This version of the article has been accepted for publication, after peer review (when applicable) and is subject to Springer Nature's AM terms of use, but is not the Version of Record and does not reflect post-acceptance improvements, or any corrections. The Version of Record is available online at: https://doi.org/10.1007/s11696-019-01010-6

Postprint of: Słupek E., Makoś P., Kucharska K., Gębicki J., Mesophilic and thermophilic dark fermentation course analysis using sensor matrices and chromatographic techniques, CHEMICAL PAPERS, Vol. 74 (2020), pp. 1573–1582

2 3	Mesophilic and thermophilic dark fermentation course analysis using sensor matrices and chromatographic techniques
4	
5	Edyta Słupek ^{*1} , Patrycja Makoś ¹ , Karolina Kucharska ¹ , Jacek Gębicki ¹
6	
7	¹ Gdańsk University of Technology, Faculty of Chemistry, Department of Process
8	Engineering and Chemical Technology, 80-233 Gdańsk, Narutowicza 11/12 street, Poland
9	
10	*Corresponding author, e-mail: edyta.slupek@pg.edu.pl
11	
12	Received [Dates will be filled in by the Editorial office]
13	
14	Abstract
15	
16	Production of biofuels from biomass is expected to benefit society and the environment. At
17	present bio waste residues processing includes hydrolysis, dark fermentation, photo
18	fermentation, pyrolysis, gasification, and chemical synthesis. As the composition and the
19	chemical structure of organic substances affect the efficiency of mentioned processes, it is
20	believed, that the glucose concentration is a crucial parameter for the evaluation of the
21	efficiency of biological processes. Also, the control of by-products formulated during each
22	stage of biomass processing affects the course of dark fermentation. Therefore model
23	processes regarding mesophilic and thermophilic dark fermentation were carried. Glucose as a
24	sole carbon source was applied as the fermentation broth and Faloye-pretreated activated
25	municipal wastewater sludge was introduced as the source of sporulating microorganisms.
26	Production of hydrogen and methane was controlled by means of sensor matrices. Obtained
27	results are comparable to those obtained using the standard method based on gas
28	chromatography and indicate the suitability of their application for on-line routine analyses of
29	hydrogen and methane during fermentation processes. In addition, the fermentation broth was
30	also examined by means of gas and liquid chromatography in the scope of glucose reduction.
31	and generation of volatile fatty acids and phenols
32	6 or , orang rang area provoid.

33 Keywords: biogas analysis; chromatographic analysis; hydrogen; methane; dark
 34 fermentation; sensor matrices

37

Introduction

The generation of biofuels from lignocellulosic biomass is a complexed process, 38 including three main stages, i.e. pretreatment, saccharification and fermentation (Kucharska et 39 al. 2018). The yield and the rate of biogas or hydrogen productivity are affected mainly by 40 process parameters i.e. pH of the pulp, temperature, composition, biomass pre-treatment 41 method and digestion time (Gomez et al. 2006; Wang et al. 2009; Chu et al. 2012; Lestinsky 42 et al. 2017). Several reports regarding the application of activated municipal wastewater 43 sludge for dark fermentation processes can be found in the literature (Wu and Chang 2007; 44 Jeppsson et al. 2007; Azbar et al. 2009; Ottaviano et al. 2017). However, the literature lacks 45 complexed experiments related to the comparison between mesophilic and thermophilic dark 46 fermentation course when the same sporulating microorganisms obtained during inoculum 47 pre-treatment from activated sludge were applied (Faloye et al. 2013, 2014). 48

The authors propose to use glucose based fermentation broths for the evaluation of 49 biofuels efficiency The analysis of efficiency associated with glucose aims to maximize the 50 technological, energy and economic benefits in production processes. Energy efficiency is 51 understood as the ratio of energy obtained from biofuels to the energy consumed in all unit 52 processes (Wu et al. 2007). In order to compare the dark fermentation process course, 53 mesophilic and thermophilic conditions were used for the culture (Ivanova et al. 2009; Yasin 54 et al. 2013). A gas mixture containing hydrogen and carbon dioxide is formed during the dark 55 fermentation process. However, reports regarding methane formulation are also published 56 (Levin et al. 2004; Cheng et al. 2010). According to the literature, several differences in the 57 hydrogen: methane ratio may occur (Lay et al. 1999; Guo et al. 2008; Manish and Banerjee 58 2008; Wu et al. 2009; Zhu et al. 2010). 59

Hydrogen and methane can be co-generated via anaerobic digestion (AD), a multi-step process carried out by highly differentiated microorganisms. Anaerobic conditions enable the transformation of organic matter into carbon dioxide and methane or hydrogen. It is found, that several types of microbial populations have specific optimal working conditions regarding pH, temperature, alkalinity, concentration ammonia, sodium and potassium ions, volatile fatty acids or heavy metals presence (Wilkie et al. 2000; Yang et al. 2006).

Biogas is composed of methane (up to 75%), carbon dioxide (up to 40%) and constituents such as ammonia, hydrogen, hydrogen sulfide, and nitrogen. Usually, consortia of highly diversified microorganisms enable the generation of biogas and liquid by-products, 69 i.e. volatile fatty acids (VFA) and other metabolic products. It is reported that metabolic 70 pathways related to biogas generation are highly complicated (De Gioannis et al. 2013; 71 Veluchamy and Kalamdhad 2017). When hydrogen generation is concerned, different 72 sporulating bacteria capable of glucose conversion to valuable acids, i.e. propionic acid, 73 succinic acid, lactic acid and alcohols, i.e. 2,3-butanediol, ethanol with simultaneous 74 liberation of hydrogen is discussed. However, if glucose fermentation is considered, every 75 liquid by-product may lead to a decrease of the overall hydrogen or methane yield (Lee et al. 76 2006; Wu et al. 2007; Panagiotopoulos et al. 2010).

Several types of main-gaseous product and liquid by-product formulation duringanaerobic digestion are presented in Table 1.

79

Table 1. Gaseous and liquid products generated during anaerobic digestion on different inoculum and broths.

Carbon source	Applied microorganisms	Main gaseous products	Main liquid products	References			
Glucose/ model process	Mixed anaerobic microflora	Hydrogen	Butyric acid, acetic acid	(Pan et al. 2010)			
Lignocellulosic hydrolysate	Anaerobic bacteria	Methane	Lactic acid, citric acid, acetic acid	(Wong et al. 2014)			
Barleystraw,cornstoverandswitchgrass	Clostridium sp.	Methane- low efficiency	ABE (acetone; butanol; ethanol)	(Qureshi et al. 2010b, a)			
Food waste	Sewage sludge	Methane	Acetate, propionate, butyrate, valerate, hexanoic acid	(Cheng et al. 2018)			
Waste paper and kitchen waste	Genera 060F05-B- SD-P93 and Thermosyntropha	Methane	Ethanol, propionic acid, lactic acid	(Tan et al. 2019)			
Food waste	Bifidobacterium, Lactobacillus	Methane	Lactic acid, ethanol, acetic acid, propionic acid, butyric acid, valeric acid	(Feng et al. 2020)			
Arthrospira platensis	Clostridium butyricum, Rhodopseudomonas palustris	Methane and hydrogen	Ethanol, acetate, propionate, butyrate, isobutyrate, valerate,	(Ding et al. 2017)			

				isovalerate, and caproate	
Biodiesel industry residue	Mixed (from sludge)	cultures activated	Methane	Butyric acid, ethanol, acetic acid, propionic acid, valeric acid	(Kumar et al. 2015)

When mixed bacterial culture is used in dark fermentation, i.e. bacteria obtained from mixed activated wastewater sludge, hydrogen is generated in the initial stage of the process (Pandu and Joseph 2012). However, methane may occur in the final stage of the fermentation process (Teplyakov et al. 2002). As it can be inferred from the data presented in Table 1, a large number of anaerobic digestion examples concerning various feeds have been published.

The fermentation process is usually monitored by pH, biogas production rate, redox 89 potential, concentration of volatile fatty acids (VFA), total phenolic content (TPC) and gas 90 composition, in order to ensure the correctness of the process. Among these indicators, VFA 91 concentration in fermentation broth, as well as biogas compositions, are widely considered as 92 the two most crucial and direct indicators of the biogas production process due to the fact that 93 the dark fermentation process leads mainly to the formation of VFA followed by gasses 94 production (hydrogen and carbon dioxide) which, in the last step, are transformed into 95 methane. However, the increase in VFA concentration is linked to the methanogenesis 96 inhibition or organic overloading and implies a risk of reducing the efficiency of biogas 97 production (Rosecrance et al. 2013). In addition, several studies have also observed that the 98 formation of phenols may also adversely affect the fermentation process (Fenske et al. 1998; 99 Luo et al. 2002; Per Persson et al. 2002). 100

For the determination of VFA in fermentation broths, the techniques of fluorescence spectroscopy, near-infrared spectroscopy, titration, high performance liquid chromatography (LC) and gas chromatography (GC) are mainly used. The concentration of phenolic compounds could be analysed using UV-Vis spectroscopy (Madsen et al. 2011), GC and HPLC analysis (Nilvebrant et al. 2001; Quéméneur et al. 2012). The analysis of gas formulated during anaerobic digestion is usually carried using gas chromatography, for the determination of the gas content and composition (Rosales-Colunga et al. 2010).

However, GC measurement has several disadvantages i.e.: manual injections and longtime analysis (Isobe et al. 2011). To analyze the processes occurring during dark fermentation, a sensor matrices consisted of sensors selective for hydrogen, methane, carbon the dioxide, hydrogen sulphide and ammonia may be applied (Hoff et al. 2006; Gebicki 2016; 112 Gebicki and Dymerski 2016). Nowadays, sensor arrays in environmental applications are 113 mainly used for air analysis. This technique belongs to dynamically developing instrumental 114 techniques and it is increasingly applied for monitoring and evaluation of the effectiveness of 115 deodorization of unpleasant odours generated by different fields of human activity 116 (Szulczyński et al. 2017).

However, they can also be used to on-line analysis of the biogas composition. In such 117 cases, the biogas characteristics can be detected using metals oxide based MOS sensors. 118 These sensors should be selective for hydrogen, methane and inorganic compounds, i.e. 119 hydrogen sulphide, ammonia, oxygen, carbon dioxide, as well as organic compounds, toluene, 120 benzene or VFA (e.g. acetic acid, butyric acid). In addition, the sensors should be 121 characterized by good selectivity for a given gas and a lack of sensitivity to the interaction of 122 other gases contained in the mixture (Ponzoni et al. 2017). In addition, the sensor matrices 123 require careful design and testing for which model conditions are used and then perform tests 124 on real samples. Continuous biogas measurements using sensor matrices are possible using 125 the flow configuration of the measurement system. 126

The paper presents a comprehensive evaluation of the mesophilic and thermophilic 127 dark fermentation processes in model conditions. As a source of carbon, glucose was selected 128 because it may be sole carbons source for most the microorganisms. The fermentation broth 129 was examined by means of gas and liquid chromatography in the scope of glucose reduction 130 as well as generation of dark fermentation by-products (i.e. VFA and TPC). The possibility of 131 using sensory matrices to investigate the composition of biogas was also examined. The 132 results obtained with sensor matrices were compared with gas chromatography. Then the 133 correlation matrices were created to better understand the course of fermentation processes. 134

Experimental

137

135

136

138 Materials and Methods

139

140 Chemicals

141

For the purposes of analytical methods, the standard substances: D (+) Glucose
(≥99.5% Sigma Aldrich), Sodium Hydroxide (99%, Sigma Aldrich), Dichloromethane
(≥99.9%, Sigma Aldrich), Buffered Peptone Water (Biomaxima, Poland), Syringol (99%
Sigma Aldrich), Formic Acid (80% POCH), Acetic Acid (>99% Sigma Aldrich), Propionic

Acid (≥99% Sigma Aldrich), Butanoic Acid (≥99% Sigma Aldrich), Isobutanoic Acid (≥99%
Sigma Aldrich), were used in the study.

Anaerobic conditions during dark fermentation were created by purging the bioreactor
with nitrogen – purity N5 (Linde Gas, Poland).

Hydrogen – purity N 5.5 from a Packard 9400 hydrogen generator (Packard, USA) 151 was used in the gas chromatography. During the analysis of the sensor matrices, N5 purity 152 compressed air was used (Linde Gas, Poland). An eluent consisting of aqueous 0.2% HCOOH 153 (POCH, Poland) was used for the high-performance liquid chromatography analysis.

154

155 Dark fermentation

156

Dark fermentation was carried out in sterile 1200 mL glass bioreactors withworking 157 volume of 1000 mL). The initial fermentation broth was composed of 900 mL of 20 g/L 158 solution of Buffered Peptone Water (Biomaxima, Poland) and 5.5 g/L of glucose (POCH, 159 Poland) as a sole carbon source. Dark fermentation was carried out with the use of activated 160 sludge after Faloye procedure. The Faloye procedure was used for inoculum preparison. The 161 pH of the activated sludge was adjusted to 8.93 with 1 M NaOH solution and further 162 autoclaved (15 minutes, 121 °C). After autoclaving the pre-treated sludge was thermostated 163 for 20 h at 37 °C with constant stirring to stabilize the culture of microorganisms. 164

The fermentation broth was adjusted to pH = 7.00 (1 M NaOH) and a constant pH was 165 maintained throughout the process, using Arduino Data Logger. The anaerobic conditions 166 were created by purging the reactor with sterile nitrogen for 20 to 60 min. After establishing 167 anaerobic conditions, inoculations were carried out using 100 mL of activated sludge after the 168 Faloy'e procedure. The fermentation in bioreactors were carried at 35 °C (mesophilic process) 169 and 65 °C (thermophilic process) with magnetic stirring of 150 RPM. Fermentation was 170 carried for 115 hours. Due to exploitation of the carbon source, after 80 hours of the process, 171 3.0 g of glucose was added to stimulate the further biogas production. 172

173

174 Sensors analysis – gas phase analysis

175

The biogas samples were analyzed using a self - constructed sensor matrice (SM). The device was equipped with commercial sensors selective for methane and hydrogen manufactured by Figaro Engineering (TGS2611, TGS2600). In the figure 1. it the scheme of the measurement system is shown. A stream of clean air flows through the measuring

chamber at a constant flow rate of 100 mL/min. The flow stream is controlled by an ADM 180 1000 flow meter (Agilent Technologies, USA). By changing the position of the valve (see 181 Fig. 1 - point 3), the biogas sample was directed to the measurement chamber. The volume of 182 the analyzed sample was 5.0 mL while the time of dosing the sample was equal to 30 s. After 183 this time the clean air was returned to the measurement chamber for the regeneration of the 184 sensors by changing the position of the valve. Signals from the sensors were recorded using 185 an AD (analog – to – digital) converter (Simex SIAi-8). Data analysis were performed using 186 SigmaPlot 11.0 software . 187 188



189

Fig. 1 Measurement system: 1 – air, 2 – flow meter, 3 – valve 4 – sensor chamber, 5 –
methane sensor, 6 - hydrogen sensor, 7- temperature sensor, 8- humidity sensor, 9- analog-todigital converter (ACD Converter), 10 – computer.

193

195

194 Gas chromatography analysis - gas phase analysis

The biogas was also analyzed by means of gas chromatography (Perkin-Elmer AutoSystem XL) with a Porapak Q column (100-120 mesh length 6.5 m, diameter 1/8 inch) and an oven temperature of 60 °C. The following conditions were used during analysis: flame ¹⁹⁹ ionization detector (FID, temperature 220 °C) and thermal conductivity detector (TCD, ²⁰⁰ temperature 100 °C). Nitrogen with a flow of 30 mL/min was used as the carrier gas. The ²⁰¹ volume of the analyzed sample was 0.5 mL. The total analysis time was 12 minutes. During ²⁰² the analysis, Turbochrom software was applied.

203 Gas chromatographic analysis - fermentation broth analysis

204

Samples from dark fermentation process were taken to determine the changes in the 205concentration of individual and total content of volatile fatty acids and phenols. During the 206 fermentation process, 2 mL samples were collected and stored frozen in the temperature of -207 18 °C. For analysis the samples melted and centrifuged (Hitachi EBA 8S) for 5 min at 3000 208 RPM, an initial removal of the solid phase was realised. The aqueous phase (1.0 mL) was 209 filtered through a 0.45 µm hydrophilic-cellulose filter (Hahnemühle FineArt HmbH, 210 Germany) and transferred to a 1.5 mL vial. 10 µL of hydrochloric acid was added to the 211 sample to adjust the pH to 2.0 and then 300 µL of dichloromethane (DCM) was added. The 212 sample was shaken vigorously in the vial for 1 minute and then centrifuged (3000 RPM) for 5 213 minutes for liquid-liquid extraction. The obtained organic phase was transferred in a volume 214 of 150 μ L by means of an automatic pipette into 2.0 mL vials. The extracted sample (1 μ L) 215 was introduced into GC-FID. Individual VFA were analyzed by gas chromatography (Varian 216 CP 3800) with a DB-WAX column (30 m x 0.53 mm x 1.0 µm). The following 217 chromatographic conditions were used: oven temperature 100 °C (5 min) - ramped at 10 218 °C/min to 250 °C (10 min); injection port temperature 280 °C; injection volume 1 µL; 219 injection mode: split 1:20; FID detector temperature 200 °C; carrier gas N5 nitrogen (flow 1 220 mL/min). During the analysis System Control software - Varian Star was used. 221

222

223 High-performance liquid chromatography analysis - fermentation broth analysis

For the determination of the glucose and TPC content in fermentation broth, liquid 224 chromatography was applied. The filtered sample (50 μ L) of fermentation broth was directly 225 introduced into the HPLC system. The analysis was provided by means of liquid 226 chromatograph (Merck - Hitachi, Germany) equipped with a pump L-7100 with the so-called 227 low-pressure gradient system was applied. The Shodex SH1011, (7 µm, 8 x 300 mm) column 228 was used. It was thermostated by means of the ACS thermostat. The system had two detectors 229 connected in series: Spectrophotometric (L-7450 - Merck - Hitachi, Germany) in the UV-VIS 230 range using photodiode (DAD) and differential refractometric sensors (RID - RI Detector 231 2100 - Knauer, Germany). In addition, the apparatus had a valve to change the direction of the 232

233 mobile phase flow in the back-flush column (Merck, Germany), controlled manually. HSM234 software was used to record and process the results.

In the HPLC studies, the eluent used was: $H_2O + 0.2\%$ HCOOH at a flow rate of 1.2 mL/min. The temperature of the thermostated column was 60 °C. The total analysis time was 30 minutes. HPLC analysis was carried out of 50 µL sample. After 7.6 minutes, a back-flush was used to elute the TPC that were determined relative to the syringol standard. The total TPC content was determined with reference to the syringol calibration curve (TPC standard) in the range from 0.9 to 6.5 mg/mL according to previous work (Słupek et al. 2018).

241

242

Results and discussion

The objective of this paper is to present the application of sensor matrices as an alternative method for gas analysis. To analyze the products obtained during mesophilic and thermophilic dark fermentation, a sensor matrices consisted of sensors selective for hydrogen and methane were constructed.

The changes in the composition of gases generated during dark fermentation are 247 presented in Fig. 2 and Fig. 3. Significantly higher concentrations of hydrogen and methane 248 were obtained for fermentation under mesophilic conditions compared to thermophilic 249 conditions. The highest concentrations of hydrogen and methane were obtained in the range 250 of 40 to 45 hours of the mesophilic process and in the range of 20 to 24 hours of the 251 thermophilic process. In both processes, the concentration of methane produced remains 252 constant, while the hydrogen concentration changes significantly during the process. In order 253 to effectively use the activated sludge after the Faloye process, 3.0 g of glucose was added for 254 stimulation of the microspheres of bacteria responsible for methanogenesis. After 85 h of both 255 processes, the termination of hydrogen production is visible, while methane production 256 remains at a constant level. 257

It was noticed, that the application of sensor matrices allows to obtain an on-line gas 258 analysis and with its application it is possible to obtain two different streams during anaerobic 259 digestion. In the first stage of the process the main gaseous products are hydrogen and carbon 260 dioxide, while after 80 hours of the process, the second stage starts and methane is formed. It 261 can be assumed that methanogens consume the acids generated by hydrogenogenic bacteria in 262 the first stage of the process. Separation of the streams may allow decreasing the costs related 263 to the separation of biohydrogen from biomethane. In order to accurately determine the end of 264 the stage production biohydrogen and the start of the stage production biomethane, on-line 265 gas analysis is necessary. 266



Fig. 2 Changes in the gas composition (hydrogen and methane) occurring during dark respect to the mesophilic process determined by means of gas chromatography (GC – green and violet line) and sensor matrices (SM – red and blue line) (n=3).





Fig. 3 Changes in gas composition (hydrogen and methane) occurring during dark fermentation with respect to the thermophilic process done by means of gas chromatography (GC - green and violet line) and sensor matrices (SM – red and blue line) (n=3).

The results obtained by commercially available selective sensors for methane and hydrogen were used and the results were compared with the results obtained during GC analysis. It can be concluded that the results obtained using sensor matrices (see blue and red line, Fig. 2 and Fig. 3) correspond with the gas chromatography results (see violet and green line, Fig. 2 and Fig. 3).

The repeatability of the analytical procedure for sensor matrices and gas 284 chromatography was determined by means of the standard deviation value (RSD) obtained as 285 a result of three analysis operations of the reference gas sample at 1000 ppm methane and 286 hydrogen. As a result of comparison of repeatability of both analytical procedures, RSD = 287 2.82% (methane) and RSD = 3.54% (hydrogen) were obtained for sensor matrices, RSD = 288 1.59% (methane) and RSD = 1.81% (hydrogen) for gas chromatography. In the real process, 289 the minimum and maximal concentration differences of the resulting biogas were found 290 between the results obtained from sensor matrices and GC. The lowest difference for the 291

methane concentration in the mesophilic process was 0.35 ppm, while the highest 52.66 ppm. 292 In the thermophilic process, the lowest difference was 0.20 ppm and the highest difference 293 was 6.52 ppm. The calculations were also made for hydrogen concentration where the lowest 294 differences of 9.47 ppm were obtained in the mesophilic process, while the highest - 1053.46 295 ppm. In the thermophilic process, the lowest concentration was 0.85 ppm, while the highest 296 difference was 56.15 ppm. The average standard deviation between the obtained results from 297 the sensor matrix and GC, which was at the RSD level = 3.89% (methane) and RSD = 8.95%298 (hydrogen) was calculated. In addition, the results were analyzed by analysis of variance 299 (ANOVA) (Table S1-S4). In ANNOVA, the values of statistical parameter p were used as 300 criteria at a 95% confidence level. All the obtained results using both methods (GC and SM) 301 were found to be statistically insignificant due to the p-value higher than 0.05. For the 302 thermophilic process, the p-value was 0.68 and 0.74 for methane and hydrogen respectively 303 and for the mesophilic process, the p-value was 0.99 and 0.97 for methane and hydrogen 304 respectively. The obtained differences in the values are acceptable and indicate the usefulness 305 of sensor matrices in the on-line control of the dark fermentation process. However, the 306 correctness of the results obtained by sensors matrices should be periodically checked using 307 gas chromatography. 308

Biogas production is a sensitive process because there are strong correlations of many factors 309 (such as substrate concentration, composition of fermentation broth, temperature and pH 310 value) that affect the efficiency of the production of biohydrogen and biomethane. These 311 additional parameters were also monitored and controlled throughout the dark fermentation. 312 Total glucose concentration used as the sole carbon source in the initial fermentation broth 313 was set at 5.5 g/L for each of the processes (mesophilic and thermophilic). Hence, the glucose 314 content for each analyzed process corresponds proportionally with the data presented in Fig. 4 315 as well as Tables S5 and S5. One way dark fermentation can occur is the conversion of 316 glucose to hydrogen and acetic acid (Eq. 1). This reaction occurs spontaneously with a 317 maximum theoretical production of 4 moles of hydrogen per mole of glucose as soon as acetic 318 acid is one of VFA. In addition, other VFAs may be formed in fermentation processes such 319 propionic acid (Eq. 2), which reduce the efficiency of the process (Manish and Banerjee 2008; 320 Luo et al. 2010). 321

$$C_{6}H_{12}O_{6} + 2H_{2}O \rightarrow 2CH_{3}COOH + 2CO_{2} + 4H_{2}$$
(1)

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$$
⁽²⁾

322





Fig. 4 Changes in the glucose, total phenolic compound (TPC) and selected volatile fatty
acids (VFA) concentration in fermentation broth during dark fermentation A) mesophilic
process, B) thermophilic process.

The production of biogas under anaerobic conditions in the digester requires the joint 329 action of many populations of microorganisms that have been extracted from the activated 330 sludge. In the fermentation processes, it can be observed that microorganisms not only 331 produce biogas but also VFA (acidogenesis stage). In the next fermentation stage 332 (acetogenesis) propionic, butanoic, and isobutanoic acids are converted to octanoic acid and 333 phenolic compounds (Fig. 4). In the mesophilic process, VFA accumulated much faster in the 334 fermentation chamber, than in thermophilic conditions. It may be a consequence of a much 335 faster loss of glucose in the mesophilic process, which led to faster biogas production but also 336 resulted in the formation of more inhibitors of dark fermentation. Previous studies showed 337 that the optimal pH in terms of biohydrogen production is within a range of 5.0-7.0 which 338 favors the activity of the hydrogenases and is also suitable for microbial development in dark 339 fermentation (Li and Fang 2007; Szulczyński et al. 2019). During the fermentation process, 340 growth of the bacteria that contribute to the formation of volatile organic acids, resulting in 341 decrease in pH. However, after a few days of the process of reaching an increase in pH due to 342 the conversion of organic acids to methane after multiplication of methanogens. Rapid pH 343 changes can adversely affect stability and efficiency process. Therefore, the process was 344 carried out with pH control (Cieślik et al. 2016). 345

Based on the data presented in Tables S5 and S6, a correlation matrix for the in Tables S5 and S

	Methane	Hydrogen	Glucose	TPC	Acetic Acid	Propionic Acid	Butanoic Acid	Isobutanoic Acid	
Methane	1	0.93			-0.2			-0.36	
Hydrogen	0.93	1	-0.13	0.06	-0.21	-0.16	-0.14	-0.36	
Glucose	-0.14	-0.13	1	-0.76	-0.28	-0.59	-0.65	-0.44	
TPC	0.07	0.06	-0.76	1	0.47	0.73	0.84	0.71	
Acetic Acid	-0.2	-0.21	-0.28	0.47	1	0.44	0.65	0.61	
Propionic Acid	-0.17	-0.16	-0.59	0.73	0.44	1	0.93	0.84	

0.8

0.6

0.4

0.2

0

-0.2

-0.4

-0.6

-0.8

-1

348 prepared. The correlation matrix was created by means of R Studio software (Fig. 5 and Fig.349 6) (RStudio 2016; RCore 2018).

350

Butanoic Acid

Isobutanoic Acid

-0.36

-0.36

351 Fig. 5 Correlation matrix for the formation of hydrogen and methane, but also for glucose,352 individual inhibitors and total phenolic compound (TPC) - mesophilic process.

0.84

0.71

0.65

0.61

0.93

0.84

1

0.9

0.9

1

-0.65

-0.44

	Methane	Hydrogen	Glucose	TPC	Acetic Acid	Propionic Acid	Butanoic Acid	Isobutanoic Acid	
Methane	1	0.4	0.31	-0.28	-0.59	-0.33	-0.26	-0.4	- (
Hydrogen	0.4	1	0.8	-0.82	-0.89	-0.89	-0.82	-0.9	- (
Glucose	0.31	0.8	1	-0.95	-0.69	-0.92	-0.93	-0.9	- (
TPC	-0.28	-0.82	-0.95	1	0.7	0.92	0.93	0.89	
Acetic Acid	-0.59	-0.89	-0.69	0.7	1	0.8	0.7	0.85	
Propionic Acid	-0.33	-0.89	-0.92	0.92	0.8	1	0.98	0.98	
Butanoic Acid	-0.26	-0.82	-0.93	0.93	0.7	0.98	1	0.93	• •
Isobutanoic Acid	-0.4	-0.9	-0.9	0.89	0.85	0.98	0.93	1	

354

Fig. 6 Correlation matrix for the formation of hydrogen and methane, but also for glucose,
individual inhibitors and total phenolic compound (TPC) - thermophilic process.

357

Correlation analysis consists in examining whether two variables (expressed in 358 numbers) are significantly related to each other. The calculated determination ratio varies 359 from -1 to 1. A positive correlation appears when the increase in the value of one variable 360 corresponds with the increase in the value of the second variable, while negative correlation 361 occurs when the increase in the value of one variable corresponds with the decrease in the 362 value of the second variable. A value of (0) means a total lack of correlation between the two 363 factors (Zhu et al. 2017). Unexpectedly, it was found that the production of hydrogen and 364 methane is negatively correlated with the concentration of glucose in the growth medium (see 365 Fig.5) - the mesophilic process, with respect to the increase of the glucose concentration. 366 ³⁶⁷ However, there is a positive correlation between hydrogen and methane generation and the

concentration of glucose during the process carried out in thermophilic conditions. 368 Thermophilic process course corresponds with the tendencies presented in the literature, for 369 diversified biofuels generation (Wilkie et al. 2000; Eskicioglu et al. 2011; Cieślik et al. 2016; 370 Łukajtis et al. 2018). The authors suppose, that these untypical results are related with the 371 sudden changes in the broth composition, i.e a significant decrease in glucose concentration 372 after 46 hours of the process, due to glucose supplementation. In the mesophilic process the 373 decrease in glucose concentration is observed after 15 h of the process. In addition, a strong 374 positive correlation is observed for methane and hydrogen generation, which indicates the 375 simultaneous formation of both gases under mesophilic and thermophilic conditions. During 376 the dark fermentation process organic compounds break down into small molecules which are 377 substrates for the hydrogen generation by hydrogenogenes, which are also able to generate 378 acetic acid. In the first stage of biogas production, hydrogen, methane, and TPC are produced. 379 The formulated acetic acid is used by methanogenes to produce methane (Bateni et al. 2017). 380 Therefore, for this stage of the process, it may be crucial to consider acetic acid as a second 381 carbon source, besides glucose in order to provide conclusions regarding chemometrical 382 analysis of the processes. Higher concentrations of acetic acid are obtained during mesophilic 383 process and therefore, its effect on the mesophilic process (Fig.4a) course is noticeable. 384 Preparing a procedure for carbon balance in the system may be a required step to be 385 considered in further research. Methane and hydrogen productivity correlation during the 386 thermophilic process is lower in comparison with the mesophilic process. The correlation 387 matrices for the mesophilic and thermophilic process demonstrate a strong negative 388 correlation of glucose concentration with TPC and VFA concentration during 389 hydrogenogenesis. Decreasing glucose concentration and an increase in TPC concentration 390 result in a decrease in biogas productivity. 391

Conclusions

The paper presents the use of chromatographic techniques and sensor matrices for the monitoring of hydrogen and methane production during the dark fermentation process carried out under mesophilic and thermophilic conditions (model conditions). In order to understand the changes occurring during the whole dark fermentation process, gas phase (methane, hydrogen) studies and fermentation broth (glucose, VFA, TPC) studies were carried out. In the first stage of dark fermentation, the production of hydrogen was mainly observed. The

392

393

394

second stage was initiated, which consisted of redirecting the process to methanisation. The 402 results indicate significantly higher concentrations of hydrogen and methane during the dark 403 fermentation process under mesophilic conditions than in the process under thermophilic 404 conditions. The concentration of biogas (methane and hydrogen) is closely related to the 405 content of glucose in the nutrient solution. In the mesophilic process, a significant decrease in 406 glucose concentration was observed. Microorganisms in the first stage of the fermentation 407 process, convert glucose to biogas, and after 17 hours to VFA, while after 20 h other 408 fermentation inhibitors (TPC) was also created. Similarly, in the thermophilic process, 409 initially, glucose is converted by bacteria into gases, in turn, both phenolic compounds and 410 VFA are formed after 46 hours of the process. In both mesophilic and thermophilic processes, 411 the decrease in the production efficiency of hydrogen and methane is associated with an 412 increase in the concentration of fermentation inhibitors (VFA and TPC). Microorganisms 413 cease to produce both hydrogen and methane after consumption of glucose. 414

Correlation of factors enabled also the selection of significant variables that should be 415 controlled on-line during processes carried out in actual real conditions. The most important 416 parameters - concentration of methane and hydrogen was monitored on-line during 417 fermentation processes by sensor matrices. The results obtained from sensor matrices are 418 comparable to those obtained with gas chromatography coupled with a TCD and FID. The 419 results indicate suitability of sensors matrices for on-line routine analyses of hydrogen and 420 methane during fermentation processes. Moreover, sensor matrices based analysis enables 421 finding the point at which the hydrogen generating bacteria culture is terminated and the 422 fermentation process tends to redirect to the anaerobic digestion and the production of 423 methane. Hydrogen and methane production using one process allows a better use of the 424 potential of bacteria contained in the activated sludge, and also significantly reduces the cost 425 of biogas production compared to individual processes. In addition, the use of sensor matrices 426 allows immediate correction of the fermentation broth composition, which allows to improve 427 the efficiency of biogas production. The use of GC techniques in "off-line" or "in-line" mode 428 results in a long delay in the results obtained, which prevents immediate action to correct the 429 process or eliminate potential system failures. 430

In the case of biogas production, i.e. from landfills, the obtained biogas stream contains much more pollutants (i.e. hydrogen sulphide, ammonia, carbon dioxide, volatile organic compounds) which can affect the process of dark fermentation and the operation and correctness of results obtained from sensor matrices. Therefore, in the future, it is planned to 435 create sensor matrices in which additional temperature, humidity, and selective pollution436 sensors will be considered.

437

Author Contributions: Edyta Słupek, Karolina Kucharska conceived and designed the
experiments. Edyta Słupek, Patrycja Makoś carried the experiments. Edyta Słupek, Patrycja
Makoś, Karolina Kucharska and Jacek Gębicki wrote the paper.

441

442 Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had 443 no role in the design of the study; in the collection, analyses, or interpretation of data; in the 444 writing of the manuscript, and in the decision to publish the results.

445 446

References

Azbar N, Dokgöz FT, Keskin T, et al (2009) Comparative Evaluation of Bio-Hydrogen
Production From Cheese Whey Wastewater Under Thermophilic and Mesophilic
Anaerobic Conditions. International Journal of Green Energy 6:192–200. doi:
10.1080/15435070902785027

451 Bateni H, Saraeian A, Able C (2017) A comprehensive review on biodiesel purification and
452 upgrading. Biofuel Research Journal 4:668–690. doi: 10.18331/brj2017.4.3.5

453 Cheng H, Hiro Y, Hojo T, Li YY (2018) Upgrading methane fermentation of food waste by
454 using a hollow fiber type anaerobic membrane bioreactor. Bioresource Technology
455 267:386–394. doi: 10.1016/j.biortech.2018.07.045

Cheng J, Xie B, Zhou J, et al (2010) Cogeneration of H2 and CH4 from water hyacinth by
two-step anaerobic fermentation. International Journal of Hydrogen Energy. doi:
10.1016/j.ijhydene.2009.07.012

Chu CFC-F, Xu K-QKQK-Q, Li YYY-Y, Inamori Y (2012) Hydrogen and methane potential
based on the nature of food waste materials in a two-stage thermophilic fermentation
process. International Journal of Hydrogen Energy 37:10611–10618. doi:
10.1016/j.ijhydene.2012.04.048

463 Cieślik M, Dach J, Lewicki A, et al (2016) Methane fermentation of the maize straw silage
464 under meso- and thermophilic conditions. Energy 115:1495–1502. doi:
465 10.1016/j.energy.2016.06.070

466 De Gioannis G, Muntoni A, Polettini A, Pomi R (2013) A review of dark fermentative
467 hydrogen production from biodegradable municipal waste fractions. Waste Management
468 33:1345–1361. doi: 10.1016/j.wasman.2013.02.019

⁴⁶⁹ Ding L, Cheng J, Lu H, et al (2017) Three-stage gaseous biofuel production combining dark
⁴⁷⁰ hydrogen, photo hydrogen, and methane fermentation using wet Arthrospira platensis
⁴⁷¹ cultivated under high CO2 and sodium stress. Energy Conversion and Management
⁴⁷² 148:394–404. doi: 10.1016/j.enconman.2017.05.079

473 Eskicioglu C, Kennedy KJ, Marin J, Strehler B (2011) Anaerobic digestion of whole stillage
474 from dry-grind corn ethanol plant under mesophilic and thermophilic conditions.
475 Bioresource Technology 102:1079–1086. doi: 10.1016/j.biortech.2010.08.061

476 Faloye FD, Gueguim Kana EB, Schmidt S (2013) Optimization of hybrid inoculum
477 development techniques for biohydrogen production and preliminary scale up.
478 International Journal of Hydrogen Energy 38:11765–11773. doi:
479 10.1016/j.ijhydene.2013.06.129

Faloye FD, Gueguim Kana EB, Schmidt S (2014) Optimization of biohydrogen inoculum
development via a hybrid pH and microwave treatment technique - Semi pilot scale
production assessment. International Journal of Hydrogen Energy 39:5607–5616. doi:
10.1016/j.ijhydene.2014.01.163

⁴⁸⁴ Feng K, Li H, Deng Z, et al (2020) Effect of pre-fermentation types on the potential of
⁴⁸⁵ methane production and energy recovery from food waste. Renewable Energy 146:1588–
⁴⁸⁶ 1595. doi: 10.1016/j.renene.2019.07.127

⁴⁸⁷ Fenske JJ, Griffin DA, Penner MH (1998) Comparison of aromatic monomers in
⁴⁸⁸ lignocellulosic biomass prehydrolysates. Journal of Industrial Microbiology and
⁴⁸⁹ Biotechnology 20:364–368. doi: 10.1038/sj.jim.2900543

Gebicki J (2016) Trends in Analytical Chemistry Application of electrochemical sensors and
 sensor matrixes for measurement of odorous chemical compounds. Trends in Analytical
 Chemistry 77:1–13. doi: 10.1016/j.trac.2015.10.005

Gebicki J, Dymerski T (2016) Application of Chemical Sensors and Sensor Matrixes to Air
Quality Evaluation. Elsevier Ltd

Gomez X, Moran A, Cuetos MJ, et al (2006) The production of hydrogen by dark
fermentation of municipal solid wastes and slaughterhouse waste: A two-phase process.
Journal of Power Sources 157:727–732. doi: 10.1016/j.jpowsour.2006.01.006

Guo WQ, Ren NQ, Wang XJ, et al (2008) Biohydrogen production from ethanol-type
fermentation of molasses in an expanded granular sludge bed (EGSB) reactor.
International Journal of Hydrogen Energy. doi: 10.1016/j.ijhydene.2008.05.033

⁵⁰¹ Hoff SJ, Bundy DS, Nelson MA, et al (2006) Emissions of ammonia, hydrogen sulfide, and ⁵⁰² odor before, during, and after slurry removal from a deep-pit swine finisher. Journal of fermentation of de-oiled Jatropha waste hydrolyzates. International Journal of Hydrogen
 Energy 40:10766–10774. doi: 10.1016/j.ijhydene.2015.06.118

Lay JJ, Lee YJ, Noike T (1999) Feasibility of biological hydrogen production from organic
fraction of municipal solid waste. Water Research 33:2579–2586. doi: Doi
10.1016/S0043-1354(98)00483-7

Lee KS, Lo YC, Lin PJ, Chang JS (2006) Improving biohydrogen production in a carrierinduced granular sludge bed by altering physical configuration and agitation pattern of
the bioreactor. International Journal of Hydrogen Energy 31:1648–1657. doi:
10.1016/j.ijhydene.2005.12.020

Lestinsky P, Grycova B, Pryszcz A, et al (2017) Hydrogen production from microwave
 catalytic pyrolysis of spruce sawdust. Journal of Analytical and Applied Pyrolysis
 124:175–179. doi: 10.1016/j.jaap.2017.02.008

Levin DB, Pitt L, Love M (2004) Biohydrogen production: Prospects and limitations to
 practical application. International Journal of Hydrogen Energy 29:173–185. doi:
 10.1016/S0360-3199(03)00094-6

Li C, Fang HHP (2007) Fermentative hydrogen production from wastewater and solid wastes
by mixed cultures. Critical Reviews in Environmental Science and Technology 37:1–39.
doi: 10.1080/10643380600729071

Management

Association

56:581-590.

doi:

the

503

Air

and

Waste

⁵³⁷ Łukajtis R, Rybarczyk P, Kucharska K, et al (2018) Optimization of saccharification
⁵³⁸ conditions of lignocellulosic biomass under alkaline pre-treatment and enzymatic
⁵³⁹ hydrolysis. Energies. doi: 10.3390/en11040886

Luo C, Brink DL, Blanch HW (2002) Identification of potential fermentation inhibitors in
conversion of hybrid poplar hydrolyzate to ethanol. Biomass and Bioenergy 22:125–138.
doi: 10.1016/S0961-9534(01)00061-7

Luo G, Xie L, Zou Z, et al (2010) Anaerobic treatment of cassava stillage for hydrogen and
methane production in continuously stirred tank reactor (CSTR) under high organic
loading rate (OLR). International Journal of Hydrogen Energy 35:11733–11737. doi:
10.1016/j.ijhydene.2010.08.033

Madsen M, Holm-Nielsen JB, Esbensen KH (2011) Monitoring of anaerobic digestion
processes: A review perspective. Renewable and Sustainable Energy Reviews 15:3141–
3155. doi: 10.1016/j.rser.2011.04.026

Manish S, Banerjee R (2008) Comparison of biohydrogen production processes. International
 Journal of Hydrogen Energy 33:279–286. doi: 10.1016/j.ijhydene.2007.07.026

Nilvebrant NO, Reimann A, Larsson S, Jönsson LJ (2001) Detoxification of lignocellulose
hydrolysates with ion-exchange resins. Applied Biochemistry and Biotechnology - Part
A Enzyme Engineering and Biotechnology 91–93:35–49. doi: 10.1385/ABAB:91-93:19:35

Ottaviano LM, Ramos LR, Botta LS, et al (2017) Continuous thermophilic hydrogen
production from cheese whey powder solution in an anaerobic fluidized bed reactor:
Effect of hydraulic retention time and initial substrate concentration. International
Journal of Hydrogen Energy. doi: 10.1016/j.ijhydene.2016.11.168

Pan C, Zhang S, Fan Y, Hou H (2010) Bioconversion of corncob to hydrogen using anaerobic
 mixed microflora. International Journal of Hydrogen Energy. doi:
 10.1016/j.ijhydene.2009.04.023

Panagiotopoulos IA, Bakker RR, De Vrije T, et al (2010) Pretreatment of sweet sorghum
bagasse for hydrogen production by Caldicellulosiruptor saccharolyticus. International
Journal of Hydrogen Energy 35:7738–7747. doi: 10.1016/j.ijhydene.2010.05.075

⁵⁶⁶ Pandu K, Joseph S (2012) Comparisons and Limitations of Biohydrogen Production
 ⁵⁶⁷ Processes: a Review. International Journal of Advances in Engineering & Technology
 ⁵⁶⁸ 2:2231–1963

Fer Persson †, Jessica Andersson †, Lo Gorton †, et al (2002) Effect of Different Forms of
Alkali Treatment on Specific Fermentation Inhibitors and on the Fermentability of

- Lignocellulose Hydrolysates for Production of Fuel Ethanol. Journal of Agricultural and
 Food Chemistry 50:5318–5325. doi: 10.1021/jf0255650
- 573 Ponzoni A, Baratto C, Cattabiani N, et al (2017) Smetal oxide gas sensors, a survey of
- selectivity issues addressed at the SENSOR lab, Brescia (Italy). Sensors (Switzerland)
- 575 17:. doi: 10.3390/s17040714
- 576 Quéméneur M, Hamelin J, Barakat A, et al (2012) Inhibition of fermentative hydrogen 577 production by lignocellulose-derived compounds in mixed cultures. International Journal
- of Hydrogen Energy 37:3150–3159. doi: 10.1016/j.ijhydene.2011.11.033
- ⁵⁷⁹ Qureshi N, Saha BC, Dien B, et al (2010a) Production of butanol (a biofuel) from agricultural
 ⁵⁸⁰ residues: Part I Use of barley straw hydrolysate. Biomass and Bioenergy 34:566–71.
 ⁵⁸¹ doi: 10.1016/j.biombioe.2009.12.024
- ⁵⁸² Qureshi N, Saha BC, Hector RE, et al (2010b) Production of butanol (a biofuel) from
 ⁵⁸³ agricultural residues: Part II Use of corn stover and switchgrass hydrolysates. Biomass
 ⁵⁸⁴ and Bioenergy 32:176–83. doi: 10.1016/j.biombioe.2009.12.023
- 585 RCore (2018) R Core, Team, https://www.r-project.org/
- ⁵⁸⁶ Rosales-Colunga LM, González-García R, De León Rodríguez A (2010) Estimation of
 ⁵⁸⁷ hydrogen production in genetically modified E. coli fermentations using an artificial
 ⁵⁸⁸ neural network. International Journal of Hydrogen Energy 35:13186–13192. doi:
 ⁵⁸⁹ 10.1016/j.ijhydene.2010.08.137
- ⁵⁹⁰ Rosecrance JC, Paulsen R, Gilkey D, et al (2013) Control of mixing step in the bread
 ⁵⁹¹ production with weak wheat flour and sourdough. Journal of Agricultural ... XLIV:10–
 ⁵⁹² 13. doi: 10.4081/jae.2013.(s1)
- 593 RStudio (2016) RStudio Team, Version: 3.5.2, http://www.rstudio.com/
- Słupek E, Makoś P, Kamiński M (2018) CAMERA SEPARATORIA Volume 10, Number 2
 / 2018, pp. 52-63 Metodyka oznaczania sumarycznej zawartości inhibitorów
 fermentacji ciemnej oraz monocukrów w brzeczkach fermentacyjnych techniką HP LCRID-UV-VIS / DAD Methodology for determining the total conte. 10:52–63
- 598 Szulczyński B, Kucharska K, Kamiński M (2019) Laboratory bioreactor with pH control
 599 system for investigations of hydrogen production in the dark fermentation process.
 600 Aparatura Badawcza i Dydaktyczna 39–46
- Szulczyński B, Wasilewski T, Wojnowski W, et al (2017) 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 (Switzerland) 17:.
 doi: 10.3390/s17112671

Tan L, Nishimura H, Wang YF, et al (2019) Effect of organic loading rate on thermophilic
methane fermentation of stillage eluted from ethanol fermentation of waste paper and
kitchen waste. Journal of Bioscience and Bioengineering 127:582–588. doi:
10.1016/j.jbiosc.2018.10.006

Teplyakov V V., Gassanova LG, Sostina EG, et al (2002) Lab-scale bioreactor integrated with
active membrane system for hydrogen production: Experience and prospects.
International Journal of Hydrogen Energy 27:1149–1155. doi: 10.1016/S03603199(02)00093-9

⁶¹³ Veluchamy C, Kalamdhad AS (2017) Enhanced methane production and its kinetics model of
 thermally pretreated lignocellulose waste material. Bioresour Technol 241:1–9. doi:
 10.1016/j.biortech.2017.05.068

⁶¹⁶ Wang B, Wan W, Wang J (2009) Effect of ammonia concentration on fermentative hydrogen
⁶¹⁷ production by mixed cultures. Bioresource Technology 100:1211–1213. doi:
⁶¹⁸ 10.1016/j.biortech.2008.08.018

Wilkie AC, Riedesel KJ, Owens JM (2000) Stillage characterization and anaerobic treatment
 of ethanol stillage from conventional and cellulosic feedstocks. Biomass and Bioenergy
 19:63–102. doi: 10.1016/S0961-9534(00)00017-9

Wong YM, Wu TY, Juan JC (2014) A review of sustainable hydrogen production using seed
sludge via dark fermentation. Renewable and Sustainable Energy Reviews 34:471–482.
doi: 10.1016/j.rser.2014.03.008

Wu KJ, Chang CF, Chang JS (2007) Simultaneous production of biohydrogen and bioethanol
 with fluidized-bed and packed-bed bioreactors containing immobilized anaerobic sludge.
 Process Biochemistry 42:1165–1171. doi: 10.1016/j.procbio.2007.05.012

Wu KJ, Chang JS (2007) Batch and continuous fermentative production of hydrogen with
 anaerobic sludge entrapped in a composite polymeric matrix. Process Biochemistry
 42:279–284. doi: 10.1016/j.procbio.2006.07.021

Wu X, Zhu J, Dong C, et al (2009) Continuous biohydrogen production from liquid swine
manure supplemented with glucose using an anaerobic sequencing batch reactor.
International Journal of Hydrogen Energy 34:6636–6645. doi:
10.1016/j.ijhydene.2009.06.058

Yang H, Shao P, Lu T, et al (2006) Continuous bio-hydrogen production from citric acid
wastewater via facultative anaerobic bacteria. International Journal of Hydrogen Energy
31:1306–1313. doi: 10.1016/j.ijhydene.2005.11.018

638 Yasin NHM, Mumtaz T, Hassan MA, Abd Rahman N (2013) Food waste and food processing

waste for biohydrogen production: A review. Journal of Environmental Management
130:375–385. doi: 10.1016/j.jenvman.2013.09.009

- ⁶⁴¹ Zhu G-F, Wu P, Wei Q-S, et al (2010) Biohydrogen production from purified terephthalic
 ⁶⁴² acid (PTA) processing wastewater by anaerobic fermentation using mixed microbial
 ⁶⁴³ communities. International Journal of Hydrogen Energy 35:8350–8356. doi:
 ⁶⁴⁴ 10.1016/j.ijhydene.2009.12.003
- ⁶⁴⁵ Zhu Q, Liu Q, Qin SJ (2017) Concurrent Monitoring and Diagnosis of Process and Quality
 ⁶⁴⁶ Faults with Canonical Correlation Analysis. IFAC-PapersOnLine 50:7999–8004. doi:
 ⁶⁴⁷ 10.1016/j.ifacol.2017.08.1222
- 648
- 649
- 650

Mesophilic and thermophilic dark fermentation course analysis using sensor matrices and chromatographic techniques

Edyta Słupek^{*1}. Patrycja Makoś¹. Karolina Kucharska¹. Jacek Gębicki¹

¹ Gdańsk University of Technology. Faculty of Chemistry. Department of Process Engineering and Chemical Technology. 80-233 Gdańsk. Narutowicza 11/12 street. Poland

*Corresponding author. e-mail: edyta.slupek@pg.edu.pl

Source of variation	SS	df	MS	F	p-value	F-critical
Between groups	2.109149	1	2.109149	0.16484	0.686707	4.061706
Within groups	562.9854	44	12.79512			
Total	565.0945	45				

Table S1 Analysis of variance (ANOVA) for the methane in thermophilic process.

Table S2 Analysis of variance (ANOVA) for the hydrogen in thermophilic process.

Source of variation	SS	df	MS	F	p-value	F-critical
Between groups	2390.174	1	2390.174	0.108959	0.742899	4.061706
Within groups	965205.1	44	21936.48			
Total	967595.3	45				

Table S3 Analysis of variance (ANOVA) for the methane in mesophilic process.

Source of variation	SS	df	MS	F	p-value	F-critical
Between groups	4.28611	1	4.28611	5.97E-05	0.99387	4.061706
Within groups	3158609	44	71786.58			
Total	3158614	45				

Table S4 Analysis of variance (ANOVA) for the hydrogen in mesophilic process.

Source of variation	SS	df	MS	F	p-value	F-critical
Between groups	154985.6	1	154985.6	0.001415	0.970167	4.061706
Within groups	4.82E+09	44	1.1E+08			
Total	4.82E+09	45				

Table	S5 Cha	anges in the glu	icos	se. total pheno	lic con	npounds	(TPC	C) and selected	volatile f	atty
acids	(VFA)	concentration	in	fermentation	broth	during	dark	fermentation	- mesopł	nilic
proces	SS									

Time	Glucosa	TPC	Acetic Acid	Propionic Acid	Butanoic Acid	Isobutanoic
TILL	Olucose	пс	Actic Acia	Toplonic Acid	Butanole Acid	Acid
[h]				[mg/mL]		
2	5.50	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
15	3.21	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
17	2.56	<lod< td=""><td><lod< td=""><td>0.020</td><td>0.034</td><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td>0.020</td><td>0.034</td><td><lod< td=""></lod<></td></lod<>	0.020	0.034	<lod< td=""></lod<>
20	2.05	1.582	<lod< td=""><td>0.020</td><td>0.031</td><td><lod< td=""></lod<></td></lod<>	0.020	0.031	<lod< td=""></lod<>
24	1.80	3.991	<lod< td=""><td>0.022</td><td>0.031</td><td><lod< td=""></lod<></td></lod<>	0.022	0.031	<lod< td=""></lod<>
26	1.44	4.160	0.011	0.022	0.147	<lod< td=""></lod<>
40	1.21	4.350	0.012	0.123	0.222	<lod< td=""></lod<>
46	0.55	4.888	0.015	0.228	0.525	<lod< td=""></lod<>
48	0.05	4.932	0.015	0.228	0.529	1.845
70	3.00	5.136	0.095	0.358	0.598	1.948
76	2.12	5.340	0.150	0.390	0.658	1.955
80	1.56	5.545	0.240	0.399	0.758	2.020
86	0.68	5.786	0.550	0.490	0.950	2.029
94	0.55	5.958	0.650	0.555	0.999	2.255
111	<lod< td=""><td>6.145</td><td>0.071</td><td>0.898</td><td>1.001</td><td>2.268</td></lod<>	6.145	0.071	0.898	1.001	2.268
115	<lod< td=""><td>6.15</td><td>0.074</td><td>0.969</td><td>1.041</td><td>2.345</td></lod<>	6.15	0.074	0.969	1.041	2.345

LOD – limit of detection;

LOD = 0.01 mg/mL. RSD = 2.15% - values calculated for a concentration of 2.5 mg/mL (glucose). LOD = 0.073 mg/mL. RSD = 1.23% - values calculated for a concentration of 3.6 mg/mL (TPC). LOD = 0.001 - 0.003 mg/mL. RSD = 2.13% - values calculated for a concentration of 5 µg/mL (organic acids)

Time	Glucose	TPC	Acetic	Propionic	Butanoic Acid	Isobutanoic
			Acid	Acid		Acid
[h]				[mg/mL]		
2	5.50	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
15	5.21	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
17	4.97	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
20	4.80	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
24	4.61	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
26	4.55	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
40	4.51	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
46	4.45	1.021	<lod< td=""><td>0.020</td><td>0.030</td><td><lod< td=""></lod<></td></lod<>	0.020	0.030	<lod< td=""></lod<>
48	2.50	1.044	<lod< td=""><td>0.020</td><td>0.049</td><td><lod< td=""></lod<></td></lod<>	0.020	0.049	<lod< td=""></lod<>
70	3.00	1.245	<lod< td=""><td>0.289</td><td>0.339</td><td>0.154</td></lod<>	0.289	0.339	0.154
76	2.21	1.355	<lod< td=""><td>0.350</td><td>0.340</td><td>0.495</td></lod<>	0.350	0.340	0.495
80	1.62	1.579	<lod< td=""><td>0.359</td><td>0.339</td><td>0.513</td></lod<>	0.359	0.339	0.513
86	1.55	1.714	0.010	0.450	0.349	0.526
94	1.41	1.849	0.020	0.468	0.398	0.759
111	1.22	1.912	0.021	0.555	0.400	0.815
115	1.20	1.985	0.030	0.581	0.434	0.828

Table S6 Changes in the glucose. total phenolic compounds (TPC) and selected volatile fatty acids (VFA) concentration in fermentation broth during dark fermentation- thermophilic process

LOD – limit of detection;

LOD = 0.01 mg/mL. RSD = 2.15% - values calculated for a concentration of 2.5 mg/mL (glucose). LOD = 0.073 mg/mL. RSD = 1.23% - values calculated for a concentration of 3.6 mg/mL (TPC). LOD = 0.001 - 0.003 mg/mL. RSD = 2.13% - values calculated for a concentration of 5 µg/mL (organic acids)

	Mesophilic Process				Thermophilic Process			
Time	GC-	MS-	GC-	MS-	GC-	MS-	GC-	MS-
	H_2	H ₂	CH ₄	CH ₄	H ₂	H_2	CH ₄	CH ₄
[h]	[mg /L]*							
2	0.043	0.042	0.061	0.054	0.030	0.029	0.052	0.050
15	0.043	0.042	0.061	0.062	0.036	0.032	0.053	0.051
17	0.038	0.040	0.060	0.064	0.035	0.033	0.057	0.056
20	0.039	0.043	0.060	0.068	0.035	0.033	0.058	0.056
21	0.038	0.043	0.060	0.070	0.035	0.031	0.053	0.056
22	0.038	0.044	0.060	0.070	0.037	0.034	0.052	0.050
23	0.043	0.044	0.061	0.071	0.037	0.040	0.052	0.050
24	0.124	0.061	0.077	0.105	0.045	0.041	0.052	0.052
25	0.225	0.241	0.096	0.108	0.037	0.038	0.052	0.053
39	0.231	0.241	0.112	0.122	0.034	0.031	0.052	0.052
40	1.649	1.743	0.372	0.341	0.035	0.030	0.052	0.053
43	3.327	3.074	0.697	0.661	0.032	0.033	0.052	0.057
44	3.126	3.072	0.658	0.646	0.031	0.030	0.052	0.056
45	0.350	0.308	0.478	0.440	0.031	0.030	0.052	0.052
46	0.050	0.069	0.062	0.071	0.034	0.031	0.052	0.052
48	0.023	0.035	0.057	0.064	0.033	0.029	0.052	0.052
70	0.025	0.023	0.058	0.064	0.029	0.030	0.052	0.057
76	0.013	0.015	0.055	0.064	0.027	0.027	0.052	0.057
80	0.006	0.008	0.059	0.064	0.028	0.028	0.052	0.056
86	0.006	0.008	0.059	0.064	0.000	0.000	0.053	0.056
94	0.000	0.000	0.059	0.057	0.000	0.000	0.053	0.047
111	0.000	0.000	0.051	0.054	0.000	0.000	0.053	0.048
115	0.000	0.000	0.051	0.051	0.000	0.000	0.051	0.048
LOD – limit of detection; RSD = 2.82% and LOD = values calculated for a								

Table S7 Changes in the glucose. total phenolic compounds (TPC) and selected volatile fatty acids (VFA) concentration in fermentation broth during dark fermentation A) mesophilic process. B) thermophilic process.

LOD – mint of detection, RSD = 2.82% and LOD = values calculated for a concentration of 1000 mg/mL CH₄ for MS; RSD = 3.54% and LOD = 0.001 mg/L values calculated for a concentration of 1000 mg/mL H₂ for MS, RSD = 1.59% and LOD = 0.002 mg/L values calculated for a concentration of 1000 mg/mL CH₄ for GC; RSD = 1.81% and LOD = 0.001 mg/L values calculated for a concentration of 1000 mg/mL H₂ for GC

*mg – (H₂ or CH₄) / L (total gas phase)