem, Spain) (40 g L^{-1}). The pH of the Sabouraud mineral salt medium was 5.6 ± 0.2.

2.3. Batch VOC biodegradation assay

Batch biodegradation tests were carried out in 1.2-L gas-tight glass

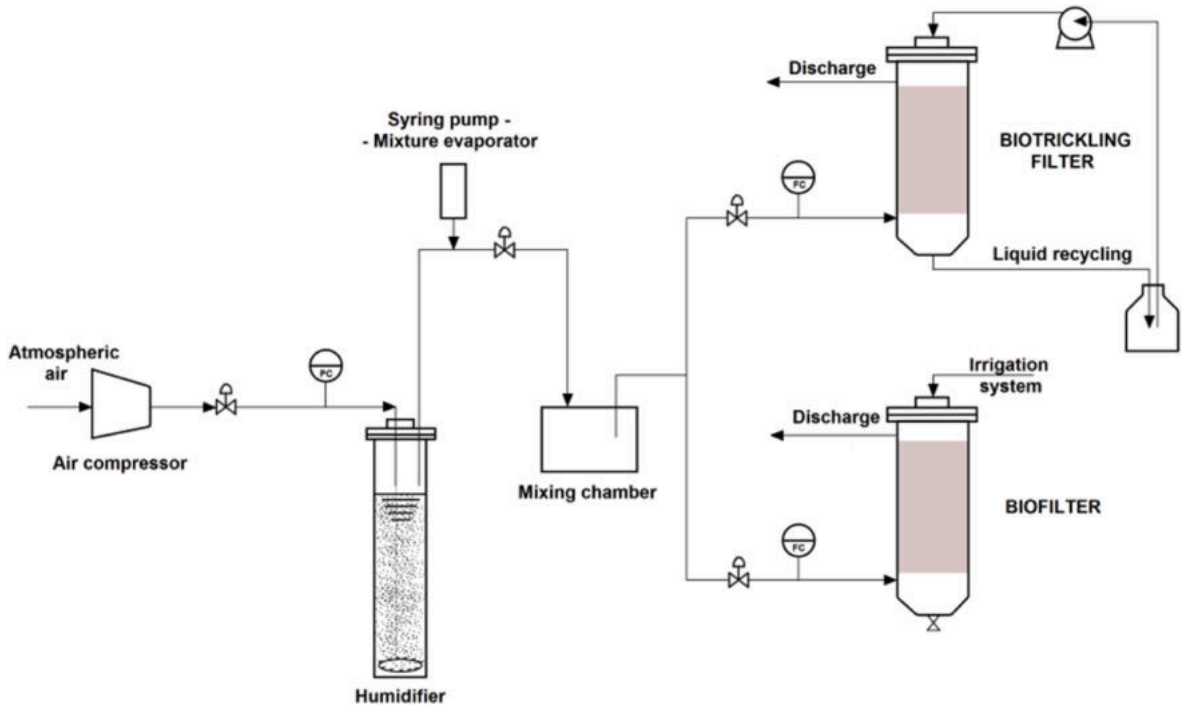


Fig. 1. Schematic representation of the experimental set-up.

bottles (closed with butyl septa and sealed with plastic caps) containing 200 mL of MSM inoculated with a loop inoculum of *C. subhashii* taken from the solid medium. *n*-hexane, TCE, toluene and α-pinene were injected into the headspace of the bottles to achieve an initial concentration of 300, 175, 150 and 145 mg m^{-3}, respectively. Positive and negative controls were also prepared with *C. subhashii* (without VOCs) and with VOCs (without *C. subhashii*), respectively. The bottles were magnetically agitated at 300 rpm and incubated for 28 days at 25 °C. All tests were conducted in duplicate. The headspace concentrations of the target VOCs were daily determined by GC-FID using a 100 μL gastight syringe (Hamilton, Australia).

2.4. Continuous VOC abatement in a fungal biofilter and a fungal biotrickling filter

A conventional biofilter (BF) and a biotrickling filter (BTF) (Fig. 1) made of clear PVC columns (10 cm internal diameter × 100 cm height) were set up. Each column was filled with 34 cm of polyurethane foam (PUF), reaching a total packed bed volume (V_p) of 2.5 L (Filtren TM 25280, Recticel Ibérica S.L., Spain). The PUF exhibited a density of 0.01 g mL^{-1}, a specific surface area of 1000 m^2 m^{-3}, a porosity of 96% and a water retention capacity of 0.12 L_{water} L_{PUF}^{-1}. Air was initially humidified in a clear PVC column (0.1 m internal diameter × 1.6 m height, filled with 1.2 m of water) and mixed with a liquid mixture of *n*-hexane, TCE, toluene and α-pinene injected at 0.3 mL h^{-1} using a syringe pump (KDS100 Legacy, Fisherbrand, USA), resulting in average concentrations of 206.6 ± 6.9, 237.3 ± 7.6, 310.3 ± 10.7 and 393.5 ± 21.2 mg m^{-3}, respectively. Both the fungal biofilter and fungal biotrickling filter were fed with the polluted air from the bottom at 5 L min^{-1}, which resulted in an empty bed residence time of 0.5 min. The air flowrates were controlled using rotameters (Aalborg, USA). The BF was periodically irrigated at 17.8 mL MSM $L_{packing}^{-1}$ d^{-1}. In the BTF, a recycling nutritive solution (MSM) was continuously agitated in an external 1-L tank and recycled at a rate of 2 m h^{-1} using a peristaltic pump (Watson Marlow, USA). In addition, a MSM renewal rate of 40 mL d^{-1} was implemented in the BTF. Gas samples were periodically collected from each module at the gas inlet and outlet using a 100-μL gastight syringe (Hamilton, Australia) in order to determine the concentration of the target VOCs and CO_2.

The pH and culture absorbance at 600 nm (OD_{600}) for the monitoring of the suspended biomass concentration were daily measured in the leachate of the BF (from aliquots of 40 mL) and in the liquid effluent of the BTF by withdrawing a 40-mL aliquot from the external 1-L mineral medium tank during the MSM renewal. The experiment lasted 48 days.

2.5. Analytical methods

CO_2 and O_2 gas concentrations were quantified using a Bruker 430 gas chromatograph (Bruker Corporation, Palo Alto, USA) equipped with a CP-Molsieve 5A and a CP-PoraBOND Q columns and a thermal conductivity detector. Oven, injector and detector temperatures were kept at 45, 150 and 200 °C, respectively, while helium was employed as a carrier gas at 13.7 mL min^{-1}. This method is described elsewhere (Estrada et al., 2014). The concentrations of VOCs were measured in a GC-FID (Varian 3900) equipped with an Agilent HP-5MSI capillary column (30 m × 0.25 mm × 0.25 μm) as described by González-Martín and co-workers (González-Martín et al., 2022). Measurements of the OD_{600} were performed in a SPECTROstar Nano spectrophotometer (BMG LABTECH, Germany). pH was determined using a pH-meter Basic 20 (Crison, Spain).

2.6. Calculations

Results from the biofiltration experiments were herein expressed in terms of VOC removal efficiency (RE, %), which was calculated according to Equation (1):

$$RE = 100 \times \frac{C_{in} - C_{out}}{C_{in}} \quad (1)$$

where C_{in} and C_{out} stand for the inlet and outlet VOCs concentrations. The average values of RE along with its standard deviation were calculated for each VOC under steady state.

The volumetric CO_2 production (g m^{-3} h^{-1}) is defined as:

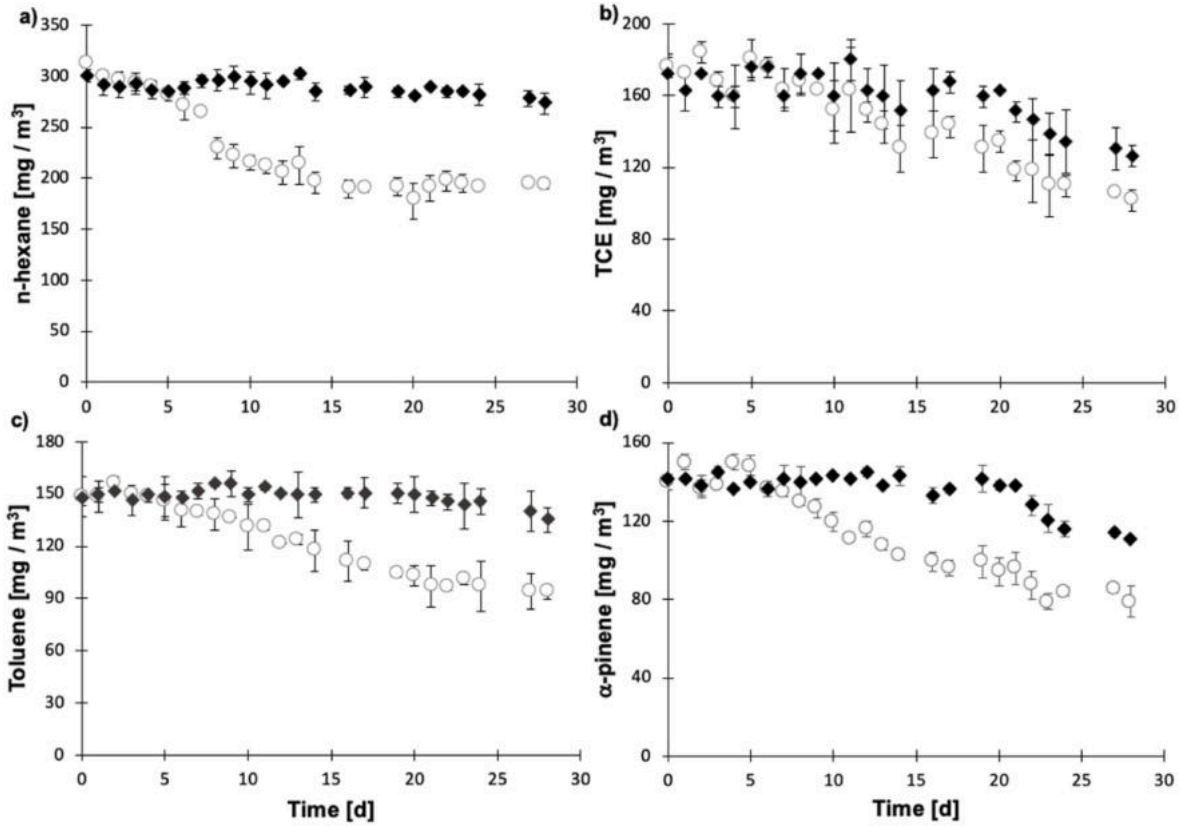


Fig. 2. Time course of the headspace concentration of (a) *n*-hexane, (b) TCE, (c) toluene, (d) α-pinene in the abiotic control (◆) and biodegradation assays (o).

$$CO_2\ production = \frac{Q\ (CO_{2\ out} - CO_{2\ in})}{V_{bed}} \quad (2)$$

where $CO_{2\ out}$ and $CO_{2\ in}$ are the CO_2 concentrations at the gas outlet and inlet of the reactor, Q is the gas volumetric flow rate and V_{bed} is the packed bed volume.

The pollutant elimination capacity (EC) is defined according to Equation (3):

$$EC = \frac{Q\ (C_{in} - C_{out})}{V_{bed}} \quad (3)$$

as a function of the air flow rate (Q), the inlet and outlet gas concentrations (C_{in} and C_{out}), and the packed bed volume (V_{bed}).

3. Results and discussion

3.1. Batch VOC biodegradation assay

The concentrations of the target VOCs in the headspace of the bottles started to decrease by day 6. The highest removal at the end of the experiment was recorded for *n*-hexane (~38%), followed by toluene (~33%), α-pinene (~26%) and TCE (~22%) (Fig. 2). An unexpected deterioration in the biodegradation capacity of *C. subshashii* was observed for *n*-hexane by day 18, and for α-pinene by day 23, which might have been due to the accumulation of inhibitory metabolites in the cultivation medium. Interestingly, the biodegradation of TCE and toluene continued until the end of the experiment. A gradual decrease in the concentration of the target VOCs in the abiotic controls was observed as a result of pollutant adsorption onto the glass wall or butyl septum. This phenomenon occurred to a greatest extent for TCE and α-pinene compared to *n*-hexane and toluene, but a more rapid decrease in pollutant concentration was recorded in the assays inoculated with *C. subshashii*.

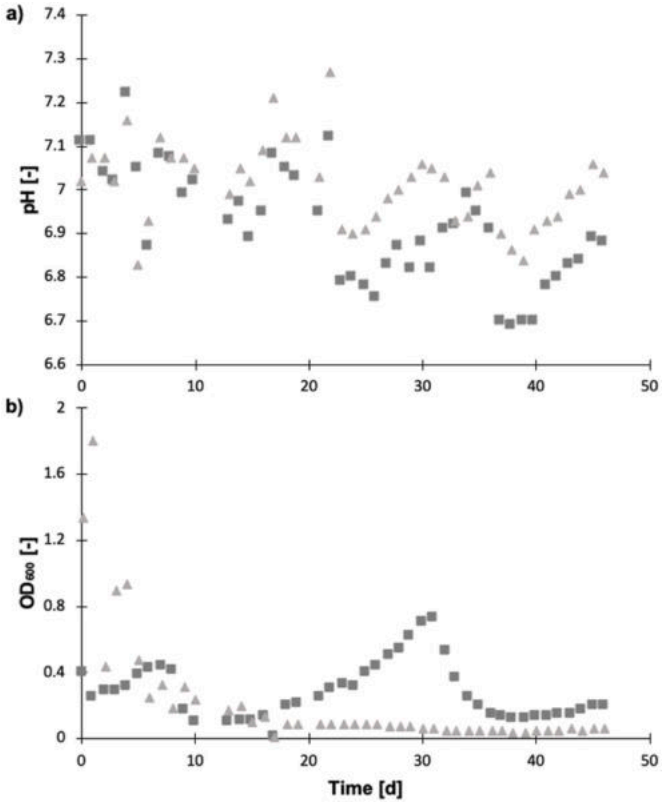


Fig. 3. Time course of (a) pH and (b) OD_{600} in the BTF trickling solution (■) and BF leachate (▲).

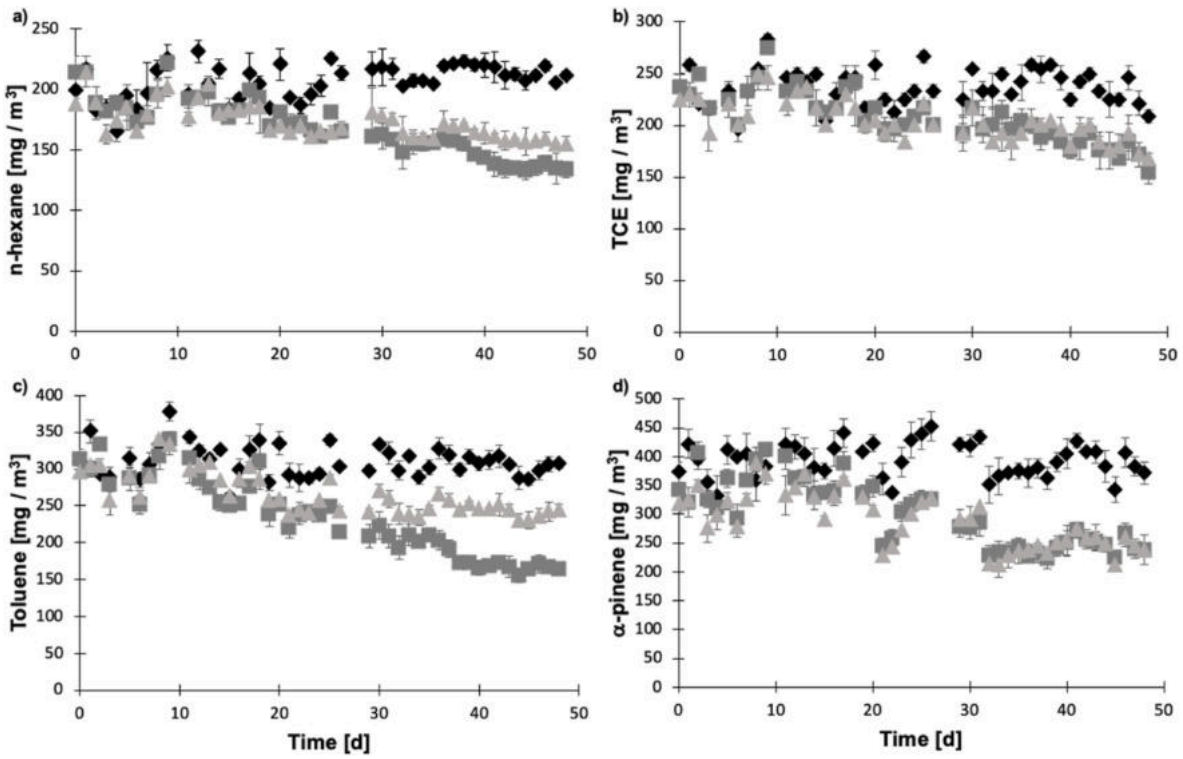


Fig. 4. Time course of the inlet (◆) and outlet concentration of (a) *n*-hexane, (b) TCE, (c) toluene, (d) α-pinene in the biofilter inoculated with *C. subhashii* (▲) and the biotrickling filter inoculated with *C. subhashii* (■) and operated at a gas residence time of 30 s.

3.2. Continuous VOC abatement in a fungal biofilter and a fungal biotrickling filter

A start-up period of 20 days, characterized by low VOC removal efficiencies, was observed in the BF and BTF inoculated with *C. subshashii* (Fig. 3). The gradual formation of the fungal biofilm in the packing material of both BF and BTF resulted in a steady increase in biofiltration performance. Rybarczyk et al. (2021) (Rybarczyk et al., 2021) presented investigations on the removal of cyclohexane from air in a BTF inoculated with *C. subshashii*, where the start-up period lasted 30–35 days. On the other hand, Vergara-Fernández et al. (2018a, 2018b) observed a start-up period of 18 days for toluene during the simultaneous abatement of formaldehyde, toluene and benzo[α]pyrene in a biofiltration reactor inoculated with *Fusarium solani* fungi and *Rhodococcus erythropolis* bacteria. In this context, the initiation of the process of biotransformation of a compound by a given species of fungus is directly related to the type of compound to be removed, the operational conditions in the bioreactor and the physiological state of the inoculum.

A slight decrease in the pH of both the BF leachate and BTF trickling solution was observed, probably due to the release of acidic fungal biodegradation metabolites (Fig. 3a). Interestingly, this drop in pH occurred in a larger extent in the BTF, with pHs fluctuating between 6.7 and 7, as a result of the higher VOC removals supported by this bioreactor configuration. Typically, pH fluctuations of ±1 do not alter *Candida* metabolism as previously reported by (Rane et al., 2019). Biomass concentration in the trickling solution of the BTF, estimated as OD_{600}, gradually increased from day 10 (OD_{600} = 0.10) to 31 (OD_{600} = 0.73) (Fig. 3b). This phenomenon was likely due to biofilm detachment from the packaging material and its subsequent washout by the trickling solution. On the other hand, biomass concentration in the leachate of BF decreased significantly to finally stabilize by day 20. From this day onwards, the OD_{600} remained constant in the BF at 0.055 ± 0.015, which suggest that cells did not detach from the biofilm formed in the polyurethane foam. The low OD_{600} and strong biofilm attachment in the BF may be likely due to the absence of trickling solution and the associated shear stress in the biofilm. In the BTF, the higher VOC removal efficiencies recorded entailed a higher biomass growth. It should be noted that the measurement of optical density also takes into account dead cells with preserved integrity of cytoplasmic membranes. Therefore, a high OD_{600} value does not fully reflect the physiological state of the culture and their potential for biofilm formation.

Maximum and stable *n*-hexane removal efficiencies of 34–37% in BTF and 25–27% in BF were recorded under steady state at 30 s of EBRT, indicating that *C. subhashii* did not only promote the mass transfer of *n*-hexane to the biofilm but contributed to *n*-hexane biodegradation (Fig. 4a). Interestingly, a similar start-up period of 20 days was observed in both biofiltration configurations. In this context, *n*-hexane has been effectively removed using *Fusarium solani* in a 2.5 L BF packed with perlite operated at EBRT of 60 s with elimination capacities of 90–130 g m^{-3} h^{-1} and a maximum RE of 100% below inlet concentrations of 1.8 g m^{-3} (which corresponded to a critical inlet load of around 70 g m^{-3} h^{-1}) (Arriaga and Revah, 2005). The discrepancy in the results was likely due to the fact that Arriaga and Revah (2005) operated a BF with a twice higher EBRT and pure *n*-hexane instead of a mixture of VOCs. In addition, process operation at hexane concentrations 10 folds higher than in the study herein presented promoted an active fungal growth and therefore an effective hexane capture and biodegradation.

The start-up period to achieve a significant TCE removal in both biofiltration units was approximately 21 days. The steady state REs of TCE in BTF fluctuated between 22 and 26% from days 38–48. Similarly, steady state REs of TCE in BF oscillated between 21 and 23% from days 33–48 (Fig. 4b). Apart from carbon and hydrogen, TCE also contains chlorine atoms, which hinders it enzymatic degradation by microorganisms. TCE abatement in the presence of methanol has been conducted with a consortium of *Fusarium verticillioides* and *Fusarium solani* in a BTF operated at an EBRT of 9 s, with eliminations capacities of 3.2–12.9 g m^{-3} h^{-1} and maximum REs of TCE of 87.1%) (Chheda and Sorial, 2017). Similarly, TCE elimination capacities of 4.9–3.6 g m^{-3} h^{-1} and maximum REs of 52.9% were recorded in a BTF inoculated with an *Ascomycota* strain and operated at a EBRT 405 s (Quan et al., 2018).

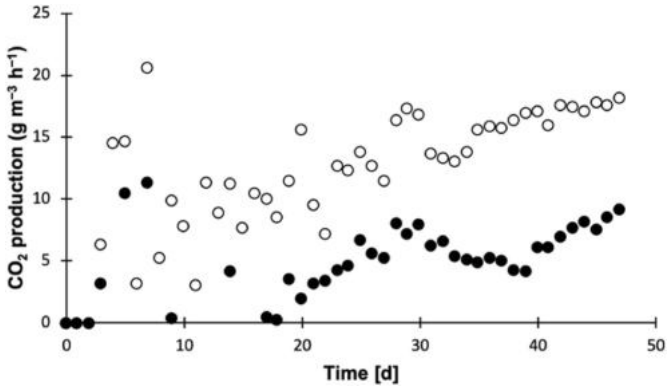


Fig. 5. Time course of the CO_2 production in the BTF (○) and in the BF (●) inoculated with *C. subhashii* and operated at a gas residence time of 30 s.

Interestingly, higher toluene REs were achieved under steady state in BTF than in BF likely due to the moderate aqueous solubility of this aromatic VOC. Thus, steady state toluene REs fluctuated in the range of 41–46% (day 40–50) with a reduced start-up period of 10 days. Toluene REs of 18–21% were reached in BF during steady state (Fig. 4c). Toluene removal has been conducted with a non-virulent consortium of black yeast *Cladophialophora* sp. in a BF operated at an EBRT of 12–48 s, with elimination capacities of 5.0–30.0 g m^{-3} h^{-1} and maximum REs of toluene of 100% (Prenafeta-Boldú et al., 2008).

Finally, steady state α-pinene REs of 25–29% and 20–27% were recorded in BTF and BF, respectively, with a start-up period of ~17 days (Fig. 4d). Preliminary studies carried out α-pinene removal with *Ophiostoma* sp. in a BF operated at EBRTs of 26–72 s, with elimination capacities of 143.0 g m^{-3} h^{-1} and REs of α-pinene of up to 95% (González-Martín et al., 2022). Similarly α-pinene REs of 89% were achieved in a BF operated at an EBRT of 31 s with *Ophiostoma* sp. (Jin et al., 2007). Finally, the operation of a BTF at an EBRT of 26–38 s with *Candida boidinii* and *Ophiostoma stenoceras* consortium resulted in pinene elimination capacities of 175.0 g m^{-3} h^{-1} and REs of 67% (López et al., 2013).

Fungal cells colonizing the packing material in the BTF produced more CO_2 than those in BF mediated by the higher efficiency in the removal of the hydrophobic VOCs in the former biofiltration unit (Fig. 5). At this point it should be highlighted that since the emission treated was a diluted VOC air stream, O_2 never limited the biodegradation process. The VOCs mineralization ratio (CO_2 production/VOCs-EC) averaged 0.12 ± 0.01 and 0.07 ± 0.02% in the BTF and the BF, respectively.

3.2.1. *Henry's law constants and Hansen solubility parameters*

The ability of a compound to be dissolved in a given solvent can be predicted through Hansen's solubility parameters. The principle of the Hansen three dimensional solubility parameters (the Hildebrand parameter) can be expressed according to Equation (4):

$$\delta_T^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \quad (4)$$

where δ_T is the total solubility parameter (the so-called Hildebrand solubility parameter) and δ_D, δ_P and δ_H are the components of the Hansen parameters of the compound due to dispersion, polar and hydrogen bonding, respectively. The δ_D, δ_P and δ_H components of the Hildebrand parameter for each individual compound were listed in Table 1.

The solubility distance (R_a) is the distance between the solvent (water) and the solute (compound) in Hansen (Hildebrand) solubility parameters and can be defined according to Equation (5) (Li et al., 2016):

$$R_a^2 = 4(\delta_{D1} - \delta_{D2})^2 + (\delta_{P1} - \delta_{P2})^2 + (\delta_{H1} - \delta_{H2})^2 \quad (5)$$

The closer the solubility parameters of the compound and solvent are, the more likely the compound is to dissolve in a given solvent. The values of the solubility distance (R_a) showed in Table 1 are consistent with the biodegradation pattern herein obtained: toluene ≈ *n*-hexane> α-pinene > TCE. Thus, the higher the R_a value calculated for a given compound and the solvent used, the better removal through biofiltration.

Hydrophobicity patterns differed from those theoretically expected according to Henry's law constants (in order of decreasing solubility: *n*-hexane > α-pinene > TCE > toluene). To date, the mechanisms of degradation of these VOCs individually by *C. subhashii* have not been investigated. In this context, it is not possible to lay the foundation of possible inhibition mechanisms using this VOC mixture. Interestingly, the presence of trickling liquid phase did not alter the patterns of degradation observed.

4. Conclusion

C. subhashii immobilized in PUF supported an effective abatement of hydrophobic VOCs at a relatively short EBRT regardless of the gas-phase bioreactor configuration evaluated. Both the batch and continuous VOC biodegradation assays showed a consistent biodegradation pattern: toluene ≈ *n*-hexane > α-pinene > TCE. The biotrickling filter supported a slightly higher VOC abatement performance, as confirmed by the higher CO_2 concentrations recorded in the BTF off-gas. The decrease in the pH of the cultivation broth and the unexpected deterioration in VOC biodegradation in the batch assays suggested the release of inhibitory metabolites from fungal metabolism during VOC mineralization. Despite the promising results herein obtained, further research on the optimization of process parameters such as EBRT, inlet load and pH, and on the elucidation of potential inhibition mechanisms in the VOC mixture, is required to enhance the efficiency of the biofiltration process for the abatement of hydrophobic compounds by *C. subhashii*.

CRediT authorship contribution statement

Milena Marycz: Conceptualization, Methodology, Investigation, Formal analysis, Roles/. **Yadira Rodríguez:** Writing – review & editing, Methodology. **Jacek Gębicki:** Supervision, Writing – review & editing. **Raúl Muñoz:** Supervision, Conceptualization, Methodology, Project administration, Writing – review & editing, Funding acquisition, Resources.

Table 1
Henry's constant and Hansen parameters for water and some VOCs.

Compounds	Henry's constant[a]	Hansen solubility parameters [MPa]			R_a (relative for a water)	Reference
		Dispersion δ_D	Polar δ_P	Hydrogen bonding δ_H		
***n*-hexane**	$1.0•10^{-3}$	15.2	0.8	2.0	43.08	Filly et al. (2014)
TCE	$9.9•10^{-2}$	18.0	3.1	5.3	39.48	Hansen (2007)
Toluene	$1.6•10^{-1}$	18.0	1.4	2.0	43.13	Hansen (2007)
α-pinene	$4.9•10^{-2}$	17.0	1.3	2.0	42.99	Filly et al. (2014)
Water	–	15.6	16.0	42.3	–	Subrahmanyam et al. (2015)

[a] Henry's law constants at 298.15 K [M/atm] (Henry's law constants n, 2021).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The present work has been sponsored by Gdansk University of Technology with a project co-financed from European Funds 'Integrated Programme of Development of Gdansk University of Technology' (the project nº POWR.03.05.00–00-Z044/17). The Regional Government of Castilla y León and the EU-FEDER programme [grant number CLU 2017-09 and UIC 315] are also gratefully acknowledged.

References

Abraham, S., Joslyn, S., Suffet IH (Mel), 2015. Treatment of odor by a seashell biofilter at a wastewater treatment plant. J. Air Waste Manage. Assoc. 65, 1217–1228. https://doi.org/10.1080/10962247.2015.1075918.

Arriaga, S., Revah, S., 2005. Removal of n-hexane by *Fusarium solani* with a gas-phase biofilter. J. Ind. Microbiol. Biotechnol. 32, 548–553. https://doi.org/10.1007/s10295-005-0247-9.

Arriaga, S., Muñoz, R., Hernández, S., Guieysse, B., Revah, S., 2006. Gaseous hexane biodegradation by *Fusarium solani* in two liquid phase packed-bed and stirred-tank bioreactors. Environ. Sci. Technol. 40, 2390–2395. https://doi.org/10.1021/es051512m.

Chheda, D., Sorial, G.A., 2017. Evaluation of co-metabolic removal of trichloroethylene in a biotrickling filter under acidic conditions. J. Environ. Sci. (China) 57, 54–61. https://doi.org/10.1016/j.jes.2016.12.008.

Cox, H., 1995. Styrene Removal from Waster Gas by the Fungus *Exophiala Jeanselmei* in a Biofilter. Rijksuniversiteit Groningen, the Netherlands.

Estrada, J.M., Hernández, S., Muñoz, R., Revah, S., 2013. A comparative study of fungal and bacterial biofiltration treating a VOC mixture. J. Hazard Mater. 250–251, 190–197. https://doi.org/10.1016/j.jhazmat.2013.01.064.

Estrada, J.M., Lebrero, R., Quijano, G., Pérez, R., Figueroa-González, I., García-Encina, P. A., et al., 2014. Methane abatement in a gas-recycling biotrickling filter: evaluating innovative operational strategies to overcome mass transfer limitations. Chem. Eng. J. 253, 385–393. https://doi.org/10.1016/J.CEJ.2014.05.053.

Filly, A., Fabiano-Tixier, A.S., Lemasson, Y., Roy, C., Fernandez, X., Chemat, F., 2014. Extraction of aroma compounds in blackcurrant buds by alternative solvents: theoretical and experimental solubility study. Compt. Rendus Chem. 17, 1268–1275. https://doi.org/10.1016/J.CRCI.2014.03.013.

González-Martín, J., Cantera, S., Lebrero, R., Muñoz, R., 2022. Optimization of acrylic-styrene latex-based biofilms as a platform for biological indoor air treatment. Chemosphere 287, 132182. https://doi.org/10.1016/J.CHEMOSPHERE.2021.132182.

Gospodarek, M., Rybarczyk, P., Szulczyński, B., Gębicki, J., 2019. Comparative evaluation of selected biological methods for the removal of hydrophilic and hydrophobic odorous VOCs from air. Processes 7, 187. https://doi.org/10.3390/pr7040187.

van Groenestijn, J., Harkes, M., Cox, H., Doddema, H., 1995. Ceramic Materials in Biofiltration. Proceedings USC-TRG Conference on Biofiltration, Los Angeles, USA.

Guieysse, B., Hort, C., Platel, V., Munoz, R., Ondarts, M., Revah, S., 2008. Biological treatment of indoor air for VOC removal: potential and challenges. Biotechnol. Adv. 26, 398–410. https://doi.org/10.1016/J.BIOTECHADV.2008.03.005.

Hansen, C.M., 2007. Methods of Characterization - Surfaces. Hansen Solubility Parameters: A Users Handbook, second ed., pp. 113–123. https://doi.org/10.1201/9781420006834/HANSEN-SOLUBILITY-PARAMETERS-CHARLES-HANSEN

Henry's law constants n.d (accessed April 2, 2021). http://www.mpch-mainz.mpg.de/~sander/res/henry.html.

Jin, Y., Guo, L., Veiga, M.C., Kennes, C., 2007. Fungal biofiltration of α-pinene: effects of temperature, relative humidity, and transient loads. Biotechnol. Bioeng. 96, 433–443. https://doi.org/10.1002/bit.21123.

Jorio, H., Jin, Y., Elmrini, H., Nikiema, J., Brzezinski, R., Heitz, M., 2009. Treatment of VOCs in biofilters inoculated with fungi and microbial consortium. Environ. Technol. 30, 477–485. https://doi.org/10.1080/09593330902778849.

Kośmider, J., Mazur-Chrzanowska, B., Wyszyński, B., 2012. Odory. Wydawnictwo Naukowe PWN.

Lebrero, R., López, J.C., Lehtinen, I., Pérez, R., Quijano, G., Muñoz, R., 2016. Exploring the potential of fungi for methane abatement: performance evaluation of a fungal-bacterial biofilter. Chemosphere 144, 97–106. https://doi.org/10.1016/j.chemosphere.2015.08.017.

Li, Z., Smith, K.H., Stevens, G.W., 2016. The use of environmentally sustainable bio-derived solvents in solvent extraction applications—a review. Chin. J. Chem. Eng. 24, 215–220. https://doi.org/10.1016/J.CJCHE.2015.07.021.

Liu, Y.-J., Mu, Y.-J., Zhu, Y.-G., Ding, H., Arens, N.C., 2007. Which ornamental plant species effectively remove benzene from indoor air? Atmos. Environ. 41, 650–654.

López, M.E., Rene, E.R., Malhautier, L., Rocher, J., Bayle, S., Veiga, M.C., et al., 2013. One-stage biotrickling filter for the removal of a mixture of volatile pollutants from air: performance and microbial community analysis. Bioresour. Technol. 138, 245–252. https://doi.org/10.1016/J.BIORTECH.2013.03.136.

Marycz, M., Brillowska-Dąbrowska, A., Gębicki, J., 2020. Evaluation of immobilization of selected peat-isolated yeast strains of the species *Candida albicans* and *Candida subhashii* on the surface of artificial support materials used for biotrickling filtration. Processes 8, 801. https://doi.org/10.3390/pr8070801.

Marycz, M., Brillowska-Dąbrowska, A., Muñoz, R., Gębicki, J., 2022. A state of the art review on the use of fungi in biofiltration to remove volatile hydrophobic pollutants. Rev. Environ. Sci. Biotechnol. 1–22. https://doi.org/10.1007/S11157-021-09608-7.

Mudliar, S., Giri, B., Padoley, K., Satpute, D., Dixit, R., Bhatt, P., et al., 2010. Bioreactors for treatment of VOCs and odours – a review. J. Environ. Manag. 91, 1039–1054. https://doi.org/10.1016/J.JENVMAN.2010.01.006.

Ój, Prado, Veiga, M.C., Kennes, C., 2008. Removal of formaldehyde, methanol, dimethylether and carbon monoxide from waste gases of synthetic resin-producing industries. Chemosphere 70, 1357–1365. https://doi.org/10.1016/J.CHEMOSPHERE.2007.09.039.

Prenafeta-Boldú, F.X., Illa, J., van Groenestijn, J.W., Flotats, X., 2008. Influence of synthetic packing materials on the gas dispersion and biodegradation kinetics in fungal air biofilters, 2008 Appl. Microbiol. Biotechnol. 79 (2), 319–327. https://doi.org/10.1007/S00253-008-1433-2, 79.

Prenafeta-Boldú, F.X., de Hoog, G.S., Summerbell, R.C., 2018. Fungal communities in hydrocarbon degradation. In: Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology, Handbook of Hydrocarbon and Lipid Microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60063-5_8-2.

Qi, B., Moe, W.M., Kinney, K.A., 2005. Treatment of paint spray booth off-gases in a fungal biofilter. J. Environ. Eng. 131, 180–189. https://doi.org/10.1061/(ASCE)0733-9372(2005)131:2(180).

Quan, Y., Wu, H., Guo, C., Han, Y., Yin, C., 2018. Enhancement of TCE removal by a static magnetic field in a fungal biotrickling filter. Bioresour. Technol. 259, 365–372. https://doi.org/10.1016/J.BIORTECH.2018.03.031.

Raboni, M., Torretta, V., Viotti, P., 2017. Treatment of airborne BTEX by a two-stage biotrickling filter and biofilter, exploiting selected bacterial and fungal consortia. Int. J. Environ. Sci. Technol. 14, 19–28. https://doi.org/10.1007/s13762-016-1127-8.

Rane, H.S., Hayek, S.R., Frye, J.E., Abeyta, E.L., Bernardo, S.M., Parra, K.J., et al., 2019. *Candida albicans* Pma1p contributes to growth, pH homeostasis, and hyphal formation. Front. Microbiol. 10, 1012. https://doi.org/10.3389/FMICB.2019.01012/BIBTEX.

Revah, S., Vergara-Fernández, A., Hernández, S., 2011. Fungal biofiltration for the elimination of gaseous pollutants from air. Mycofactories 109–120. Bentham Science Publishers Ltds.

Rybarczyk, P., Szulczyński, B., Gębicki, J., Hupka, J., 2019a. Treatment of malodorous air in biotrickling filters: a review. Biochem. Eng. J. 141, 146–162. https://doi.org/10.1016/J.BEJ.2018.10.014.

Rybarczyk, P., Szulczyński, B., Gospodarek, M., Gębicki, J., 2019b. Effects of n-butanol presence, inlet loading, empty bed residence time and starvation periods on the performance of a biotrickling filter removing cyclohexane vapors from air. Chem. Pap. 74, 1039–1047. https://doi.org/10.1007/s11696-019-00943-2.

Rybarczyk, P., Marycz, M., Szulczyński, B., Brillowska-Dąbrowska, A., Rybarczyk, A., Gębicki, J., 2021. Removal of cyclohexane and ethanol from air in biotrickling filters inoculated with Candida albicans and Candida subhashii. Arch. Environ. Protect. 47 https://doi.org/10.24425/aep.2021.136445.

Subrahmanyam, R., Gurikov, P., Dieringer, P., Sun, M., Smirnova, I., 2015. On the road to biopolymer aerogels—dealing with the solvent. Gels 1, 291–313. https://doi.org/10.3390/GELS1020291, 2015; 1:291–313.

Szulczyński, B., Rybarczyk, P., Gospodarek, M., Gębicki, J., 2019. Biotrickling filtration of n-butanol vapors: process monitoring using electronic nose and artificial neural network. Monatsh. Fur Chem. 150, 1667–1673. https://doi.org/10.1007/s00706-019-02456-w.

van Groenestijn, J.W., Hesselink, P.G.M., 1993. Biotechniques for air pollution control. Biodegradation 4, 283–301. https://doi.org/10.1007/BF00695975.

Vergara-Fernández, A., Scott, F., Moreno-Casas, P., Díaz-Robles, L., Muñoz, R., 2016. Elucidating the key role of the fungal mycelium on the biodegradation of n-pentane as a model hydrophobic VOC. Chemosphere 157, 89–96. https://doi.org/10.1016/J.CHEMOSPHERE.2016.05.034.

Vergara-Fernández, A., Revah, S., Moreno-Casas, P., Scott, F., 2018a. Biofiltration of volatile organic compounds using fungi and its conceptual and mathematical modeling. Biotechnol. Adv. 36, 1079–1093. https://doi.org/10.1016/j.biotechadv.2018.03.008.

Vergara-Fernández, A., Yánez, D., Morales, P., Scott, F., Aroca, G., Diaz-Robles, L., et al., 2018b. Biofiltration of benzo[α]pyrene, toluene and formaldehyde in air by a consortium of *Rhodococcus erythropolis* and *Fusarium solani*: effect of inlet loads, gas flow and temperature. Chem. Eng. J. 332, 702–710. https://doi.org/10.1016/J.CEJ.2017.09.095.

Yang, D.S., Pennisi, S., Son, K., Kays, S.J., 2009. Screening indoor plants for volatile organic pollutant removal efficiency. Am. Soc. Hortic. Sci. 44, 1377–1381.

3.6. Publication 6: Fungal co-culture improves the biodegradation of hydrophobic VOCs gas mixtures in conventional biofilters and biotrickling filters

As part of the second scientific internship at the University of Valladolid, a comparative study was carried out on the efficiency of BF and BTF for removing a mixture of hydrophobic VOCs using *C. subhashii*, compared to another fungus species. Based on the literature review, the *F. solani* species was selected for comparison as one of the fungi most effectively used in biofiltration to remove hydrophobic VOCs. Thus, *C. subhashii*, *F. solani* and their consortium were used to populate the columns. In order to maintain the continuity of the research, n-hexane, TCE, toluene and α-pinene were also selected as the model hydrophobic VOC mixture, as in the previous experiment.

The obtained results showed that, regardless of the tested hydrophobic compound, the BTF configuration is able to achieve better degradation compared to BF. Based on this information, the process was started with the BF configuration, and when a steady state was noted, the configuration was changed to BTF. With this treatment, the effectiveness of reducing hydrophobic VOCs in each of the variants increased.

The results indicate that the species *C. subshashii*, effectively removes selected hydrophobic pollutants from the air at a level comparable to the fungus species most commonly used for this purpose so far, regardless of the bioreactor configuration. Interestingly, the best VOC removal efficiency was achieved in each case for a consortium of both species. Additional biofilm studies showed that in all columns, both those immobilized by single species and their consortium, *C. subshashii* dominated the fungal populations at the end of the experiment.

As proposed in the previous article, the Hansen solubility parameters were used to determine the role of the hydrophobicity of the removed VOCs in their mass transfer to the liquid phase, and thus the elimination achieved by the fungi. In order to carry out a complete analysis of the obtained results, the knowledge of the characteristics of fungi species was also used. In BF1/BTF1, inhabited by *C. subhashii*, the biodegradation course was very similar to that presented in P5. Interestingly, also in BF1/BTF1, the presence of the trickling liquid phase did not change the observed degradation patterns. In contrast, the degradation patterns obtained with BF2/BTF2 and BF3/BTF3 colonized by *F. solani* and the consortium, respectively, were different. The observed phenomenon is explained by the fact that the discussed biofilters were inhabited by different species of fungi. As a result, *C. subhashii* (yeast) is characterized by a different morphology, and therefore hydrophobicity of cells, than *F. solanii* (mould). These results and observations confirm the legitimacy of expanding research in the future by understanding the metabolic pathways of fungi used in biofiltration processes. This research will allow entering a new level of designing and optimizing processes dedicated to air removal.

The publication presents experiments leading to the implementation of task T6. *Comparison of selected fungus with the species most effectively used in biofiltration (according to prior literature) in their ability to use a mixture of hydrophobic VOCs from the gas phase as a carbon source.*

The novelty presented in it is:

- *C. subshashii* effectively removes selected hydrophobic VOCs from the air at a comparable level with *F. solani*, regardless of the gas-phase bioreactor configuration assessed.

Chemosphere 313 (2023) 137609

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Fungal co-culture improves the biodegradation of hydrophobic VOCs gas mixtures in conventional biofilters and biotrickling filters

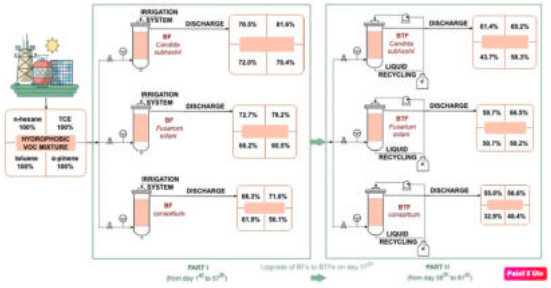
Milena Marycz [a,b], Anna Brillowska-Dąbrowska [c], Sara Cantera [d], Jacek Gębicki [b], Raúl Muñoz [a,*]

[a] Institute of Sustainable Processes, Universidad de Valladolid, Dr. Mergelina S/n, 47011, Spain
[b] Department of Process Engineering and Chemical Technology, Faculty of Chemistry, Gdansk University of Technology, 11/12 G. Narutowicza Street, 80-233, Gdansk, Poland
[c] Department of Molecular Biotechnology and Microbiology, Faculty of Chemistry, Gdansk University of Technology, 11/12 G. Narutowicza Street, 80-233, Gdansk, Poland
[d] Laboratory of Microbiology, Wageningen University and Research, Stippeneng 4, 6708 WE, Wageningen, Netherlands

HIGHLIGHTS

- Fungal co-culture in biofiltration improves the removal of hydrophobic VOCs.
- Biotrickling filters supported a superior VOCs removal performance than biofilters.
- Effective removal of VOCs at a EBRT of 77 s regardless of bioreactor configuration.
- Highest steady state REs for all VOCs in the BTF inhabited by the consortium.
- Community structure analyses confirmed the dominance of the inoculated fungi.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: Vincenzo Naddeo

Keywords:
Biofiltration
Candida subhashii
Fusarium solani
Fungi consortium
Mixed VOCs

ABSTRACT

The present study systematically evaluated the potential of *Candida subhashii*, *Fusarium solani* and their consortium for the abatement of *n*-hexane, trichloroethylene (TCE), toluene and α-pinene in biofilters (BFs) and biotrickling filters (BTFs). Three 3.2 L BFs packed with polyurethane foam and operated at a gas residence time of 77 s with an air mixture of hydrophobic volatile organic compounds (VOCs) were inoculated with *C. subhashii*, *F. solani* and a combination of thereof. The systems were also operated under a BTF configuration with a liquid recirculating rate of 2.5 L h^{-1}. Steady state elimination capacities (ECs) of total VOCs of 17.4 ± 0.7 g m^{-3} h^{-1} for *C. subhashii*, 21.2 ± 0.8 g m^{-3} h^{-1} for *F. solani* and 24.4 ± 1.4 g m^{-3} h^{-1} for their consortium were recorded in BFs, which increased up to 27.2 ± 1.6 g m^{-3} h^{-1}, 29.2 ± 1.9 g m^{-3} h^{-1}, 37.7 ± 3.3 g m^{-3} h^{-1} in BTFs. BTFs supported a superior biodegradation performance compared to BF, regardless of the VOCs. Moreover, a more effective VOC biodegradation was observed when *C. subhashii* and *F. solani* were grown as a consortium. The microbial analysis conducted revealed that the fungi initially introduced in each BF represented the dominant species by the end of the experiment, with *C. subhashii* gradually overcoming *F. solani* in the system inoculated with the fungal consortium.

* Corresponding author.
E-mail address: mutora@iq.uva.es (R. Muñoz).

https://doi.org/10.1016/j.chemosphere.2022.137609
Received 29 April 2022; Received in revised form 6 December 2022; Accepted 17 December 2022
Available online 22 December 2022

1. Introduction

In recent years, biofiltration has become the leading technique for the abatement of volatile organic compounds (VOCs) in polluted air emissions with high flow rates (<400 000 m^3 h^{-1}) and low pollutant contaminants (<1 g m^{-3}) (Gospodarek et al., 2019a; Vergara-Fernández et al., 2007). Biological gas treatment is based on the ability of key microorganisms to biotransform under ambient pressure and temperature VOCs into CO_2, water and biomass. Biofiltration exhibits many advantages over the physical-chemical techniques traditionally used for air treatment, including low energy demand and environmentally friendliness since biotechnologies do not require the use of hazardous reagents or extreme operational conditions (Gospodarek et al., 2019a). However, the efficiency of conventional biofiltration for the removal of hydrophobic VOCs in gas mixtures is low, due to the high water content of bacterial biofilms that limits the gas-liquid mass transfer of these VOCs. Moreover, bacterial biofilms exhibit a limited surface area to transfer the hydrophobic VOCs from the gas phase, which hinders absorption of these compounds (van Groenestijn and Kraakman, 2005).

In this context, filamentous fungi have emerged as a simple and straightforward solution to overcome VOC mass transfer limitations in biofiltration (van Groenestijn and Kraakman, 2005; van Groenestijn and Liu, 2002; Arriaga and Revah, 2005a, 2005b). Compared to bacteria, fungi are more resistant to low humidity and acidic conditions, and can support a superior VOC abatement as a result of their large surface aerial mycelia and more effective partitioning of the VOC in the fungal biomass (Marycz et al., 2022a). This hydrophobic nature of the fungal mycelium is partly due to the presence of hydrophobins in the fungal cell wall (Vergara-Fernández et al., 2006; Lugones et al., 1998). Hydrophobins are a family of surface-active proteins produced by filamentous fungi that play an important role in fungal metabolism. Hydrophobins are characterized by a specific arrangement of cysteine residues, which form four disulfide bridges in the amino acid sequence. This characteristic structure provides fungi the ability to spontaneously form amphipathic monolayers between the hydrophobic and hydrophilic environments (Tymiński et al., 2018). In a biotrickling filter, such a surface is formed at the gas-liquid interface. Hydrophobins play an important role in fungal cell adhesion, formation of protective surface coatings and pathogenicity (Vergara-Fernández et al., 2006; Wösten, 2001). Based on these properties, fungi can be used in conventional biofilters and biotrickling filters for highly efficient removal of hydrophobic VOCs directly from the gas phase, which allows overcoming pollutants mass transfer resistance in the liquid phase (Marycz et al., 2022a; Wösten, 2001). In addition, using the dimensionless Henry law and the inlet concentration, the aqueous concentration of the different VOCs can be estimated to highlight that the potential maximum VOC concentration in the recycling medium are low.

Biotrickling filters are the most cost-effective configuration for biodegradation of hydrophobic VOCs, among all known biofiltration techniques. The development of fungal biotrickling filters would allow greater control of process variables such as biofilm thickness and pH, and thus gas pollutant abatament can be used at higher VOC loading rates than with conventional biofilters (Marycz et al., 2022a).

The use of BTFs for the removal of gas mixtures of hydrophobic VOCs has great potential and is a great technological challenge in terms of selecting the optimal conditions for controlling the trickling solution flow. To the best of our knowledge, there are currently no universal process standards that would allow for the design of an optimal water control strategy during fungal BTF operation (Lee et al., 2021). The trickling solution flow plays a critical role in supporting the physiological processes of microorganisms, both by buffering the system and by continuously supplying nutrients (Lebrero et al., 2014; López et al., 2016). However, an excessive flow of aqueous medium, eventually forming a thick layer of water over the biofilm growing on the packing material, can inhibit the internal physiological processes of microorganisms and limit the mass transfer of some hydrophobic compounds and oxygen (Lebrero et al., 2014; Lee and Heber, 2010; Lee et al., 2013). Therefore, in order to effectively carry out the biodegradation of hydrophobic VOCs in BTFs, special attention should be paid to select the appropriate trickling flow rate. For this purpose, the following should be taken into account: the type of microorganisms used, the properties of the support material (e.g. porosity), the characteristics of the target VOCs and the biological conditions prevailing in the biofilter (thickness of the created biofilm) (Lee and Heber, 2010; Alonso and Suidan, 1997). When discussing the impact of the presence of water on the efficiency of biofiltration, one should also mention the problem of "water stress", which sometimes occurs in BF, but not in BTF. The phenomenon of "water stress" refers to the situation when the microorganisms inhabiting the BF column experience a water activity deficit. As already mentioned, fungi are much more resistant to such stress than bacteria. "Water stress" impairs biofilms, which significantly affects the damage to microbial cells, thereby reducing the efficiency of the process (Lee et al., 2021).

Arriaga and Revah (2005) (Arriaga and Revah, 2005a), Zehraoui et al. (2014) (Zehraoui et al., 2014), Chheda and Sorial (2017) (Chheda and Sorial, 2017) and Arellano-García et al. (2018) (Arellano-García et al., 2018) demonstrated the superior capacity of fungi to abate hydrophobic VOCs in biofilters compared to bacteria. *Fusarium solani* is one of the most effective hydrophobic VOC degrading fungi used in biofiltration (Marycz et al., 2022a).

On the other hand, *Candida subhashii* (López et al., 2016) was also examined for its ability to decompose multiple VOCs (Marycz et al., 2020). To the current knowledge of the authors, *C. subhashii* was first used in biotrickling filters (BTF) to remove hydrophobic compound (cyclohexane) by Rybarczyk et al., in 2021 (Rybarczyk et al., 2021). Marycz and co-workers (2022) initially tested the ability of *C. subhashii* to remove a mixture of hydrophobic VOCs (*n*-hexane, TCE, toluene and α-pinene) in biofiltration systems (Marycz et al., 2022b). With a relatively short gas residence times (30 s), *C. subhashii* immobilized in PUF supported a higher abatement efficiency of hydrophobic VOCs under a BTF configuration compared to the performance of a conventional biofilter (BF) (Marycz et al., 2022b). Additionally, *Candida* spp. Have been successfully used in BTFs to remove hydrophobic VOCs (including styrene and α-pinene in cometabolism with methanol, and cyclohexane with ethanol) (Rybarczyk et al., 2021; Li et al., 2019; López et al., 2013). In this context, although the potential of fungi to remove hydrophobic VOCs has been previously explored, there is still a lack of systematic studies comparing the ability of fungi for VOC abatement in different operational systems. In the context of the studies received so far, the authors decided to compare the potential of *C. subhashii* with *F. solanii* as one of the fungal species most effectively used in biofiltration to remove hydrophobic VOCs. In addition, the co-culture functioning of *F. solani* and *C. subhashii* has never been investigated in the context of VOC biofiltration.

The present study systematically assessed the potential of *Candida subhashii*, *Fusarium solani* and a combination of thereof for the treatment of *n*-hexane, TCE, toluene and α-pinene in biofilters and biotrickling filters. Diversity and community structure analyses of the fungal populations at the end of the experiment confirmed the dominance of the inoculated fungi.

2. Materials and methods

2.1. Microorganisms and inocula

Fusarium solani CBS 117476 was purchased to the Centraalbureau voor Schimmelcultures (The Netherlands), while the strain of *C. subhashii* used in this study was isolated by the authors of this publication from peat (Gospodarek et al., 2019b). The methodology applied for colonization of polyurethane foam (PUF) by *C. subhashii* and *F. solani* was developed according to Marycz et al. (2020). The PUF inoculated with the fungal consortium was first colonized with *F. solani* as above

described and then immersed in a *C. subhashii* liquid culture for 48 h at 24 °C. The entire PUF inoculation process by selected fungal species was carried out under aseptic conditions, but BF and BTF operation was not carried out under sterile conditions. The technical specifications of the PUF employed as a packing material for immobilization of the selected fungi are (Marycz et al., 2020): specific surface area of 1000 m^2 m^{-3}, density of 0.01 g mL^{-1}, porosity of 96% and water retention capacity of 0.12 L_{water} L_{PUF}^{-1}. PUF has been widely used in biofiltration for years as the currently most popular inert filling of biotrickling filters. Adsorption onto the surface of this packing material was negligible due to the inert nature of the packed bed, as previously confirmed in ad-hoc abiotic tests.

2.2. VOCs and mineral salt medium

N-hexane (Sigma-Aldrich, South Korea) (30% v/v), TCE (Panreac AppliChem, Spain) (15% v/v), toluene (Sigma-Aldrich, USA) (30% v/v) and α-pinene (Sigma-Aldrich, USA) (25% v/v) were used as model hydrophobic VOCs pollutants. The minimal salt medium (MSM) used to support fungal growth in the biofiltration columns was previously described by Marycz et al. (2020). This medium was composed of: Na_2HPO_4 $2H_2O$ (Panreac AppliChem, Spain) (15.2 g L^{-1}), KH_2PO_4 (Panreac AppliChem, Spain) (3 g L^{-1}), NaCl (Panreac AppliChem, Spain) (0.5 g L^{-1}) and NH_4Cl (Sigma-Aldrich, USA) (1 g L^{-1}). After mixing all components, the pH of the medium was ~7.

2.3. Continuous VOC degradation in biofilters and biotrickling filters

Three BFs (Fig. 1) were set-up in clear PVC columns (11 cm of internal diameter and 40 cm of height). Each column was filled with 33.85 cm of PUF, reaching a total packed bed volume (V_p) of 3.22 L (Filtren TM 25280, Recticel Ibérica S.L., Spain). Air was initially humidified in a clear PVC column (0.1 m internal diameter × 1.6 m height, filled with 1.2 m of water) and mixed with a liquid mixture of *n*-hexane, TCE, toluene and α-pinene injected at 0.5 mL h^{-1} using a syringe pump (KDS100 Legacy, Fisherbrand, USA). This VOC injection resulted in average concentrations of 266.9 ± 11.6, 317.1 ± 9.0, 431.4 ± 16.2 and 386.3 ± 15.0 mg m^{-3} of *n*-hexane, TCE, toluene and α-pinene, respectively. The BFs and BTFs were fed with the polluted air from the bottom at 2.5 L min^{-1}, which resulted in an empty bed residence time (EBRT) of 77 s. The air flow rates were controlled using rotameters (Aalborg, USA). The BFs were periodically irrigated with 120 mL MSM per day using a 205S/CA4 peristaltic pump (Watson Marlow, USA).

The BFs were upgraded on day 57th to biotrickling filters (BTFs) using 1 L stirred tank reactors and 520S peristaltic pumps (Watson Marlow, USA) (Fig. 2). The BTFs were operated at a liquid recirculating rate of 0.025 m^3 h^{-1} with a MSM renewal rate of 120 mL d^{-1} for 24 days. The liquid medium, both in BF and BTF, was always renewal after daily gas sampling.

Gas samples were periodically collected from each module at the gas inlet and outlet using a 100-μL gastight syringe (Hamilton, Australia) in order to determine the concentration of the target VOCs, CO_2 and O_2. The pH and culture absorbance at 600 nm (OD_{600}) in the leachate of the BF and the liquid effluent of the BTF were also daily measured. Samples (1 mL) of the packing beds were drawn at the end of the experiment (day 81st) and preserved at −20 °C to determine the structure of the fungal populations.

The results from the biofiltration experiments are expressed in terms of VOC removal efficiency (*RE*, %), the pollutant inlet load (*IL*, g m^{-3}

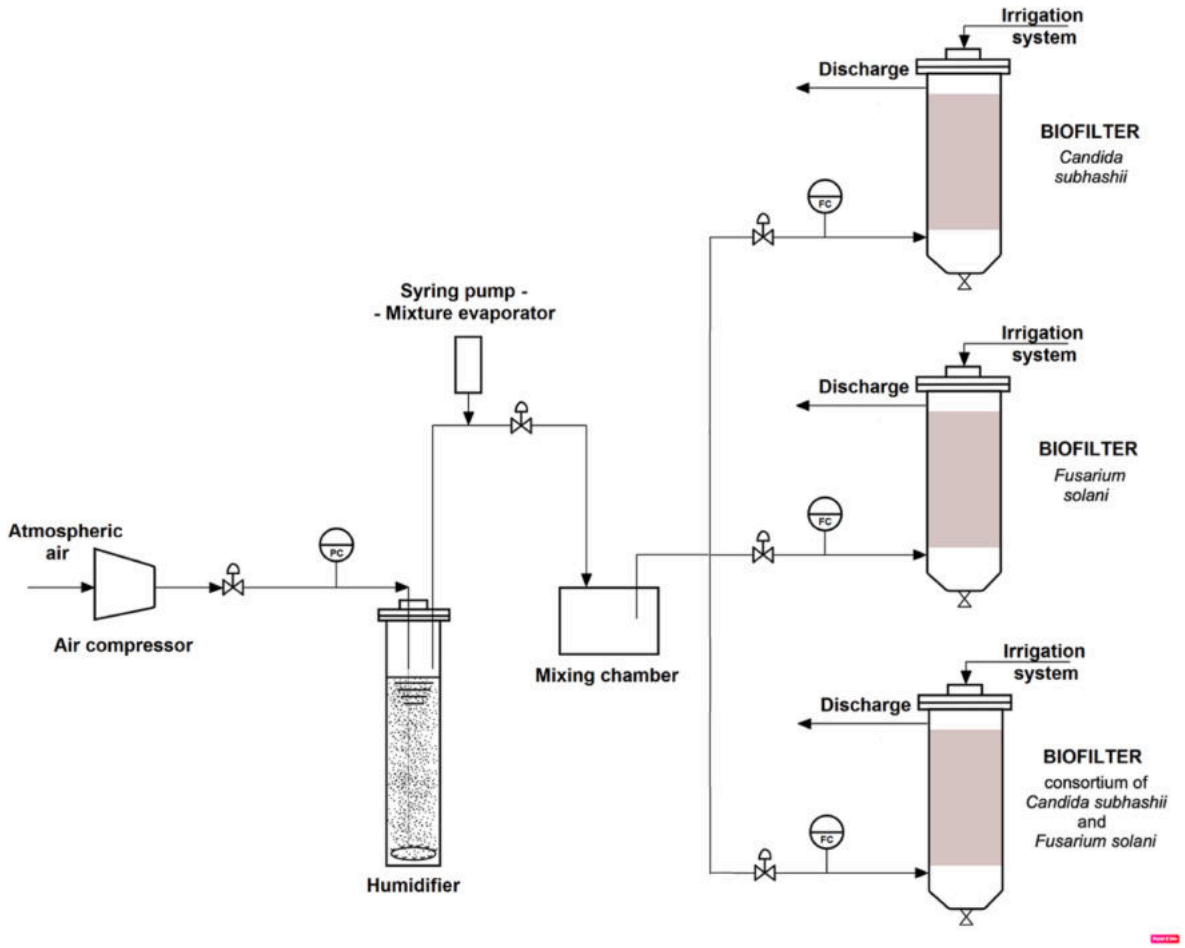


Fig. 1. Schematic representation of the BF experimental set-up during the first 56 days of experiment.

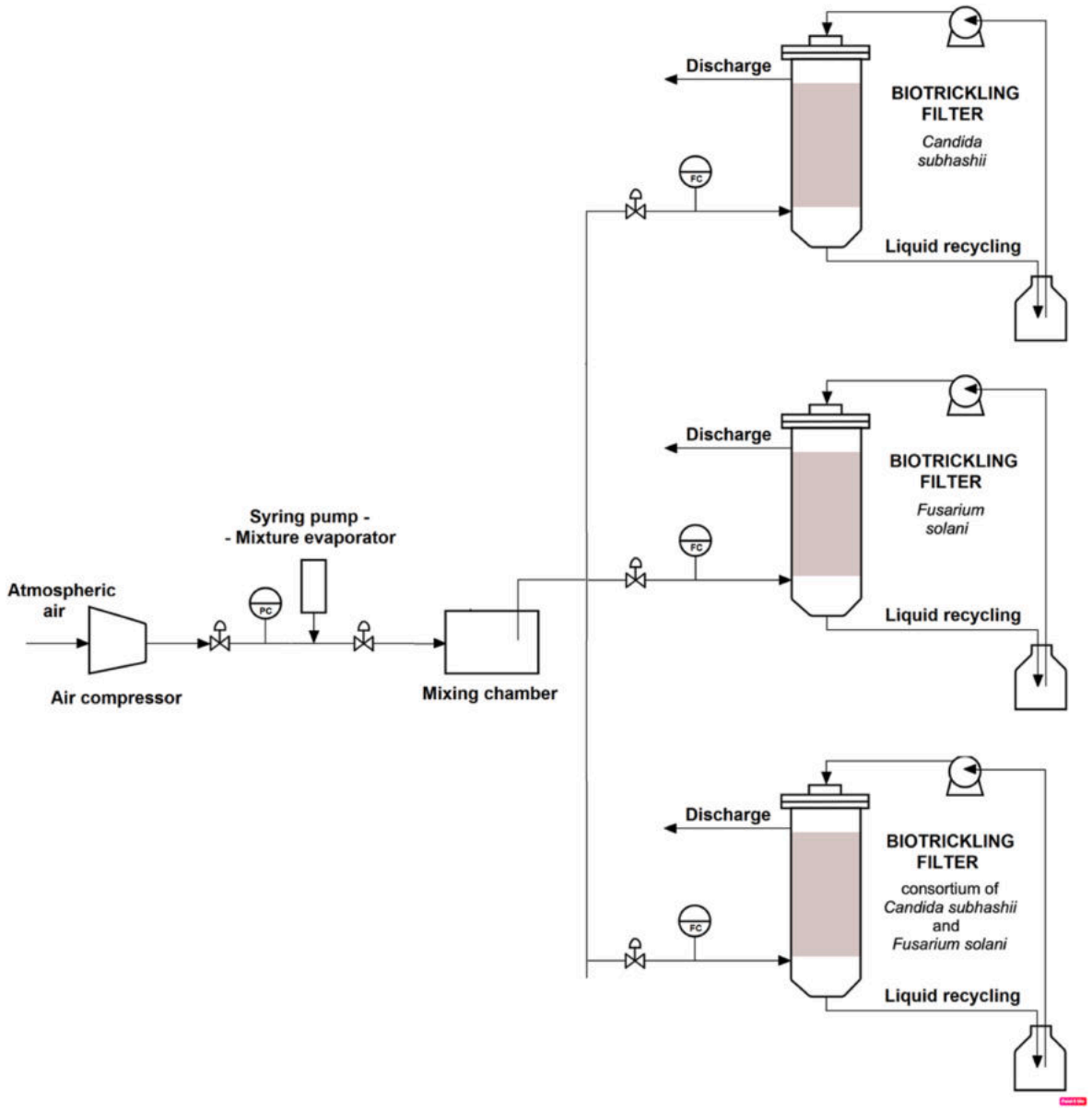


Fig. 2. Schematic representation of the BTF experimental set-up used from the 57th day of operation onwards.

h^{-1}), total inlet load (IL_T, g m^{-3} h^{-1}), elimination capacity (EC, g m^{-3} h^{-1}), total elimination capacity (EC_T, g m^{-3} h^{-1}) and volumetric CO_2 production (g m^{-3} h^{-1}):

$$RE = 100 \cdot \frac{C_{in} - C_{out}}{C_{in}} \quad (1)$$

$$IL = \frac{Q \bullet C_{in}}{V_{bed}} \quad (2)$$

$$IL_T = \frac{Q \bullet C_{inT}}{V_{bed}} \quad (3)$$

$$EC = \frac{Q \bullet (C_{in} - C_{out})}{V_{bed}} \quad (4)$$

$$EC_T = \frac{Q \bullet (C_{inT} - C_{outT})}{V_{bed}} \quad (5)$$

$$CO_2\ production = \frac{Q \bullet (CO_{2\ out} - CO_{2\ in})}{V_{bed}} \quad (6)$$

where C_{in} and C_{out} are the inlet and outlet gas concentrations, C_{inT} and C_{outT} are the total inlet and total outlet gas concentrations, $CO_{2\ out}$ and $CO_{2\ in}$ are the CO_2 concentrations at the gas outlet and inlet of the reactor, Q is the gas volumetric flow rate and V_{bed} is the packed bed volume. The average values of VOC-RE and VOC-EC along with its standard deviation were calculated for each VOC under steady state in both BF and BTF configurations.

The results were compared on the basis using analysis of variance (ANOVA) and paired *t*-Student test, which was performed with a 5% level of significance (p-value $\leq$0.05).

2.4. Analytical methods

CO_2 and O_2 gas concentrations were quantified using a Bruker 430 gas chromatograph (Bruker Corporation, Palo Alto, USA) equipped with a CP-Molsieve 5A and a CP-PoraBOND Q columns and a thermal conductivity detector. Oven, injector and detector temperatures were kept at 45, 150 and 200 °C, respectively, while helium was employed as a carrier gas at 13.7 mL min^{-1} (Estrada et al., 2014). The concentrations of

VOCs were measured in a GC-FID (Varian 3900) equipped with an Agilent HP-5MSI capillary column (30 m × 0.25 mm × 0.25 μm) as described by González-Martín and co-workers (González-Martín et al., 2022). Measurements of the OD_{600} were performed in a SPECTROstar Nano spectrophotometer (BMG LABTECH, Germany). pH was determined using a pH-meter Basic 20 (Crison, Spain).

2.5. Fungal community analysis

DNA extraction and Illumina Miseq amplicon sequencing were carried out by ADM Biopolis (Valencia, Spain). The target regions to study the fungal community were the internal transcribed spacer (ITS) regions in the ribosomal RNA (rRNA) operon, ITS3 and ITS4 (Nash et al., 2017). Amplification was developed using universal linker sequences (which allowed amplicons for incorporation indexes), sequencing primers (Nextera XT Index kit, ILLUMINA) and the corresponding primers of the specific region of the internal transcribed spacer (ITS) of nuclear DNA. ITS based libraries were quantified by fluorimetry using Quant-iT™ PicoGreen™ dsDNA Assay Kit (Thermofisher). Libraries were pooled before sequencing on the MiSeq platform (Illumina). The size and quantity of the pool were assessed in the Bioanalyzer 2100 (Agilent) and with the Library Quantification Kit for Illumina (Kapa Biosciences), respectively. The library was spiked with 10% PhiX (Illumina) and sequenced on an Illumina MiSeq instrument with 300 cycles paired reads configuration. The ITS regions were processed and quality filtered using the DADA2 plugin on QIIME2 (Callahan et al., 2016). Amplicon sequence variants (ASVs) from the forward and reverse flows were merged. Chimeric regions were removed and those clean ASVs were annotated against the NCBI database (version 2021) using blastn version 2.2.29+ (Sayers et al., 2019). The ASVs assigned with an identity of at least 97% were launched against UNITE v.8.3 for fungi using NBAYES (Bokulich et al., 2018). Data was normalized, and alpha and beta diversity analyses were calculated using vegan R package (Oksanen et al., 2019). The main species of the fungal population are shown in a heatmap plotted using the package for R *pheatmap* (Kolde, 2019). The main fungal genera are shown in a stacked bar graph created with R using the package *ggplot2* (Wickham, 2016). The nucleotide sequence dataset obtained in this study has been deposited at DDBJ/ENA/GenBank as bioproject: PRJNA823533.

3. Results and discussion

3.1. Continuous VOC degradation in the BF and BTF operated with the different fungal species

Stable total VOCs RE of 26.9 ± 0.7% (EC = 17.4 ± 0.6 g m^{-3} h^{-1}) in BF1 (from day 34th to 57th), 32.9 ± 0.8% (EC = 21.2 ± 0.8 g m^{-3} h^{-1}) in BF2 (from day 36th to 57th) and 37.8 ± 1.6% (EC = 24.4 ± 1.4 g m^{-3} h^{-1}) in BF3 (from day 34th to 57th) were recorded under BF operation (Fig. 3). Steady state total VOCs REs of 42.6 ± 1.4% (EC = 27.7 ± 1.6 g m^{-3} h^{-1}) for *C. subhashii*, 44.9 ± 1.7% (EC = 29.2 ± 1.9 g m^{-3} h^{-1}) for *F. solani* and 58.0 ± 2.1% (EC = 37.7 ± 3.3 g m^{-3} h^{-1}) for the combination of thereof were recorded under BTF operation. A steady elimination was achieved from day 66th in BTF1, from day 65th in BTF2 and from day 66th in BTF3 until the end of biofilters operation (day 81st).

3.1.1. N-hexane removal

An acclimation period of 13 days, characterized by low VOC removal efficiencies, was observed in the BF inoculated with *C. subhashii* (BF1) (Figs. 4–7, S1-S4). This lag phase was 3 days longer in the BF inoculated with *F. solani* (BF2) and the consortium (BF3). REs of *n*-hexane ranging from 28 to 31% (RE = 29.5 ± 1.3%, EC = 3.6 ± 0.2 g m^{-3} h^{-1}) in BF1, 25–29% (RE = 27.3 ± 1.9%, EC = 3.6 ± 0.3 g m^{-3} h^{-1}) in BF2 and 31–36% (RE = 33.7 ± 1.6%, EC = 4.3 ± 0.2 g m^{-3} h^{-1}) in BF3 were recorded under steady state at 77 s of EBRT from day 23rd to day 57th (Fig. 4, S1). On the other hand, process operation under a BTF

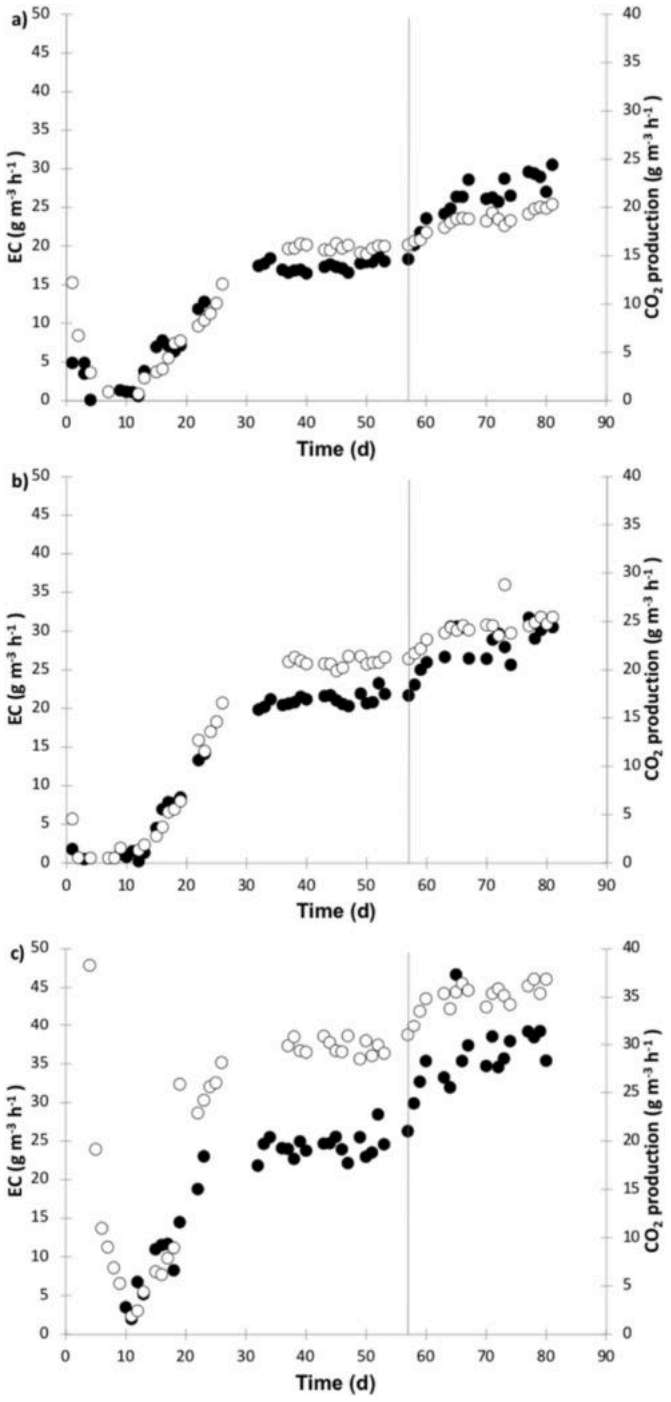


Fig. 3. Time course of the CO_2 production (solid symbols) and Total Elimination Capacity (empty symbols) in the bioreactors containing (a) *Candida subhashii*, (b) *Fusarium solani*, (c) and the consortium *C. subhashii-F. solani*, operated at a gas residence time of 77 s. Bioreactor configuration was shifted from BF to BTF by day 57th (vertical line).

configuration resulted in a rapid enhancement in *n*-hexane removal. *n*-hexane REs ranged from 37 to 42% (RE = 38.6 ± 2.2%, EC = 5.0 ± 0.3 g m^{-3} h^{-1}) in *C. subhashii* BTF (BTF1), 40–42% (RE = 40.3 ± 3.0%, EC = 5.2 ± 0.4 g m^{-3} h^{-1}) in *F. solani* BTF (BTF2) and 42–48% (RE = 45.0 ± 2.1%, EC = 5.8 ± 0.4 g m^{-3} h^{-1}) in the fungal consortium BTF (BTF3) during steady state (from day 60th to 81st in BTF1, 64th-81st in BTF2 and 59th-81st in BTF3). Thus, BTFs supported higher hexane removals than BFs regardless of the fungal species present in the packed bed. The gradual increase in the removal of *n*-hexane when the operation of the

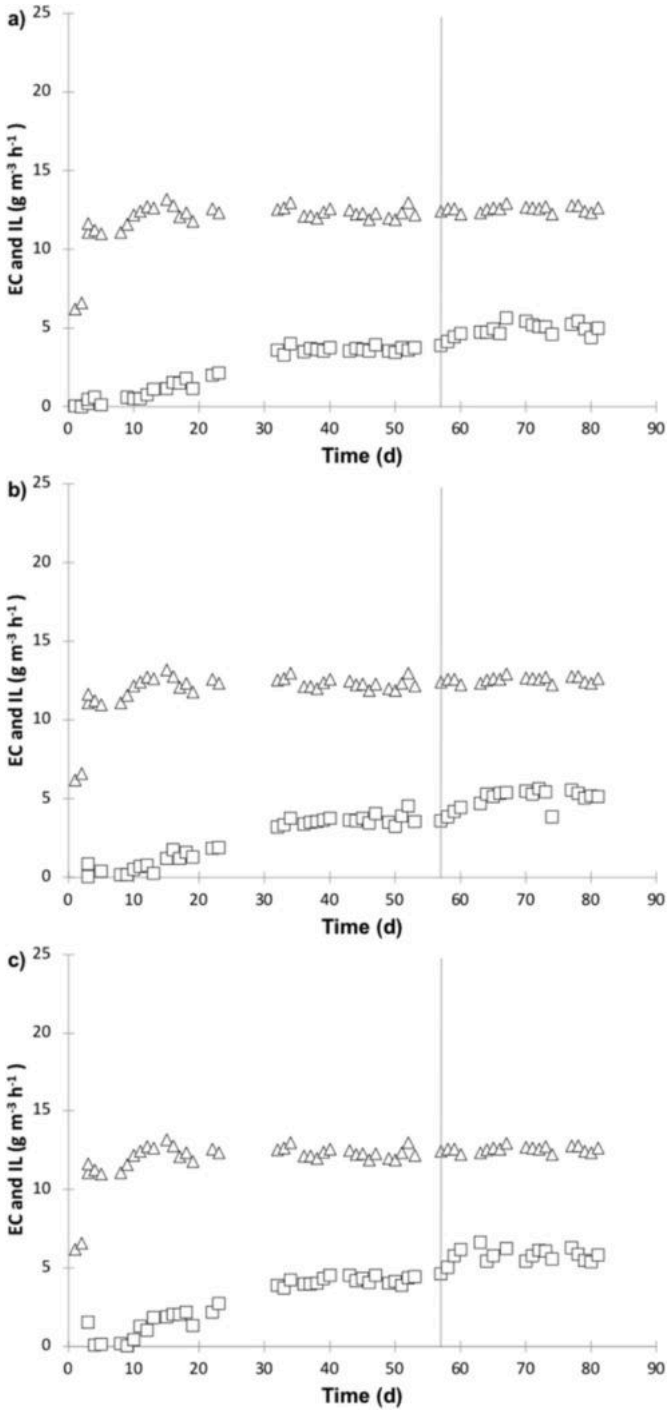


Fig. 4. Time course of the *n*-hexane Inlet Load (empty triangles) and the *n*-hexane Elimination Capacity (empty squares) in the bioreactors containing (a) *Candida subhashii*, (b) *Fusarium solani*, (c) and the consortium *C. subhashii-F. solani*, operated at a gas residence time of 77 s. Bioreactor configuration was shifted from BF to BTF by day 57th (vertical line).

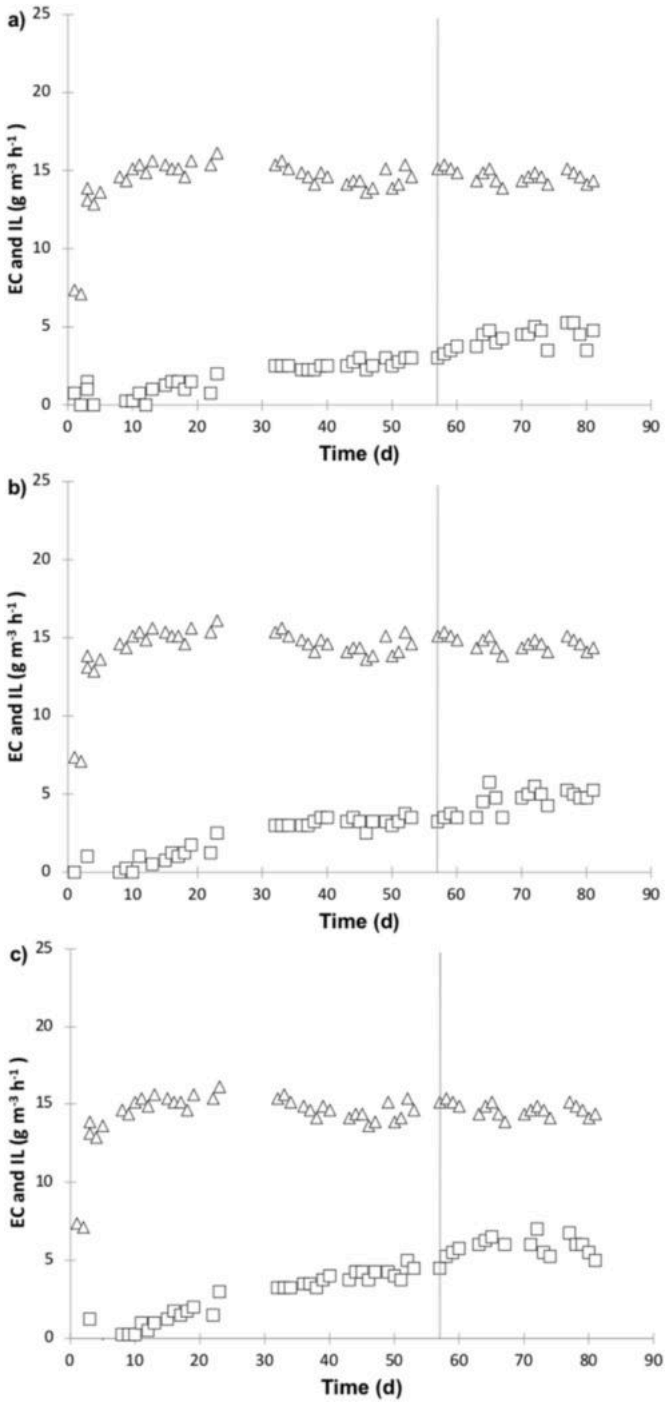


Fig. 5. Time course of the TCE Inlet Load (empty triangles) and the TCE Elimination Capacity (empty squares) in the bioreactors containing (a) *Candida subhashii*, (b) *Fusarium solani*, (c) and the consortium *C. subhashii-F. solani*, operated at a gas residence time of 77 s. Bioreactor configuration was shifted from BF to BTF by day 57th (vertical line).

packed bed bioreactor was shifted to a BTF configuration might be explained by the enhanced fungal colonization of the packed bed as a result of the continuous liquid recirculation (Caicedo et al., 2018). Interestingly, the fungal consortium supported a superior hexane elimination than the individual fungal strains in both BFs and BTFs (p-value $\geq$0.05). Studies of the synergism of microbial species in biofiltration have proven that a properly selected microbial consortium can improve both the practical feasibility of such processes and their performance (Arriaga and Revah, 2005a). These fungi were probably involved in the removal of VOCs by co-metabolism, and a significant mineralization was achieved through the synergistic interaction of *C. subhashii* and *F. solani* (Prenafeta-Boldú et al., 2004; MJJVis and Field, 1998; Boonchan et al., 2000). Previous studies in literature have also reported a superior removal of hexane in BTFs compared to BF. Likewise, BTF operation at pH 7 with the fungal species *Aspergillus niger*, *F. solani* and *Fusarium verticillioides* (*F. verticillioides* genus became the dominant species) supported *n*-hexane REs <64% at a critical inlet load of 36.3 g m^{-3} h^{-1}

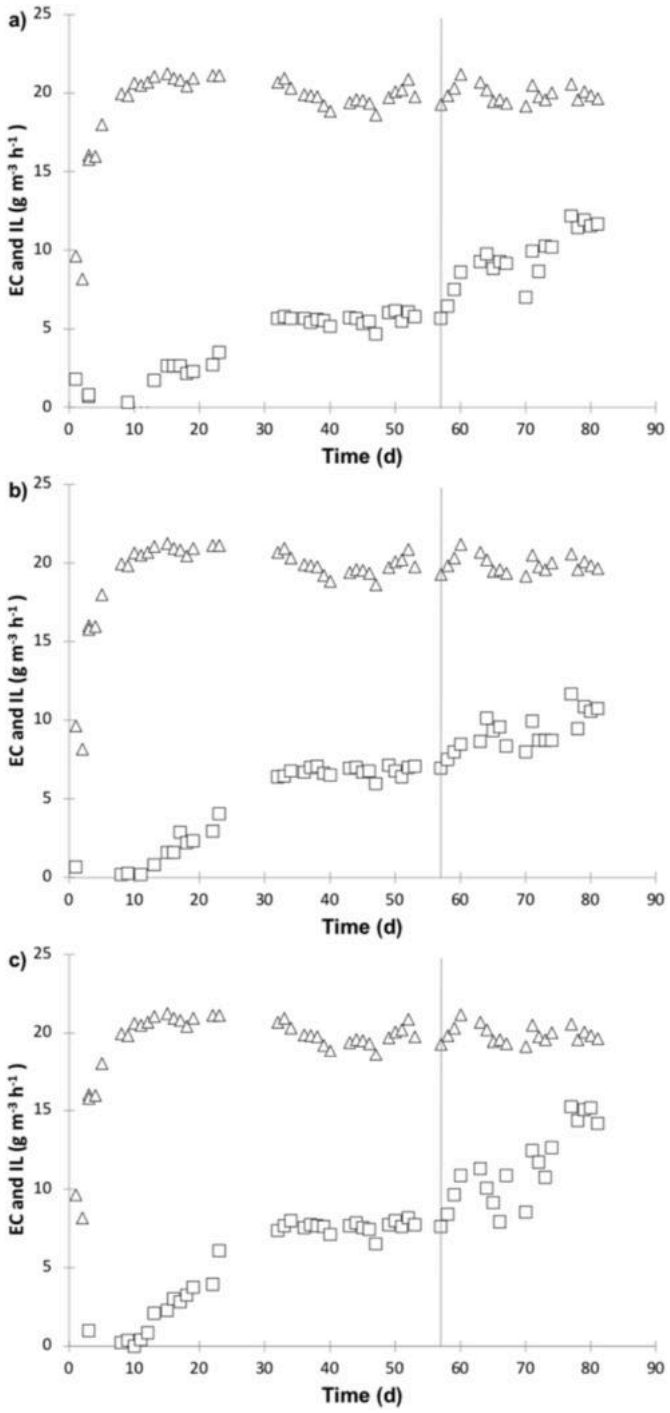


Fig. 6. Time course of the toluene Inlet Load (empty triangles) and the toluene Elimination Capacity (empty squares) in the bioreactors containing (a) *Candida subhashii*, (b) *Fusarium solani*, (c) and the consortium *C. subhashii-F. solani*, operated at a gas residence time of 77 s. Bioreactor configuration was shifted from BF to BTF by day 57th (vertical line).

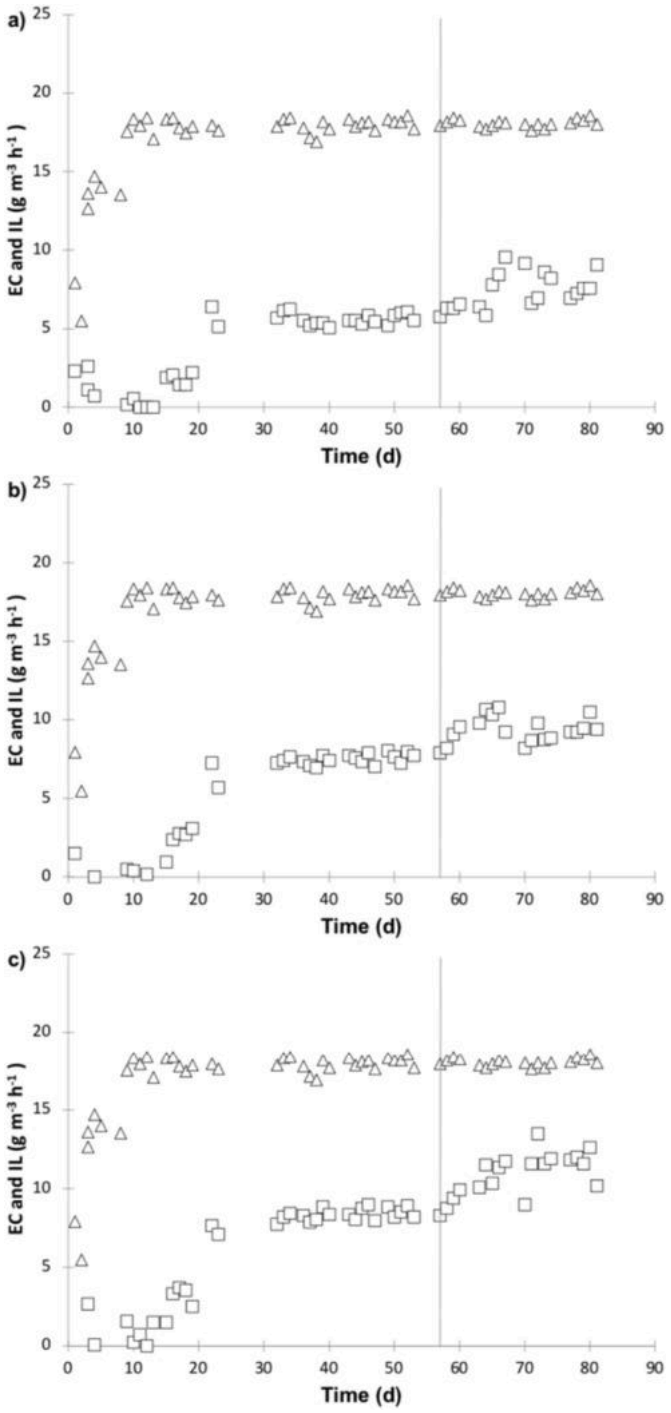


Fig. 7. Time course of the α-pinene Inlet Load (empty triangles) and the α-pinene Elimination Capacity (empty squares) in the bioreactors containing (a) *Candida subhashii*, (b) *Fusarium solani*, (c) and the consortium *C. subhashii-F. solani*, operated at a gas residence time of 77 s. Bioreactor configuration was shifted from BF to BTF by day 57th (vertical line).

(Wösten, 2001). A maximum *n*-hexane RE of 100% was also observed by Arriaga and Revah (van Groenestijn and Liu, 2002) using *F. solani* in a 3.22-L perlite-based BF operating at an EBRT of 60 s with an inlet load of approximately 70 g m^{-3} h^{-1}. This superior *n*-hexane removal compared to the study herein presented was probably caused by the fact that Arriaga and Revah (2005a) operated with pure hexane concentrations 10 times higher, which increased the carbon and energy available and likely promoted an intensive fungal biomass growth.

3.1.2. TCE removal

High chloride ions concentration can interfere with fungal metabolisms. However, during the experiment, no negative influence of the presence of chloride ions was noted, which was liketly due to the low TCE biodegradation performance. Steady state TCE removal efficiencies of 16–21% (RE = 18.4 ± 1.6%, EC = 2.7 ± 0.3 g m^{-3} h^{-1}) for *C. subhashii*, 19–24% (RE = 21.8 ± 1.9%, EC = 3.2 ± 0.3 g m^{-3} h^{-1}) for *F. solani* and 25–32% (RE = 28.4 ± 2.0%, EC = 4.2 ± 0.4 g m^{-3} h^{-1}) for their consortium were achieved under BF configuration (Fig. 5, S2). The

fastest stabilization (day 32nd) was achieved in BF1, while 36 and 39 days were needed to reach steady states in BF2 and BF3, respectively. During the first week under a BTF configuration, an increase in the RE of TCE was observed regardless of the fungal community. Stable TCE RE of 30–35% (RE = 30.8 ± 3.5%, EC = 4.5 ± 0.6 g m^{-3} h^{-1}) in BTF1 (from day 67th to 81st), 31–37% (RE = 33.5 ± 3.3%, EC = 4.9 ± 0.6 g m^{-3} h^{-1}) in BTF2 (from day 64th to 81st) and 41–45% (RE = 43.4 ± 1.5%, EC = 6.1 ± 0.5 g m^{-3} h^{-1}) in BTF3 (from day 63rd to 81st) were recorded under BTF operation. Similarly to the observations in *n*-hexane removal, the highest TCE abatement performance was supported by the combination of *C. subhashii* and *F. solani,* and process operation under a BTF configuration. Similarly, maximum TCE REs of 52.9% were recorded in a 2.7-L BTF packed with wood chips inoculated with an *Ascomycota* strain and operated at an EBRT of 405 s under a magnetic field (Quan et al., 2018). It was hypothesized that process operation under a magnetic field improved the relative abundance of *Ascomycota* and therefore the performance of the fungal biofilter. However, the high EBRT (5 times higher than in our particular study) was likely the main responsible of the higher TCE-REs reported (Quan et al., 2018).

3.1.3. Toluene degradation

During process start-up, the inlet toluene concentrations were gradually adjusted to the target values. In this period, higher toluene concentrations were recorded in the outlet than in the inlet stream, which were caused by experimental errors during toluene determination and the low biodegradation performance of the BF right after inoculation. Steady state toluene REs of 27–30% (RE = 28.0 ± 1.3%, EC = 5.6 ± 0.3 g m^{-3} h^{-1}) for *C. subhashii*, 31–36% (RE = 33.8 ± 1.7%, EC = 6.7 ± 0.3 g m^{-3} h^{-1}) for *F. solani* and 35–40% (RE = 38.1 ± 1.4%, EC = 7.6 ± 0.4 g m^{-3} h^{-1}) for the combination of thereof were recorded under BF operation (Fig. 6, S3). A steady elimination was achieved from day 32nd in BF1, from day 34th in BF3 and from day 36th in BF2 until the end of biofilters operation (day 57th). Toluene removal gradually increased when the bioreactors were shifted to BTF operation. Toluene removal efficiencies accounted for 58.8 ± 3.9% (EC = 11.3 ± 0.8 g m^{-3} h^{-1}) in BTF1, 56.2 ± 4.7% (EC = 9.9 ± 1.1 g m^{-3} h^{-1}) in BTF2 and 75.8 ± 0.4% (EC = 13.5 ± 1.7 g m^{-3} h^{-1}) in BTF3 by the end of the operation. Toluene REs of up to 100% have been previously observed in a 0.48-L BF packed with perlite granules and PUF cubes operated at an EBRT of 12–48 s and inoculated with a non-virulent consortium of the black yeast *Cladophialophora* sp., (Prenafeta-Boldú et al., 2008). In contrast, Estrada and co-workers (Estrada et al., 2013) used *Paecilomyces variotii* to remove a VOC mixture (propanal, methyl isobutyl ketone, toluene and hexanol) in a 2-L BTF packed with vermiculite (EBRT = 60 s and inlet concentration of toluene 1.0 ± 0.3 g m^{-3}). However, the toluene REs achieved averaged only 5.8 ± 4.7%. This significantly lower removal of toluene was most likely related to the shorter gas residence time in the BTF and the (Bokulich et al., 2018) inhibitory effect of propanal on the biodegradation of the remaining VOCs. Similarly, Zhang and co-workers (Zhang et al., 2019) investigated the toluene abatement performance of a BTF packed with ceramic particles and inoculated with a dominant *Fusarium oxysporum* culture. BTF operation at 77 s of EBRT and inlet toluene concentrations of 0.2–0.3 g m^{-3} resulted in steady state REs higher than 92.5%. In this work, *Fusarium* was gradually replaced by *Paramicrosporidium saccamoebae* and a very diverse fungal community, which likely contributed to the achievement of the high toluene abatement performance recorded.

3.1.4. α-pinene removal

During process start-up, the inlet α-pinene concentrations were gradually adjusted to the target values. In this period, higher α-pinene concentrations were recorded in the outlet than in the inlet stream, which were caused by the experimental errors during α-pinene determination and the low biodegradation performance of the BF right after inoculation. Finally, steady state α-pinene REs in the BFs of 28–32% (RE = 29.6 ± 1.8%, EC = 5.6 ± 0.4 g m^{-3} h^{-1}) for *C. subhashii*, 38–42% (RE = 39.5 ± 1.2%, EC = 7.5 ± 0.3 g m^{-3} h^{-1}) for *F. solani* and 41–46% (RE = 43.9 ± 1.5%, EC = 8.4 ± 0.4 g m^{-3} h^{-1}) for their consortium were recorded (Fig. 7, S4). A steady pinene elimination was achieved from day 22nd in BF1 and from day 32nd in BF2 and BF3 until the end of BF operation (day 57th). The shift in bioreactor configuration to BTF also induced a gradual enhancement in α-pinene removal up to day 65th in BTF1, day 59th in BTF2 and up to day 63rd in BTF3. Steady state α-pinene REs ranged between 37 and 48% (RE = 41.7 ± 5.0%, EC = 8.0 ± 0.9 g m^{-3} h^{-1}) for *C. subhashii*, 54–57% (RE = 49.8 ± 3.8%, EC = 9.5 ± 0.7 g m^{-3}) for *F. solani* and 54–64% (RE = 59.6 ± 5.6%, EC = 11.4 ± 1.1 g m^{-3} h^{-1}) for their consortium. López and co-workers (López et al., 2013) reported a similar α-pinene RE of 67% in a 4.55-L BTF, inoculated with pure strains of *Candida boidinii*, *Ophiostoma stenoceras* and *Rhodococcus erythropolis,* and operated at an EBRT of 26–38 s and α-pinene concentrations of 0.05–2.7 g m^{-3}. Similarly, the operation of a 1.1-L BF packed with lava rock inhabited by *Ophiostoma* sp. at an EBRT of 26–72 s resulted in α-pinene REs of 95% (Jin et al., 2006). Finally, α-pinene REs of up to 89% were achieved in a 4.71-L BF packed with polypropylene pall rings and perlite operated at EBRTs of 65 s using *Ophiostoma* sp. as a model fungus (Jin et al., 2007).

3.1.5. CO_2 production and oxygen consumption during VOC degradation

The concentrations of CO_2 during BF and BTF operation was linearly correlated with the VOC removal efficiencies recorded (Fig. S5a). The co-culture interactions in the packing material of BF3 generated ≈110 mg CO_2 m^{-3} more than *Fusarium solani* metabolism in BF2, while BF1 produced ≈50 mg CO_2 m^{-3} less than BF2. Overall, an increase in the CO_2 concentration of ≈50%, 64% and 92% compared to the CO_2 in the inlet polluted air was observed during operation of BF1, BF2 and BF3. This increase in CO_2 concentration accounted for 56–62%, 73–78% and 107–112% in the outlet of BTF1, BTF2 and BTF3, respectively. On the other hand, the consumption of O_2 in BF1 and BF2 accounted for 10–12% of the input oxygen, and 11–13% in BF3 (Fig. S5b). Similarly, fungal O_2 consumption under BTF operation increased up to 15–20%, 17–18%, and 16–19% of the input oxygen in BTF1, BTF2 and BTF3, respectively. At this point it should be highlighted that since the polluted emission treated was a diluted VOC air stream, O_2 did not limit the biodegradation process regardless of the bioreactor configuration tested.

3.1.6. pH and fungal biomass dettachment

On the other hand, a slight decrease from 7.0 to 6.8–6.9 was observed in the pH of the leachate from both *C. subhashii* and *F. solani* BFs, and down to 6.7 in the recirculating solution of the BTFs, likely due to the release of acid metabolites during fungal VOC biodegradation (Fig. 8a). Overall, the difference between the pH in the leachate from BF2 was on average 0.1 lower than the pH in the leachate from BF1. Interestingly, high pH values ranging from 7.1 to 7.4 were recorded in the leachate during the start-up of BF3 (up to day 20th), concomitantly with high OD_{600} values. Indeed, OD_{600} dropped from 2.6 at day 1st to 0.25 at day 22nd (Fig. 8b) as a result of cell washout by the trickling solution from the packaging material. From day 20th to 40th, the pH in the leachate from the BF inoculated with the fungal consortium remained constant at 6.9 and rapidly decreased to 6.5 by day 50th. Biomass washout in the BF and BTF inhabited by *C. subhashii* and *F. solani* remained low ($OD_{600} \leq 0.2$), except in the column inhabited by *C. subhashii*, where a significant increase in biomass concentration was observed from day 60th onwards. Indeed, OD_{600} increased from 0.278 by day 60th to 0.943 by day 81st mediated by biofilm detachment from the packaging material inhabited by *C. subhashii* and its subsequent washout by the trickling solution of the BTF. It should be noted that the measurement of OD also takes into account dead cells with preserved integrity of cytoplasmic membranes. Therefore, a high OD_{600} value does not fully reflect the physiological state of the culture and their potential for biofilm formation. However, the accurate determination of biomass content in the packing media of the bioreactors treating hydrophobic VOCs is not as crucial as in bioreactors treating hydrophilic VOCs, which

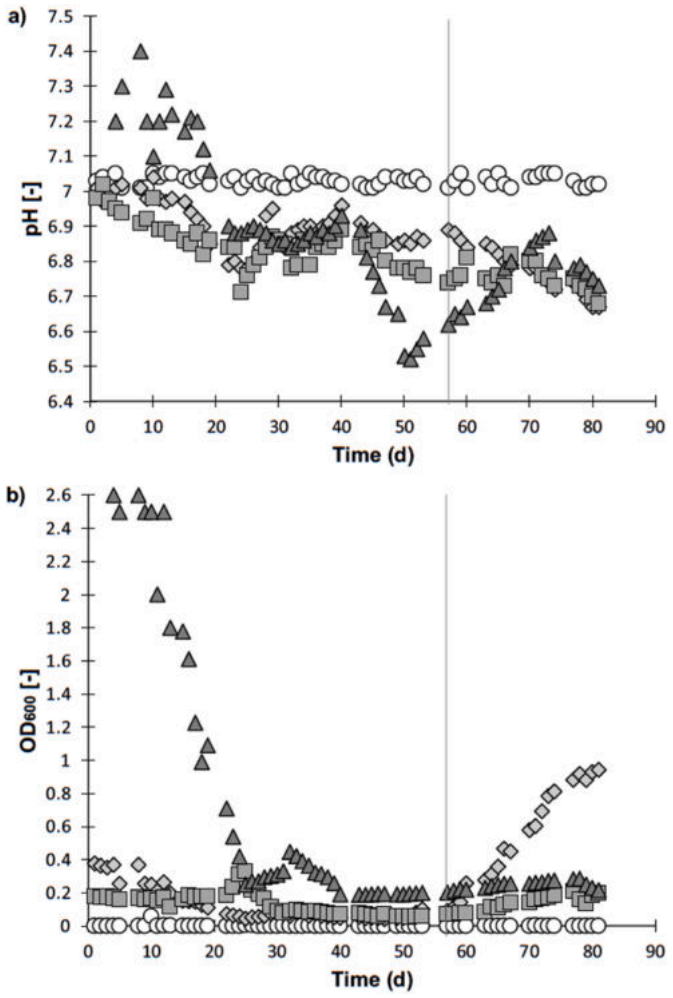


Fig. 8. Time course of (a) pH and (b) OD_{600} in the leachate of the BFs and effluent of the BTFs inoculated with *Candida subhashii* (◆), *Fusarium solani* (■), and the consortium *C. subhashii-F. solani* (▲), and in the pure MSM (O).

are more prone to be limited by microbial activity.

3.2. Fungal diversity and community structure

The analysis of the fungal community in the sample set displayed a total of 255 537 sequences belonging to 23 ASVs identified as fungal species. A total of 11 ASVs were found in BF1/BTF1, 21 ASVs in BF2/BTF2 and 8 ASVs in the BF3/BTF3. According to the alpha diversity at ASV level, the highest richness was obtained by the end of the operation of BF2/BTF2 operated with *F. solani* and the lower richness was found in the BF3/BTF3 operated with the consortium. Beta diversity showed that the most dissimilar biofilter was BF2/BTF2, inoculated with *F. solani,* while BF1/BTF1 and BF3/BTF3 were more similar due to the dominance of *C. subhashii.* Nevertheless, the diversity indexes and the total ASV detected were low regardless of the bioreactor configuration and fungi inoculated, which indicated a fungal specialization towards VOC biodegradation and a lack of contamination.

The main fungal species identified are shown in Fig. 9. In BF1/BTF1, inoculated with *C. subhashii*, this species was the most dominant representing 99.8% of the fungal population by the end of operation. Some members of *F. solani*, and the genera *Penicillium* and *Simplicillum,* were also detected in very small numbers in BF1/BTF1. In BF2/BTF2, inoculated with *F. solani*, a 5% fungal contamination was detected by the end of operation. *Aspergillus versicolor* and *Exophiala equina* represented 1.7 and 1.6% of the fungal community. In the case of BF3/BTF3, originally inoculated with both fungal species, *C. subhashii* efficiently outcompeted *Fusarium solani* and was the most representative species by the end of operation in BTF3 (78.8%). In this reactor, *F. solani* members accounted for 21.2% of the fungal population. The detection of different fungal species was negligible in BF3/BTF3, probably related to the production of antifungals by *C. subhashii* and *F. solani* as a result of the competition for niche overlap between both fungal species (El-Mehalawy, 2004). *C. subhashii* can exert antagonistic and inhibitory effects on the growth of filamentous fungi such as *Fusarium* or *Aspergillus* (Hilber-Bodmer et al., 2017).

Overall, these results showed that Thus, *C. subhashii* was the most resilient fungi, dominating the fungal community in BF1/BTF1 and BF3/BTF3 originally inoculated with this fungal strain and with a combination of this strain and *F. solani*. Yeast from this genus are very versatile and resists a wide range of pollutants and environmental conditions (Mixao et al., 2021; Moreno et al., 2019). *C. subhashii* has the metabolic pathways for the degradation of persistent compounds such as phenol (Marycz et al., 2022a; Filipowicz et al., 2020). However, there is a lack of studies implementing this species for the treatment of VOCs, and the ones that have been carried out involved co-metabolism (Rybarczyk et al., 2021).

The maximum elimination capacities of *n*-hexane, TCE, toluene and α-pinene were obtained in the BTFs operated with the consortium of *C. subhashii* and *F. solani*. They accounted for 5.8 ± 0.4, 6.1 ± 0.5, 13.5 ± 1.7, 11.4 ± 1.1 g m^{-3} h^{-1} for each abovementioned VOC, respectively. However, these elimination capacities were lower than those previously reported in BFs treating VOCs with different *Fusarium* species. For example, ECs of 50 g m^{-3} h^{-1} at inlet *n*-hexane concentrations of 1 g m^{-3} were obtained in perlite-based biofilters operated with *F. solani* (Arriaga and Revah, 2005a) and ECs of 47.7 g *n*-hexane m^{-3} h^{-1} have been obtained by *F. verticillioides* in a biofilter packed with perlite (Zehraoui et al., 2014). Moreover, *F. verticillioides* and *F. solani* were able to remove TCE co-metabolically with methanol in a biofilter packed with diatomaceous earth pellets at ECs of 3.2–12.9 g m^{-3} h^{-1}.

This lower performance was likely related to the lower inlet concentrations tested in this research, and the associated microbial population enriched in each condition. The microbial population associated with the fungal consortium exhibits a key role on the final performance of waste treatment bioreactors. However, there is still a lack of studies considering the whole ecological niche in waste treatment processes (Gonzalez-Martinez et al., 2018).

In order to determine the role of the hydrophobicity of the target VOCs in their mass transfer to the liquid phase, and thus the elimination achieved by fungi, the Hansen solubility parameters and the knowledge of the characteristics of the fungi species were used. Marycz and co-workers (2022) presented the affinity of the target VOCs to dissolve in water on the basis of Hansen's solubility parameters (Marycz et al., 2022b). Based on the analysis of the experimental results, a biodegradation pattern of the removed compounds was obtained, which was consistent with the fact that the closer the solubility parameters of the compound and solvent are, the more likely the compound dissolved in a given solvent (Marycz et al., 2022b). Interestingly, for BF1/BTF1 a very similar biodegradation pattern was obtained to that presented in (Marycz et al., 2022b). In (Marycz et al., 2022b) the same species of *C. subhashii* as in BF1/BTF1 was used to colonize the biofilters. However, the biodegradation patterns obtained for BF2/BTF2 and BF3/BTF3 were different. Interestingly, the presence of trickling liquid phase did not alter the patterns of degradation observed for BT1/BTF1 only. The authors explain this phenomenon by the use of a different species of fungus (*F. solani*) to colonize both columns, which is characterized by a different morphology and cell hydrophobicity. *C. subhashii*, as a representative of yeast, forms a thin layer (biofilm) on the surface of the packing material, which plays a crucial role in the biofiltration process. During the BF process, pollutants contained in the air are absorbed on the surface of the biofilm, while during BTF, VOCs must first dissolve in the trickling liquid medium flowing through the column, and then penetrate into the

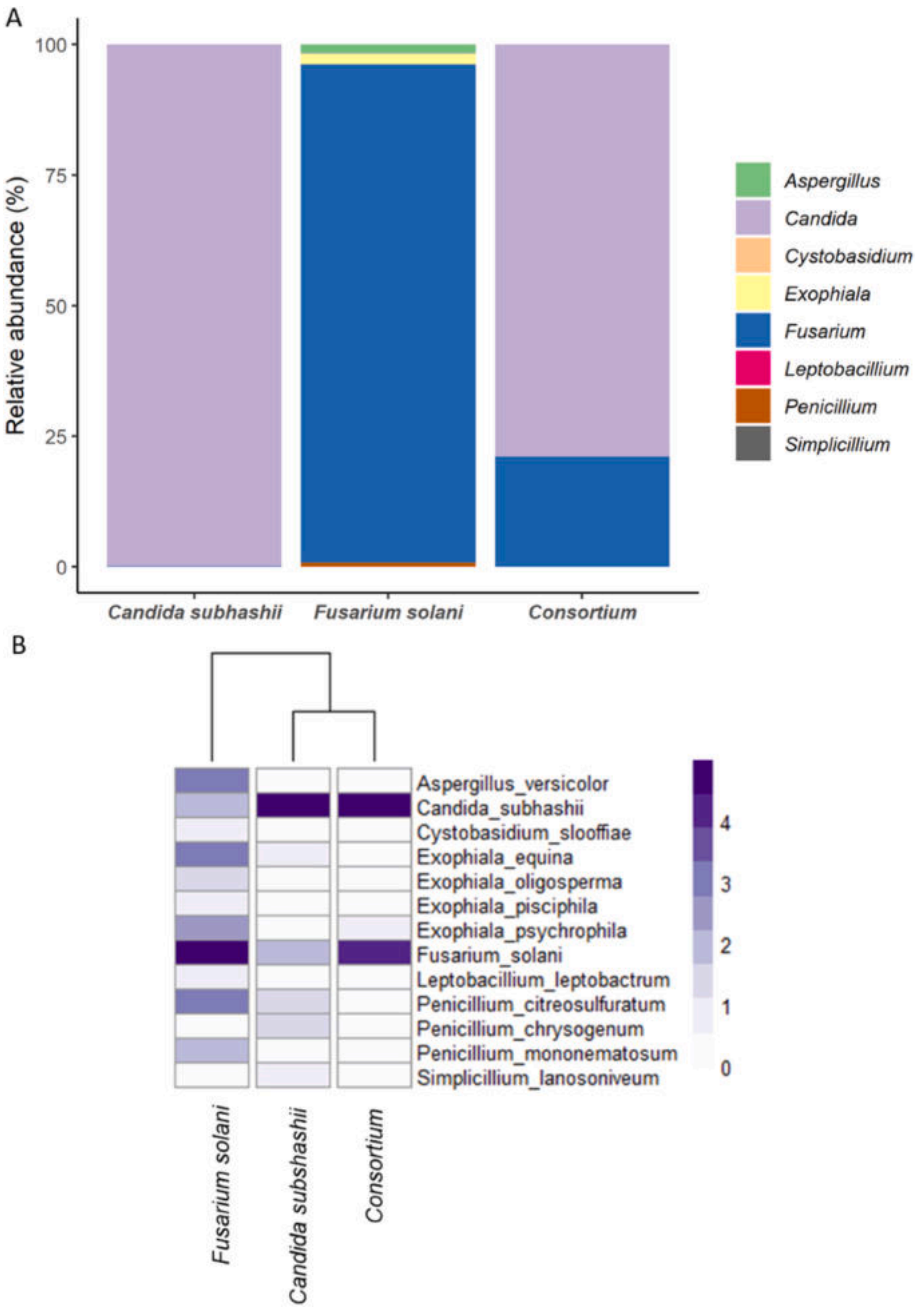


Fig. 9. (a) Stacked bar graph of the total fungal genera found in the three BFs/BTFs in the last day of the process. (b) Heat map of the total fungal species found in the BFs/BTFs in the last day of the process. Data were log transformed to show small abundances of different fungal species. The dendrogram on top represents hierarchical clustering of the sample replicates.

biofilm (Marycz et al., 2022a). This association shows that the biodegradation efficiency of *C. subhashii* is closely related to the solubility of the gases, resulting from the Hansen parameters. On the other hand, *F. solanii* has an aerial mycelium that is in direct contact with the gas phase, enabling an effective adsorption and biodegradation of hydrophobic VOCs (Marycz et al., 2022a). Thus, regarding *F. solani*, the biodegradation efficiency of hydrophobic VOCs is not directly related to the solubility of gases in the liquid phase. To date, the mechanisms of degradation of these VOCs individually by *C. subhashii, F. solani* and their consortium have not been investigated. In this context, it is not possible to lay the foundation of potential inhibition mechanisms using this VOC mixture.

Pressure drop across the packed bed was not measured in this study, but under the low VOC concentrations tested at 77s of EBRT, not excessive biomass accumulation and therefore not high pressure drop was expected in the BF and BTF over the 3 month of operation, regardless of the fungal inoculum tested. A mass balance based on a saturated liquid for each VOC illustrates that the leaching effect of VOCs in the aqueous phase can be considered negligible (Table 1).

This work was devoted to the systematic comparison of *C. subhashii* with *F. solani* and their consortium, under two gas-phase reactor configurations. In the previous work by Marycz and co-workers (2022) only *C. subhashii* was evaluated, and the performance achieved was lower than when *C. subhashii* was grown in symbiosis with *F. solani*. Therefore, this work represents a contribution beyond the state of the art of the fungal biofiltration field.

Overall, these results demonstrated the superior performance of a consortium in comparison to a single dominant species and provided

Table 1
The summary of the concentrations of each VOC in the aqueous and gas phases during the process.

Compounds	Henry's constant (dimensionless)[a]	Concentration [mg m^{-3}]		VOS mass flow [mg day^{-1}]	
		in the gas phase	in the aqueous phase	Input the gas phase	Leaching the aqueous phase
n-hexane	$1.0•10^{-3}$	266.9	0.27	960.84	$3.20•10^{-5}$
TCE	$9.9•10^{-2}$	317.1	31.39	1141.56	$3.77•10^{-3}$
toluene	$1.6•10^{-1}$	431.4	69.02	1553.04	$8.28•10^{-3}$
α-pinene	$4.9•10^{-2}$	386.3	18.93	1390.68	$2.22•10^{-3}$

[a] Henry's law constants at 298.15 K (Henry's law constants n, 2021).

proof of the important role of *C. subhashii* in the treatment of hydrophobic VOCs.

4. Conclusion

F. solani and *C. subhashii* supported an effective abatement of hydrophobic VOCs at an EBRT of 77 s regardless of the gas-phase bioreactor configuration evaluated. The highest steady state REs for all VOCs were achieved in the biotrickling filter inhabited by the *F. solani* and *C. subhashii* consortium. Under steady state, REs of 42–48% (RE = 45.0 ± 2.1%, EC = 5.8 ± 0.4 g m^{-3} h^{-1}), 41–45% (RE = 43.4 ± 1.5%, EC = 6.1 ± 0.5 g m^{-3} h^{-1}), 60–76% (RE = 75.8 ± 0.4%, EC = 13.5 ± 1.7 g m^{-3} h^{-1}) and 54–64% (RE = 59.6 ± 5.6%, EC = 11.4 ± 1.1 g m^{-3} h^{-1}) were recorded for *n*-hexane, TCE, toluene and α-pinene, respectively. The consistent steady states achieved also showed the robustness of the fungal biodegradation process. The biotrickling filter configuration was able to achieve better degradation compared to the biofilters regardless of the VOC tested. In addition, better VOC biodegradation performance was observed when *F. solani* and *C. subhashii* were grown as a consortium. Finally, *C. subhashii* clearly dominated the fungal population at the end of the experiment when inoculated alone and in combination with *F. solani*. These results suggest that *Candida* species are good candidates for degradation of hydrophobic VOCs in gas-phase bioreactors.

Author contribution statement

Milena Marycz: Conceptualization; Methodology; Investigation; Formal analysis; Roles/Writing – original draft; Funding acquisition. **Anna Brillowska-Dąbrowska:** Supervision, Writing – review & editing. **Sara Cantera:** Formal analysis; Writing – review & editing; Funding acquisition. **Jacek Gębicki:** Supervision, Writing – review & editing. **Raúl Muñoz:** Supervision; Conceptualization; Methodology; Project administration; Writing – review & editing; Funding acquisition; Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The present work has been sponsored by Gdansk University of Technology with a project co-financed from European Funds 'Integrated Programme of Development of Gdansk University of Technology' (the project nº POWR.03.05.00–00-Z044/17). The Regional Government of Castilla y León and the EU-FEDER program [grant number CLU 2017–09 and UIC 315] are also gratefully acknowledged. The European Commission-H2020- MSCA–IF–2019 is also gratefully acknowledged for the financial support of the project ENHANCEMENT (897284).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.137609.

References

Alonso, C., Suidan, M., 1997. Gas treatment in trickle-bed biofilters: biomass, how much is enough? Biotechnol. Bioeng. 54.6, 583–594.
Arellano-García, L., le Borgne, S., Revah, S., 2018. Simultaneous treatment of dimethyl disulfide and hydrogen sulfide in an alkaline biotrickling filter. Chemosphere 191, 809–816. https://doi.org/10.1016/j.chemosphere.2017.10.096.
Arriaga, S., Revah, S., 2005a. Removal of n-hexane by Fusarium solani with a gas-phase biofilter. J. Ind. Microbiol. Biotechnol. 32, 548–553. https://doi.org/10.1007/s10295-005-0247-9.
Arriaga, S., Revah, S., 2005b. Improving hexane removal by enhancing fungal development in a microbial consortium biofilter. Biotechnol. Bioeng. 90, 107–115. https://doi.org/10.1002/bit.20424.
Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., et al., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6, 1–17. https://doi.org/10.1186/S40168-018-0470-Z/TABLES/3.
Boonchan, S., Britz, M.L., Stanley, G.A., 2000. Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. Appl. Environ. Microbiol. 66, 1007–1019. https://doi.org/10.1128/AEM.66.3.1007-1019.2000/ASSET/DFD92721-A661-4396-A32C-09F6713E7ABC/ASSETS/GRAPHIC/AM0301507007 (JPEG).
Caicedo, F., Estrada, J.M., Silva, J.P., Muñoz, R., Lebrero, R., 2018. Effect of packing material configuration and liquid recirculation rate on the performance of a biotrickling filter treating VOCs. J. Chem. Technol. Biotechnol. 93, 2299–2306. https://doi.org/10.1002/JCTB.5573.
Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.
Chheda, D., Sorial, G.A., 2017. Evaluation of co-metabolic removal of trichloroethylene in a biotrickling filter under acidic conditions. J. Environ. Sci. (China) 57, 54–61. https://doi.org/10.1016/j.jes.2016.12.008.
El-Mehalawy, A.A., 2004. The rhizosphere yeast fungi as biocontrol agents for Wilt disease of kidney bean caused by *Fusarium oxysporum*. Int. J. Agric. Biol. 6, 310–316.
Estrada, J.M., Hernández, S., Muñoz, R., Revah, S., 2013. A comparative study of fungal and bacterial biofiltration treating a VOC mixture. J. Hazard Mater. 250–251, 190–197. https://doi.org/10.1016/j.jhazmat.2013.01.064.
Estrada, J.M., Lebrero, R., Quijano, G., Pérez, R., Figueroa-González, I., García-Encina, P. A., et al., 2014. Methane abatement in a gas-recycling biotrickling filter: evaluating innovative operational strategies to overcome mass transfer limitations. Chem. Eng. J. 253, 385–393. https://doi.org/10.1016/J.CEJ.2014.05.053.
Filipowicz, N., Momotko, M., Boczkaj, G., Cieśliński, H., 2020. Determination of phenol biodegradation pathways in three psychrotolerant yeasts, *Candida subhashii* A011, *Candida oregonensis* B021 and *Schizoblastosporion starkeyi-henricii* L012, isolated from Rucianka peatland. Enzyme Microb Technol 141, 109663. https://doi.org/10.1016/J.ENZMICTEC.2020.109663.
González-Martín, J., Cantera, S., Lebrero, R., Muñoz, R., 2022. Optimization of acrylic-styrene latex-based biofilms as a platform for biological indoor air treatment. Chemosphere 287, 132182. https://doi.org/10.1016/J.CHEMOSPHERE.2021.132182.
Gonzalez-Martinez, A., Sihvonen, M., Muñoz-Palazon, B., Rodriguez-Sanchez, A., Mikola, A., Vahala, R., 2018. Microbial ecology of full-scale wastewater treatment systems in the polar arctic circle: archaea, bacteria and fungi. Sci. Rep. 8 https://doi.org/10.1038/S41598-018-20633-5.
Gospodarek, M., Rybarczyk, P., Szulczyński, B., Gębicki, J., 2019a. Comparative evaluation of selected biological methods for the removal of hydrophilic and hydrophobic odorous VOCs from air. Processes 7, 187. https://doi.org/10.3390/pr7040187.
Gospodarek, M., Rybarczyk, P., Brillowska-Dąbrowska, A., Gębicki, J., 2019b. The use of various species of fungi in biofiltration of air contaminated with odorous volatile organic compounds. E3S Web of Conferences 100, 00021. https://doi.org/10.1051/e3sconf/201910000021.
Henry's law constants n.d. http://www.mpch-mainz.mpg.de/~sander/res/henry.html (accessed April 3, 2021).
Hilber-Bodmer, M., Schmid, M., Ahrens, C.H., Freimoser, F.M., 2017. Competition assays and physiological experiments of soil and phyllosphere yeasts identify *Candida subhashii* as a novel antagonist of filamentous fungi. BMC Microbiol. 17 https://doi.org/10.1186/S12866-016-0908-Z.
Jin, Y., Veiga, M.C., Kennes, C., 2006. Performance optimization of the fungal biodegradation of α-pinene in gas-phase biofilter. Process Biochem. 41, 1722–1728. https://doi.org/10.1016/j.procbio.2006.03.020.

Jin, Y., Guo, L., Veiga, M.C., Kennes, C., 2007. Fungal biofiltration of α-pinene: effects of temperature, relative humidity, and transient loads. Biotechnol. Bioeng. 96, 433–443. https://doi.org/10.1002/bit.21123.

Kolde, R., 2019. Pheatmap: Pretty Heatmaps. R Package version 1.0.12. https://CRAN.R-project.org/package=pheatmap. (Accessed 29 March 2022). accessed.

Lebrero, R., Gondim, A.C., Pérez, R., García-Encina, P.A., Muñoz, R., 2014. Comparative assessment of a biofilter, a biotrickling filter and a hollow fiber membrane bioreactor for odor treatment in wastewater treatment plants. Water Res. 49, 339–350. https://doi.org/10.1016/J.WATRES.2013.09.055.

Lee, S hun, Heber, A.J., 2010. Ethylene removal using biotrickling filters: part II. Parameter estimation and mathematical simulation. Chem. Eng. J. 158, 89–99. https://doi.org/10.1016/J.CEJ.2009.12.034.

Lee, S hun, Li, C., Heber, A.J., Ni, J., Huang, H., 2013. Biofiltration of a mixture of ethylene, ammonia, n-butanol, and acetone gases. Bioresour. Technol. 127, 366–377. https://doi.org/10.1016/J.BIORTECH.2012.09.110.

Lee, S.H., Kurade, M.B., Jeon, B.H., Kim, J., Zheng, Y., Salama, E.S., 2021. Water condition in biotrickling filtration for the efficient removal of gaseous contaminants 41, 1279–1296. https://doi.org/10.1080/07388551.2021.1917506.

Li, K., Zhou, J., Wang, L., Mao, Z., Xu, R., 2019. The styrene purification performance of biotrickling filter with toluene-styrene acclimatization under acidic conditions. J. Air Waste Manage. Assoc. 69, 944–955. https://doi.org/10.1080/10962247.2019.1604450.

López, M.E., Rene, E.R., Malhautier, L., Rocher, J., Bayle, S., Veiga, M.C., et al., 2013. One-stage biotrickling filter for the removal of a mixture of volatile pollutants from air: performance and microbial community analysis. Bioresour. Technol. 138, 245–252. https://doi.org/10.1016/J.BIORTECH.2013.03.136.

López, L.R., Bezerra, T., Mora, M., Lafuente, J., Gabriel, D., 2016. Influence of trickling liquid velocity and flow pattern in the improvement of oxygen transport in aerobic biotrickling filters for biogas desulfurization. J. Chem. Technol. Biotechnol. 91, 1031–1039. https://doi.org/10.1002/JCTB.4676.

Lugones, L.G., Wösten, H.A.B., Wessels, J.G.H., 1998. A hydrophobin (ABH3) specifically secreted by vegetatively growing hyphae of *Agaricus bisporus* (common white button mushroom). Microbiology (N. Y.) 144, 2345–2353. https://doi.org/10.1099/00221287-144-8-2345/CITE/REFWORKS.

Marycz, M., Brillowska-Dąbrowska, A., Gębicki, J., 2020. Evaluation of immobilization of selected peat-isolated yeast strains of the species *Candida albicans* and *Candida subhashii* on the surface of artificial support materials used for biotrickling filtration. Processes 8, 801. https://doi.org/10.3390/pr8070801.

Marycz, M., Brillowska-Dąbrowska, A., Muñoz, R., Gębicki, J., 2022 2022. A state of the art review on the use of fungi in biofiltration to remove volatile hydrophobic pollutants. Rev. Environ. Sci. Biotechnol. https://doi.org/10.1007/S11157-021-09608-7, 1–22.

Marycz, M., Rodríguez, Y., Gębicki, J., Muñoz, R., 2022b. Systematic comparison of a biotrickling filter and a conventional filter for the removal of a mixture of hydrophobic VOCs by *Candida subhashii*. Chemosphere 306.

Mixao, V., Hegedusova, E., Saus, E., Pryszcz, L.P., Cillingova, A., Nosek, J., et al., 2021. Genome analysis of *Candida subhashii* reveals its hybrid nature and dual mitochondrial genome conformations. DNA Res. 28 https://doi.org/10.1093/DNARES/DSAB006.

Mjj, Kotterman, Vis, E.H., Field, J.A., 1998. Successive mineralization and detoxification of benzo[a]pyrene by the white rot fungus *Bjerkandera* sp. strain BOS55 and indigenous microflora. Appl. Environ. Microbiol. 64, 2853–2858. https://doi.org/10.1128/AEM.64.8.2853-2858.1998/ASSET/21DCB737-7B87-4CA3-8662-A5A5CB305909/ASSETS/GRAPHIC/AM0880164005 (JPEG).

Moreno, A., Demitri, N., Ruiz-Baca, E., Vega-González, A., Polentarutti, M., Cuéllar-Cruz, M., 2019. Bioreduction of precious and heavy metals by *Candida* species under oxidative stress conditions. Microb. Biotechnol. 12, 1164–1179. https://doi.org/10.1111/1751-7915.13364.

Nash, A.K., Auchtung, T.A., Wong, M.C., Smith, D.P., Gesell, J.R., Ross, M.C., et al., 2017. The gut mycobiome of the Human Microbiome Project healthy cohort. Microbiome 5, 153. https://doi.org/10.1186/S40168-017-0373-4/TABLES/3.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. Vegan: community ecology package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan 13:34 2019. (accessed March 29, 2022).

Prenafeta-Boldú, F.X., Ballerstedt, H., Gerritse, J., Grotenhuis, J.T.C., 2004. Bioremediation of BTEX hydrocarbons: effect of soil inoculation with the toluene-growing fungus *Cladophialophora* sp. strain T1. Biodegradation 15, 59–65.

Prenafeta-Boldú, F.X., Illa, J., van Groenestijn, J.W., Flotats, X., 2008. Influence of synthetic packing materials on the gas dispersion and biodegradation kinetics in fungal air biofilters. Appl. Microbiol. Biotechnol. 79, 319–327. https://doi.org/10.1007/S00253-008-1433-2, 79:2 2008.

Quan, Y., Wu, H., Guo, C., Han, Y., Yin, C., 2018. Enhancement of TCE removal by a static magnetic field in a fungal biotrickling filter. Bioresour. Technol. 259, 365–372. https://doi.org/10.1016/J.BIORTECH.2018.03.031.

Rybarczyk, P., Marycz, M., Szulczyński, B., Brillowska-Dąbrowska, A., Rybarczyk, A., Gębicki, J., 2021. In: Removal of Cyclohexane and Ethanol from Air in Biotrickling Filters Inoculated with Candida Albicans and Candida Subhashii, vol. 47. Archives of Environmental Protection. https://doi.org/10.24425/aep.2021.136445.

Sayers, E.W., Cavanaugh, M., Clark, K., Ostell, J., Pruitt, K.D., GenBank, Karsch-Mizrachi I., 2019. Nucleic Acids Res. 47, D94–D99. https://doi.org/10.1093/NAR/GKY989.

Tymiński, Ł.P., Znajewska, Z., Dąbrowska, G.B., 2018. Characteristics and functions of hydrophobins and their use in manifold industries. Postepy Mikrobiol. 4.

van Groenestijn, J.W., Kraakman, N.J.R., 2005. Recent developments in biological waste gas purification in Europe. Chem. Eng. J. 113, 85–91. https://doi.org/10.1016/J.CEJ.2005.03.007.

van Groenestijn, J.W., Liu, J.X., 2002. Removal of alpha-pinene from gases using biofilters containing fungi. Atmos. Environ. 36, 5501–5508.

Vergara-Fernández, A., van Haaren, B., Revah, S., 2006. Phase partition of gaseous hexane and surface hydrophobicity of *Fusarium solani* when grown in liquid and solid media with hexanol and hexane. Biotechnol. Lett. 28, 2011–2017. https://doi.org/10.1007/s10529-006-9186-4.

Vergara-Fernández, A., Lara Molina, L., Pulido, N.A., Aroca, G., 2007. Effects of gas flow rate, inlet concentration and temperature on the biofiltration of toluene vapors. J. Environ. Manag. 84, 115–122. https://doi.org/10.1016/J.JENVMAN.2006.04.009.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis, R Books. Springer-Verlag, New York, NY.

Wösten, H.A.B., 2001. Hydrophobins: multipurpose proteins. Annu. Rev. Microbiol. 55, 625–646. https://doi.org/10.1146/annurev.micro.55.1.625.

Zehraoui, A., Wendell, D., Sorial, G.A., 2014. Treatment of hydrophobic VOCs in trickling bed air biofilter: emphasis on long-term effect of initial alternate use of hydrophilic VOCs and microbial species evolution. J. Air Waste Manage. Assoc. 64, 1102–1111. https://doi.org/10.1080/10962247.2014.925522.

Zhang, Y., Liu, J., Qin, Y., Yang, Z., Cao, J., Xing, Y., et al., 2019. Performance and microbial community evolution of toluene degradation using a fungi-based biotrickling filter. J. Hazard Mater. 365, 642–649. https://doi.org/10.1016/J.JHAZMAT.2018.11.062.

Appendix A. Supplementary data

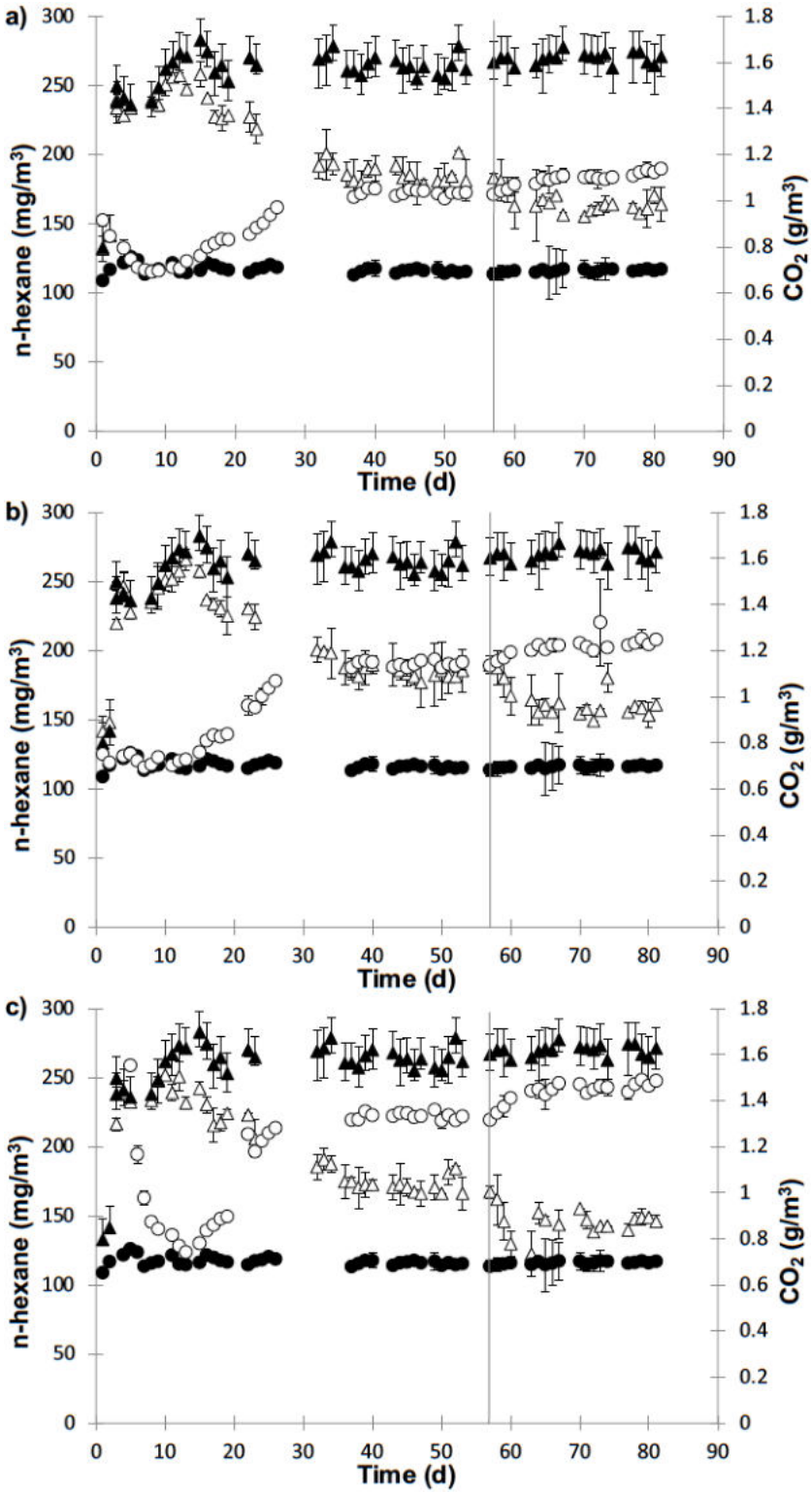


Figure S1. Time course of the inlet (solid symbols) and outlet (empty symbols) n-hexane (triangles) and CO_2 (circles) concentrations in the bioreactors containing (a) *Candida subhashii*, (b) *Fusarium solani*, (c) and the consortium *C. subhashii-F. solani*, operated at a gas residence time of 77 s. Bioreactor configuration was shifted from BF to BTF by day 57th (vertical line).

Appendix A. Supplementary data

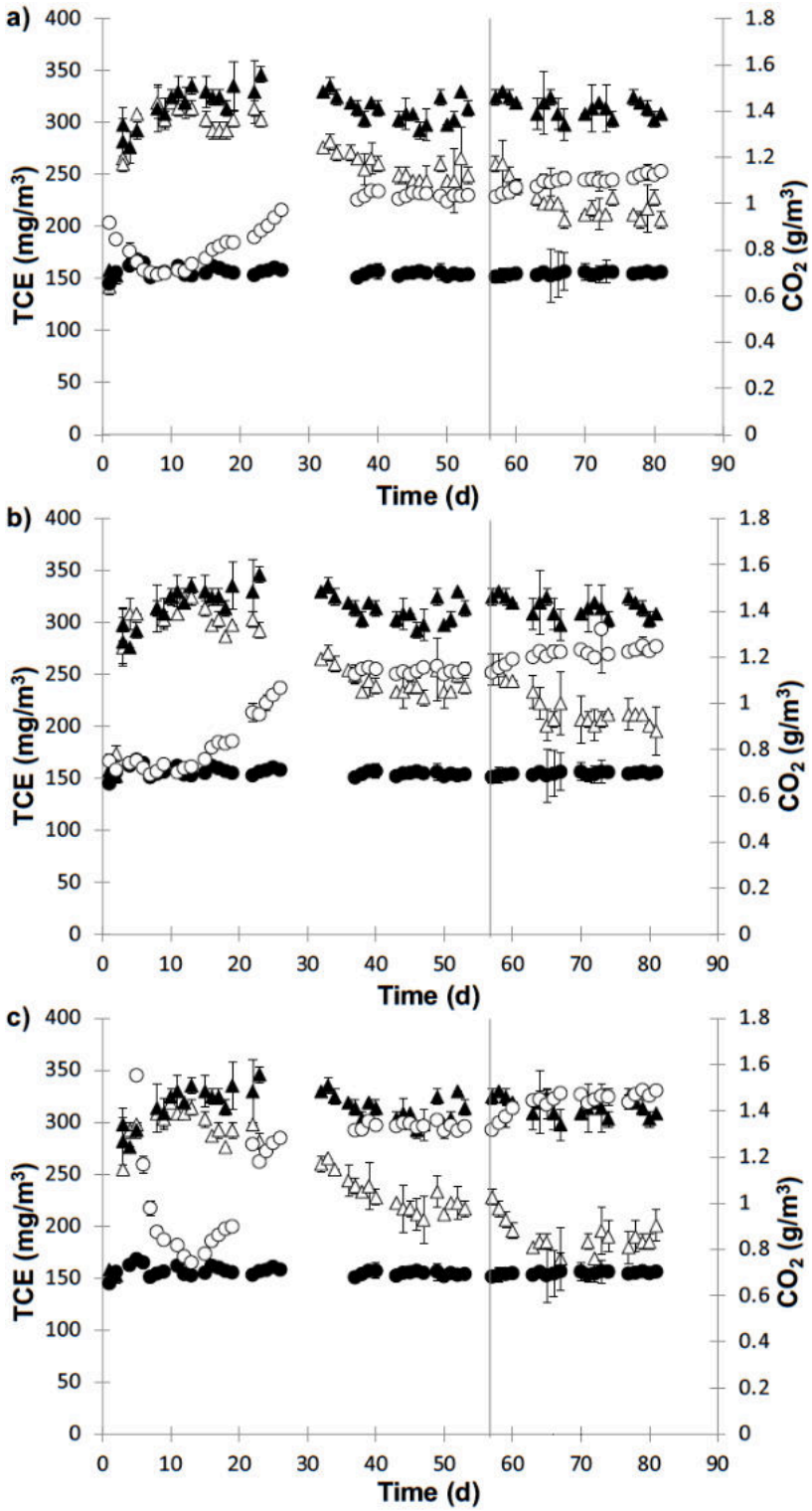


Figure S2. Time course of the inlet (solid symbols) and outlet (empty symbols) TCE (triangles) and CO_2 (circles) concentrations in the bioreactors containing (a) *Candida subhashii*, (b) *Fusarium solani*, (c) and the consortium *C. subhashii-F. solani* operated at a gas residence time of 77 s. Bioreactor configuration was shifted from BF to BTF by day 57th (vertical line).

Appendix A. Supplementary data

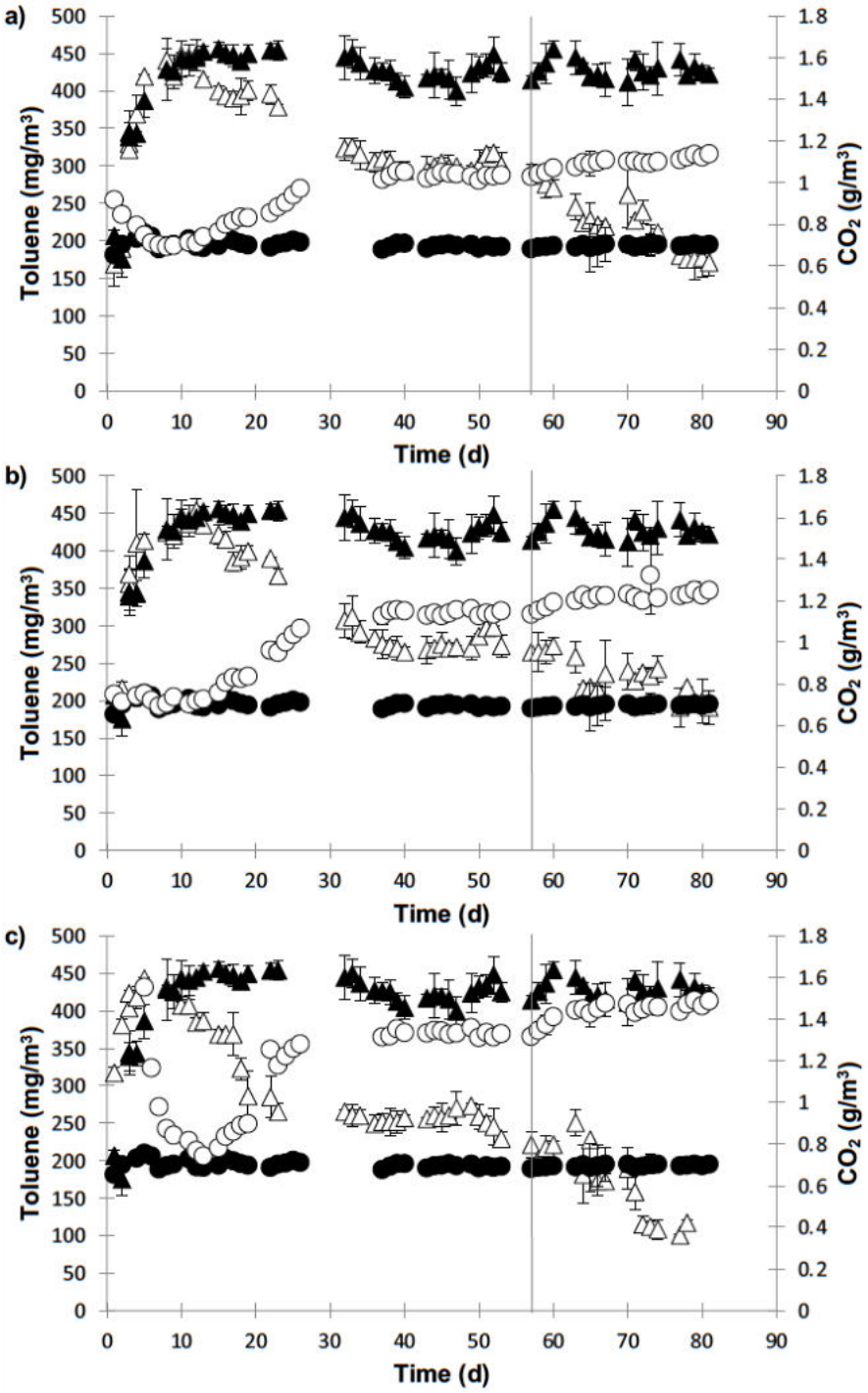


Figure S3. Time course of the inlet (solid symbols) and outlet (empty symbols) toluene (triangles) and CO_2 (circles) concentrations in the bioreactors containing (a) *Candida subhashii*, (b) *Fusarium solani*, (c) and the consortium *C. subhashii-F. solani,* operated at a gas residence time of 77 s. Bioreactor configuration was shifted from BF to BTF by day 57th (vertical line).

Appendix A. Supplementary data

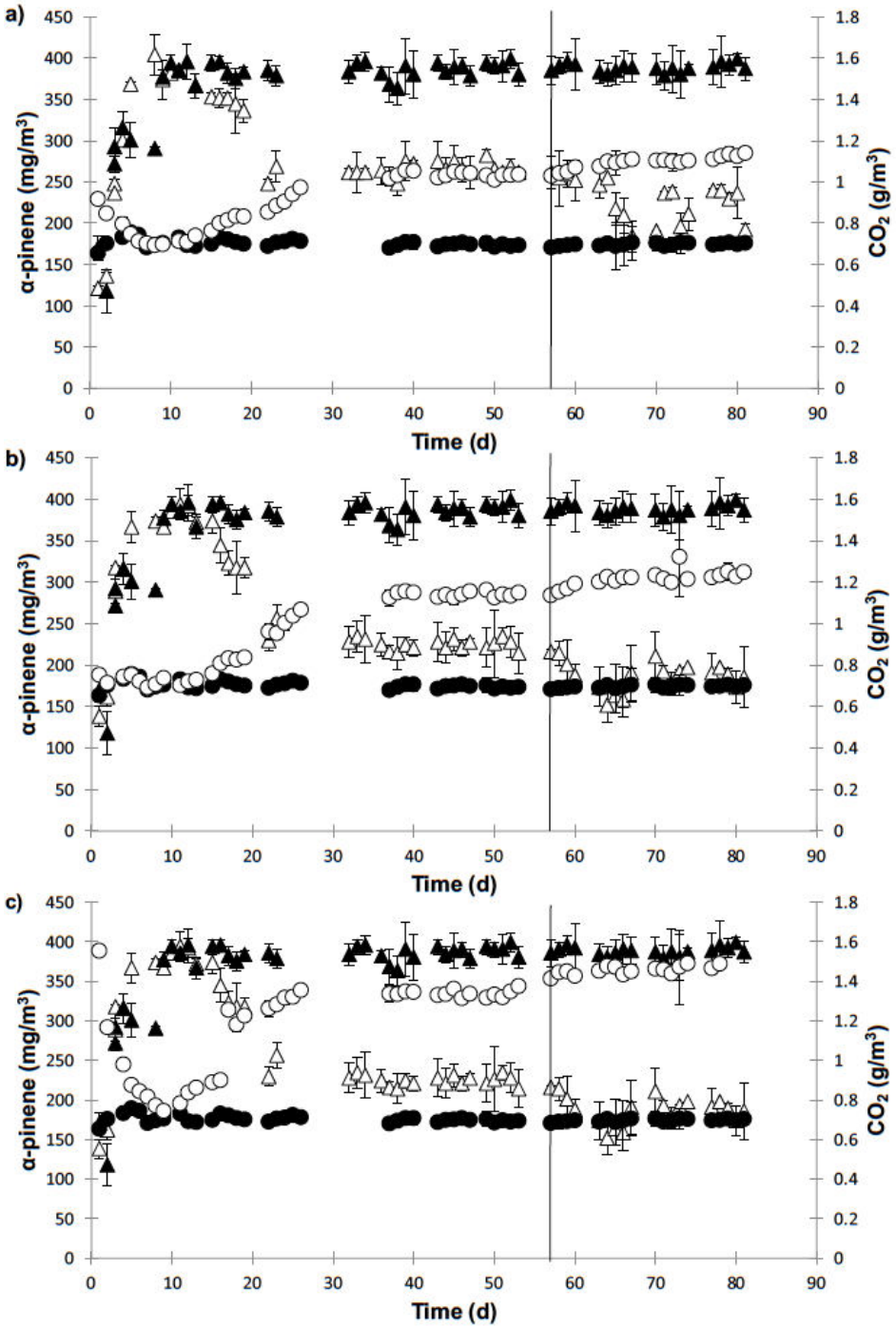


Figure S4. Time course of the inlet (solid symbols) and outlet (empty symbols) α-pinene (triangles) and CO_2 (circles) concentrations in the bioreactors containing (a) *Candida subhashii*, (b) *Fusarium solani*, (c) and the consortium *C. subhashii-F. solani,* operated at a gas residence time of 77 s. Bioreactor configuration was shifted from BF to BTF by day 57th (vertical line).

Appendix A. Supplementary data

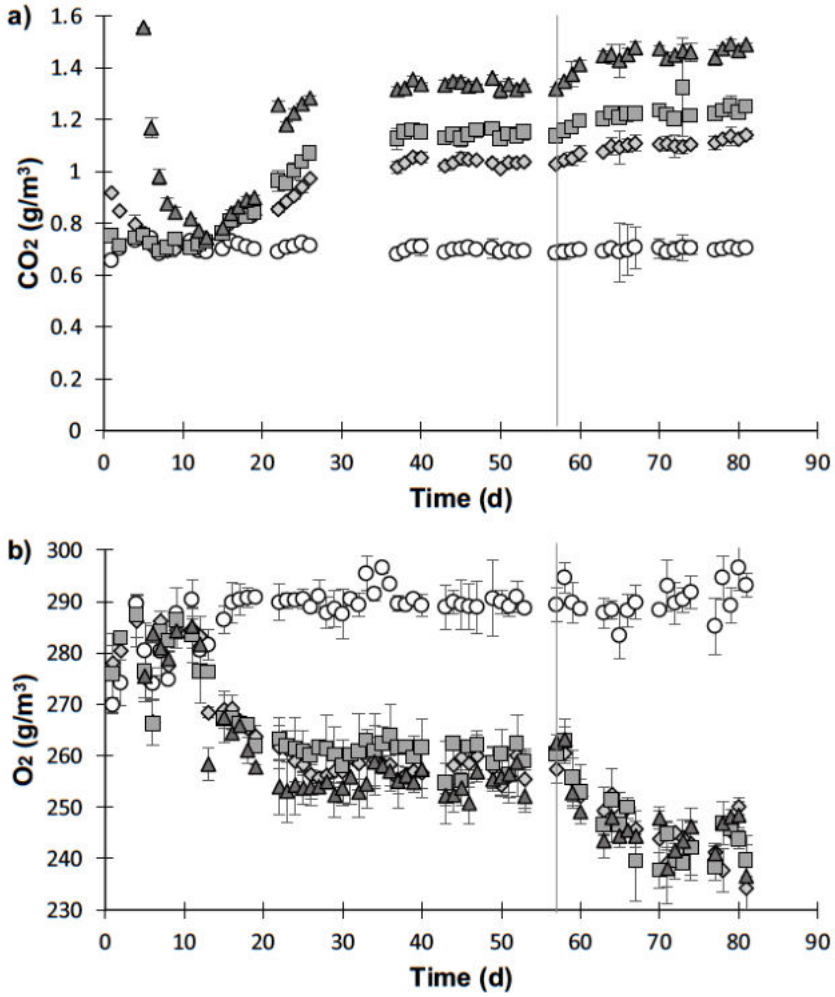


Figure S5. Time course of the inlet (o) and outlet concentration of (a) CO_2 and (b) O_2 in the bioreactors inoculated with *Candida subhashii* (◆), *Fusarium solani* (■), and the consortium *C. subhashii-F. solani* (▲) operated at a gas residence time of 77 s.

4. Summary

The main problem encountered in biofiltration is the removal of hydrophobic VOCs due to their low solubility in aqueous biofilm, which limits both reaction rate and mass transfer. Fungi have several advantages over bacteria when it comes to removing these types of compounds from the air. Fungi are resistant to low humidity, tolerate low pH, have hydrophobic proteins called hydrophobins and in some cases, have air hyphae that occupy the free spaces of the biofilter column, allowing penetration of the biofilter packing material and increasing the availability of nutrients. Acidification and drying of the filter bed are the most frequently encountered obstacles when using bacterial biofilters. The immobilization of fungi on the surface of an inert BTF material avoids these problems. Based on the literature review enriched with the decision trees method and the pairwise comparison model, it was shown that BTFs are suitable for both hydrophilic and hydrophobic compounds removal. In addition, the use of fungi in biofiltration to remove hydrophobic VOCs is in line with the principles of green engineering, the aim of which is to develop processes that reduce pollution, promote sustainable development and-minimize risks to human health and the environment.

During the experiments, an environmental isolate of *C. subhashii* was isolated, which had never been used in biofiltration before. This isolate showed high efficiency of carbon assimilation from selected hydrophobic VOCs. All further research utilized this *C. subhashii* isolate. Throughout the study, experiments on biofiltration processes were successfully used to confirm the effectiveness of *C. subhashii* immobilized in polyurethane foam in removing a mixture of hydrophobic VOCs with a relatively short EBRT, both in BF and BTF. To aid in the research, a quick and simple method of immobilizing various species of fungi on the surface of biofilter packing materials was developed and tested. Polyurethane foam was selected as the best packing material in BTF for immobilization of various species of fungi. The conducted research showed better degradation of the applied mixture of hydrophobic VOCs with a fungal configuration of BTF than BF. Subsequently, it was shown that *C. subshashii* effectively removes selected hydrophobic VOCs from the air at a level comparable to the fungus species most often used for this purpose (*F. solani*) regardless of the assessed bioreactor configuration in the gas phase.

One of the most important conclusions obtained from this research is that the most efficient processes for all tested compounds involved using biofilters inhabited by a consortium of *C. subhashii* and *F. solani*. These results clearly suggest that *C. subhashii* is a good candidate to remove hydrophobic VOCs in BTFs, as well as in BFs. In addition, the microbiological results of the fungal population studies favor *C. subhashii*, which indicates its dominance in fungal populations in all the columns studied.

During the research, attention was paid to the ubiquitous problem of the lack of monitoring of the condition of microorganism cells, both immediately after their immobilization on the surface of the filling and during the process. Currently most research on air deodorization focusses mainly on the study and accurate measurements of the processing aspect, such as the concentration at the inlet and outlet of the removed compounds, gas flow rates, velocity and remove of the circulating liquid (BTF), pH. Some research groups also perform quantitative measurements on biofilm mass gain, fungal diversity and community structure,

with no qualitative analysis. In contrast, the new research method proposed in P3 and tested in P4, based on the use of flow cytometry, enables easy, quick and relatively cheap testing of the distribution in the life cycle of fungi and the state of viability after immobilization in the tested biofilter at each stage of the process. Thus, the study of the diversity of fungi and the structure of the community inhabiting the biofilter, in terms of quantity and quality, is possible immediately after the immobilization of fungal cells on the internal material, during the biofiltration process, and at its completion. At the same time, these tests allowed for quick adjustments to be made to selected process parameters when a problem was detected. Without this type of research, it was only possible to react to the symptoms, and not the causes of any deterioration in the condition of the microorganisms. Typically, this situation is associated with a deterioration in process performance. So far, tests based on flow cytometry have been widely used in the study of cancer cells, while their slight modification by the use of sodium deoxycholate to increase the diffusion of PI through the fungal cell wall, increasing its penetration into damaged yeast cell membranes, has made it possible to know the condition of cells involved in the process biofiltration. The results of such tests enable the opportunity to quickly learn and interpret the condition of the fungi used, which can allow the process parameters to be optimized to suit the needs of microorganisms, and thus increase the efficiency of the entire biofiltration process. Therefore, the proposed method has the potential to be used on a large scale during biofiltration processes, both on a laboratory and industrial scale.

Another very rarely approach during the analysis of the obtained experimental results was to present the assessment of the affinity of VOCs used for dissolution in water based on the Hansen solubility parameters, instead of the Henry's law commonly used in biofiltration. The obtained biodegradation pattern of the removed compounds was consistent with the Hansen solubility parameters, i.e. the more similar the solubility parameters of the compound and the solvent are, the greater the probability of dissolution of the compound in a given solvent. Therefore, a promising approach in the new research on fungal biofiltration of VOC hydrophobic mixtures is the use of Hansen solubility parameters to predict the biodegradation patterns of compounds obtained as a result of the process.

Another significant problem in the current approach to biofiltration was also noticed, which is the lack of research devoted to understanding the mechanisms responsible for the use of removed compounds as a carbon source by given species of fungi. Analyzing experimental results, it was observed that different patterns of biodegradation were obtained during the removal of mixture of hydrophobic VOCs, depending on the fungi species used. This suggests that understanding the mechanisms occurring in the biofiltration process will allow for the design and optimization of processes with much greater efficiency and usefulness than at present. However, most current research into the use of specific microorganisms to remove selected compounds involves carrying out biofiltration processes without tracking the microorganism's metabolic pathways. Despite the promising results obtained, further research is needed to elucidate the potential inhibitory mechanisms in the mixture to increase the efficiency of the biofiltration process of reducing hydrophobic VOCs by *C. subhashii*. Understanding the mechanisms involved in this process will aid in its

industrial implementation, as well as minimize potential problems associated with the design of new processes.

In conclusion, in this PhD thesis all assumed research tasks were completed, which contributed to the achievement of the main aim, which was to develop an effective air deodorization system from a mixture of hydrophobic VOCs in biofiltration processes inhabited by *C. subhashii* and *F. solani* species.

It should be emphasized, however, that the conducted research is merely a pilot study that requires further improvements.

5. Conclusions on all research carried out in the dissertation

The results from the research presented in the publications P3-P6 allows us to draw the following conclusions.

- Possibility of application for the first time *C. subhashii* to remove hydrophobic VOCs (n-hexane, TCE, toluene and α-pinene) under aerobic conditions in BF and BTF.
- Based on performed comparative analyses presented in the article **P3**, it was shown that the choice of polyurethane foam as the most attractive support material for fungi inoculated in BTF.
- In the article **P3** a new method for studying the diversity and viability of fungi during the biofiltration process was proposed. Proposed method is based on optical microscopy, low cytometry and the tests employing propidium iodide and annexin V, which is fast, simple and relatively cheap. Afterwards in the article **P4** conducted tests led to conclusion that this method makes it possible to optimize the process parameters in accordance with the needs of microorganisms, and thus increase the efficiency of the entire biofiltration process, both at the beginning of the process and during its duration.
- The proposed method in the article **P3** of immobilization of fungi and its evaluation appeared to be effective (viability of the fungi isolated on support materials' surfaces at the average level of 95%), cheap and fast.
- Research presented in the article **P5** showed that *C. subhashii* immobilized on polyurethane foam supported steady state removal efficiencies (REs) of **n-hexane** of 35.7 ± 0.9% (EC = 8.8 ± 0.1 g m^{-3} h^{-1}) in BTF and 25.4 ± 0.9% (EC = 6.4 ± 0.1 g m^{-3} h^{-1}) in BF, **TCE** of 24.0 ± 1.6% (EC = 6.8 ± 0.3 g m^{-3} h^{-1}) in BTF and 20.5 ± 1.0% (EC = 6.3 ± 0.1 g m^{-3} h^{-1}) in BF, **toluene** of 44.0 ± 1.7% (EC = 16.2 ± 0.1 g m^{-3} h^{-1}) in BTF and 19.6 ± 1.5% (EC = 7.3 ± 0.2 g m^{-3} h^{-1}) in BF and **α-pinene** of 26.2 ± 1.8% (EC = 12.7 ± 0.4 g m^{-3} h^{-1}) in BTF and 25.6 ± 2.8% (EC = 11.1 ± 0.7 g m^{-3} h^{-1}) in BF, at relatively short EBRT (30 s).
- The articles **P5** and **P6** show that, BTFs supported a superior biodegradation performance compared to BF, regardless of the VOCs, according to the results of the literature review analysis from the article **P1**.
- The most important conclusion of the article **P6** is reported that *C. subhashii* supported an effective abatement of hydrophobic VOCs at relatively short EBRT regardless (77 s) of the gas-phase bioreactor configuration evaluated, with an efficiency similar to that of *F. solani*. Moreover, the highest efficiency of VOC biodegradation was observed when *C. subhashii* and *F. solani* were grown as a consortium.
  - Steady state ECs of **total VOCs** of 17.4 ± 0.7 g m^{-3} h^{-1} for *C. subhashii*, 21.2 ± 0.8 g m^{-3} h^{-1} for *F. solani* and 24.4 ± 1.4 g m^{-3} h^{-1} for their consortium were recorded in BFs, which increased up to 27.2 ± 1.6 g m^{-3} h^{-1}, 29.2 ± 1.9 g m^{-3} h^{-1}, 37.7 ± 3.3 g m^{-3} h^{-1} in BTFs.
  - Steady state REs of **n-hexane** of 29.5 ± 1.3% (EC = 3.6 ± 0.2 g m^{-3} h^{-1}) for *C. subhashii*, 27.3 ± 1.9% (EC = 3.6 ± 0.3 g m^{-3} h^{-1}) for *F. solani* and 33.7 ± 1.6% (EC = 4.3 ± 0.2 g m^{-3} h^{-1}) for their consortium were recorded in BFs, which increased up to 38.6±2.2% (EC = 5.0 ± 0.3 g m^{-3} h^{-1}), 40.3 ± 3.0% (EC = 5.2 ± 0.4 g m^{-3} h^{-1}), 45.0 ± 2.1% (EC = 5.8 ± 0.4 g m^{-3} h^{-1}) in BTFs, respectively.

The BFs supported steady state REs of **TCE** of 18.4 ± 1.6% (EC = 2.7 ± 0.3 g m^{-3} h^{-1}) for *C. subhashii*, 21.8 ± 1.9% (EC = 3.2 ± 0.3 g m^{-3} h^{-1}) for *F. solani* and 28.4 ± 2.0% (EC = 4.2 ± 0.4 g m^{-3} h^{-1}) for their consortium, which increased up to 30.8 ± 3.5% (EC = 4.5 ± 0.6 g m^{-3} h^{-1}), 33.5±3.3% (EC = 4.9 ± 0.6 g m^{-3} h^{-1}) and 43.4 ± 1.5% (EC = 6.1 ± 0.5 g m^{-3} h^{-1}) in BTFs, respectively. Steady state REs of **toluene** of 28.0 ± 1.3% (EC = 5.6 ± 0.3 g m^{-3} h^{-1}) for *C. subhashii*, 33.8 ± 1.7% (EC = 6.7 ± 0.3 g m^{-3} h^{-1}) for *F. solani*, 38.1 ± 1.4% (EC = 7.6 ± 0.4 g m^{-3} h^{-1}) for their consortium were achieved in BFs. Process operation under BTF configuration resulted in toluene REs of 58.8 ± 3.9% (EC = 11.3 ± 0.8 g m^{-3} h^{-1}), 56.2 ± 4.7% (EC = 9.9 ± 1.1 g m^{-3} h^{-1}), 75.8 ± 0.4% (EC = 13.5 ± 1.7 g m^{-3} h^{-1}) in the BFs inoculated with *C. subhashii*, *F. solani* and their consortium, respectively. Finally, steady state REs of **α-pinene** increased from 29.6 ± 1.8% (EC = 5.6±0.4 g m^{-3} h^{-1}) for *C. subhashii*, 39.5 ± 1.2% (EC = 7.5 ± 0.3 g m^{-3} h^{-1}) for *F. solani* and 43.9 ± 1.5% (EC = 8.4 ± 0.4 g m^{-3} h^{-1}) for their consortium in BFs, up to 41.7 ± 5.0% (EC = 8.0 ± 0.9 g m^{-3} h^{-1}), 49.8 ± 3.8% (EC = 9.5 ± 0.7 g m^{-3} h^{-1}) and 59.6 ± 5.6% (EC = 11.4 ± 1.1 g m^{-3} h^{-1}) in BTFs, respectively.

- The microbial analysis from the article **P6** revealed that the fungi initially introduced in each biofilter represented the dominant fungal species by the end of the experiment, but also *C. subhashii* clearly dominated the fungal population at the end of the experiment when inoculated alone and in combination with *F. solani*.
- All results of the thesis suggest that *C. subhashii* is perspective candidate for degradation of hydrophobic VOCs in gas-phase bioreactors.

Extending the results developed in this thesis, a potential future improvement to these methods would be the use consortium of multiple fungi species in a single BTF, rather than a single strain. A synergistic consortium of fungi could be chosen such that cross-feeding interactions, or the excretion of surfactants to the trickling solution, could increase the effectiveness of hydrophobic VOC removal, due to mutual interactions among the fungi.

To maximize the effectiveness of this method, an increased understanding of both the metabolic pathways involved in removing VOCs in fungi, and the action of the extracellular polymeric substance in the presence of hydrophobic VOCs will be needed to enable us to choose the most effective fungi consortia, as well as to optimize the biofiltration process.

Finally, the environmental benefits of using fungi in the biofiltration of hydrophobic VOCs cannot be overlooked, following as it does the primary principles of green engineering - sustainable development, pollution reduction, and minimizing health and environmental risks.

6. References

[1] Lauderdale CV, Aldrich HC, Lindner AS. Isolation and characterization of a bacterium capable of removing taste- and odor-causing 2-methylisoborneol from water. Water Res 2004; 38: 4135–42. https://doi.org/10.1016/j.watres.2004.08.014.

[2] Jońca J, Pawnuk M, Arsen A, Sówka I. Electronic noses and their applications for sensory and analytical measurements in the waste management plants - A review. Sensors 2022; 22: 1510. https://doi.org/10.3390/S22041510.

[3] Miller U, Sówka I, Grzelka A, Pawnuk M. Application of biological deodorization methods in the aspect of sustainable development. SHS Web Conf., vol. 57, 2018. https://doi.org/10.1051/shsconf/20185702006.

[4] Iranpour R, Cox HH, Deshusses MA, Schroeder ED. Literature review of air pollution control biofilters and biotrickling filters for odor and volatile organic compound removal. Environmental Progress 2005; 24: 254–67. https://doi.org/10.1002/EP.10077.

[5] Nanda S, Sarangi KP, Abraham J. Microbial biofiltration technology for odour abatement: An introductory review. Journal of Soil Science and Environmental Management 2012; 3: 28–35. https://doi.org/10.5897/JSSEM11.090.

[6] WHO. Burden of disease from Household Air Pollution for 2012. Geneva: World Health Organization: 2014. https://doi.org/10.1146/annurev-publhealth-032013-182356.

[7] WHO. Ambient air pollution: a global assessment of exposure and burden of disease. Geneva: World Health Organization: 2016.

[8] WHO. Evolution of WHO air quality guidelines: past, present and future. Geneva: World Health Organization: 2017.

[9] Environment Agency European. Air quality in Europe –2019 report. Geneva: World Health Organization: 2019.

[10] WHO. Burden of disease from the joint effects of household and ambient Air pollution for 2016. Geneva: World Health Organization: 2018.

[11] Śmiełowska M, Marć M, Zabiegała B. Indoor air quality in public utility environments—A review. Environmental Science and Pollution Research 2017; 24: 11166–76. https://doi.org/10.1007/S11356-017-8567-7/FIGURES/1.

[12] Ng LC, Musser A, Persily AK, Emmerich SJ. Indoor air quality analyses of commercial reference buildings. Build Environ 2012; 58: 179–87. https://doi.org/10.1016/J.BUILDENV.2012.07.008.

[13] Righi E, Aggazzotti G, Fantuzzi G, Ciccarese V, Predieri G. Air quality and well-being perception in subjects attending university libraries in Modena (Italy). Science of The Total Environment 2002; 286: 41–50. https://doi.org/10.1016/S0048-9697(01)00960-3.

[14] Lebrero R, Rodríguez E, Estrada JM, García-Encina PA, Muñoz R. Odor abatement in biotrickling filters: Effect of the EBRT on methyl mercaptan and hydrophobic VOCs removal. Bioresour Technol 2012; 109: 38–45. https://doi.org/10.1016/J.BIORTECH.2012.01.052.

[15] Brancher M, Griffiths KD, Franco D, de Melo Lisboa H. A review of odour impact criteria in selected countries around the world. Chemosphere 2017; 168: 1531–70. https://doi.org/10.1016/J.CHEMOSPHERE.2016.11.160.

[16] Dague RR. Fundamentals of Odor Control. J Water Pollut Control Fed 1972; 44: 583–94.

[17] Wise PM, Olsson MJ, Cain WS. Quantification of Odor Quality. Chem Senses 2000; 25: 429–43. https://doi.org/10.1093/CHEMSE/25.4.429.

[18] Kaeppler K, Mueller F. Odor Classification: A review of factors influencing perception-based odor arrangements. Chem Senses 2013; 38: 189–209. https://doi.org/10.1093/CHEMSE/BJS141.

[19] Cantera S, López M, Muñoz R, Lebrero R. Comparative evaluation of bacterial and fungal removal of indoor and industrial polluted air using suspended and packed bed bioreactors. Chemosphere 2022; 308. https://doi.org/10.1016/J.CHEMOSPHERE.2022.136412.

[20] Ghanizadeh F, Godini H. A review of the chemical and biological pollutants in indoor air in hospitals and assessing their effects on the health of patients, staff and visitors. Rev Environ Health 2018; 33: 231–45. https://doi.org/10.1515/REVEH-2018-0011/MACHINEREADABLECITATION/RIS.

[21] Lebrero R, Bouchy L, Stuetz R, Muȕoz R. Odor assessment and management in wastewater treatment plants: a review. Crit Rev Environ Sci Technol 2011; 41: 915–50. https://doi.org/10.1080/10643380903300000.

[22] Jiang C, Li D, Zhang P, Li J, Wang J, Yu J. Formaldehyde and volatile organic compound (VOC) emissions from particleboard: Identification of odorous compounds and effects of heat treatment. Build Environ 2017; 117: 118–26. https://doi.org/10.1016/J.BUILDENV.2017.03.004.

[23] Mackie RI, Stroot PG, Varel VH. Biochemical identification and biological origin of key odor components in livestock waste. J Anim Sci 1998; 76: 1331–42. https://doi.org/10.2527/1998.7651331X.

[24] Van Horn HH, Wilkie AC, Powers WJ, Nordstedt RA. Components of dairy manure management systems. J Dairy Sci 1994; 77: 2008–30. https://doi.org/10.3168/JDS.S0022-0302(94)77147-2.

[25] Monteny GJ, Bannink A, Chadwick D. Greenhouse gas abatement strategies for animal husbandry. Agric Ecosyst Environ 2006; 112: 163–70. https://doi.org/10.1016/J.AGEE.2005.08.015.

[26] Xu L, Hu Y, Liang W. Composition and correlation of volatile organic compounds and odor emissions from typical indoor building materials based on headspace analysis. Build Environ 2022; 221: 109321. https://doi.org/10.1016/J.BUILDENV.2022.109321.

[27] Piccardo MT, Geretto M, Pulliero A, Izzotti A. Odor emissions: a public health concern for health risk perception. Environ Res 2022; 204: 112121. https://doi.org/10.1016/J.ENVRES.2021.112121.

[28] Ministry of Climate and Environment (Ministerstwo Klimatu i Środowiska - Portal gov.pl) https://www.gov.pl/web/klimat (accessed March 20, 2023).

[29] Ministry of Climate and Environment (Ministerstwo Klimatu i Środowiska) Kodeks przeciwdziałania uciążliwości zapachowej. https://www.gov.pl/web/klimat/uciazliwosc-zapachowa (accessed March 12, 2023).

[30] Lewkowska P, Cieślik B, Dymerski T, Konieczka P, Namieśnik J. Characteristics of odors emitted from municipal wastewater treatment plant and methods for their identification and deodorization techniques. Environ Res 2016; 151: 573–86. https://doi.org/10.1016/j.envres.2016.08.030.

[31] Wysocka I, Gębicki J, Namieśnik J. Technologies for deodorization of malodorous gases. Environmental Science and Pollution Research 2019: 1–26. https://doi.org/10.1007/s11356-019-04195-1.

[32] Rybarczyk P, Szulczyński B, Gębicki J, Hupka J. Treatment of malodorous air in biotrickling filters: A review. Biochem Eng J 2019; 141: 146–62. https://doi.org/10.1016/J.BEJ.2018.10.014.

[33] Kośmider J, Mazur-Chrzanowska B, Wyszyński B. Odory. Wydawnictwo Naukowe PWN; 2012.

[34] Bruchet A, Duguet JP. Role of oxidants and disinfectants on the removal, masking and generation of tastes and odours. Water Science and Technology 2004; 49: 297–306. https://doi.org/10.2166/WST.2004.0589.

[35] Kowalczyk A, Piecuch T, Andriyevska L. Zastosowanie olejku eterycznego z sosny do maskowania uciążliwych zapachów wydzielających się w procesie mechanicznego odwadniania komunalnych. Annual Set The Environment Protection 2013; 15: 807–22.

[36] Zhu JC, Chen F, Wang LY, Niu YW, Xiao ZB. Evaluation of the synergism among volatile compounds in Oolong tea infusion by odour threshold with sensory analysis and E-nose. Food Chem 2017; 221: 1484–90. https://doi.org/10.1016/J.FOODCHEM.2016.11.002.

[37] Bindra N, Dubey B, Dutta A. Technological and life cycle assessment of organics processing odour control technologies. Science of The Total Environment 2015; 527–528: 401–12. https://doi.org/10.1016/J.SCITOTENV.2015.05.023.

[38] Zhao Y, Lu W, Wang H. Volatile trace compounds released from municipal solid waste at the transfer stage: Evaluation of environmental impacts and odour pollution. J Hazard Mater 2015; 300: 695–701. https://doi.org/10.1016/J.JHAZMAT.2015.07.081.

[39] Decottignies V, Filippi G, Bruchet A. Characterisation of odour masking agents often used in the solid waste industry for odour abatement. Water Science and Technology 2007; 55: 359–64. https://doi.org/10.2166/WST.2007.198.

[40] Kamal MS, Razzak SA, Hossain MM. Catalytic oxidation of volatile organic compounds (VOCs) – A review. Atmos Environ 2016; 140: 117–34. https://doi.org/10.1016/J.ATMOSENV.2016.05.031.

[41] Kim BR. VOC Emissions from Automotive Painting and Their Control: A Review 2011; 16: 1–9. https://doi.org/10.4491/eer.2011.16.1.001.

[42] Lewandowski DA. Design of thermal oxidation systems for volatile organic compounds. CRC Press; 2017.

[43] Cannon BJ. Dual-chamber RTO oxidizers provide cost-effective VOC compliance for metal finishers and coaters. Metal Finishing 2003; 101: 53–6. https://doi.org/10.1016/S0026-0576(03)80022-3.

[44] Kolta T. Selecting equipment to control air pollution from automotive painting operations. SAE Technical Papers 1992. https://doi.org/10.4271/920189.

[45] Schlegelmilch M, Streese J, Stegmann R. Odour management and treatment technologies: An overview. Waste Management 2005; 25:928–39. https://doi.org/10.1016/J.WASMAN.2005.07.006.

[46] Matros YS, Bunimovich GA, Strots VO, Roach CR, Lorensen CM, Wherrett MR. Retrofitting a regenerative thermal oxidizer in an automotive assembly plant. Metal Finishing 1999; 97: 10–6. https://doi.org/10.1016/S0026-0576(00)82138-8.

[47] Li WB, Wang JX, Gong H. Catalytic combustion of VOCs on non-noble metal catalysts. Catal Today 2009; 148: 81–7. https://doi.org/10.1016/J.CATTOD.2009.03.007.

[48] Hayes RE. Introduction to catalytic combustion. Routledge; 2021.

[49] Zhang Z, Jiang Z, Shangguan W. Low-temperature catalysis for VOCs removal in technology and application: A state-of-the-art review. Catal Today 2016; 264: 270–8. https://doi.org/10.1016/J.CATTOD.2015.10.040.

[50] Everaert K, Baeyens J. Catalytic combustion of volatile organic compounds. J Hazard Mater 2004; 109: 113–39. https://doi.org/10.1016/J.JHAZMAT.2004.03.019.

[51] Szynkowska MI, Wojciechowska E, Weglinska A, Paryjczak T. Odours emission. An environmental protection issue. Przemysł Chemiczny 2009; 88: 712–20.

[52] Miller U, Grzelka A, Romanik E, Kuriata M. Analysis of the application of selected physico-chemical methods in eliminating odor nuisance of municipal facilities. E3S Web of Conferences, vol. 28, 2018. https://doi.org/10.1051/e3sconf/20182801023.

[53] Stryszewska D. Technologie zimnej plazmy. Wytwarzanie, modelowanie, zastosowanie. Elektryka 2011; 1.

[54] Stryczewska D. Technologie plazmowe w energetyce i inżynierii środowiska. Wydawnictwo Politechniki Lubelskiej; 2009.

[55] Yepez X V., Misra NN, Keener KM. Nonthermal plasma technology. Food Engineering Series 2020: 607–28. https://doi.org/10.1007/978-3-030-42660-6_23/FIGURES/4.

[56] Yamamoto T, Okubo M. Nonthermal plasma technology. Advanced Physicochemical Treatment Technologies 2007: 135–293. https://doi.org/10.1007/978-1-59745-173-4_4.

[57] Vandenbroucke AM, Morent R, De Geyter N, Leys C. Non-thermal plasmas for non-catalytic and catalytic VOC abatement. J Hazard Mater 2011; 195: 30–54. https://doi.org/10.1016/J.JHAZMAT.2011.08.060.

[58] Mizuno A. Generation of non-thermal plasma combined with catalysts and their application in environmental technology. Catal Today 2013; 211: 2–8. https://doi.org/10.1016/J.CATTOD.2013.03.029.

[59] Guieysse B, Hort C, Platel V, Munoz R, Ondarts M, Revah S. Biological treatment of indoor air for VOC removal: Potential and challenges. Biotechnol Adv 2008; 26: 398–410. https://doi.org/10.1016/J.BIOTECHADV.2008.03.005.

[60] Prabhat Kumar T, Anil Kumar M, Chandrajit B. Biofiltration of Volatile Organic Compounds (VOCs)-An Overview. Research Journal of Chemical Sciences 2011; 1: 83–92.

[61] López ME, Rene ER, Veiga MC, Kennes C. Biogas technologies and cleaning techniques. Environmental Chemistry for a Sustainable World 2012; 2: 347–77. https://doi.org/10.1007/978-94-007-2439-6_9/FIGURES/11.

[62] Freudenthal K, Otterpohl R, Behrendt J. Absorption of odourous substances using selective gas–liquid separation processes. Waste Management 2005; 25: 975–84. https://doi.org/10.1016/J.WASMAN.2005.07.007.

[63] States Environmental Protection Agency - Office of Water U. Biosolids and Residuals Management Fact Sheet Odor Control in Biosolids Management. 2000.

[64] Riva G, Sacco A. Sewage and wastewater odor control. Ozone News 2006; 34: 17–20.

[65] Boon AG, Boon K. Catalytic-iron filters for effective and low-cost treatment of odorous air. Water and Environment Journal 1999; 13: 189–94. https://doi.org/10.1111/J.1747-6593.1999.TB01033.X.

[66] Żarczyński A, Rosiak K, Anielak P, Ziemiński K, Wolf W. Praktyczne metody usuwania siarkowodoru z biogazu. II, Zastosowanie roztworów sorpcyjnych i metod biologicznych. Acta Innovations 2015; 15: 57–71.

[67] Gholami Z, Vakili M, Gholami F. Removal of hydrogen sulfide from gaseous streams by a chemical method using ferric sulfate solution. World Appl Sci J 2012; 19: 241–5. https://doi.org/10.5829/idosi.wasj.2012.19.02.1541.

[68] Darlington A, Chan M, Malloch D, Pilger C, Dixon MA. The biofiltration of indoor air: implications for air quality. Indoor Air 2000; 10: 39–46. https://doi.org/10.1034/j.1600-0668.2000.010001039.x.

[69] Barbusinski K, Kalemba K, Kasperczyk D, Urbaniec K, Kozik V. Biological methods for odor treatment – A review. J Clean Prod 2017; 152: 223–41. https://doi.org/10.1016/J.JCLEPRO.2017.03.093.

[70] van Groenestijn JW, Hesselink PGM. Biotechniques for air pollution control. Biodegradation 1993; 4: 283–301. https://doi.org/10.1007/BF00695975.

[71] Prado ÓJ, Veiga MC, Kennes C. Removal of formaldehyde, methanol, dimethylether and carbon monoxide from waste gases of synthetic resin-producing industries. Chemosphere 2008; 70: 1357–65. https://doi.org/10.1016/J.CHEMOSPHERE.2007.09.039.

[72] Devinny JS, Ramesh J. A phenomenological review of biofilter models. Chemical Engineering Journal 2005; 113: 187–96. https://doi.org/10.1016/j.cej.2005.03.005.

[73] Jasiczak J. Kryteria doboru powłok ochronnych w betonowych obiektach oczyszczania ścieków. Materiały budowlane 2019; 1: 31–4. https://doi.org/10.15199/33.2019.05.04.

[74] Estrada JM, Kraakman B, Lebrero R, Muñoz R. A sensitivity analysis of process design parameters, commodity prices and robustness on the economics of odour abatement technologies. Biotechnol Adv 2012; 30: 1354–63. https://doi.org/10.1016/j.biotechadv.2012.02.010.

[75] Estrada JM, Bart Kraakman NJR, Muñoz R, Lebrero R. A comparative analysis of odour treatment technologies in wastewater treatment plants. Environ Sci Technol 2011; 45: 1100–6. https://doi.org/10.1021/es103478j.

[76] Cohen Y. Biofiltration – the treatment of fluids by microorganisms immobilized into the filter bedding material: a review. Bioresour Technol 2001; 77: 257–74. https://doi.org/10.1016/S0960-8524(00)00074-2.

[77] Cox HHJ, Deshusses MA. Biotrickling Filters, Springer, Dordrecht; 2001, p. 99–131. https://doi.org/10.1007/978-94-017-0930-9_4.

[78] Gao L, Keener TC, Zhuang L, Siddiqui KF. A technical and economic comparison of biofiltration and wet chemical oxidation (scrubbing) for odor control at wastewater treatment plants. Environ Eng Policy 2001:203–12. https://doi.org/10.1007/s100220100036.

[79] McNevin D, Barford J. Biofiltration as an odour abatement strategy. Biochem Eng J 2000; 5: 231–42. https://doi.org/10.1016/S1369-703X(00)00064-4.

[80] Fernández M, Ramírez M, Pérez RM, Gómez JM, Cantero D. Hydrogen sulphide removal from biogas by an anoxic biotrickling filter packed with Pall rings. Chemical Engineering Journal 2013; 225: 456–63. https://doi.org/10.1016/J.CEJ.2013.04.020.

[81] Devinny JS, Deshusses MA, Webster TS. Biofiltration for air pollution control. Lewis publishers; 1999.

[82] Bajpai P, Bajpai PK, Kondo R. Biotechnology for environmental protection in the pulp and paper industry. Springer-Verlag Berlin Heidelberg, Germany; 1999.

[83] Barbusiński K, Kalemba K. Use of biological methods for removal of H_2S from biogas in wastewater treatment plants–a review. Architecture Civil Engineering Environment 2016:103–12.

[84] Khan FI, Ghoshal A. Removal of Volatile Organic Compounds from polluted air. J Loss Prev Process Ind 2000; 13: 527–45. https://doi.org/10.1016/S0950-4230(00)00007-3.

[85] Oliva G, Zarra T, Naddeo V, Munoz R, Lebrero R, Angeles R, et al. Comparative analysis of AOPs and biological processes for the control of VOCs industrial emissions. Chem Eng Trans, vol. 68, 2018, p. 451–6. https://doi.org/10.3303/CET1868076.

[86] Yang C, Qian H, Li X, Cheng Y, He H, Zeng G, et al. Simultaneous removal of multicomponent VOCs in biofilters. Trends Biotechnol 2018; 36: 673–85. https://doi.org/10.1016/J.TIBTECH.2018.02.004.

[87] Mohseni M, Allen DG. Biofiltration of mixtures of hydrophilic and hydrophobic volatile organic compounds. Chem Eng Sci 2000; 55: 1545–58. https://doi.org/10.1016/S0009-2509(99)00420-0.

[88] Cheng Y, He H, Yang C, Zeng G, Li X, Chen H, et al. Challenges and solutions for biofiltration of hydrophobic volatile organic compounds. Biotechnol Adv 2016; 34: 1091–102. https://doi.org/10.1016/J.BIOTECHADV.2016.06.007.

[89] Lebrero R, Hernández M, Quijano G, Muñoz R. Hexane biodegradation in two-liquid phase biofilters operated with hydrophobic biomass: Effect of the organic phase-packing media ratio and the irrigation rate. Chemical Engineering Journal 2014; 237: 162–8. https://doi.org/10.1016/J.CEJ.2013.10.016.

[90] Deshusses MA, Johnson CT. Development and validation of a simple protocol to rapidly determine the performance of biofilters for VOC treatment. Environ Sci Technol 2000; 34: 461–7. https://doi.org/10.1021/ES9909172/ASSET/IMAGES/LARGE/ES9909172F00008.JPEG.

[91] Rybarczyk P. Removal of volatile organic compounds (VOCs) from air: focus on biotrickling filtration and process modeling. Processes 2022; 10. https://doi.org/10.3390/PR10122531.

[92] Miller U, Sówka I, Adamiak W. The effect of betaine on the removal of toluene by biofiltration. SN Appl Sci 2019; 1: 1–8. https://doi.org/10.1007/S42452-019-0832-6/FIGURES/4.

[93] Avalos Ramirez A, García-Aguilar BP, Jones JP, Heitz M. Improvement of methane biofiltration by the addition of non-ionic surfactants to biofilters packed with inert materials. Process Biochemistry 2012; 47: 76–82. https://doi.org/10.1016/J.PROCBIO.2011.10.007.

[94] Bailón L, Nikolausz M, Kästner M, Veiga MC, Kennes C. Removal of dichloromethane from waste gases in one- and two-liquid-phase stirred tank bioreactors and biotrickling filters. Water Res 2009; 43: 11–20. https://doi.org/10.1016/J.WATRES.2008.09.031.

[95] Arriaga S, Muñoz R, Hernández S, Guieysse B, Revah S. Gaseous hexane biodegradation by *Fusarium solani* in two liquid phase packed-bed and stirred-tank bioreactors. Environ Sci Technol 2006; 40: 2390–5. https://doi.org/10.1021/es051512m.

[96] Ralebitso-Senior TK, Senior E, Di Felice R, Jarvis K. Waste gas biofiltration: Advances and limitations of current approaches in microbiology. Environ Sci Technol 2012; 46: 8542–73. https://doi.org/10.1021/ES203906C/ASSET/IMAGES/LARGE/ES-2011-03906C_0001.JPEG.

[97] Vergara-Fernández A, Hernández S, Revah S. Phenomenological model of fungal biofilters for the abatement of hydrophobic VOCs. Biotechnol Bioeng 2008; 101: 1182–92. https://doi.org/10.1002/BIT.21989.

[98] Lamprea Pineda PA, Demeestere K, Toledo M, Van Langenhove H, Walgraeve C. Enhanced removal of hydrophobic volatile organic compounds in biofilters and biotrickling filters: A review on the use of surfactants and the addition of hydrophilic compounds. Chemosphere 2021; 279: 130757. https://doi.org/10.1016/J.CHEMOSPHERE.2021.130757.

[99] Kwarciak-Kozłowska A, Bańka B. Biofiltracja jako metoda unieszkodliwiania odorów powstających podczas kompostowania frakcji biodegradowalnej odpadów komunalnych i przemysłowych. Inżynieria i Ochrona Środowiska 2014; 17: 631–45.

[100] Soccol CR, Woiciechowski AL, Vandenberhe LPS, Kaskantis GN, Soccol VT. Biofiltration: An emerging technology. Indian J Biotechnol 2003; 2: 396–410.

[101] Szklarczyk M, Czemarmazowicz M, Adamiak W. Biologiczne oczyszczanie gazów stan obecny i perspektywy rozwoju. Biotechnologia 1997; 36: 108–16.

[102] Šimončicová J, Kryštofová S, Medvecká V, Ďurišová K, Kaliňáková B. Technical applications of plasma treatments: Current state and perspectives. Appl Microbiol Biotechnol 2019; 103: 5117–29.

[103] Pomeroy RD. Deodorizing gas streams by the use of microbiological growth. US Patent 2793096, 1957.

[104] Kołwzan B, Adamiak W, Dziubek AM. Możliwości zastosowania grzybów w technologiach oczyszczania i remediacji wybranych elementów środowiska. Ochrona Środowiska 2018;40.

[105] Vitko TG, Cowden S, Suffet IH (Mel). Evaluation of bioscrubber and biofilter technologies treating wastewater foul air by a new approach of using odor character, odor intensity, and chemical analyses. Water Res 2022; 220: 118691. https://doi.org/10.1016/J.WATRES.2022.118691.

[106] Deshusses MA. Biological waste air treatment in biofilters. Curr Opin Biotechnol 1997; 8: 335–9. https://doi.org/10.1016/S0958-1669(97)80013-4.

[107] Malhautier L, Khammar N, Bayle S, Fanlo JL. Biofiltration of volatile organic compounds. Appl Microbiol Biotechnol 2005; 68: 16–22. https://doi.org/10.1007/S00253-005-1960-Z/FIGURES/1.

[108] Toledo-Cervantes A, Lebrero R, Cavinato C, Muñoz R. Biogas upgrading using algal-bacterial processes. Microalgae-Based Biofuels and Bioproducts 2017: 283–304. https://doi.org/10.1016/B978-0-08-101023-5.00012-1.

[109] Estrada JM, Hernández S, Muñoz R, Revah S. A comparative study of fungal and bacterial biofiltration treating a VOC mixture. J Hazard Mater 2013; 250–251: 190–7. https://doi.org/10.1016/J.JHAZMAT.2013.01.064.

[110] Chmiel K, Jastrzębski AB, Palica M. Biofiltracja lotnych związków organicznych. Przemysł Chemiczny 2005; 6: 442–5.

[111] Wu H, Yan H, Quan Y, Zhao H, Jiang N, Yin C. Recent progress and perspectives in biotrickling filters for VOCs and odorous gases treatment. J Environ Manage 2018; 222: 409–19. https://doi.org/10.1016/J.JENVMAN.2018.06.001.

[112] Kim S, Deshusses MA. Determination of mass transfer coefficients for packing materials used in biofilters and biotrickling filters for air pollution control. 1. Experimental results. Chem Eng Sci 2008; 63: 841–55. https://doi.org/10.1016/J.CES.2007.10.011.

[113] Cabrol L, Malhautier L. Integrating microbial ecology in bioprocess understanding: the case of gas biofiltration. Applied Microbiology and Biotechnology 2011; 90: 837–49. https://doi.org/10.1007/S00253-011-3191-9.

[114] García-Peña I, Hernández S, Auria R, Revah S. Correlation of biological activity and reactor performance in biofiltration of toluene with the fungus *Paecilomyces variotii* CBS115145. Appl Environ Microbiol 2005; 71: 4280. https://doi.org/10.1128/AEM.71.8.4280-4285.2005.

[115] De Vela RJL, Gostomski PA. Minimising biomass accumulation in biotrickling filters. Reviews in Environmental Science and Bio/Technology 2018 17:3 2018; 17: 417–30. https://doi.org/10.1007/S11157-018-9471-4.

[116] Cox HH, Deshusses MA. Biological waste air treatment in biotrickling filters. Curr Opin Biotechnol 1998; 9: 256–62. https://doi.org/10.1016/S0958-1669(98)80056-6.

[117] Lalanne F, Malhautier L, Roux J-C, Fanlo J-L. Absorption of a mixture of volatile organic compounds (VOCs) in aqueous solutions of soluble cutting oil. Bioresour Technol 2008; 99: 1699–707. https://doi.org/10.1016/J.BIORTECH.2007.04.006.

[118] Srivastava NK, Majumder CB. Novel biofiltration methods for the treatment of heavy metals from industrial wastewater. Ournal of Hazardous Materials 2008; 151: 1–8.

[119] López ME, Rene ER, Malhautier L, Rocher J, Bayle S, Veiga MC, et al. One-stage biotrickling filter for the removal of a mixture of volatile pollutants from air: Performance and microbial community analysis. Bioresour Technol 2013; 138: 245–52. https://doi.org/10.1016/J.BIORTECH.2013.03.136.

[120] Delhoménie M-C, Bibeau L, Gendron J, Brzezinski R, Heitz M, Delhomé M-C, et al. Degradation of toluene, xylene, and trimethylbenzene vapors by biofiltration: a comparison. J Air Waste Manage Assoc 2012; 53: 217–26. https://doi.org/10.1080/10473289.2003.10466137.

[121] Machnik G. Techniki molekularne wykorzystywane w analizie mutacji i polimorfizmów. PPM 2020.

[122] Etscheid M, Riesner D. TGGE and DGGE. Molecular Tools for Screening Biodiversity: Plants and Animals 1998.

[123] Chung YC. Evaluation of gas removal and bacterial community diversity in a biofilter developed to treat composting exhaust gases. J Hazard Mater 2007; 144: 377–85. https://doi.org/10.1016/j.jhazmat.2006.10.045.

[124] Xie B, Liang SB, Tang Y, Mi WX, Xu Y. Petrochemical wastewater odor treatment by biofiltration. Bioresour Technol 2009; 100: 2204–9. https://doi.org/10.1016/J.BIORTECH.2008.10.035.

[125] Revah S, Vergara-Fernández A, Hernández S. Fungal biofiltration for the elimination of gaseous pollutants from air. Mycofactories. Bentham Science Publishers Ltds, 2011, p. 109–20.

[126] Detchanamurthy S, Gostomski PA. Biofiltration for treating VOCs: An overview. Rev Environ Sci Biotechnol 2012; 11: 231–41. https://doi.org/10.1007/S11157-012-9288-5/FIGURES/3.

[127] Wu X, Lin Y, Wang Y, Yang C. Interactive effects of dual short-chain n-alkanes on removal performances and microbial responses of biotrickling filters. Chemical Engineering Journal 2023; 461. https://doi.org/10.1016/J.CEJ.2023.141747.

[128] Vergara-Fernández A, Revah S, Moreno-Casas P, Scott F. Biofiltration of volatile organic compounds using fungi and its conceptual and mathematical modeling. Biotechnol Adv 2018; 36: 1079–93. https://doi.org/10.1016/j.biotechadv.2018.03.008.

[129] Lebrero R, López JC, Lehtinen I, Pérez R, Quijano G, Muñoz R. Exploring the potential of fungi for methane abatement: Performance evaluation of a fungal-bacterial biofilter. Chemosphere 2016; 144: 97–106. https://doi.org/10.1016/J.CHEMOSPHERE.2015.08.017.

[130] Vergara-Fernandez A, Scott F, Carreno-Lopez F, Aroca G, Moreno-Casas P, Gonzalez-Sanchez A, et al. A comparative assessment of the performance of fungal-bacterial and fungal biofilters for methane abatement. J Environ Chem Eng 2020; 8: 104421. https://doi.org/10.1016/J.JECE.2020.104421.

[131] Rodríguez E, García-Encina PA, Muñoz R, Lebrero R. Microbial community changes during different empty bed residence times and operational fluctuations in an air diffusion reactor for odor abatement. Sci Total Environ 2017; 590–591: 352–60. https://doi.org/10.1016/J.SCITOTENV.2017.01.161.

[132] Delhoménie M-C, Heitz M. Biofiltration of air: a review. Crit Rev Biotechnol 2005; 25: 53–72.

[133] Vergara-Fernández A, Hernández S, Revah S. Elimination of hydrophobic volatile organic compounds in fungal biofilters: Reducing start-up time using different carbon sources. Biotechnol Bioeng 2011; 108: 758–65. https://doi.org/10.1002/BIT.23003.

[134] Zhang Y, Liu J, Xing H, Li J. Effect of liquid supply on the performance of a fungal bio-trickling filter treating hydrophobic VOC. Biochem Eng J 2020;161:107658. https://doi.org/10.1016/J.BEJ.2020.107658.

[135] Vergara-Fernández A, Salgado-Ísmodes V, Pino M, Hernández S, Revah S. Temperature and moisture effect on spore emission in the fungal biofiltration of hydrophobic VOCs. Journal of Environmental Science and Health, Part A 2012; 47: 605–13. https://doi.org/10.1080/10934529.2012.650581.

[136] Zheng M, Li C, Liu S, Gui M, Ni J. Potential application of aerobic denitrifying bacterium *Pseudomonas aeruginosa* PCN-2 in nitrogen oxides (NOx) removal from flue gas. J Hazard Mater 2016; 318: 571–8. https://doi.org/10.1016/J.JHAZMAT.2016.07.047.

[137] Giri BS, Kim KH, Pandey RA, Cho J, Song H, Kim YS. Review of biotreatment techniques for volatile sulfur compounds with an emphasis on dimethyl sulfide. Process Biochemistry 2014; 49: 1543–54. https://doi.org/10.1016/J.PROCBIO.2014.05.024.

[138] Li Y, Zhang W, Xu J. Siloxanes removal from biogas by a lab-scale biotrickling filter inoculated with *Pseudomonas aeruginosa* S240. J Hazard Mater 2014; 275: 175–84. https://doi.org/10.1016/J.JHAZMAT.2014.05.008.

[139] He S, Ni Y, Lu L, Chai Q, Liu H, Yang C. Enhanced biodegradation of n-hexane by *Pseudomonas* sp. strain NEE2. Scientific Reports 2019 9:1 2019; 9: 1–9. https://doi.org/10.1038/s41598-019-52661-0.

[140] Zare H, Najafpour G, Rahimnejad M, Tardast A, Gilani S. Biofiltration of ethyl acetate by *Pseudomonas putida* immobilized on walnut shell. Bioresour Technol 2012; 123: 419–23. https://doi.org/10.1016/J.BIORTECH.2012.07.036.

[141] Kumar A, Dewulf J, Luvsanjamba M, Van Langenhove H. Continuous operation of membrane bioreactor treating toluene vapors by *Burkholderia vietnamiensis* G4. Chemical Engineering Journal 2008; 140: 193–200. https://doi.org/10.1016/J.CEJ.2007.09.039.

[142] Zampolli J, Orro A, Manconi A, Ami D, Natalello A, Di Gennaro P. Transcriptomic analysis of *Rhodococcus opacus* R7 grown on polyethylene by RNA-seq. Scientific Reports 2021; 11: 1–14. https://doi.org/10.1038/s41598-021-00525-x.

[143] Zampolli J, Di Canito A, Manconi A, Milanesi L, Di Gennaro P, Orro A. Transcriptomic Analysis of *Rhodococcus opacus* R7 Grown on o-Xylene by RNA-Seq. Front Microbiol 2020; 11: 1808. https://doi.org/10.3389/FMICB.2020.01808/BIBTEX.

[144] Balasubramanian P, Philip L, Murty Bhallamudi S. Biotrickling filtration of VOC emissions from pharmaceutical industries. Chemical Engineering Journal 2012; 209: 102–12. https://doi.org/10.1016/J.CEJ.2012.04.020.

[145] Cox H. Styrene removal from waster gas by the fungus *Exophiala jeanselmei* in a biofilter. Rijksuniversiteit Groningen, the Netherlands, 1995.

[146] van Groenestijn J, van Heininge W, Kraakman N. Biofilters based on the action of fungi. Water Sci Technol 2001; 44: 227–32.

[147] van Groenestijn J, Harkes M, Cox H, Doddema H. Ceramic materials in biofiltration. Proceedings USC-TRG Conference on Biofiltration, Los Angeles, USA: 1995.

[148] Kennes C, Veiga MC. Bioreactors for Waste Gas Treatment environmental pollution. Dordrecht: 2001.

[149] Jin Y, Guo L, Veiga MC, Kennes C. Fungal biofiltration of α-pinene: Effects of temperature, relative humidity, and transient loads. Biotechnol Bioeng 2007; 96: 433–43. https://doi.org/10.1002/bit.21123.

[150] Shareefdeen Z, Herner B, Singh A. Biotechnology for Air Pollution Control. Springer, Berlin-Heidelberg-New York: 2005.

[151] Vergara-Fernández A, van Haaren B, Revah S. Phase partition of gaseous hexane and surface hydrophobicity of *Fusarium solani* when grown in liquid and solid media with hexanol and hexane. Biotechnol Lett 2006; 28: 2011–7. https://doi.org/10.1007/s10529-006-9186-4.

[152] Tymiński ŁP, Znajewska Z, Dąbrowska GB. Characteristics and functions of hydrophobins and their use in manifold industries. Postępy Mikrobiologii 2018; 4.

[153] Wösten HAB. Hydrophobins: Multipurpose Proteins. Annu Rev Microbiol 2001; 55: 625–46. https://doi.org/10.1146/annurev.micro.55.1.625.

[154] Jorio H, Jin Y, Elmrini H, Nikiema J, Brzezinski R, Heitz M. Treatment of VOCs in biofilters inoculated with fungi and microbial consortium. Environ Technol 2009; 30: 477–85. https://doi.org/10.1080/09593330902778849.

[155] Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbiology and Molecular Biology Reviews 1997; 61: 47–64. https://doi.org/10.1128/MMBR.61.1.47-64.1997.

[156] Souza EC, Vessoni-Penna TC, De Souza Oliveira RP. Biosurfactant-enhanced hydrocarbon bioremediation: An overview. Int Biodeterior Biodegradation 2014; 89: 88–94. https://doi.org/10.1016/J.IBIOD.2014.01.007.

[157] Van Hamme JD, Singh A, Ward OP. Physiological aspects: Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnol Adv 2006; 24: 604–20. https://doi.org/10.1016/J.BIOTECHADV.2006.08.001.

[158] Mulligan CN, Yong RN, Gibbs BF. Surfactant-enhanced remediation of contaminated soil: a review. Eng Geol 2001; 60: 371–80. https://doi.org/10.1016/S0013-7952(00)00117-4.

[159] Pacwa-Płociniczak M, Płaza GA, Piotrowska-Seget Z, Cameotra SS. Environmental applications of biosurfactants: recent advances. International Journal of Molecular Sciences 2011, Vol 12, Pages 633-654 2011; 12: 633–54. https://doi.org/10.3390/IJMS12010633.

[160] Mulligan CN. Environmental applications for biosurfactants. Environmental Pollution 2005; 133: 183–98. https://doi.org/10.1016/J.ENVPOL.2004.06.009.

[161] Sobrinho HBS, Luna JM, Rufino RD, Porto A, Sarubbo LA. Biosurfactants: classification, properties and environmental applications. Recent Developments in Biotechnology 2014; 11: 1–29.

[162] Miller U, Sówka I, Skrętowicz M. Zastosowanie surfaktantów w biotechnologii środowiska. EKO-DOK 11th conference on interdisciplinary problems in environmental protection and engineering, Polanica-Zdrój: 2019.

[163] Liu Q, Li M, Chen R, Li Z, Qian G, An T, et al. Biofiltration treatment of odors from municipal solid waste treatment plants. Waste Management 2009; 29: 2051–8. https://doi.org/10.1016/J.WASMAN.2009.02.002.

[164] Prenafeta-Boldú FX, Roca N, Villatoro C, Vera L, de Hoog GS. Prospective application of melanized fungi for the biofiltration of indoor air in closed bioregenerative systems. J Hazard Mater 2019; 361 :1–9. https://doi.org/10.1016/J.JHAZMAT.2018.08.059.

[165] Kraakman NJR, Rocha-Rios J, van Loosdrecht MCM. Review of mass transfer aspects for biological gas treatment. Appl Microbiol Biotechnol 2011; 91: 873–86. https://doi.org/10.1007/s00253-011-3365-5.

[166] Vergara-Fernández A, Scott F, Moreno-Casas P, Díaz-Robles L, Muñoz R. Elucidating the key role of the fungal mycelium on the biodegradation of n-pentane as a model hydrophobic VOC. Chemosphere 2016; 157: 89–96. https://doi.org/10.1016/J.CHEMOSPHERE.2016.05.034.

[167] Vergara-Fernández A, Yánez D, Morales P, Scott F, Aroca G, Diaz-Robles L, et al. Biofiltration of benzo[α]pyrene, toluene and formaldehyde in air by a consortium of *Rhodococcus erythropolis* and *Fusarium solani*: Effect of inlet loads, gas flow and temperature. Chemical Engineering Journal 2018; 332: 702–10. https://doi.org/10.1016/J.CEJ.2017.09.095.

[168] Vergara-Fernández A, Fernández F, Hernández S, Hernández H, Ul Muñoz R, Muñoz M, et al. Influence of the inlet load, EBRT and mineral medium addition on spore emission by *Fusarium solani* in the fungal biofiltration of hydrophobic VOCs. Journal of Chemical Technology & Biotechnology 2012; 87: 778–84. https://doi.org/10.1002/JCTB.3762.

[169] Baig ZT, Meng L, Saingam P, Xi J. Ozonation and depolymerization of extracellular polymeric substances (EPS) extracted from a biofilter treating gaseous toluene. Polymers 2018, Vol 10, Page 763 2018;10:763. https://doi.org/10.3390/POLYM10070763.

[170] Flemming HC, Wingender J. Relevance of microbial extracellular polymeric substances (EPSs) - Part I: Structural and ecological aspects. Water Science and Technology, vol. 43, IWA Publishing; 2001, p. 1–8. https://doi.org/10.2166/wst.2001.0326.

[171] Späth R, Flamming HC, Wuertz S. Sorption properties of biofilms. Water Science and Technology 1998; 37: 207–10. https://doi.org/10.1016/S0273-1223(98)00107-3.

[172] Flemming HC, Neu TR, Wingender J. The perfect slime: microbial extracellular polymeric substances (EPS). IWA Publishing; 2016.

[173] Gospodarek M, Rybarczyk P, Brillowska-Dąbrowska A, Gębicki J. The use of various species of fungi in biofiltration of air contaminated with odorous volatile organic compounds. E3S Web of Conferences 2019; 100: 00021. https://doi.org/10.1051/e3sconf/201910000021.

[174] Brillowska-Dąbrowska A, Świerkowska A, Lindhardt Saunte DM, Arendrup MC. Diagnostic PCR tests for *Microsporum audouinii*, *M. canis* and *Trichophyton* infections. Med Mycol 2010; 48: 486–90. https://doi.org/10.3109/13693780903312454.

[175] Lehrer RI, Cline MJ. Interaction of *Candida albicans* with human leukocytes and serum. J Bacteriol 1969; 98: 996–1004.

[176] Sudbery PE. Growth of *Candida albicans* hyphae. Nature Reviews Microbiology 2011 9:10 2011; 9: 737–48. https://doi.org/10.1038/nrmicro2636.

7. Scientific achievements

$\Sigma_{IF} = 45.186$

Total N° of citations (Scopus): 97

H index (Scopus): 5

7.1. Publications from the JCR list

1. **Marycz, M.**, Brillowska-Dąbrowska, A., Cantera, S., Gębicki, J., & Muñoz, R. (2023). Fungal co-culture improves the biodegradation of hydrophobic VOCs gas mixtures in conventional biofilters and biotrickling filters. *Chemosphere*, *313*, 137609. doi.org/10.1016/j.chemosphere.2022.137609 (IF=8.943)
2. **Marycz, M.**, Rodríguez, Y., Gębicki, J., Muñoz, R. (2022). Systematic comparison of a biotrickling filter and a conventional filter for the removal of a mixture of hydrophobic VOCs by *Candida subhashii*. *Chemosphere*, 135608. doi.org/10.1016/j.chemosphere.2022.135608 (IF=8.943)
3. **Marycz, M.**, Brillowska-Dąbrowska, A., Muñoz R., Gębicki, J. (2022). A state of the art review on the use of fungi in biofiltration to remove volatile hydrophobic pollutants. *Reviews in Environmental Science and Bio/Technology*, 21(1), 225-246. doi.org/10.1007/s11157-021-09608-7 (IF=14.284)
4. Rybarczyk, P., **Marycz, M.**, Szulczyński, B., Brillowska-Dąbrowska, A., Rybarczyk, A., Gębicki, J. (2021). Removal of cyclohexane and ethanol from air in biotrickling filters inoculated with *Candida albicans* and *Candida subhashii*. *Archives of Environmental Protection*, 47(1), 16-34. doi.org/10.24425/aep.2021.136445 (IF=1.872)
5. **Marycz, M.**, Brillowska-Dąbrowska, A., Gębicki, J. (2020). Evaluation of immobilization of selected peat-isolated yeast strains of the Species *Candida albicans* and *Candida subhashii* on the surface of artificial support materials used for biotrickling filtration. *Processes*, *8*(7), 801. doi.org/10.3390/pr8070801 (IF=3.352)
6. Szulczyński, B., Rybarczyk, P., **Marycz, M.**, Gębicki, J. (2020). Enhancing the removal efficiency of cyclohexane in biotrickling filtration process monitored by electronic nose. *Chemical Engineering Transactions*, *82*, 433-438. doi.org/10.3303/CET2082073 (IF=0.681)
7. **Gospodarek, M**., Rybarczyk, P., Szulczyński, B., & Gębicki, J. (2019). Comparative evaluation of selected biological methods for the removal of hydrophilic and hydrophobic odorous VOCs from air. *Processes*, 7(4), 187. doi.org/10.3390/pr7040187 (IF=3.352)
8. Szulczyński, B., Rybarczyk, P., **Gospodarek, M.**, & Gębicki, J. (2019). Biotrickling filtration of n-butanol vapors: process monitoring using electronic nose and artificial neural network. *Monatshefte für Chemie-Chemical Monthly*, 150(9), 1667-1673. doi.org/10.1007/s00706-019-02456-w (IF=1.613)
9. Rybarczyk, P., Szulczyński, B., **Gospodarek, M.**, & Gębicki, J. (2020). Effects of n-butanol presence, inlet loading, empty bed residence time and starvation periods on the performance of a biotrickling filter removing cyclohexane vapors from air. *Chemical Papers*, 74(3), 1039-1047. doi.org/10.1007/s11696-019-00943-2 (IF=2.146)

7.2. Other publications in peer-reviewed journals

1. **Marycz, M.** (2020). The use of natural and synthetic packing materials in conventional biofilters and in biotrickling filters. Laborant, 14(1).
2. **Gospodarek, M.**, Rybarczyk, P., Brillowska-Dąbrowska, A., & Gębicki, J. (2019). The use of various species of fungi in biofiltration of air contaminated with odorous volatile organic compounds. In E3S Web of Conferences (Vol. 100, p. 00021). EDP Sciences. doi.org/10.1051/e3sconf/201910000021.
3. Rybarczyk, P., **Gospodarek, M.**, Szulczyński, B., Gębicki, J., & Namiesnik, J. (2019). Laboratory biotrickling filter for the removal of odorous volatile compounds from air. Scientific and didactic equipment, 24.
4. **Gospodarek, M.**, Rybarczyk, P., Gębicki, J. (2018). Porównanie skuteczności dezodoryzacji powietrza metodami biologicznymi. *XIV Konferencja DLA MIASTA I ŚRODOWISKA – Problemy Unieszkodliwiania Odpadów*, 1-6.
5. **Gospodarek, M.**, Rybarczyk, P., Gębicki, J. (2018). Aktualny stan prawny w zakresie przeciwdziałania uciążliwościom zapachowym. *XIV Konferencja DLA MIASTA I ŚRODOWISKA – Problemy Unieszkodliwiania Odpadów*, 1-6.

7.3. Chapters in book monographs

1. Augustin, E., Kwaśniewska, A., **Marycz, M.**, Mazerska, Z., Mieszkowska, A., Potęga, A., Pilch, J., Wandas. A. Biochemia: materiały do zajęć laboratoryjnych: praca zbiorowa. Wydawnictwo Politechniki Gdańskiej, 2022, ISBN 8373488499, 9788373488496

7.4. Conference presentations

1. The role of microorganisms in air deodorization by biofiltration, IV Interdisciplinary Academic Conference on Environmental Protection (IAKOS), 05-07.04.2019, Gdansk, Poland.
2. Biological methods of air deodorization, XIV Conferences for the city and the environment - problems of waste disposal, 26.11.2018, Warsaw, Poland.
3. Removal of VOCs from air and assessment of dominant species in a peat-perlite biotrickling filter, 14th Summer School for Graduate Students and Young Researchers 'Interfacial Phenomena in Theory and Practice' organized by the Department of Process Engineering and Chemical Technology of the Gdansk University of Technology in Waglikowice, 24-28.06.2019.

7.5. Posters at scientific conferences

1. **Marycz M**., Brillowska-Dąbrowska A., Deshusses M. Analysis of biofilm on the surface of artificial support materials used for biotrickling filtration by flow cytometry, EBRC 2022 ANNUAL MEETING, 19-20.05.2022, Berkeley, CA, USA.
2. **Gospodarek, M.,** Brillowska-Dąbrowska, A., Rybarczyk, P., Gębicki, J. Selection of microorganisms using n-butanol and cyclohexane as a source of carbon for biofiltration process, 46th International

Conference of the Slovak Society of Chemical, 19-23.05.2019, Tatranske Matliare, Slovakia, **Student Poster Award**

3. Rybarczyk, P., **Gospodarek, M.**, Szulczyński, B., Gębicki, J. Simultaneous removal of n-butanol and cyklohexane from air in a biotrickling filter, 46th International Conference of the Slovak Society of Chemical, 19-23.05.2019, Tatranske Matliare, Slovakia.
4. **Gospodarek, M.,** Rybarczyk, P., Brillowska-Dąbrowska, A., Gębicki, J. Rola mikroorganizmow w dezodoryzacji powietrza metoda biofiltracji [The role of microorganisms in air deodorization by biofiltration], IV Interdisciplinary Academic Conference on Environmental Protection (IAKOŚ), 05-07.04.2019, Gdansk, Poland.
5. **Gospodarek, M.,** Rybarczyk, P., Brillowska-Dąbrowska, A., Gębicki, J. The use of various species of fungi in biofiltration of air contaminated with odorous volatile organic compounds, 11th Conference on Interdisciplinary Problems in Environment Protection and Engineering, 08.-10.04.2019, Polanica Zdroj, Poland.
6. **Gospodarek, M.**, Rybarczyk, P., Gębicki, J. Comparison of the effectiveness of air deodorization by biological methods, XIV Conferences for the city and the environment - problems of waste disposal, 26.11.2018, Warsaw, Poland.
7. **Gospodarek, M.**, Rybarczyk, P., Gębicki, J. Current legal status in the area of odour nuisance prevention, XIV Conferences for the city and the environment - problems of waste disposal, 26.11.2018, Warsaw, Poland.
8. **Gospodarek, M.**, Pawlowska, M., Augustin, E. The cellular response of human colon and lung cancer cells to treatment with unsymmetrical bisacridine derivatives, 3rd Congress of Polish Biosciences BIO2018, 21.09.2018, Gdansk, Poland.
9. **Gospodarek, M.**, Holowacz, I. Optimization of the mixing process in the airlift reactor, BIOOPEN IV National Conference of Doctoral Students in Life Sciences, 25.05.2018, Lodz, Poland.

7.6. Scientific internships

1. 25.10.2020-24.01.2021 and 28.05-27.09.2021 - Academic training in the laboratory Department of Chemical Engineering and Environmental Technology at the University of Valladolid under supervision of Prof Raúl Muñoz Torre (https://orcid.org/0000-0003-1207-6275)
2. 04.01-20.07.2022 - Academic training in the laboratory Department of Civil and Environmental Engineering, Duke University under supervision of Prof Marc A. Deshusses (https://orcid.org/0000-0003-0639-7865) - *High-performance biogas modernization process*
3. 06.01-08.06.2022 - Academic training in the laboratory Department of Civil, Structural and Environmental Engineering at UCC and Director of the MaREI center for energy, climate and marine under supervision of Prof Jerry D Murphy (https://orcid.org/ 0000-0003-2120-1357) - *The development of ex-situ biological CO_2 methanation using a bespoke cascading system*

Cork, 08.05.2023 r.

Milena Marycz, M.Sc. Eng.
Department of Process Engineering and Chemical Technology
Faculty of Chemistry, Gdansk University of Technology
80-233 Gdańsk (Poland)
Narutowicza 11/12 Street

Author's statement

As a co-author of the article:

1. **Gospodarek, M**., Rybarczyk, P., Szulczynski, B., & Gebicki, J. (2019). Comparative Evaluation of Selected Biological Methods for the Removal of Hydrophilic and Hydrophobic Odorous VOCs from Air. *Processes*, 7(4), 187. doi.org/10.3390/pr7040187

   I declare that my contribution consisted of:
   - conducting a literature review and collecting a literature dataset,
   - preparation a method of comparison and performation the calculations
   - preparation of drawings and preparation of the original content of the manuscript,
   - development and interpretation of the obtained results,
   - acquiring funding.

2. **Marycz, M.**, Brillowska-Dąbrowska, A., Muñoz R., Gębicki, J. (2022). A state of the art review on the use of fungi in biofiltration to remove volatile hydrophobic pollutants. *Reviews in Environmental Science and Bio/Technology*, 21(1), 225-246. doi.org/10.1007/s11157-021-09608-7

   I declare that my contribution consisted of:
   - conducting a literature review and collecting a literature dataset,
   - preparation of drawings and preparation of the original content of the manuscript,
   - development and interpretation of the obtained results,
   - acquiring funding.

3. **Marycz, M.**, Brillowska-Dabrowska, A., Gebicki, J. (2020). Evaluation of Immobilization of Selected Peat-Isolated Yeast Strains of the Species *Candida albicans* and *Candida subhashii* on the Surface of Artificial Support Materials Used for Biotrickling Filtration. *Processes*, *8*(7), 801. doi.org/10.3390/pr8070801

   I declare that my contribution consisted of:
   - isolation from peat and species identification of all isolates presented in the article,
   - designing the method for studying the diversity and viability of fungi during the biofiltration process, based on optical microscopy, flow cytometry and the tests employing propidium iodide and annexin V,
   - development of a new method of immobilization of fungi on inert packing materials constituting the filling of biofilters,
   - preparation and exchange of microbiological media,
   - developing research methodology,
   - conducting research and performing calculations,
   - preparation of drawings and preparation of the original content of the manuscript,
   - development and interpretation of the obtained results.

4. Rybarczyk, P., **Marycz, M.**, Szulczynski, B., Brillowska-Dabrowska, A., Rybarczyk, A., Gebicki, J. (2021). Removal of Cyclohexane and Ethanol From Air in Biotrickling Filters Inoculated with *Candida albicans* and *Candida subhashii*. *Archives of Environmental Protection*, 47(1), 16-34. doi.org/10.24425/aep.2021.136445

   I declare that my contribution consisted of:
   - immobilization of fungi on inert packing materials constituting the filling of biofilters,
   - conducting research on the diversity and viability of fungi in the biofiltration process based on optical microscopy, flow cytometry and tests using propidium iodide and annexin V,
   - preparation and exchange of microbiological media,
   - designing and conducting microbiological control of biofiltration processes,
   - developing research methodology (as part of microbiological experiments),
   - preparation of drawings and preparation of the original content of the manuscript (as part of microbiological experiments),
   - development and interpretation of the obtained results (as part of microbiological experiments).

5. **Marycz, M.**, Rodríguez, Y., Gębicki, J., Muñoz, R. (2022). Systematic comparison of a biotrickling filter and a conventional filter for the removal of a mixture of hydrophobic VOCs by *Candida subhashii*. *Chemosphere*, 135608. doi.org/10.1016/j.chemosphere.2022.135608

   I declare that my contribution consisted of:
   - design and construction of biofilters,
   - developing research methodology,
   - immobilization of fungi on polyurethane foam constituting the filling of biofilters,
   - designing and conducting microbiological control of biofiltration processes,
   - preparation and exchange of microbiological media,
   - performing chromatographic analyses,
   - conducting research and performing calculations,
   - preparation of drawings and preparation of the original content of the manuscript,
   - development and interpretation of the obtained results,
   - acquiring funding.

6. **Marycz, M**., Brillowska-Dąbrowska, A., Cantera, S., Gębicki, J., & Muñoz, R. (2023). Fungal co-culture improves the biodegradation of hydrophobic VOCs gas mixtures in conventional biofilters and biotrickling filters. *Chemosphere*, *313*, 137609. doi.org/10.1016/j.chemosphere.2022.137609

   I declare that my contribution consisted of:
   - design and construction of biofilters,
   - developing research methodology,
   - immobilization of fungi on polyurethane foam constituting the filling of biofilters,
   - designing and conducting microbiological control of biofiltration processes,
   - preparation and exchange of microbiological media,
   - performing chromatographic analyses,
   - conducting research and performing calculations,
   - preparation of drawings and preparation of the original content of the manuscript,
   - development and interpretation of the obtained results,
   - acquiring funding.

Milena Marycz

Elektronicznie podpisany przez Milena Marycz
Data: 2023.05.15 15:44:52 +01'00'

Gdańsk, 08.05.2023 r.

PhD. DSc. Eng. Jacek Gębicki, Assoc. Prof
Department of Process Engineering and Chemical Technology
Faculty of Chemistry, Gdansk University of Technology
80-233 Gdańsk (Poland)
Narutowicza 11/12 Street

Author contribution statement

As a co-author of the article:

1. Gospodarek, M., Rybarczyk, P., Szulczynski, B., & **Gebicki, J.** (2019). Comparative Evaluation of Selected Biological Methods for the Removal of Hydrophilic and Hydrophobic Odorous VOCs from Air. *Processes*, 7(4), 187. doi.org/10.3390/pr7040187

   I declare that my contribution consisted of:
   - development of the concept of the article,
   - reviewing, editing and proofreading of manuscripts sent for printing.
   - funding acquisition.

2. Marycz, M., Brillowska-Dąbrowska, A., Muñoz R., **Gębicki, J.** (2022). A state of the art review on the use of fungi in biofiltration to remove volatile hydrophobic pollutants. *Reviews in Environmental Science and Bio/Technology*, 21(1), 225-246. doi.org/10.1007/s11157-021-09608-7

   I declare that my contribution consisted of:
   - development of the concept of the article,
   - reviewing, editing and proofreading of manuscripts sent for printing.
   - funding acquisition.

3. Marycz, M., Brillowska-Dabrowska, A., **Gebicki, J.** (2020). Evaluation of Immobilization of Selected Peat-Isolated Yeast Strains of the Species *Candida albicans* and *Candida subhashii* on the Surface of Artificial Support Materials Used for Biotrickling Filtration. *Processes*, *8*(7), 801. doi.org/10.3390/pr8070801

   I declare that my contribution consisted of:
   - development of the concept of the article,
   - coordinating, administrating and supervising research (process part of the experiments),
   - substantive assessment of the results and conclusions obtained (process part of the experiments),
   - reviewing, editing and proofreading of manuscripts sent for printing.
   - funding acquisition,
   - resources.

4. Rybarczyk, P., Marycz, M., Szulczynski, B., Brillowska-Dabrowska, A., Rybarczyk, A., **Gebicki, J.** (2021). Removal of Cyclohexane and Ethanol From Air in Biotrickling Filters Inoculated with *Candida albicans* and *Candida subhashii*. *Archives of Environmental Protection*, 47(1), 16-34. doi.org/10.24425/aep.2021.136445

   I declare that my contribution consisted of:
   - development of the concept of the article,

- coordinating, administrating and supervising research (process part of the experiments),
- substantive assessment of the results and conclusions obtained (process part of the experiments),
- reviewing, editing and proofreading of manuscripts sent for printing.
- funding acquisition,
- resources.

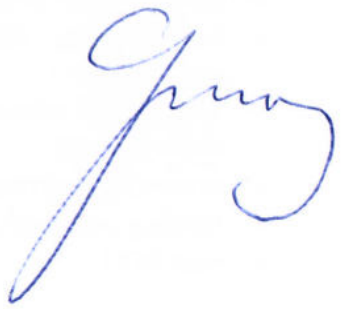
5. Marycz, M., Rodríguez, Y., **Gębicki, J.**, Muñoz, R. (2022). Systematic comparison of a biotrickling filter and a conventional filter for the removal of a mixture of hydrophobic VOCs by *Candida subhashii*. *Chemosphere*, 135608. doi.org/10.1016/j.chemosphere.2022.135608

   I declare that my contribution consisted of:
   - participation in the development of the concept of the article,
   - substantive assessment of the results and conclusions,
   - reviewing, editing and proofreading of manuscripts sent for printing.
   - acquiring funding.

6. Marycz, M., Brillowska-Dąbrowska, A., Cantera, S., **Gębicki, J.**, & Muñoz, R. (2023). Fungal co-culture improves the biodegradation of hydrophobic VOCs gas mixtures in conventional biofilters and biotrickling filters. *Chemosphere*, *313*, 137609. doi.org/10.1016/j.chemosphere.2022.137609

   I declare that my contribution consisted of:
   - participation in the development of the concept of the article,
   - substantive assessment of the results and conclusions,
   - reviewing, editing and proofreading of manuscripts sent for printing.
   - acquiring funding.

0805-2023



Gdańsk, 08.05.2023 r.

PhD. DSc. Eng. Anna Brillowska-Dąbrowska, Assoc. Prof.
Department of Molecular Biotechnology and Microbiology
Faculty of Chemistry, Gdansk University of Technology
80-233 Gdańsk (Poland)
Narutowicza 11/12 Street

Author contribution statement

As a co-author of the article:

1. Marycz, M., **Brillowska-Dąbrowska, A.**, Muñoz R., Gębicki, J. (2022). A state of the art review on the use of fungi in biofiltration to remove volatile hydrophobic pollutants. *Reviews in Environmental Science and Bio/Technology*, 21(1), 225-246. doi.org/10.1007/s11157-021-09608-7

   I declare that my contribution consisted of:
   - development of the concept of the article,
   - reviewing, editing and proofreading of manuscripts sent for printing.

2. Marycz, M., **Brillowska-Dabrowska, A.**, Gebicki, J. (2020). Evaluation of Immobilization of Selected Peat-Isolated Yeast Strains of the Species *Candida albicans* and *Candida subhashii* on the Surface of Artificial Support Materials Used for Biotrickling Filtration. *Processes*, *8*(7), 801. doi.org/10.3390/pr8070801

3. Rybarczyk, P., Marycz, M., Szulczynski, B., **Brillowska-Dabrowska, A.**, Rybarczyk, A., Gebicki, J. (2021). Removal of Cyclohexane and Ethanol From Air in Biotrickling Filters Inoculated with *Candida albicans* and *Candida subhashii*. *Archives of Environmental Protection*, 47(1), 16-34. doi.org/10.24425/aep.2021.136445

   I declare that my contribution consisted of:
   - development of the concept of the article,
   - coordinating, administrating and supervising research (as part of microbiological experiments),
   - substantive assessment of the results and conclusions obtained (as part of microbiological experiments),
   - reviewing, editing and proofreading of manuscripts sent for printing.
   - acquiring funding,
   - resources.

4. Marycz, M., **Brillowska-Dąbrowska, A.**, Cantera, S., Gębicki, J., & Muñoz, R. (2023). Fungal co-culture improves the biodegradation of hydrophobic VOCs gas mixtures in conventional biofilters and biotrickling filters. *Chemosphere*, *313*, 137609. doi.org/10.1016/j.chemosphere.2022.137609

   I declare that my contribution consisted of:
   - reviewing, editing and proofreading of manuscripts sent for printing,
   - acquiring funding.

Valladolid, 08.05.2023 r.

Professor Raúl Muñoz Torre
Chemical Engineering and Environmental Technology
Universidad de Valladolid
Dr. Mergelina s/n, 47011, Valladolid (Spain)

Author contribution statement

As a co-author of the article:

1. Marycz, M., Brillowska-Dąbrowska, A., **Muñoz R.**, Gębicki, J. (2022). A state of the art review on the use of fungi in biofiltration to remove volatile hydrophobic pollutants. *Reviews in Environmental Science and Bio/Technology*, 21(1), 225-246. doi.org/10.1007/s11157-021-09608-7

   I declare that my contribution consisted of:
   - reviewing, editing and proofreading of manuscripts sent for printing.
   - acquiring funding.

2. Marycz, M., Rodríguez, Y., Gębicki, J., **Muñoz, R.** (2022). Systematic comparison of a biotrickling filter and a conventional filter for the removal of a mixture of hydrophobic VOCs by *Candida subhashii*. *Chemosphere*, 135608. doi.org/10.1016/j.chemosphere.2022.135608
3. Marycz, M., Brillowska-Dąbrowska, A., Cantera, S., Gębicki, J., & **Muñoz, R.** (2023). Fungal co-culture improves the biodegradation of hydrophobic VOCs gas mixtures in conventional biofilters and biotrickling filters. *Chemosphere*, *313*, 137609. doi.org/10.1016/j.chemosphere.2022.137609

   I declare that my contribution consisted of:
   - development of the concept of the article,
   - coordinating, administrating and supervising research,
   - substantive assessment of the results and conclusions obtained,
   - reviewing, editing and proofreading of manuscripts sent for printing.
   - acquiring funding,
   - resources.

MUÑOZ TORRE RAUL - 16811991A
Firmado digitalmente por MUÑOZ TORRE RAUL - 16811991A
Fecha: 2023.05.08 06:52:47 +02'00'

Gdańsk, 08.05.2023 r.

Piotr Rybarczyk, PhD
Department of Process Engineering and Chemical Technology
Faculty of Chemistry, Gdansk University of Technology
80-233 Gdańsk (Poland)
Narutowicza 11/12 Street

Author contribution statement

As a co-author of the article:

1. Gospodarek, M., **Rybarczyk, P.**, Szulczynski, B., & Gebicki, J. (2019). Comparative Evaluation of Selected Biological Methods for the Removal of Hydrophilic and Hydrophobic Odorous VOCs from Air. *Processes*, 7(4), 187. doi.org/10.3390/pr7040187

   I declare that my contribution consisted of:
   - revised the literature for the introduction and co-discussed of the results,
   - reviewing, editing and proofreading of manuscripts sent for printing,
   - linguistic proofreading of the manuscript.

2. **Rybarczyk, P.**, Marycz, M., Szulczynski, B., Brillowska-Dabrowska, A., Rybarczyk, A., Gebicki, J. (2021). Removal of Cyclohexane and Ethanol From Air in Biotrickling Filters Inoculated with *Candida albicans* and *Candida subhashii*. *Archives of Environmental Protection*, 47(1), 16-34. doi.org/10.24425/aep.2021.136445

   I declare that my contribution consisted of:
   - conceptualization and preparation of the part of the manuscript concerning the biotrickling filtration process part,
   - conducting the biotrickling filtration processes (process part of the experiments),
   - performing chromatographic analyses,
   - reviewing, editing and proofreading of manuscript sent for printing (introduction and biotrickling filtration process part of the experiments).

8-05-2023

Piotr Rybarczyk

Gdańsk, 08.05.2023

Bartosz Szulczyński, PhD
Department of Process Engineering and Chemical Technology
Faculty of Chemistry, Gdansk University of Technology
80-233 Gdańsk (Poland)
Narutowicza 11/12 Street

Author contribution statement

As a co-author of the article:

- Gospodarek, M., Rybarczyk, P., **Szulczyński, B.**, & Gębicki, J. (2019). Comparative Evaluation of Selected Biological Methods for the Removal of Hydrophilic and Hydrophobic Odorous VOCs from Air. *Processes*, 7(4), 187. doi.org/10.3390/pr7040187

  I declare that my contribution consisted of:
  - conducting data analysis using a decision tree model for the initial selection of the deodorization method,
  - preparation of the manuscript and figures for the section presenting the decision tree model results for the initial selection of the deodorization method.

- Rybarczyk, P., Marycz, M., **Szulczyński, B.**, Brillowska-Dąbrowska, A., Rybarczyk, A., Gębicki, J. (2021). Removal of Cyclohexane and Ethanol From Air in Biotrickling Filters Inoculated with *Candida albicans* and *Candida subhashii*. *Archives of Environmental Protection*, 47(1), 16-34. doi.org/10.24425/aep.2021.136445

  I declare that my contribution consisted of:
  - design and construction of bioreactors,
  - conducting the biofiltration process (process part of the experiment),
  - performing chromatographic analyses.

08.05.2023

Valladolid, 08.05.2023 r.

Yadira Rodríguez, PhD
(1) Department of Chemical Engineering
and Environmental Technology
School of Industrial Engineering
University of Valladolid
Dr. Mergelina, s/n, 47011 Valladolid, Spain
(2) Institute of Sustainable Processes
Dr. Mergelina s/n, 47011 Valladolid, Spain

Author contribution statement

As a co-author of the article:

1. Marycz, M., **Rodríguez, Y.**, Gębicki, J., Muñoz, R. (2022). Systematic comparison of a biotrickling filter and a conventional filter for the removal of a mixture of hydrophobic VOCs by *Candida subhashii*. *Chemosphere*, 135608. doi.org/10.1016/j.chemosphere.2022.135608

   I declare that my contribution consisted of:
   - assistance in the design and construction of biofilters,
   - assistance in developing research methodology (process part of the experiment),
   - reviewing, editing and proofreading of manuscript sent for printing.

RODRIGUEZ MUÑOZ YADIRA - 53155622T

Firmado digitalmente por RODRIGUEZ MUÑOZ YADIRA - 53155622T
Fecha: 2023.05.09 10:47:22 +02'00'

Wageningen, 08.05.2023 r.

Sara Cantera, PhD
Laboratory of Microbiology
Wageningen University & Research
Stippeneng 4, 6708 WE,
Wageningen, Netherlands

Author contribution statement

As a co-author of the article:

1. Marycz, M., Brillowska-Dąbrowska, A., **Cantera, S.**, Gębicki, J., & Muñoz, R. (2023). Fungal co-culture improves the biodegradation of hydrophobic VOCs gas mixtures in conventional biofilters and biotrickling filters. *Chemosphere*, *313*, 137609. doi.org/10.1016/j.chemosphere.2022.137609

   I declare that my contribution consisted of:
   - conducting a fungal communities analysis based on their sequence,
   - preparation of figures and preparation of the manuscript in the section on the analysis of fungal communities (*3.2. Fungal diversity and community structure*),
   - acquiring funding.

CANTERA RUIZ DE PELLON SARA - 72078628D

Firmado digitalmente por CANTERA RUIZ DE PELLON SARA - 72078628D
Fecha: 2023.05.08 10:01:21 +02'00'

Gdańsk, 08.05.2023 r.

Agnieszka Rybarczyk, PhD
Department of Histology
Faculty of Medicine, Medical University of Gdańsk
80-211 Gdańsk (Poland)
Dębinki 1 Street

Author contribution statement

As a co-author of the article:

1. Rybarczyk, P., Marycz, M., Szulczynski, B., Brillowska-Dabrowska, A., **Rybarczyk, A.**, Gebicki, J. (2021). Removal of Cyclohexane and Ethanol From Air in Biotrickling Filters Inoculated with *Candida albicans* and *Candida subhashii*. *Archives of Environmental Protection*, 47(1), 16-34. doi.org/10.24425/aep.2021.136445

   I declare that my contribution consisted of:
   - linguistic proofreading of the manuscript.

8/05/2023
A. Rybarczyk