



## Biomass in biogas production: Pretreatment and codigestion

Archana Kasinath<sup>a,\*\*</sup>, Sylwia Fudala-Ksiazek<sup>b</sup>, Malgorzata Szopinska<sup>a</sup>, Hubert Bylinski<sup>a,c,\*\*\*</sup>, Wojciech Artichowicz<sup>d</sup>, Anna Remiszewska-Skwarek<sup>a</sup>, Aneta Luczkiewicz<sup>a,\*</sup>

<sup>a</sup> Department of Water and Wastewater Technology, Faculty of Civil and Environmental Engineering, Gdansk University of Technology, 11/12 Narutowicza St., Gdansk, 80-233, Poland

<sup>b</sup> Department of Sanitary Engineering, Faculty of Civil and Environmental Engineering, Gdansk University of Technology, 11/12 Narutowicza St., Gdansk, 80-233, Poland

<sup>c</sup> Department of Concrete Structures, Faculty of Civil and Environmental Engineering, Gdansk University of Technology, 11/12 Narutowicza St., Gdansk, 80-233, Poland

<sup>d</sup> Department of Hydraulic Engineering, Faculty of Civil and Environmental Engineering, Gdansk University of Technology, 11/12 Narutowicza St., Gdansk, 80-233, Poland

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### ABSTRACT

Globally, there is increasing awareness that the implementation of ‘waste to energy’ technology is one of the best means to achieve sustainable energy development. The most popular approach is the conversion of organic-rich compounds into clean and renewable products by anaerobic digestion (AD). Biogas can be produced from agricultural residues, municipal/industrial biowastes, and sustainable biomass, especially materials that are locally available. However, in many cases, the methane yields obtained from the conventional AD process are regarded as having limited profitability. This paper summarizes the recent knowledge regarding the different strategies that are used to enhance AD efficiency and the methods to strengthen the existing incentives to overcome today’s barriers to biogas production. Special attention was given to several approaches used to improve the biodegradability of organic matter and the methane potential of feedstocks, mainly codigestion and pretreatment of single/mixed substrates. The statistical analyses indicated enormous variability among biogas systems, thus, there is a need for unification of the methods applied for process control and the parameters used for the discussion of results. A synchronized methodology is also needed to understand the environmental advantages and drawbacks of selected utilization pathways in biogas production. Currently, the underestimated potential of AD is of growing interest, and pretreatment/codigestion can directly increase the effectiveness of this technology and lead to its optimization. Nonetheless, a proper evaluation of the environmental (e.g., sustainable biomass) and social (e.g., bioaerosol nuisance) aspects is also needed.

### 1. Introduction

Fossil fuels (coal, oil, gas) have driven the industrial revolution, which indirectly caused simultaneous technological, economic and social progress but negatively impacted and influenced the environment, e.g., climate change [1]. Thus, currently, to reduce the use of fossil fuels, the development and utilization of renewable energy resources have become a major component of sustainable global energy strategies [2]. Renewable technologies produce power, heat or mechanical energy

using biomass (energy crops, agricultural or forestry residues, biogenic municipal waste, etc.), wind, solar (thermal and photovoltaic), hydro (river flow, tides, waves), and geothermal energy. The Renewable Energy Directive (Directive 2009/28/EC) has set the goal that in the European Union (EU), the abovementioned sources should reach 20% of the overall final energy consumption sources by 2020 [3,4]. Note that each EU member has its own 2020 target according to Annex 1 of Directive 2009/28/EC, which depends on several factors (mainly the renewable energy starting point, the potential of increase and the economic performance) and varies from 10% in Malta, 15% in Poland, to

\* Corresponding author. Department of Water and Wastewater Technology, Faculty of Civil and Environmental Engineering, Gdansk University of Technology, 11/12 Narutowicza St., Gdansk, 80-233, Poland.

\*\* Corresponding author.

\*\*\* Corresponding author. Department of Water and Wastewater Technology, Faculty of Civil and Environmental Engineering, Gdansk University of Technology, 11/12 Narutowicza St., Gdansk, 80-233, Poland.

E-mail addresses: [archana.kasinath@pg.edu.pl](mailto:archana.kasinath@pg.edu.pl) (A. Kasinath), [hubert.bylinski@pg.edu.pl](mailto:hubert.bylinski@pg.edu.pl) (H. Bylinski), [aneta.luczkiewicz@pg.edu.pl](mailto:aneta.luczkiewicz@pg.edu.pl) (A. Luczkiewicz).

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Abbreviations			
AD	anaerobic digestion	KVIC	Khadi and Village Industries Commission
BSP	Biogas Support Programme	LCA	life cycle assessment
CCS	carbon capture storage	MLBPs	medium-level biogas plants
CIPAV	Centre for Research on Sustainable Agricultural Production Systems	MSW	municipal solid waste
COD	chemical oxygen demand	MW	megawatts
DCM	dairy cow manure	NBMMP	The National Biogas and Manure Management Programme
DM	dry mass	P2G	power-to-gas technologies
DMS	dimethyl sulfide	SS	sewage sludge
DNA	deoxyribonucleic acid	T-CM	treated chicken manure
EBA	Environmental Biogas Agency	TPAD	temperature - phased anaerobic digestion
EU	European Union	TS	total solids
F	Fenton oxidation	U	ultrasounds
FAO	Food and Agriculture Organization	UK	United Kingdom
FOG	fats, oils and grease	VACs	volatile aromatic compounds
GC-MS	gas chromatography - mass spectrometry	VOCs	volatile organic compounds
GHG	greenhouse gases	VS	volatile solids
KS test	The Kolmogorov–Smirnov test	VSCs	volatile sulfur compounds
		VSS	volatile suspended solids
		WAS	waste activated sludge
		WWTP	wastewater treatment plant

49% in Sweden. According to Eurostat, in 2019, the gross final energy consumption as a proportion of the energy from renewable sources reached 19.73% in the EU (compared with 8.5% in 2004) [5], which confirmed that the abovementioned national goals have already been achieved by some member states (14 out of 27), while others (including *inter alia* Poland, Slovenia, France and Netherlands) are still far from reaching their goals (for details, see Fig. 1 and Fig. S1). Worldwide, the total final energy consumption from renewable sources has also been increasing and reached 10.6% (with 4% thermal energy, 3.6% – hydropower, 2% – wind power and solar, and 1% – transport biofuels), while nuclear energy and fossil fuels accounted for 2.2% and 79.7%, respectively; the remaining 7.5% of the total final energy consumption was biomass, which is traditionally used for cooking and heating in developing countries [6]. Note that due to both global economic growth

and higher cooling/heating demands in some parts of the world, in 2018, the total energy demand increased by 2.3%, which was the greatest increase in this decade. Energy demand mainly increased in China, the United States, and India (together nearly 70% of the total increase in energy demand). In Europe, despite economic expansion, demand increased by only 0.2% and is explained mainly by an increase in energy efficiency [7].

Access to renewable energy and other energy-related issues (e.g., energy efficiency) have been recognized as a global priority for sustainable development and a key factor for achieving the Sustainable Development Goals (SDG, included in a United Nations Resolution, 2030 Agenda) [9]. The EU also remains quite ambitious in the area of zero carbon emissions and in achieving the aims of the Paris Agreement. This was confirmed on November 28, 2018 when the European Commission

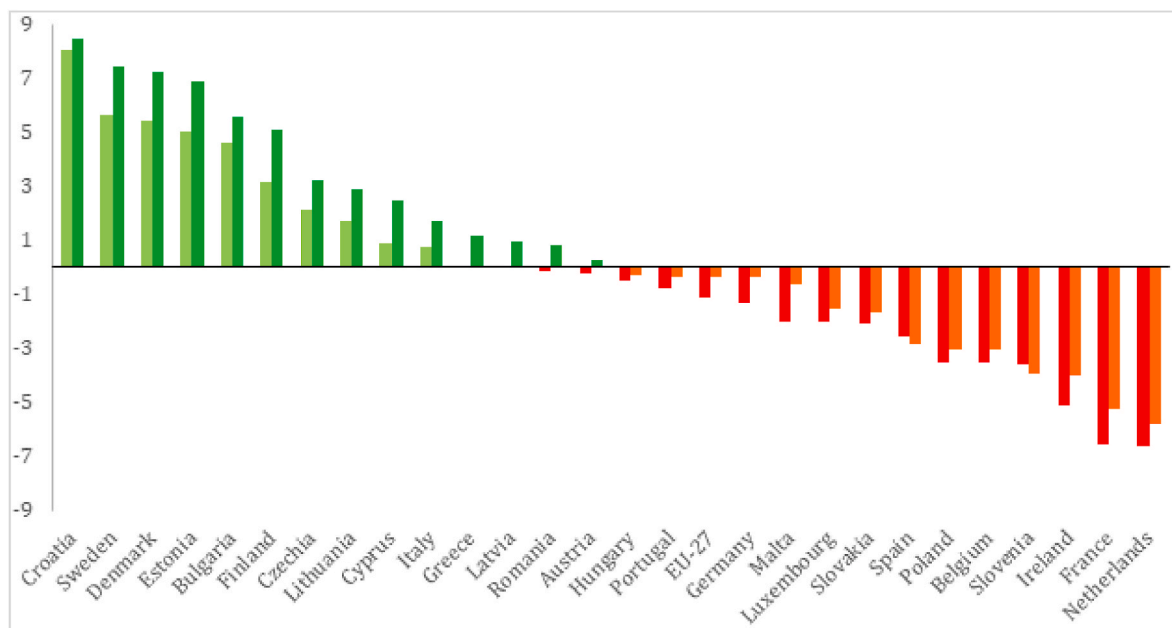


Fig. 1. Differences between the actual proportion of renewable energy (relative to the gross final energy consumption) and the targeted values estimated for each of the 27 EU countries in 2018 (first column) and 2019 (second column); positive values indicate that the actual value was higher than the target value, and negative values indicate that the actual value was lower than the target value [8].

announced a long-term strategy to meet climate-neutral goals by 2050 and, importantly, to simultaneously maintain a prosperous, modern and competitive economy [10]. The basis for this new system is the national energy and climate plans for the next 10 years (from 2021 to 2030) and the national long-term strategies, which all EU members were asked to prepare and submit to the Commission [11].

The transition of industry, transport, agriculture, and other sectors from fossil fuels to zero carbon emissions by 2050 will be supported by the European Green Deal Investment Plan (also referred to as the Sustainable Europe Investment Plan), the new flagship initiative of the European Commission. Under the “Green Deal”, EU countries are expected to jointly achieve 10-year term targets and reduce overall emissions by at least 50–55% by 2030. Since the 2030 target requires concrete efforts across the economy, through the EU budget and associated instruments, approximately €1 trillion will be spent on (I) identifying, structuring, and executing sustainable projects in the power, heating/cooling and transport sectors, (II) facilitating and supporting sustainable, fully decarbonized technological solutions, and (III) maximizing the energy efficiency, smart network infrastructure and circular economy benefits over the next decade [10]. In addition to investments, achieving climate neutrality will require support and close cooperation between the European Commission, EU member states and a wide range of stakeholders. Of high importance is the interconnection and sectoral integration of authorities and public administrations at regional and local levels (this is also required to mitigate the tensions between different ministries, e.g., environment and energy), as well as cooperation between business sectors, research organizations, non-governmental organizations and the public. However, it is uncertain to what extent public engagement may drive the transition of the future energy system; some countries, e.g., France and Germany, have already used stakeholder consultation in the preparation of national long-term strategies (e.g., Ref. [12]). The increasing awareness of climate change and the broader public debate and consultation may

extend the concept of a climate-neutral society, where the consumer choice for products/services with a lower carbon footprint has a powerful role in shaping the future moderation of demand and diversifying de-carbonization pathways.

In the EU, the updated bioeconomy strategy and the 2050 climate-neutral goals will force the energy sector to decarbonize, which means that there is no place for raw fossil fuels [13,14]. In the EU, the transition to a clean energy system and to a carbon-neutral economy and renewable gases (Fig. 2) are expected to play a crucial role. In the literature, various names are used to refer to the different types of renewable gases; thus, renewable gases are regarded as follows: (I) biogas produced through anaerobic digestion (AD), (II) biomethane produced through thermal gasification or after the purification of biogas, (III) hydrogen produced from natural gas using carbon capture storage (CCS) or produced from the electrolysis of water using renewable electricity and (IV) methane from hydrogen produced after the methanation of renewable electricity-sourced hydrogen [15]. According to the above, the general definition of renewable gases is presented in Fig. 2.

Renewable gas production, unlike other renewable energy sources, can be easily transported and stored, can serve to produce heat and electricity [16,17], can be used to power vehicles and can be transported through gas networks [18]. Additionally, their production is highly in line with the circular bioeconomy, which may help to manage biomass resources (food industry/agricultural side products, municipal waste) locally [19]. However, the abovementioned advantage is also a type of limitation because it means that biogas production is not suitable for every location.

In the EU Green Deal strategy, renewable gases are expected to play a key role in achieving climate-neutral objectives by 2050. However, to increase the share of renewable gases in the renewable energy sector, institutional support and favourable conditions are needed. One of the most important legal acts published in 2018 by the EU is the Directive of the European Parliament and of the Council on the promotion of the use

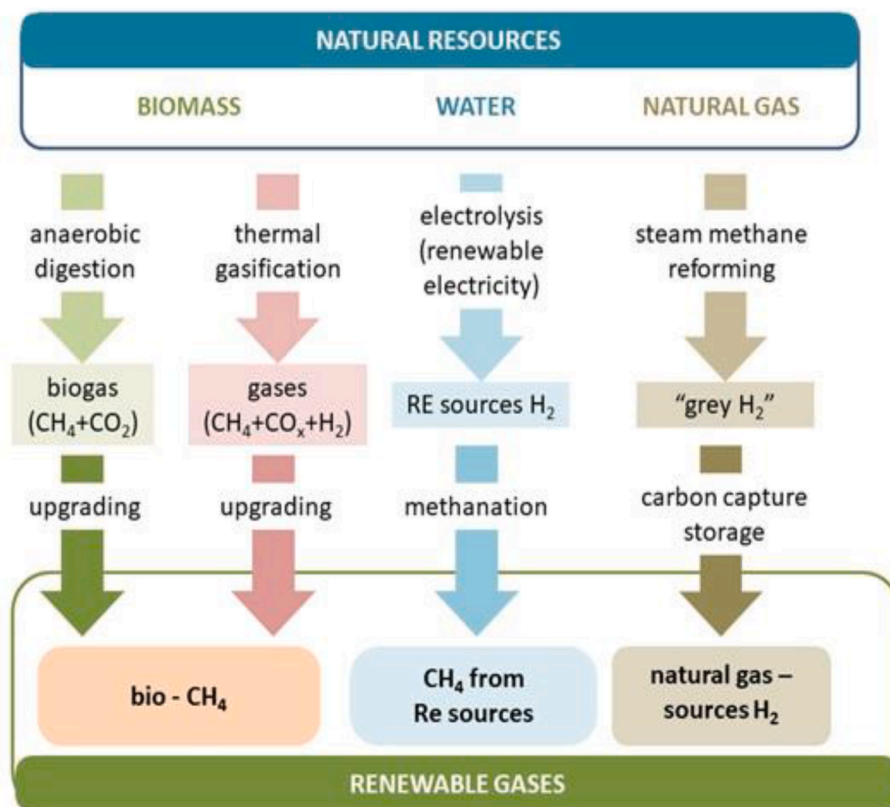


Fig. 2. Definition of renewable gases according to Ref. [15].

of energy from renewable sources [20]. This document establishes a binding EU target for the overall share of renewable energy in gross final energy consumption in 2030. It also sets rules regarding financial support for the use of energy in the transport sector and regional cooperation between member states and other countries. The important aspect described in this directive is the need to reduce greenhouse gas emissions through the use of biofuels, bioliquids and biomass fuels according to the sustainability strategy. The International Energy Agency released a report in 2020 that pointed to the huge, untapped potential of organic waste and sustainable biomass in clean energy production [21]. Both of the abovementioned sources combine bioenergy with carbon capture and storage and, thus, are important for building EU climate neutrality, along with wind and solar energy [22]. The other EU legislation framework related to biogas implementation is mentioned in Table S1 (see the supplementary materials).

Apart from legislation issues, in many countries, different types of limitations exist, such as technical, economic, sociocultural, and environmental limitations [23]. Even in the EU, where the flagship initiative is to accelerate integrated climate actions by, e.g., green investments, an integrated approach and stable support scheme for renewable gases are also strongly needed at the national level. Among different countries, the relevant policies differ, as do energy producer and consumer engagement. Moreover, an important issue is the engagement of non-profit organizations that support renewable gas as a sustainable and flexible (on demand) energy source. A good example is the European Biogas Association (EBA), which was founded in 2009 [24].

AD has a strong heritage and the potential to meet current energy needs, limit greenhouse gas (GHG) emissions and recover nutrients. However, despite the above, and despite the supporting legislative regulations and appropriate financing tools (for the EU and other countries), less than 2.5% of the AD potential is utilized [25]. Thus, in this paper, historical and current aspects of AD are combined with the most important biogas technology issues (the process by which the studies included in the review were explored and selected, is provided in the supplementary materials). Special focus was given to biomass pretreatment and codigestion, as well as optimization of AD processes as a response to the needs of individual entrepreneurs. The opportunities and challenges of AD were combined with the barriers faced by the biogas industry and the upgrading options. The factors that control the effectiveness of biomass digestion and methane yield at the laboratory and industrial scales were analysed through statistical methods. The possibility of comparing the different biogas plants and various AD technologies is vital to understanding the key parameters and biomass footprint of biogas production chains.

## 2. Historical background of biogas production

The history of biogas probably dates back to the 10th century BC in Assyria, where biogas was used for heating bath water [26,27]. In 1630, the Flemish chemist and physician Jan Baptista van Helmont introduced the term “gas” to describe the byproduct of organic matter decomposition emitted to air. Then, in the late 18th century, Alessandro Volta, an Italian physicist, inspired by Benjamin Franklin’s essay on the “flammable air” topic, isolated gaseous bubbles from Maggiore Lake’s marsh and discovered their flammability in a closed vessel. A few years later, in 1808, the presence of methane (biogas) in the AD of cattle manure was detected by Humphrey Davey [28]. In 1875, a Dutch farmer, Wouter Sluys, used natural gas for the first time for illumination purposes, and almost at the same time (in 1884), a student of Louis Pauster, Ulysses Gayon, confirmed in front of the Academy of Science in Paris that the fermentation process could also provide an effective source fuel for heating and lighting (he obtained approximately 100 L of biogas per cubic metre of manure fermented at 35 °C) [29]. Ten years later, in 1895, the first wastewater sludge digester was built in Exeter, UK, which fuelled the streetlamps. In 1897, biogas from human waste was also used for lighting in Matinga Leper Asylum in Mumbai, India. By the 1900s,

AD technology was used in many parts of the world. The most important historical aspects of the development of biogas technology throughout the world are presented in Fig. 3. More detailed information, which is divided into selected regions of the world, is presented in Table S2 (see supplementary materials).

The first-ever attempt to build a plant to produce biogas from manure was constructed in Bombay, India in 1900, but it was not very successful until 1937, when Desai, a microbiologist at the Indian Agricultural Research Institute (IARI, then the Imperial Agricultural Research Institute), led the commissioning of an AD plant, which worked satisfactorily for several years [30]. In 1906, German engineer Karl Imhoff patented a chamber (Imhoff tank), which was used for the reception and AD of the extracted sludge. Importantly, the AD process was also studied as an important part of microbial activity, and in the 1930s, the first anaerobic bacteria were identified, and some conditions to promote methane production were established [31].

Public biogas supply facilities were developed in Europe especially quickly after World War II, which drove the search for alternative sources of energy [32]. For instance, in 1957, the British inventor Harold Bate modified a car so that it could use biogas produced from chicken manure from his farm [28]. The use of AD for treating industrial wastewater has grown tremendously, and it is estimated that European plants comprise 44% of the installed bases, while 14% of systems are located in North America, and a considerable number of systems are located in South America [33]. In Asian, Latin American and African countries, the growth of biogas use was most evident in the 1970s [34]. Additionally, in North America, AD began to be used in the 1970s [35], and the main focus was on farm biogas plants; the number increased from 25 in 2000 to 176 in 2011. In 2006, the Canadian government implemented a Renewable Energy Standard programme, which provided higher rates for biogas-produced electricity and financially assisted farmers in reducing the cost of constructing digesters [36].

In 2000, there were approximately 850 farm-based digesters in Germany, which increased to approximately 7,800 plants in 2014 [37]. Denmark committed to an increased energy initiative that would double biogas production by 2000 and triple it by 2005 through AD [38]. The United States has made significant progress towards the commercial use of municipal solid waste (MSW) processing facilities. In 2003, the United States produced 147 trillion BTUs (British thermal units) of energy from landfill gas, approximately 0.6% of the total U.S. energy requirement [35,39,40].

In 2014, it was estimated that China had 100,000 biogas plants and 43 million residential-scale digesters, generating approximately 15 billion m<sup>3</sup> of biogas. In 2014, India had approximately 4.75 million farm-size biogas plants, in comparison to its potential of 12 million biogas plants, which could generate approximately 10 billion m<sup>3</sup> biogas/year. India also planned to install 110,000 biogas plants from 2014 to 2019. Nepal has one of the most successful biogas programmes in the world, with more than 330,000 household biogas plants installed [41]. In Africa, attempts have been made by international organizations and foreign agencies to promote biogas technology. It was estimated by the SNV Netherlands Development Organisation (based on the FAO-STAT – the Food and Agriculture Organization Corporate Statistical Database) that the households that could be qualified for installation of small digesters amounted to 32.9 million in 2018 (a 78% increase compared to 2006), mainly in Ethiopia, Nigeria and Uganda (5.4; 3.5; 3.1 million households, respectively) and approximately 2 million households in Tanzania, Kenya, Sudan and Burkina Faso [42]. This rise in the “technical potential” for households to run a biodigester was driven by an increase in access to both the growth of the dairy sector in Africa (availability of dung) and access to water. The minimum standards for biogas stove burners have been estimated to be 0.38 m<sup>3</sup>/h in China, 0.45 m<sup>3</sup>/h in India and 0.5 m<sup>3</sup>/h in Kenya, while to generate approximately 1 m<sup>3</sup> of biogas daily, at least 20–30 kg of fresh dung is needed. For this reason, the household should theoretically have available 2 mature cattle; however, in reality, at least 3 or 4 mature cattle



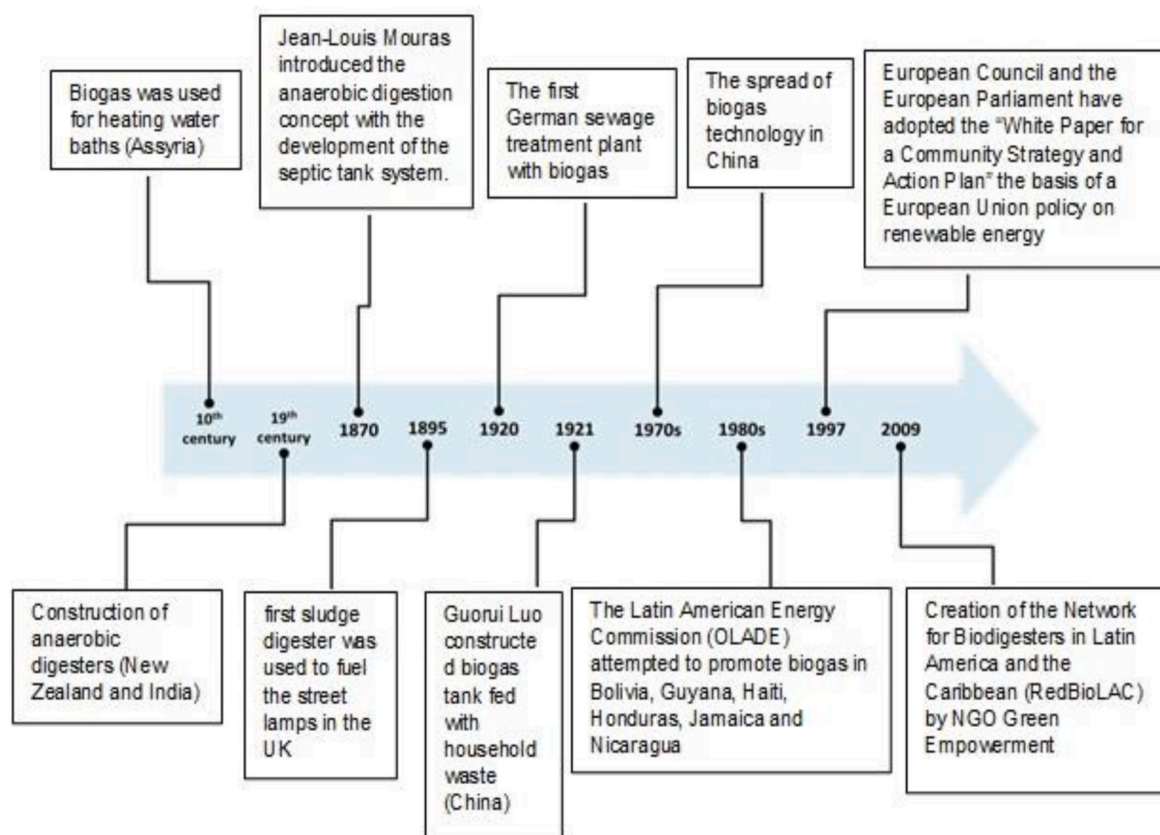


Fig. 3. The historical events that occurred throughout the world in the biogas sector.

should be available [43].

However, even though small-scale biogas plants have been established across the continent, only a few are in use due to insufficient knowledge of AD and the inadequate potential of the installed plants [44]. Deficient understanding is an important barrier in the implementation, safe operation and maintenance of biogas plants in many developing countries. Additionally, the high investment costs of AD systems, even though the operating expenses are very low, are considered critical factors affecting the implementation of biogas projects [45–48]. Currently, in rural and semiurban areas of developing countries, family-type biogas plants are promoted and are suspected to simultaneously provide education to the householders [49].

As already mentioned, AD technology contributes to the production of renewable energy and a circular economy, stimulates sustainable economic development and simultaneously mitigates climate change. However, even though such technologies are widely applied worldwide, their use by industry is still in the early stages [25]. Biogas production and utilization are still facing several problems in developing countries, among which, a lack of funds and a lack of knowledge (of effective biogas production and utilization methods, as well as process maintenance) seem to be the most important obstacles to overcome. The current status of the industry and the deployment of technology are discussed in Section 3.

### 3. Implementation of biogas technology—technical aspects

Various substances are used for biogas production, such as wheat straw, corn stover, sugarcane bagasse, forest residues, switchgrass, energy cane, sorghum, food waste, sewage sludge, livestock residues, manure, source sorted municipal waste and wastewater with a high organic content [15]. In general, it is estimated that in the EU, the majority of biogas plants (approximately 70%) operate using

agricultural substrates [50], followed by energy crops [51], organic waste (including municipal waste), sewage sludge and manure [52]. In some countries, access to feedstock is a crucial barrier in the biogas industry, or there is a gap in legislation, such as a lack of mandatory food waste collection. Nonetheless, a clear increase in biogas production worldwide and in the EU was noted from the 19th century, despite unfavourable legal conditions. However, taking into account the number of biogas plants per 1 million capita, Germany (136 plants) is a leading country in this field, followed by Switzerland (74), the Czech Republic (54) and Luxembourg (50); additionally, Poland has 8 plants per 1 million people [50].

The total installed electric capacity of biogas plants in Europe reached a total of 10,532 MW (megawatts) in 2017, with a trend towards installations of higher capacities [50]. In general, this increase was primarily from agricultural biogas plants (digestion of agricultural residues of plants and animal manure, as well as energy crops and catch crops), followed by landfill biogas plants (rotting of the deposited organic waste fraction) and sewage sludge biogas plants [50].

In the case of biomethane from biogas, this sector has been developing rapidly in the EU in recent years, even though the cost of biomethane is generally higher than that of natural gas (however, this varies substantially across EU countries) [15]. Biomethane benefits the energy system as natural gas but lacks CO<sub>2</sub> emissions and mitigates the emission of methane. Recognition of those values together with technological improvement and supportive policies should increase the cost competitiveness of biomethane [25]. Currently, the costs of biomethane through AD are highly dependent on the price of the feedstock employed. Excluding feedstock costs, the break-even price for biomethane produced through AD, including upgrading and injection, is approximately 100 €/MWh. Feedstock costs vary greatly among regions, and as a result, so do the unit total cost estimates of biomethane. Data from various European countries reveal break-even price estimates

ranging from 60 to 120 €/MWh [15].

Upgraded biogas technology results in improved electricity/heat efficiencies in biogas plants and provides new opportunities for biomethane use in the transport sector. The total number of biomethane plants increased almost threefold between 2011 and 2017, from 187 to 540 installations [50]. Germany is a leader in this field (195 biomethane plants); however, the biomethane market is quite stable there, followed by the UK (92) and Sweden (70), which, in terms of the number of plants per million capita, is at the top of the list. In total, 15 European countries reported biomethane production in 2017 (Austria, Switzerland, Germany, Denmark, Spain, Finland, France, Hungary, Iceland, Italy, Luxembourg, the Netherlands, Norway, Sweden and the United Kingdom), with a total biomethane production of 19,352 GWh (1.94 bcm, billion cubic metres). The market was joined by three more countries in 2018 (Belgium, Estonia and Ireland) [50]. New science—business cooperation and new opportunities for jobs are also expected, especially in the local area, where biomethane could be used as fuel in natural gas-powered vehicles (NGVs) or injected into the natural gas grid as a substitute for natural gas to supply traditional end-users (power plants, industries and households) [41]. However, the selection of optimal renewable energy sources requires a long-term perspective, which is essential to overcome investment barriers. Even now, there is limited knowledge about the energy efficiency investments needed, especially at the national/global level, to increase both renewable energy efficiency and its share in the energy sector. The reduction of the power demand is particularly crucial. In the case of biogas production, the consumption of energy production summarizes the energy inflow and outflow in an AD system. As previously mentioned, in developing countries, biogas is mainly produced in small, domestic digesters, providing fuel for cooking, heating or electricity, while in developed countries, large-scale, farm- or commercial-based electricity and heat biogas plants are implemented. In Europe, biogas is produced mainly (approximately 74% of the primary biogas energy output) from agricultural waste and energy crops, followed by landfill biogas recovery (approximately 17%), sewage sludge, food waste and other sources [41]. The energy demand of AD plants depends on their scale, the technological advancement of the process and the sources of the biogas. Note that the energy calculation should include different aspects, which combined form the technological and market framework as follows:

- Biomass-specific factors—cultivation and harvesting (important for energy crops), as well as transport to the AD plant;
- Process-specific factors—biomass pretreatment demands (mechanical/thermal/chemical/biological/hybrid), anaerobic reaction (mesophilic, thermophilic), pH control, mixing, pumping, and services;
- Final digestate (AD byproduct) deposition factors—land application (transport) or incineration (transport and ash handling); and
- Market factors—energy price regulation, taxes, subsidies and quotas.

The cost–benefit analysis of waste-based biogas production differs from that of crop-based biogas production. In the case of energy crops, important energy consumption is connected with field preparation, planting, biomass cultivation (fertilizers and pesticides), harvesting, storage in bunker silos and sometimes transport to the biogas plant [53]. It is also suggested that farm-based systems seem to be more energetically efficient than large-scale commercial systems, especially when the transport distance is greater than 75 km and they are not used for the return of digested biomass [54]. It is also possible that the energy consumed in different biomass pretreatment types does not provide the required energy output (expressed by, e.g., an increase in methane yield). Additionally, in energy crop cultivation for biogas production, undesirable ecological impacts, such as soil erosion, nitrate leaching or loss of biodiversity, need to be taken into account [55,56].

Nonetheless, biogas production enables increasing self-sufficiency in energy production at the farm level, as well as improving nutrient recycling, both of which could improve the feasibility of local

agriculture and create new job opportunities in rural communities [57].

The environmental impacts that are associated with producing and utilizing biogas as an energy carrier are also assessed through the life cycle assessment (LCA) method [58]. LCA studies focusing on biogas production systems in Europe and throughout the world are usually prepared according to ISO14040, with assumptions based on the following predominant factors:

- functional units and system boundaries, which allow us to compare different configurations of biogas plants, as well as alternative fossil fuels;
- assessment of impact categories, such as primary energy, greenhouse gases, acidification and eutrophication; these factors help to mitigate climate change and to evaluate the negative impacts of each biogas plant configuration on the air quality; and
- biogas plant design and description of the biogas plant configuration [59].

Worldwide, various biogas systems can be found with different parameters (designs, plant scales, input materials, geographical regions and infrastructure, and social, economic and environmental pressures). Additional diversity is linked to hot spots of concern, which may focus on the production of feedstocks and transport (cradle-to-gate), biogas production from different feedstocks (gate-to-gate), and biogas utilization with the final disposal of byproducts (gate-to-grave). However, the energy efficiency is not studied using a harmonized methodology and, in most cases, cannot be directly compared (for details please see Section 7). Thus, there is a clear need for references to assess the reliable bioenergy potential and energy balance and correctly estimate the environmental and economic impacts [58].

Despite the uncertainty of the biogas market, its development, especially in waste-to-energy systems, has been favoured in several countries by positive policy framework conditions, programmes, administrative procedures and financial support, such as feed-in tariffs, which are long-term contracts to renewable energy producers and other investment support, especially for electricity generated from biogas [41]. Note that AD is also a source of heat. The use of the derived heat, even for own purposes, may increase the income and profitability of AD technology, but it is rarely utilized in biogas plants.

#### 4. Renewability of biogas

As shown in Fig. 4, biogas is generated via an anaerobic biological process (AD). In the absence of oxygen, organic matter is broken down to form a gas mixture composed mainly of methane (50%–75%) and carbon dioxide (25%–50%) [60] and, depending on the substrate being digested, minor amounts of hydrogen sulfide (<0.8%) and ammonia (<1%) [26,61]. Importantly, AD technology is in line with the circular economy and plays a vital role in the “waste conversion into a resource philosophy”, since it mainly converts nonmarket biomass and biowaste (agricultural residuals, sewage sludge, wastewater, and animal slurries) into methane-rich biogas (see Fig. 4) [59,62].

The AD of organic matter is a four-step process (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) carried out by syntrophic associations of bacterial consortia (see Fig. 4). During the first step, hydrolysis, the hydrolytic enzymes excreted by bacteria break up insoluble polymers, carbohydrates, lipids and protein into soluble mono- and oligomers, which are directly available to microorganisms [63]. In acidogenesis (the second step), simple sugars, amino acids, and fatty acids are further degraded into acetate, carbon dioxide, and hydrogen and into volatile fatty acids and alcohols, while in acetogenesis (the third step), volatile fatty acids and alcohols are further degraded into H<sub>2</sub> and acetic acid. Finally, methanogenesis transforms the mixture of CO<sub>2</sub>, H<sub>2</sub>, formate, methanol, and acetate into the final product, which is methane. This final step is performed mainly through acetoclastic, hydrogenotrophic, and methylotrophic pathways [64]. The known

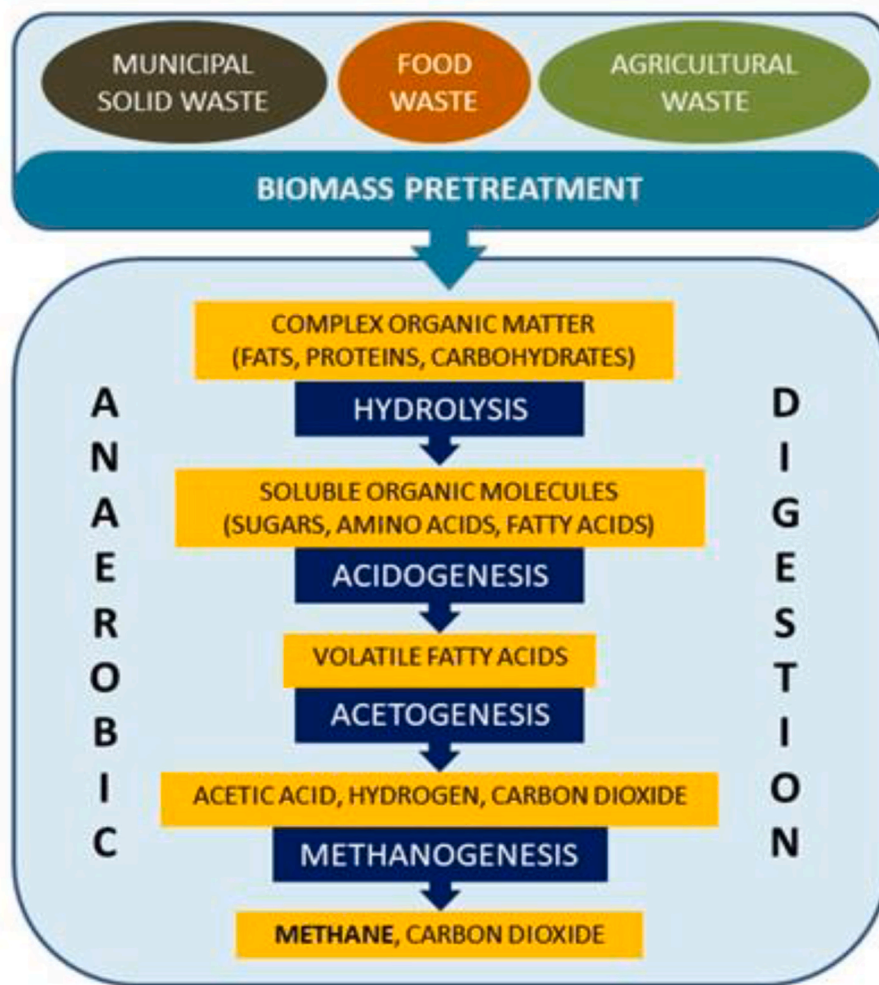


Fig. 4. The AD process.

methanogenic members belong to the *Archaea* domain, mainly six orders (*Methanosarcinales*, *Methanobacteriales*, *Methanomicrobiales*, *Methanococcales*, *Methanopyrales*, and *Methanocellales*) of the *Euryarchaeota* phylum. Recently, however, putative methane-metabolizing genes were also detected among members of the phyla *Bathyarchaeota* and *Verstraetearchaeota* [65,66].

AD can be performed using mesophilic or thermophilic treatment. Thermophilic treatment is operated optimally between 49 °C and 57 °C up to 70 °C [67], and mesophilic treatment is operated optimally between 30 °C and 35 °C [68,69]. Thermophilic digestion has a few advantages over mesophilic digestion, such as (I) faster fermentation than mesophilic digestion, (II) a higher biogas yield (shorter retention time) due to elevated biochemical reactions, (III) effective reduction of organic matter and volatile suspended solids (VSS), (IV) improvement of the rheological properties of the digested sludge [70,71], (V) the ability to withstand higher organic loads due to faster reaction rates [72,73], and (VI) a high pathogen inactivation efficiency [74]. There are also some disadvantages of thermophilic treatment, e.g., (I) the process requires more energy to maintain the temperature, (II) the feeding biomass must enter at a high temperature and (III) the temperature fluctuation is problematic for biogas production. The benefits of ensuring a proper temperature during AD show that the mesophilic digester performance presents better parameter stability and economic benefits, including stable biogas production. Therefore, more than 90% of biodigesters worldwide use mesophilic digestion [75].

To obtain a higher methane yield, several modifications of one-stage

thermophilic or one-stage mesophilic AD have been suggested, such as temperature—phased anaerobic digestion (TPAD), where the thermophilic stage, which is used to enhance hydrolysis, is followed by the mesophilic stage, which is believed to be more conducive for methanogenesis [76]. In addition to this and other multistage systems [77, 78], codigestion and substrate pretreatment have also been tested (for details, see Section 5).

The important advantage of biogas technology is its scalability. Biogas can be produced in large-scale installations, which require expert knowledge in the design and construction phase, as well as during operation and maintenance since a lack of knowledge in this area would lead to the failure of the biogas plant [23]. However, biogas can be produced decentrally in household biodigesters, which have become increasingly popular in rural regions (as already discussed in Section 2). Another important aspect of biogas production is the process byproduct—digestate. Digestate is a nutrient-rich stabilized biomass that can be further used as an organic fertilizer to supplement or replace the use of mineral fertilizers. It should be mentioned, however, that the agronomic use of digestate, usually regionally regulated, may depend on the feedstock used in the biogas unit [79].

The AD process, which occurs in landfill sites, strongly depends on the deposited waste material, as well as the landfill cell age (the biogas production decreases with time). To some extent, EU regulations, which limit the deposition of biodegradable municipal solid waste, will reduce landfill biogas production over time (emissions are expected to expire in 30 years). Note that currently, the organic municipal solid waste fraction



is increasingly used as a substrate or cosubstrate in AD [80]. However, AD plants treating the organic fraction of municipal solid waste are usually dedicated plants equipped, e.g., with a pretreatment step tailored to a specific waste stream with a particular composition.

For wastewater treatment plants (WWTPs), AD is regarded as an appropriate technique for biogas production from sewage sludge (composed of primary and secondary sludge), which serves as the main feedstock. Since approximately 50% of the organic matter is transformed into biogas, AD is also simultaneously used as a stabilization stage of sewage sludge [81]. Digested sludge is usually dewatered to the level required by its further utilization (composting or incineration), while the rejected liquid fraction is reintroduced to the WWTPs. Note that AD is regarded as a multistep and complex process that can be effectively applied only in large- and medium-scale WWTPs; AD is currently at a developing stage at small-scale WWTPs. In some cases, depending on national legislation, other locally available organic materials (from households or industries) can also be codigested with sewage sludge, mainly to increase biogas production and cost effectiveness [25].

## 5. Biomass mono- and codigestion

As mentioned earlier in Section 3, biogas is produced under anaerobic conditions by microorganisms metabolizing organic-rich biomass (e.g., agricultural, industrial and human wastes) into methane with limited amounts of carbon dioxide and other gases [82]. It is also well known that the bacterial consortia performing AD are easily influenced by operational parameters such as the substrate characteristics (e.g., biodegradability, C/N ratio, water content), temperature, pH, mixing ratios, additives and other factors [83,84]. Thus, the study of biomass characteristics is important prior to AD. In general, (I) the biomass should have a high nutritional value, which yields a higher production of biogas (e.g., set the C/N ratio to the optimum value of 25:1; higher C/N ratios result in lower methane concentrations in biogas), (II) the biomass moisture and pH should be appropriate, (III) the amount of possible toxic substances should be limited, (IV) the biogas produced from digestion should have further applications and be useable and (V) the residue from digestion should be useful as a fertilizer [85]. In Table 1, the biogas production and methane yield from selected substrates are presented. Based on these data, pig manure and sewage sludge are the most profitable substrates for AD. Pig manure yields a high amount of methane; however, the ammonium content should be monitored due to its possible inhibition of biogas production. Thus, many studies have already focused on protein-, lipid-, and cellulose-rich substrates to evaluate their combined potential for biogas production and methane yields.

Regardless of the feedstock characteristics, in most cases, the first step of AD—hydrolysis—can be a rate-determining process, since

biomass composed of cellulose, hemicellulose, and lignin can hardly be accessible to microbial degradation. Thus, different methods that can shorten the duration of this stage (and at the same time increase the bioavailability of soluble substances for methanogenic bacteria) have already been tested and implemented, mainly the pretreatment of feedstock or codigestion. Biomass pretreatment is adapted according to the feedstock structure to help solubilize and hydrolyse complex organic matter [89]; however, this step can also be used to extract more biogas from the same amount of feedstock. In the case of the codigestion process, (I) energy-rich organic materials are added to digesters with excess capacity or (II) to supplement the AD process with macro- and micro-nutrients (to meet the species-specific requirements of microorganisms involved in degradation, as mentioned above with respect to the C/N ratio). Some examples of the methane yields from cosubstrates are presented in Table 2. Each biomass shows different biogas production results, which may vary because of the treatment used, the different mix ratios used and the characteristics of the biomass.

As reported in Table 2 and discussed in Section 7, a suitable codigestion process can significantly enhance the biogas production compared with monodigestion. However, in a real-case scenario, the biogas produced from biomass is strongly linked to the local market and even the seasons. Thus, in certain cases, to increase methane production, locally available substrates can be pretreated prior to AD.

## 6. Biomass pretreatment

The main goal of pretreatment is to overcome the digestion barriers. The first step of AD (hydrolysis) is often considered to be the rate-limiting step, because complex organic matter (e.g., cellulose, hemicellulose, and lignin, proteins, polysaccharides and lipids) needs to be solubilized and hydrolysed into simple components (for details see Fig. 4) (e.g., long-chain fatty acids, sugars and alcohols) [89]. The duration of this AD stage may be shortened, e.g., by different biomass pretreatment methods, for both mono- and codigestion. In such a case, it has to be adapted according to the biomass structure and characteristics; thus, feedstock-based pretreatment methods are needed to facilitate the AD process. Then, pretreatment (also referred to as the conditioning process) is used to speed up and enhance digestion, as well as to improve dewatering and the quality of the digestate.

The pretreatment process facilitates microbial digestion by removing the barriers and making the organic content of the substrate easily accessible and utilizable by the microbial community [94]. Among the pretreatment technologies, the following methods can be differentiated: mechanical (ultrasonic, microwave, electrokinetic and high-pressure homogenization), thermal (low and high temperatures), chemical (acidic, alkali, ozonation, Fenton and Fe(II)-activated persulfate oxidation), and biological (temperature-phased AD and microbial electrolysis cells) [89,95–99]. In general, all the pretreatment methods mentioned

**Table 1**  
Biogas production from selected substrates.

Substrate	% DM	Biogas yield	Methane content [%]	Methane yield [m <sup>3</sup> CH <sub>4</sub> /kg VS]	Source
Pig manure	8–17	3.6–4.8 (m <sup>3</sup> /kg DM)	70–80	0.25–0.35	[26,41,54,87]
Cow manure	8–16	0.2–0.3 (m <sup>3</sup> /kg DM)	55–75	0.20–0.25	[26,41,55,87]
Chicken manure	25	0.35–0.8 (m <sup>3</sup> /kg DM)	60–80	0.30–0.35	[26,41,87]
Sewage sludge	20	0.35–0.50 (m <sup>3</sup> /kg DM)	65–70	0.30–0.40	[26,41]
Straw, grass	~80	0.35–0.40 (m <sup>3</sup> /kg DM)/0.53–0.60 (Nm <sup>3</sup> /kg VS)	54	0.20–0.25	[26,41,51,55,86,87]
Maize	20–48	0.25–0.40 (m <sup>3</sup> /kg DM)/0.56–0.65 (Nm <sup>3</sup> /kg VS)	52	0.25–0.45	[26,41,51,86,87]
Rye	33–46	0.67–0.68 (m <sup>3</sup> /kg DM)/0.56–0.78 (Nm <sup>3</sup> /kg VS)	53	–	[26,50,86,87]
Triticale	27–41	0.68–0.77 (m <sup>3</sup> /kg DM)/0.59–0.62 (Nm <sup>3</sup> /kg VS)	54	–	[26,50,86,87]
Sugar beet	19–22	0.39–0.76 (m <sup>3</sup> /kg DM)	53	0.23–0.38	[26,41,51,55,86,87]
Rice straw hull (husks)	86	0.014–0.018 (m <sup>3</sup> /kg DM)	–	0.20–0.25	[26,41]
Bagasse	33	0.165 (m <sup>3</sup> /kg organic DM)	–	–	[26,88]
Wheat	88.9	0.65–0.7 (Nm <sup>3</sup> /kg VS)	54	–	[87]



**Table 2**  
Methane yield from cosubstrates.

Cosubstrates	Mixture ratio	Methane yield	Source
Pig manure: corn stover	75:25 (VS basis)	0.21 (Nm <sup>3</sup> /kg VS added), specific methane yield – 0.22 (m <sup>3</sup> /kg VS added)	[87,90]
Pig manure: wheat straw	75:25 (VS basis)	0.24 (Nm <sup>3</sup> /kg VS added), specific methane yield – 0.26 (m <sup>3</sup> /kg VS added)	[87,90]
Pig manure: potato waste	80:20 (VS basis)	0.30–0.33(Nm <sup>3</sup> /kg VS added), specific methane yield – 0.32–0.35 (m <sup>3</sup> /kg VS added)	[87,91]
OFMSW: vegetable oil	83:17 (DM basis)	0.70 ± 0.01 (Nm <sup>3</sup> /kg VS added)	[87]
OFMSW: animal fat	83:17 (DM basis)	0.51 ± 0.02 (Nm <sup>3</sup> /kg VS added)	[87]
OFMSW: cellulose	83:17 (DM basis)	0.25 ± 0.01 (Nm <sup>3</sup> /kg VS added)	[87]
OFMSW: protein	83:17 (DM basis)	0.29 ± 0.01 (Nm <sup>3</sup> /kg VS added)	[87]
Buffalo manure: maize silage	70:30 (VS basis)	0.36 ± 0.04 (Nm <sup>3</sup> /kg VS added)	[87]
Cow manure: straw	70:30 (VS basis)	0.21 ± 0.02 (Nm <sup>3</sup> /kg VS added), specific methane yield – 0.26 (m <sup>3</sup> /kg VS added)	[87,91]
Cow manure: barley straw	80:20 (Volume basis)	0.16 (Nm <sup>3</sup> /kg VS added), specific methane yield – 0.17 (m <sup>3</sup> /kg VS added)	[87,91]
Cow manure: fruit and vegetable waste	50:50 (DM basis)	0.45 (Nm <sup>3</sup> /kg VS added), specific methane yield – 0.48 (m <sup>3</sup> /kg VS added)	[87,91]
Cow manure and distillery wastewater	81:19 (wet mass basis)	specific methane yield – 0.12 (m <sup>3</sup> /kg VS)	[92]
Cow manure: forage beet silage	80:20 (DM basis)	0.40 (Nm <sup>3</sup> /kg VS added), specific methane yield – 0.42 (m <sup>3</sup> /kg VS added)	[87,91,92]
Organic kitchen waste: cow manure	75:25 (VS basis)	0.15 (Nm <sup>3</sup> /kg VS added)	[87]
Algal sludge: waste paper	50:50 (VS basis)	1.17 ± 0.07 (Nm <sup>3</sup> /kg VS added)	[87]
Food waste: cow manure	67:33 (VS basis)	0.39 (Nm <sup>3</sup> /kg VS added)	[87]
Dairy manure: potato waste	75:25 (VS basis)	0.23 (Nm <sup>3</sup> /kg VS added)	[87]
Dairy manure: used oil	75:25 (VS basis)	0.36 (Nm <sup>3</sup> /kg VS added)	[87]
Dairy manure: cheese whey	75:25 (VS basis)	0.25 (Nm <sup>3</sup> /kg VS added)	[87]
Dairy manure: switchgrass	75:25 (VS basis)	0.21 (Nm <sup>3</sup> /kg VS added)	[87]
Microalgae and wheat straw	80:20	0.29 ± 0.01 (m <sup>3</sup> /kg VS) – pretreated	[90]
Microalgae and wheat straw	50:50	0.30 ± 0.01 (m <sup>3</sup> /kg VS) – pretreated	[90]
Microalgae and wheat straw	20:80	0.31 ± 0.01 (m <sup>3</sup> /kg VS) – pretreated	[90]
Fish waste and sisal pulp	50:50	0.31 (m <sup>3</sup> /kg VS)	[93]
Fish waste and sisal pulp	33:67	0.62 (m <sup>3</sup> /kg VS)	[93]
Fish waste and sisal pulp	25:75	0.48 (m <sup>3</sup> /kg VS)	[93]
Fish waste and sisal pulp	20:80	0.44 (m <sup>3</sup> /kg VS)	[93]
Sewage sludge (SS) and (fats, oils and grease – FOG)	40:60	specific methane yield – 0.49 (m <sup>3</sup> /kg VS)	[92]
Waste-activated sludge (WAS) and FOG	34.5:65.5	specific methane yield – 0.75 (m <sup>3</sup> /kg VS)	[92]
SS and grease trap waste	77:23	specific methane yield – 0.63 (m <sup>3</sup> /kg VS)	[92]
Sewage sludge and food waste	60:40	specific methane yield – 0.18 (m <sup>3</sup> /kg VS)	[92]

above improve the feedstock accessibility for microorganisms by increasing the surface area, biomass porosity, decrystallization and solubilization [96].

The biomass pretreatment efficiency itself may be expressed as an increase in the methane yield or an increase in biogas. However, indirectly, the efficiency of pretreatment through the increase in the aforementioned soluble components can also be examined. The increase in the biogas yield in the AD process is enhanced by diverse substrate pretreatments, which are also important factors in biogas production. In turn, proper evaluation of the substrate pretreatment technology in AD is of high importance in the economic justification of its implementation [96]. In Table 3, the advantages and disadvantages of biomass pretreatment prior to AD are shown, while in Table 4 presents the pretreatment of particular mono- or codigested feedstock.

### 6.1. Pretreatment of sewage sludge from WWTPs

Legal requirements limiting nitrogen and phosphorus discharge by WWTPs demand more effective treatment methods and new infrastructures to address advanced nutrient removal. As a consequence, considerable production of activated sludge has been observed, which has to be managed in a sustainable manner. Dewatering and AD are the common methods for sewage sludge management prior to the final disposal of sludge, which is usually land application or incineration (landfilling is no longer permitted, at least in EU countries) [111]. However, the complex microstructure of sewage sludge makes dewatering and hydrolysis difficult to conduct effectively. This is mainly due to the presence of extracellular polymeric substances (EPS), such as polysaccharides, proteins and DNA, which entrap water and have high viscosities [112,113].

The pretreatment of sludge is expected to rupture the flock structure, as well as some bacterial cell walls, resulting in the release of intercellular matter in the aqueous phase [114,115]. Thus, pretreatment of

sewage sludge helps to reduce its high resistance to both dewatering and biodegradation. The increase in nutrients accessible to microbes enhances the digestion rates, reduces the retention time, and increases biogas production [89]. For this reason, the effectiveness of the pretreatment performance, in addition to the biogas productivity, may also be expressed as an increase in soluble components. However, since biomass solubilization and biogas productivity are not always directly linked with methane production [116–118], it is suggested that the AD performance is expressed as the methane yield, i.e., the volumetric methane production under standard conditions (m<sup>3</sup> CH<sub>4</sub>/day) per unit of material fed, such as total solids (TS), volatile solids (VS), chemical oxygen demand (COD) or wet weight.

The thermal, thermal-alkaline, alkaline and electrochemical pretreatment types are reported as the most effective methods for solubilizing sewage sludge, and they greatly increase further biogas production [119]. However, other types of sewage sludge pretreatment, including ultrasonication, microwave and high-pressure homogenization, have been tested. Examples of sewage sludge pretreatment methods are presented in Fig. 5.

The first commercially used thermal pretreatments for sewage sludge were “Porteous” and “Zimpro”, which were implemented in the 1960s. Both processes were typically operated between 200 and 250 °C [120], but due to the generated odours, the production of high-strength reject water and extensive corrosion under installation, they were terminated in the early 1970s or modified to lower temperatures and, subsequently used to enhance the dewaterability of sewage sludge [120].

During the 1980s, various combinations of thermal and pH-based (acid- and alkaline) technologies were tested (e.g., Synox and Protox), but none were successfully commercialized, mainly due to insufficient cost-effectiveness [121]. In the 1996 CambiTHP™ process, a combination of thermal hydrolysis and high pressure was implemented to increase biogas production and digester loading [122]. In this three-stage process, sewage sludge (primary and secondary) comprised of 16–18%

**Table 3**  
Advantages and disadvantages of biomass pretreatment prior to AD [100–102].

Pretreatment type	Advantages	Sources	Disadvantages	Sources
Physical	<ul style="list-style-type: none"> <li>Reduces process severity, water consumption and co-product formation when combined with thermochemical treatments.</li> <li>Possibility to ensure anaerobic process stabilization.</li> <li>High efficiency in improving organic matter solubilization.</li> <li>High efficiency in improving organic matter solubilization and methane production from the anaerobic process.</li> </ul>	[100, 101], [102], [102], [102]	<ul style="list-style-type: none"> <li>Increases power consumption.</li> <li>Possible formation of compounds that are difficult to degrade, with an overall reduction in methane yields.</li> <li>High energy consumption for thermal pretreatment.</li> </ul>	[101], [102], [102]
Chemical	<ul style="list-style-type: none"> <li>Low capital costs.</li> <li>Methane production up to 100% higher than that of the control.</li> <li>Strong oxidizing power ensuring a short reaction time.</li> <li>High solubilization improvement.</li> <li>No addition of chemicals to the substrate in the ozonation method.</li> </ul>	[101, 102], [102], [102], [102]	<ul style="list-style-type: none"> <li>High capital cost.</li> <li>Possible formation of less biodegradable byproducts. Limited application for wet digestion systems (TS &lt; 10%).</li> <li>High operating costs if large amounts of waste have to be treated.</li> <li>Possible formation of toxic compounds.</li> <li>Hazardous, toxic and corrosive chemicals require neutralization, detoxification and chemical recovery steps, as well as anti-corrosive materials.</li> </ul>	[100, 102], [102], [102], [102], [101, 102]
Biological	<ul style="list-style-type: none"> <li>No chemical addition.</li> <li>Low capital and operating cost requirements.</li> <li>No restriction to specific AD technologies.</li> <li>The pretreatment is selective, requires no chemical addition, uses low energy and has low severity.</li> </ul>	[100, 102], [102], [102], [101]	<ul style="list-style-type: none"> <li>Long reaction time.</li> <li>Increase in methane production.</li> <li>Difficult to apply very complex substrates.</li> <li>Enzymatic hydrolysis has a long incubation time, low production rate and high sensitivity to inhibition.</li> <li>Loss of cell activity, requires highly controlled conditions.</li> </ul>	[100, 102], [100], [102], [101]

dry solids is homogenized and preheated to approximately 100 °C in a pulper tank. Then, from the pulper tank, the warm sludge is fed to the reactor, where for 20–30 min, it is exposed to a temperature of approximately 180 °C and a pressure of approximately 6 bars. Hydrolysed and sterilized sludge is then directed to the Flash tank, which operates at atmospheric pressure. The sudden pressure drop causes further substantial cell destruction and the release of dissolved organic matter. This solubilized sludge is cooled to the temperature of (mesophilic) AD (by heat exchangers and the addition of water) and pumped to the AD reactor [122]. Another thermal hydrolysis process of municipal or industrial sludge coupled with AD (BIOTHELYS®) was introduced by Veolia in 2006 in the WWTP of Saumur, France (60,000 PE). In BIOTHELYS®, the dewatered sludge is first exposed for approximately 30 min to the thermal hydrolysis batch phase, with steam injected under a pressure of 6–8 bars and a temperature of approximately 165 °C. The continuous, adjustable feed-rate option of BIOTHELYS® is called Exelys. Both Veolia processes are advertised for thermal pretreatment of a wide range of industrial and municipal sludges, including those containing fats, oils and grease (FOG) [123,124].

In addition to the obvious advantages connected with thermal sewage sludge pretreatment by the CambiTHP™, BIOTHELYS® or Exelys processes, some issues also need to be considered, mainly process maintenance, especially the presence of staff qualified to use, service and inspect the pressure and steam devices. Another issue is connected with the treatment of rejected water, as well as the presence of ammonia in the recycled stream and the cost efficiency of the process. Thus, there is still a need to lower the costs of sewage sludge pretreatment to make it practically applicable for industrial AD, especially for smaller municipal WWTPs [90]. For this reason, the low-temperature (<100 °C) thermal pretreatment of sewage sludge has been extensively studied in combination with other processes. The abovementioned topic has been extensively studied by the authors, and low thermal disintegration has been developed under pending patent numbers P.430820 and P.430821 [125,126]. Application of this technology to sewage sludge (up to 55 °C) prior to AD allowed to obtain a final methane yield that reached 75% [125,126].

In addition, the economic feasibility of each implemented process needs to be comprehensively evaluated. In the case of sewage sludge pretreatment, detailed information is needed, which includes, in addition to the biogas (methane) increase, the energy input for any type of related activity. Thus, considering the pretreatment method, all aspects

(economic and exploitation feasibility) need to be addressed, including sewage sludge management prior to and after AD, as well as the final disposal.

## 6.2. Pretreatment of agricultural waste, food and municipal solid waste

The composition of agricultural and food waste, as well as the organic (biodegradable) fraction of municipal solid waste will differ significantly [127]. In terms of food waste, it is suggested that monopretreatment (mechanical, ultrasound, microwave, chemical, thermal and biological) is not as effective as hybrid biological-physiochemical treatment, which can enhance biogas production by 208% [128].

Agricultural waste pretreatment processing will mainly depend on the resources of a given country, although waste such as cattle, cows, pigs or poultry manure is usually accessible to biogas plants. Based on availability, lignocellulosic biomass (energy crops/plant residues) may also be a good feedstock for biogas production, but since this biomass is mainly composed of cellulose, hemicelluloses and lignin (in different ratios), it is resistant to microbial degradation and oxidation [96,129]. Thus, pretreatment is often applied, most frequently a combination of elevated temperature and chemical treatment, while thermal and other mechanical pretreatment methods are also considered. The pretreatment efficiency with respect to lignocellulosic biomass depends mainly on the lignin content of the treated material [130]. Note that any type of manure consists of lignocellulose fibres; hence, pretreatment of this substrate is similar to that for energy crops/plant residues. Detrimental effects include the formation of refractory compounds, mainly from high-temperature thermal pretreatment. There is also the risk of sugar degradation byproducts (e.g., furfural formation), which may enhance the biogas production at a low concentration (appx. 1.4 g/L); however, at higher concentrations (>2 g/L), the methanogenic activity may be reduced during the AD process [131]. Thermoacid pretreatment, especially of lignocellulose/cellulose-rich biomass, might also generate AD inhibitors, such as furans and phenolic compounds, which may hinder the microbial activity [132,133]. The aforementioned data on the biogas production and methane yields from different substrates (codigested and/or pretreated) are further discussed in Chapter 7 to identify the most significant features and to optimize the AD process.

**Table 4**  
Results of the pretreatment of selected mono- or codigested feedstocks.

Feedstock	Pretreatment methods	Results	Source
Dairy cow manure	Thermochemical	Thermal-alkali pretreatment improved the methane potential compared to the test with a raw substrate. The methane potential was enhanced by 23.6% after pretreatment with 10% NaOH at 100 °C for 5 min. The maximum production rate was improved under all studied conditions.	[82]
Treated chicken manure and maize silage	Mechanical and co-fermentation	In the batch reactors, approximately 27% more methane was produced from treated chicken manure (T-CM) than from chicken manure. Codigestion of T-CM with maize silage further increased the methane production, presumably due to the improved C/N.	[103]
Sugar beet pulp	Enzymatic hydrolysates and thermal pressure	The highest cumulative biogas productivity, i.e., 898.7 mL/gVS, was obtained from enzymatic hydrolysates of ground and thermal-pressure pretreated sugar beet pellets. This value was slightly higher compared to the biogas yield from enzymatic hydrolysates of thermal-pressure pretreated but not ground SBP (890.5 mL/g VS).	[104]
Sunflower stalks, corn stover	Chemical	Pretreatment with 4% H <sub>2</sub> O <sub>2</sub> under a thermophilic condition enhanced the anaerobic biodegradability of sunflower stalks along with an increase in methane.	[82,105,106]
Bamboo	Steam explosion	A 67% increase in the biodegradation rate.	[105]
Harvest residue and dairy cow manure (DCM)	Thermal pretreatment and anaerobic codigestion	The highest biogas and methane yields (491.37 cm <sup>3</sup> /g VS and 306.96 cm <sup>3</sup> /g VS, respectively) were obtained after anaerobic codigestion with DCM and thermally pretreated corn stover at 175 °C for 30 min; these values were 24 and 23% higher than the biogas and methane yields (372.42 and 234.62 cm <sup>3</sup> /g VS, respectively) of monodigested DCM.	[107]
Horse manure	Mechanical	A 26.5% increase in methane production in comparison to the untreated variant.	[108]
Waste-activated sludge (WAS)	Ultrasonic	Methane generation increased with the pretreatment time, and the increase in methane exceeded 64%.	[109]
Corn stover	Steam explosion	Methane yield increased by 22% at 160 °C, while harsher pretreatment conditions led to a lower methane yield.	[110]

## 7. Influence of biomass codigestion and biomass pretreatment on methane yield production

The dataset considered in this study was gathered from 33 different literature sources, which are reported in Tables 1, 2 and 4. The aim of the analysis was to identify the best possible substrate – cosubstrate – pretreatment setup to maximize the amount of the produced methane. Currently, however, there is a lack of standardized procedures for experimental design, description and reporting in the field of biogas production. This issue results in experimental reports presented in scientific papers that do not include the full set of important process characteristics. The missing information includes the experimental setup (serum bottles, mixing conditions, methane measurement devices, replicates, etc.), as well as the operating conditions (e.g., inoculum characteristics, food-to-microorganism ratios, pH, nutrients) and others. Therefore, the gathered dataset is inhomogeneous with respect to the utilized substrate, cosubstrate and pretreatment type.

In Fig. 6, the total frequencies (marginal frequencies) of observations for each possible substrate – cosubstrate – pretreatment setup are displayed. Most of the setups were missing (white empty cells in Fig. 6). This causes the dataset to be sparse, which has a negative impact on the ability to perform robust statistical analysis.

Due to the structure of the dataset, a proper statistical analysis could not be performed because the required assumptions of many statistical procedures were not met. Additionally, due to the small number of elements falling in each setup, it was impossible to use bootstrapping or Monte Carlo Markov chains to estimate the required statistical parameters. For the abovementioned reasons, the analysis has been limited to data visualization and a thorough description of the observed data.

Fig. 7 presents the combined plots of the methane yield [ML CH<sub>4</sub>/g VS] histogram and the boxplots created for each substrate category. In addition to the box plots, the exact data points were also plotted to reveal the structure of the dataset in a more detailed way. Additionally, labels that indicate the names of the cosubstrates and the colours that represent the pretreatment types are presented.

Although formal statistical analysis could not be performed due to the dataset structure, some plausible patterns can be noticed in the graphical representation of the dataset (Fig. 7). The outcomes of this analysis allow us to postulate some hypotheses; however, further investigation and research are required to confirm or reject them.

The highest methane yield was observed in the case of the sewage

sludge substrate (Fig. 7); however, the observed point can be considered an outlier because most of the observed values for this substrate provided significantly lower amounts of methane. Thus, thermal pretreatment seems to be a more effective step prior to AD, inducing progressive solubilization of sewage sludge via the disruption of cell membranes, lysis, and the release of intracellular materials (for details see also Section 6). In the manure category (Fig. 7), the overall highest amount of methane was observed in the case of chicken manure with the addition of meadow silage grass cosubstrate and thermal pretreatment. This conclusion seems to be confirmed by the fact that the addition of meadow silage grass to pig or mink manure with thermal pretreatment resulted in a higher methane amount when compared to the same substrate but without a cosubstrate. Additionally, thermal pretreatment and the addition of a cosubstrate in the form of fruit/vegetable waste resulted in more methane produced when using cow manure as a substrate. This indicates that the addition of the fibre-rich cosubstrate (meadow silage grass, fruit/vegetable waste) to manure rebalances the C/N ratio and decreases the ammonia toxicity. In the case of plant substrates (meadow grass silage, sugar beet and straw), mechanical pretreatment seems to positively influence the amount of methane produced by increasing the surface area and biomass porosity, and this method also provides accessibility for microorganisms conducting AD. On the other hand, the addition of corn straw or no cosubstrate resulted in the lowest methane yields. In the case of corn straw, soybean straw and sunflower stalk substrates with a manure cosubstrate, thermal pretreatment slightly increased the methane yields. A similar pattern was observed for the microalgal biomass substrate, in which the points representing thermochemically pretreated feedstock with a wheat straw cosubstrate had a higher methane yield than the observations that were not pretreated or had no cosubstrate addition.

The above statistical analysis of literature data has highlighted the need for high-quality scientific data and to address this problem, the design of experiments (DOE) methodology [134] should be used. The application of DOE gives the ability to maximize the amount of information obtained from an experiment while minimizing the cost. Additionally, extensive metadata regarding performed experiments or technical measurements should be included. Such metadata should contain researchers' comments, as well as all key parameters and easily obtained supporting data.

As the biogas production process is very complicated and there are many possible features influencing the methane yield, the only way to

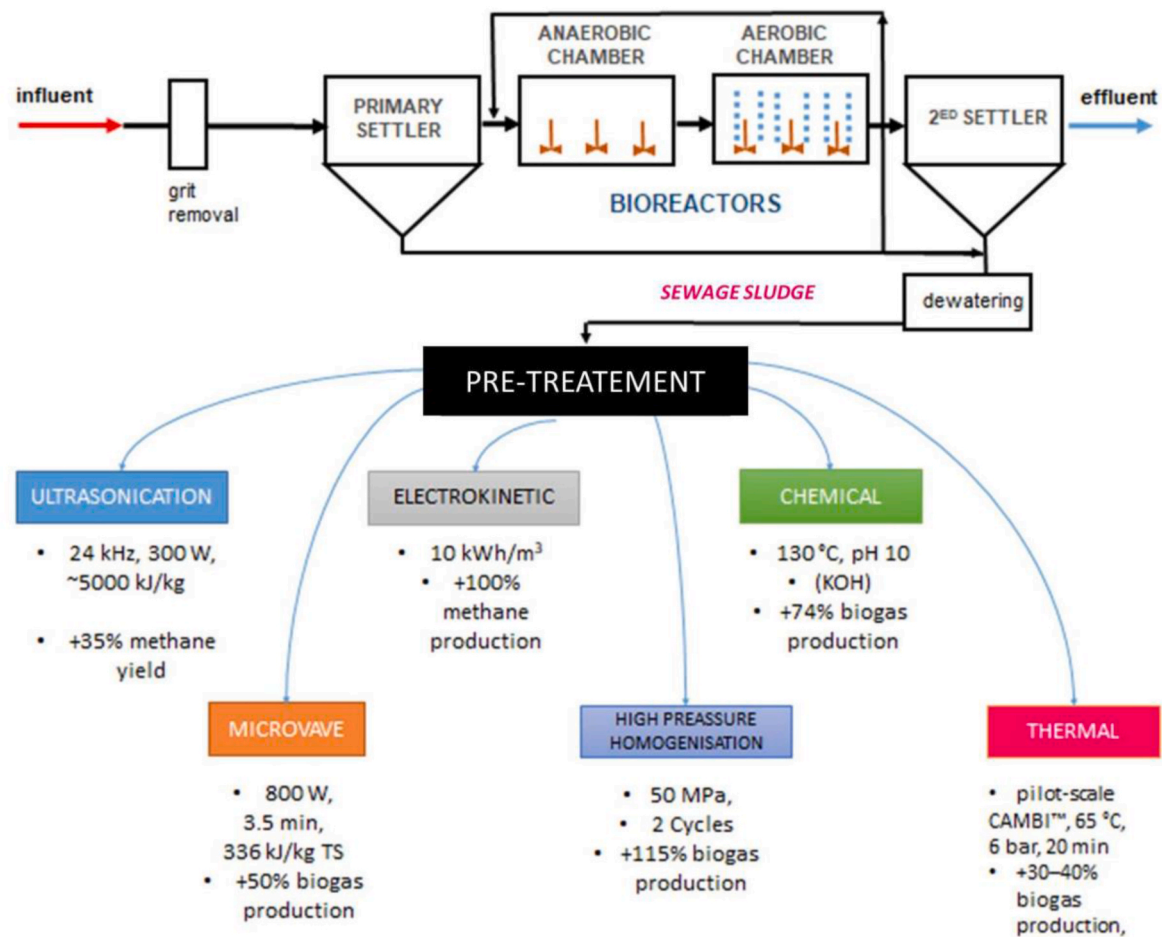


Fig. 5. Examples of the effectiveness of sewage sludge pretreatment methods for biogas production [89].

gain full control over it is to use data exploration and machine learning techniques. High-quality datasets would provide the ability to fully optimize the biogas production process by identifying the most significant features or their combinations.

## 8. Biogas production—opportunities and challenges

Biogas technology has various benefits. However, in some cases, simple economic arguments (cost-effectiveness, commercial and business potential, market principles, etc.) regarding renewable energy criteria may indicate that biogas production is not economically lucrative. Nonetheless, it has been proven that biogas technology has some environmental benefits and can help combat environmental problems. This process provides a way to treat and reuse various human, animal, agricultural, industrial and municipal wastes and side streams. AD, which relies strongly on waste and side streams, provides the lowest cost feedstocks and the highest GHG savings. In this area, it is of high importance to increase the stream of uneaten food and food residues, which can be recycled through AD, in addition to methane production transferring nutrients back into the soil with the digestate [135]. Note that according to the Food and Agriculture Organization (FAO) of the United Nations, one-third of all food produced for human consumption is wasted (approximately 1.3 billion tons of food waste per year) [122, 135, 136]. The AD of food and other organic waste increases the availability of nitrogen and phosphorus, which can be particularly beneficial in organic farming and can limit the use of inorganic fertilizers. AD can also reduce odours [41] and potential pathogens [74]. Therefore, the digestate applied to the field is safer in terms of sanitary aspects than, for instance, untreated slurry used as fertilizer.

It is also expected that technological developments might lower the cost of biogas, especially biomethane production. In this area, small-scale purification units for traffic gas production, an extension of biogas filling station network technologies for small-scale purification units, and an increase in the number of gas cars and smart grids (enhancing the role of consumers as small producers) are especially expected. In renewable power-to-gas (P2G) technologies, biogas, which typically contains approximately 60% methane (and CO<sub>2</sub> in the remaining content), can be upgraded to biomethane using renewably generated electricity. Thus, P2G technologies can be efficiently combined with AD plants [79]. The produced biomethane can be injected into the grid. The cost of biomethane production is highly dependent on the cost of the electricity used in the electrolysis process; thus, the operating costs of P2G systems are still relatively high [137]. Additionally, the biomethane injection points need to be identified in the gas distribution network [79].

Currently, biogas production can be enhanced by pretreatment, codigestion or the implementation of new technologies to obtain a variety of commercially important products from AD-treated biomass (fuels, materials and chemicals originally obtained from fossil refineries) [7, 138]. In the case of cofermentation, the feedstock can be selected based on the effectiveness and local availability of biomass [139].

In the case of biogas production from sewage sludge originating from WWTPs, the major advantages of this technology are (I) low cost energy resources due to the availability of easily affordable raw materials (sewage sludge), (II) independent energy supply for the sewage treatment plant, provided by a stable and reliable AD process, (III) sewage sludge stabilization due to the reduction of the organic content by approximately 50%, which is converted into biogas, (IV) sewage sludge





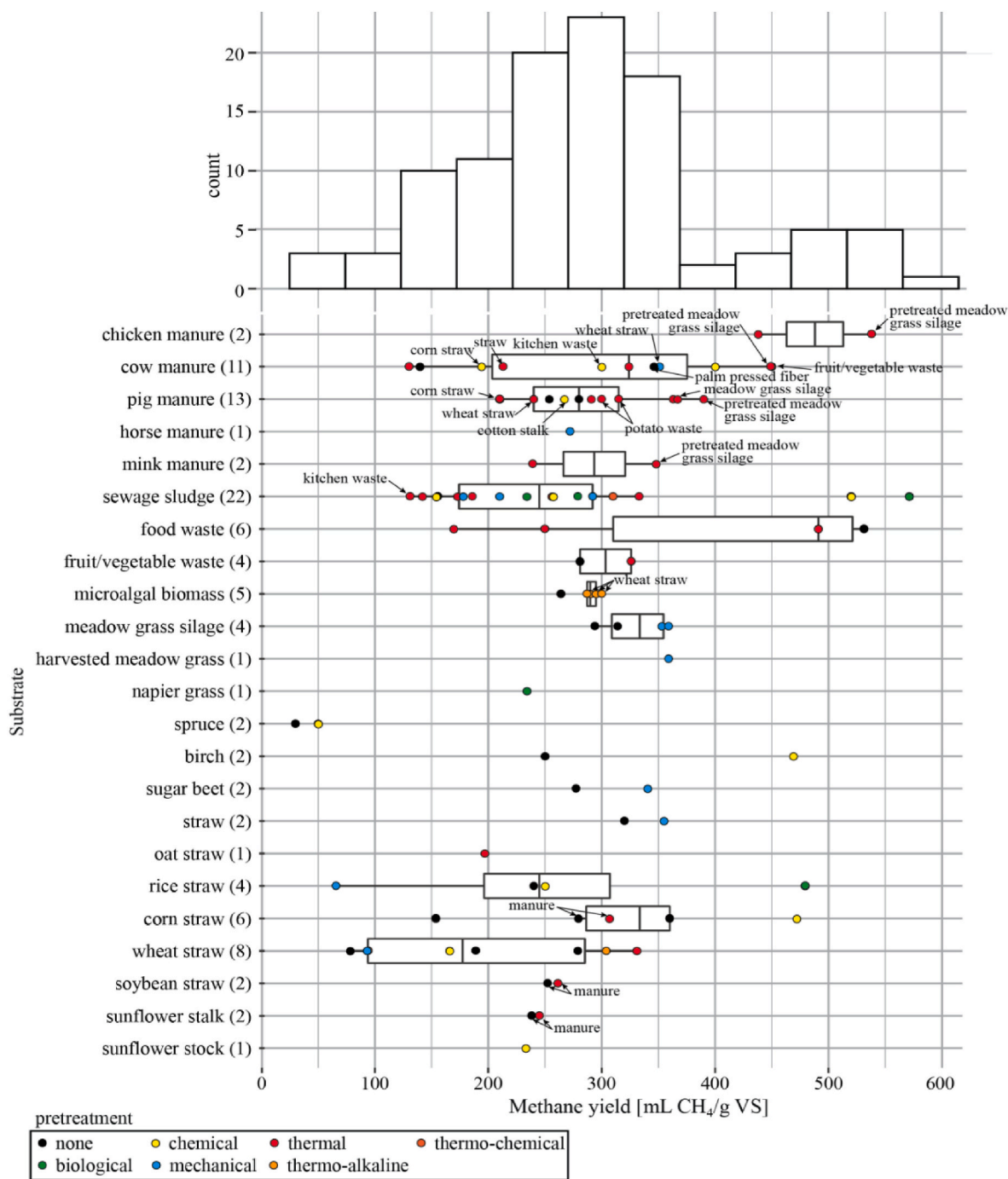


Fig. 7. Plot displaying the variability of the methane yield in the dataset. The top part of the plot presents the histogram (frequencies), whereas the bottom part displays the box plots for each substrate. The labels denote the cosubstrate type (no label means no cosubstrate). The colours of the points denote the pretreatment types. The numbers in parentheses denote the number of samples in the considered substrate category. Box plots were created only if there were at least four points in the category.

which illustrates the concentration at which the risk of odour recognition is 50% [159]. Thus, even though these compounds are present in very low concentrations (e.g., ppb v/v), they can significantly contribute to the occurrence of an odour nuisance.

The process of anaerobic biomass degradation can generate odour compounds, such as hydrogen sulfide, volatile organic sulfur compounds (VSCs), volatile aromatic compounds (VACs), aldehydes, ketones, amines and alcohols [160–163]. The decomposition of proteins and amino acids leads to the formation of significant amounts of ammonia, the emission of which can range from 18 to 150 g per ton of sludge

[164]. The odour nuisance resulting from the presence of volatile organic and inorganic compounds is a complex problem since a large group of odourous compounds can cause various odour effects occurring as a mixture (intensification or masking of the odorous intensity) [165].

The emission of odour compounds can be evaluated in two ways [166]. On the one hand, it is possible to determine the chemical concentration of individual compounds. For this purpose, chromatographic techniques, mainly gas chromatography coupled with mass spectrometry (GC-MS), are the most commonly used in this research area [167]. The second approach is based on the determination of the odour

**Table 5**  
Barriers to biogas technology.

Barriers	Source
<b>Financial and Economic</b>	
1) High investment costs include the construction cost of the biogas plant, procurement of equipment, hiring of technical staff, and introduction of the technology.	[144–146]
2) Long-term financing options, high interest rate and a high-risk perception by financial institutions are identified as the most prominent barriers to biogas dissemination in urban areas.	
3) High capital costs and low revenue accrual act as entry barriers for small private players/developers, and this technology is not economically feasible in the existing competitive market.	
<b>Market</b>	
1) The high price of biogas and the lower price of fossil fuels are critical, as is the low-priced electricity produced from coal and natural gas-fired power plants.	[145,147]
2) The electricity from other renewable sources, such as solar, hydro and wind, are also cheaper than AD-based power generation; the operation and maintenance costs of biogas-based power plants are quite high.	
<b>Social and Cultural</b>	
1) A lack of waste segregation even in urban cities increases the operation costs.	[148]
2) Limited awareness of environmental protection, economy of resources and health improvement among rural households.	[149]
<b>Regulatory and Institutional</b>	
1) Lack of political support and specific programmes to promote biogas technologies.	[23]
2) The energy sector has not received significant attention in developing countries.	[150]
3) A lack of private sector participation and poor coordination between the private and public sectors are challenges for the uptake of biogas.	[23,145]
4) The policy landscape is dynamic and uncertain, which is perceived as a problem in itself.	[151,152]
<b>Environmental</b>	
1) Potential negative environmental aspects are noise pollution, odour complaints, and the abundant water resource needs of biogas digesters.	[150]
2) Broken digester caps and gas valves that are not airtight can cause significant environmental problems.	[152]
3) Negligence regarding failed and abandoned biogas projects can pollute groundwater, nearby lakes and rivers.	[23,149]
<b>Technical and Infrastructural</b>	
1) A lack of standards and equipment for biogas systems, especially for countries that import technology.	[146]
2) Adequate water and substrate supplies are two crucial factors for the effective functioning of biogas plants.	[153]
3) Feedstock availability could also be an issue, e.g., where there are too few cattle to generate enough animal manure to produce a sufficient amount of biogas.	[154]
4) Temperature is a crucial factor influencing the rate of biogas production. During winter, the biogas production rate decreases considerably due to low temperature, which inhibit methanogenesis, thereby increasing the hydraulic retention time.	[23,145]
5) Unavailability of local biogas technology is another problem, e.g., in countries such as Malaysia where there is a lack of local biogas technology.	[145]
<b>Policy</b>	
1) The policy barriers may vary according to each country. The policy instrument in the EU favours the implementation of biogas systems.	[146,
2) At the community level, the funding support for biogas installations does not go to suitable candidates to manage the technology, and corruption is a major barrier in the policy environment.	155, 156]
3) Existing policies should focus more on improving the development of various sources of energy and technologies. The low price of LPG and available subsidies encourage the use of LPG rather than biomass and impose a barrier on modern biomass technology.	

concentration using olfactometry techniques [168].

The biomass pretreatment process can have a significant impact on the emission of odour compounds during AD. Thermal pretreatment is mainly associated with a reduction in the water content of the sludge, and this most often increases the strength of the perceived smell [158]. At high temperatures, decomposition of less stable compounds responsible for the formation of unpleasant odours can be observed. Chemical pretreatment can change the composition of biomass and, as a result, the chemical composition of biogas [89]. However, biomass pretreatment operations are still insufficiently researched to determine their effectiveness levels with simultaneous evaluations of the odour properties of biogas streams. Table 6 contains some examples of works in which the odour aspect was taken into account during the pretreatment operation of sewage sludge before AD.

Codigestion of sewage sludge and other appropriate substrates can increase the overall methane yield. However, the addition of food, agricultural, agro-industrial or municipal solid wastes can significantly change the chemical compositions of biogas streams. These types of substrates can contain many odorous compounds, which are emitted during aerobic or anaerobic processes [173]. For example, Ni et al. [174] detected some VOCs during food waste treatment operations, mainly biogenic compounds such as oxygenated compounds, hydrocarbons, terpenes, and organo-sulfur compounds, as well as abiogenic compounds (aromatic hydrocarbons and halocarbons) [174]. Based on the results describing the odour compound emissions during the treatment of potential substrates used during codigestion, more than 300 odorous substances have been identified in swine production facilities [175], more than 70 have been identified in animal manure [176] and more than 100 have been identified in dairy facilities [177]. Many of the identified substances have low odour detection thresholds. Therefore,

there is a high probability that some of these substances can be emitted from substrates during the codigestion process.

Both biomass pretreatment and codigestion of substrates have some economic and environmental advantages. One of the potential methods to increase the efficiency of biogas is anaerobic codigestion combined with substrate pretreatment. To the best of the authors' knowledge, this approach has scarcely been studied in the scientific literature compared with anaerobic codigestion without pretreatment. For this reason, further research combining pretreatment and codigestion can be interesting, but in the opinion of authors, this research should also consider issues related to the odour of biogas.

## 9. Conclusions and recommendation for further research

Bioenergy is one of the most significant but still undervalued renewable energy source. However, this technology effectively combats different environmental problems (e.g., carbon capture and storage) and supports EU climate neutrality as well as the 'waste to energy' philosophy. Different organic-rich waste streams, low-value residues and sustainable biomass sources can pave the way for the success of biogas production as an alternative to fossil fuels, and the energy produced from those sources is useable in different forms, such as heat, steam and electricity. This study provides a novel contribution to the literature by analysing the existing knowledge about the possibility of enhancing the AD potential. However, there are many local and national factors and site-specific experiences, which cannot be underestimated, and the improvement of AD technology should be sought with codigestion and the development of new pretreatment methods. Implementation of proper monitoring and control systems is crucial for effective biogas production with the available biomass and improved cost performance.

**Table 6**  
Examples of odour control using some disintegration methods.

Biomass type	Disintegration methods used	Sludge digestion type	Odour compounds/ions monitored	Main results	References
Waste activated sludge	ultrasounds (U), Fenton oxidation (F) and ultrasounds coupled with Fenton oxidation (U+F)	anaerobic digestion	hydrogen sulfide, methyl mercaptan, dimethyl sulfide, dimethyl disulfide, ammonia, dissolved sulfide and dissolved sulfate	ultrasounds coupled with Fenton oxidation can effectively decrease potential odour release compared to the U and F alone	[169]
Waste activated sludge	thermo – oxidative pretreatment (60 °C in presence of 0.6 mg H <sub>2</sub> O <sub>2</sub> + 1.5 mg FeCl <sub>2</sub> /mg S <sup>2-</sup> as oxidants	anaerobic digestion	hydrogen sulfide, methyl mercaptan, dimethyl sulfide,	hydrogen sulfide (H <sub>2</sub> S) and dimethyl sulfide (DMS) concentrations in biogas significantly decreased by an average of 75% and 40%, respectively	[170]
Waste activated sludge	oxidation by Fe (VI)	anaerobic digestion	hydrogen sulfide, methyl mercaptan and sum of odour compounds using the dynamic olfactometry technique (determined by odour concentration and emission potential)	Fe (VI) can successfully remove odour in sludge	[171]
Combined primary sludge and waste activated sludge	thermally pretreated wastewater solids	anaerobic digestion	methyl mercaptan, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide	from 52 to 92% odour reduction	[172]

For this reason, laboratory-scale studies followed by pilot-scale in situ studies are needed to properly evaluate the type of substrate, cosubstrate and the effectiveness of feedstock pretreatment in terms of the AD process and final digestate disposal.

National and regional strategies should stimulate further growth of biogas technology by applying biodegradable waste streams and/or sustainable biomass that does not deplete local resources, which would lead to ecological problems. The future use of biomass should be focused more on the local market and pretreatment/codigestion to enhance biogas production via AD. The economic and energy aspects of the appropriate use of biomass and pretreatments should be also considered. Moreover, the statistical analysis of data from the literature demonstrates the need to produce metadata by using the DOE methodology (a common framework for standardized experimental design and data collection). Only high-quality datasets would provide the ability to implement data exploration and machine learning techniques to identify the most significant features of AD (or their combinations), to fully optimize biogas production and to better understand the nature of this process.

#### 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.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rser.2021.111509>.

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