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Over the past decades, anaerobic biotechnology is commonly used for treating high-strength wastewaters from different industries. This biotechnology depends on interactions and co-operation between microorganisms in the anaerobic environment where many pollutants' transformation to energy-rich biogas occurs. Properties of wastewater vary across industries and significantly affect microbiome composition in the anaerobic reactor. Methanogenic archaea play a crucial role during anaerobic wastewater treatment. The most abundant acetoclastic methanogens in the anaerobic reactors for industrial wastewater treatment are *Methanosarcina* sp. and *Methanotrix* sp. Hydrogenotrophic representatives of methanogens presented in the anaerobic reactors are characterized by a wide species diversity. *Methanoculleus* sp., *Methanobacterium* sp. and *Methanospirillum* sp. prevailed in this group. This work summarizes the relation of industrial wastewater composition and methanogen microbial communities present in different reactors treating these wastewaters.

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Review

Methanogenic Microorganisms in Industrial Wastewater Anaerobic Treatment

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Abstract: Over the past decades, anaerobic biotechnology is commonly used for treating high-strength wastewaters from different industries. This biotechnology depends on interactions and co-operation between microorganisms in the anaerobic environment where many pollutants' transformation to energy-rich biogas occurs. Properties of wastewater vary across industries and significantly affect microbiome composition in the anaerobic reactor. Methanogenic archaea play a crucial role during anaerobic wastewater treatment. The most abundant acetoclastic methanogens in the anaerobic reactors for industrial wastewater treatment are *Methanosarcina* sp. and *Methanotrix* sp. Hydrogenotrophic representatives of methanogens presented in the anaerobic reactors are characterized by a wide species diversity. *Methanoculleus* sp., *Methanobacterium* sp. and *Methanospirillum* sp. prevailed in this group. This work summarizes the relation of industrial wastewater composition and methanogen microbial communities present in different reactors treating these wastewaters.

Keywords: wastewater treatment; industrial wastewater; anaerobic reactor; anaerobic digestion; methanogenesis; biogas; microbial community

1. Introduction

Today, water is an integral part of the course of our lives, from the operation and maintenance of households to extensive industrial and agricultural use. Ecological, political and ethical aspects today force us to constantly think about streamlining processes and managing natural resources as gently as possible. It is now very important to protect the high quality of water as a renewable resource due to the loss of its share under the surface and in the landscape and watercourses.

Industrial wastewater is an environmental pressure even if these waters are, in some cases, collected by a local sewer system, treated in an urban wastewater treatment plant (UWWTP) and subsequently released to the environment [1]. There are also cases, however, in which these waters are directly released to a water body, generally after treatment at the industrial facility where the wastewater is generated. There is a large and diverse range of economic activities affecting wastewater production. Global data on water uptake per region in 2016 are presented in Figure 1. It shows that industry in Europe is a major consumer of water in relation to other sectors. The global average water uptake by industry is around 19% [2]. According to the Food and Agriculture Organization of the United Nations (FAO) [3], industrial water uptake in Europe has, however, decreased in recent years. The overall uptake of water by industry is around 200 billion m³ per year, dominated by sea water abstraction for cooling systems, which uses around 50 billion m³ per year. Water uptake for industrial

manufacturing processes (not cooling) has experienced a 40% reduction in Europe since 1990 (from around 50 to 30 billion m³ per year).

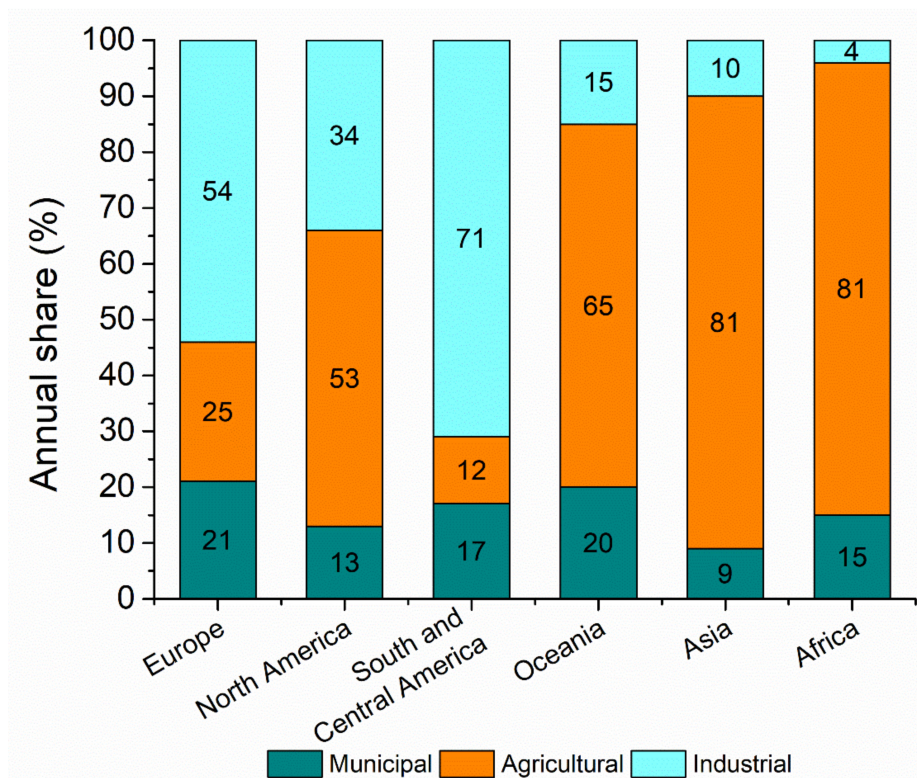


Figure 1. Annual share of global water uptake by activity and region [2], revised by authors.

The release of industrial wastewater is regulated in Europe both directly as part of the environment law on industry and indirectly by the EU policies that tackle water issues horizontally. Industrial wastewater generation and management is regulated by Water Framework Directive (WFD, 2000/60/EC). Industry's direct or indirect releases of pollution to the environment are among the key aspects regulated by the Industrial Emissions Directive (IED, 2010/75/EU). Currently, the IED regulates 31 industrial sectors and over 50,000 installations in Europe. To meet the legislation requirements, an approach known as best available techniques (BAT) is adapted. To specify processes and activities for individual industrial sectors, BAT reference documents (BREFs) are used.

The anaerobic wastewater treatment system has been known and used since the end of the 19th century [4]. Systematic research work and deeper anaerobic process understanding caused anaerobic digestion (AD), a biological process in which organic matter is converted to CH₄ and CO₂, to become a more attractive technology for wastewater treatment due to its low capital and operation cost compared to the other technologies available in last decades. Today, anaerobic techniques are generally utilized in industries with high level of soluble and readily biodegradable organic material. Many types of industrial wastewaters believed to be unsuitable for anaerobic treatment are today treated with advanced anaerobic reactor systems [5]. The role of microorganisms and their formation into complex communities is an essential pillar of the whole anaerobic biotechnology, but so far there are few laboratories and publications that deal with this issue in detail. Interactions between microorganisms, their co-operation or competition, with other aspects, have a significant impact on the final functionality of the whole process. Therefore, in addition to an external understanding of processes and technological parameters, it is important to examine the composition of microbial communities. Awareness of the general composition of the microbial community may be very important in the future for the typing of anaerobic biotechnologies, their implementation in industrial areas and the potential solution of functional defects and problems.

The main objective of this study was to investigate the complex issues of the composition of microbial communities in anaerobic reactors, especially in relation to the composition of wastewater. This review contains a basic description of anaerobic technology and the composition of selected types of industrial wastewater. It summarizes information on the occurrence of microorganisms and tries to define the composition of methanogenic communities' present in this environment.

2. Industrial Wastewater

2.1. Industrial Wastewater Types

Industrial wastewater varies in composition and cannot be simply characterized. Three major types of wastewater can be defined, processing, cleaning and sanitary. Different industrial sectors generate different compositions and quantities of these wastewaters at the effluent [6]. It may be highly biodegradable or not at all and may or may not contain compounds recalcitrant to treatment. The main concern with industrial wastewater is the increasing amount (in quantity and variety) of synthetic compounds contained in and discharged to the environment. The main industrial wastewater types are presented in Table 1.

Table 1. Industrial wastewater types depending on the industrial sectors [7], edited by the authors.

Category	Common Features	Pollutants	Typical Industrial Sectors
Minimal contamination (can be land spread)	Wastewater contains no pollutants, nutrients can be useful for agricultural plants development, levels of toxic substances is very low	Nitrogen, phosphorus	Food and drink
Equivalent to domestic-type effluents	Organic pollutants similar content as in municipal wastewater	Degradable organic matter	Food and drink
Low flow and non-domestic type pollutants at low concentrations	Wastewater containing small concentrations of other pollutants not present in urban effluents	Pesticides, hormones, nano-plastics and endocrine disrupters	Chemicals
Metals	Wastewater containing metals or metalloids from industry	Metals	Metal processing and mineral industry
High nutrient loading	Wastewater containing high concentrations of nitrogen compounds, phosphates, with higher conductivity	Substances increasing eutrophication	Chemicals: fertilizers
Effluent streams requiring pH adjustment	Wastewater streams with very low or very high pH	Acids or alkalis	Chemicals and mineral industry
Persistent organics content	Wastewater containing not easily degradable organic pollutants (persistent hydrocarbons or bioaccumulative organic toxic substances)	Persistent organics	Textiles and chemicals
Emerging substances	Wastewater contains new pollutants or has characteristics that are not currently monitored	New parameters or compounds not frequently measured	Pharmaceuticals

2.2. Industrial Water Suitable for Anaerobic Treatment and Methane Production

Anaerobic techniques are typically used for high-loaded wastewaters, expressed in chemical oxygen demand (COD), typically greater than 1500–2000 mg/L. The application of anaerobic wastewater treatment is largely confined to relatively heavily polluted wastewater with a COD between 3000 and 40,000 mg/L, e.g., in the sugar, starch, fruit and vegetable and alcoholic drinks sectors. There has recently been some success in using certain anaerobic systems even for less heavily polluted wastewater with a COD between 1500 and 3000 mg/L, e.g., in breweries, dairies and in the fruit juice, mineral water and the soft drinks sectors. Some success in using anaerobic systems for less polluted wastewater with a COD lower than 1500 mg/L was reported [7–9]. It is clear that only industrial wastewater with significant carbon loading that is treated under intended or unintended anaerobic conditions will produce methane [10]. Assessment of methane production potential from industrial wastewater streams is based on the concentration of biodegradable organic matter in the wastewater, Tables 2 and 3 [11,12], edited by the authors.

Table 2. Chemical compounds amenable to anaerobic biotechnology.

Acetaldehyde	Crotonic acid	Isobutyric acid	Isopropyl alcohol
Acetic anhydride	Diacetone gulusonic acid	Isopropanol	Propionate
Acetone	Dimethoxy benzoic acid	Lactic acid	Propylene glycol
Acrylic acid	Ethanol	Maleic acid	Protocatechuic acid
Adipic acid	Ethyl acetate	Methyl acetate	Resorcinol
Aniline	Ethyl acrylate	Methyl acrylate	Sec-butanol
1-amino-2-propanol	Ferulic acid	Methyl ethyl ketone	Sec-butylamine
4-amino butyric acid	Formaldehyde	Methyl formate	Sorbic acid
Benzoic acid	Formic acid	Nitrobenzene	Syringaldehyde
Butanol	Fumaric acid	Pentaerythritol	Syringic acid
Butyraldehyde	Glutamic acid	Pentanol	Succinic acid
Butylene glycerol	Glutaric acid	Phenol	Tert-butanol
Catechol	Glycerol	Phthalic acid	Vanillic acid
Cresol	Hexanoic acid	Propanal	Vinyl acetate
Crotonaldehyde	Hydroquinone	Propanol	

Table 3. Industrial wastes amenable to anaerobic digestion.

Agriculture wastes	Corn processing wastes	Chemical industry wastes	Seafood and shellfish wastes
Alcohol stillage	Dairy wastes	Meat packing wastes	Slaughterhouse and meat packing
Animal wastes	Egg processing wastes	Pear wastes	Sugar processing wastes
Bagasse	Fruit Leachate	Peat wastes	Tannery wastes
Bean blanching water	Giant kelp wastes	Pectin wastes	Vegetable processing wastes
Beverage production wastes	Guar gum wastes	Petroleum wastes	Wheat and grain processing wastes
Brewery wastes	H ₂ -CO pyrolysis wastes	Pharmaceutical	Wine processing wastes
Canning wastes	Heat-treated activated sludge	Potato processing wastes	Wood processing wastes
Coking mill wastes	Cheese processing wastes	Pulp and paper wastes	Wool scouring wastes

The volume of wastewater, and the propensity of the industrial sector to treat their wastewater in anaerobic systems are also important. Using these criteria, major industrial wastewater sources with high methane production potential can be identified as follows:

- Meat and poultry processing (slaughterhouses)
- Alcohol, beer, starch production
- Pulp and paper manufacture
- Chemical industry waste
- Other food and drink processing (dairy products, vegetable oil, fruits and vegetables, canneries, juice making, etc.)

Recent development and knowledge showed that anaerobic processes might be an economically feasible alternative for different types of industrial wastewaters treatment. The number of wastes that are amenable to anaerobic digestion is quite large (Tables 2 and 3). However, the feasibility of the anaerobic digestion of an industrial waste is determined by several factors. These factors are waste concentration; waste stream temperature; anaerobic process inhibitors presence in waste stream; expected biogas yield and treatment efficiency [11]. The anaerobic process has many advantages over conventionally used and proven aerobic processes. The most significant positive of the technology is the fact that energy is generated in the form of biogas by means of anaerobic decomposition from unnecessary waste in the form of pollutants in the wastewater.

2.3. Composition of Selected Industrial Wastewater

The composition of industrial wastewater will be characterized by both the substance load and the proportion of biodegradable substances and the values of other, more specific parameters (Table 4).

Table 4. Composition characteristics of individual industrial wastewaters (values are given in mg/L except pH).

Wastewater Type	COD	BOD	TS	SS	VSS	TN	TP	N-NH ₄ ⁺	pH	Reference
Slaughterhouse	2000–11,588	1300–4635 (BOD ₅)	6394	850–6300	660–5250	850	15–48	20–66	6.3–6.98	[13,14]
Poultry slaughterhouse	2790–5520	1558–2988	-	-	-	62–313 (KN)	-	16–95	6.8–7.8	[15]
Poultry processing	1140	570 (BOD ₅)	-	264	-	-	-	2.7	-	[16]
Meat processing	5160	-	2028	1820	1380	-	-	-	7.5	[17]
Livestock breeding	6190–78,600	3940–34,600	-	1850–29,000	-	1530–6500	116–1770	-	-	[18]
Dairy industry	-	10,000–50,000	-	220–340	200–300	188	100	18	9–10.5	[19]
Milk plant	2000–6000	1200–4000 (BOD ₅)	-	350–1000	330–940	50–60 (KN)	-	-	8.0–11.0	[20]
Butter production	52,000	-	-	1500	-	1120 (KN)	-	-	4.3–5.9	[20,21]
Brewery	2000–6000	1200–3600	5100–8750	2901–3000	-	25–80 (KN)	10.0–50.0	-	3.0–12.0	[22]
Raw distillery wastewater	80,000–120,000	45,000–60,000	100,000	10,000	100–2800	100–64,000	240–65,000	-	3.5–5.2	[23,24]
Sugar factory	572–6612	-	3840–5780	30–170	560–6470	-	2.0–4.0	3.7–10.1	4.7–5.2	[25]
Cellulose Processing	600–10,400	221–3700	-	20–3200	-	-	-	-	6.3–9.0	[26–28]
Fruits and Vegetables Processing	1500–4300	500–2500	400–1200	-	-	-	-	-	6–10	[29]
Vegetable oil mills	1355–1987	712–1136 (BOD ₅)	-	-	-	-	-	3.6–14.4	0.9–2.3	[30]
Olive oil mills	57,200	-	49,100	-	-	1600	300	-	4.9	[31]
Refining of vegetable oils	17,688–24,787	4120–4560 (BOD ₅)	-	791–3544	-	<10	-	<10	10.0–10.4	[32]
Blackberry processing	930	-	840	-	-	92	-	-	5.9	[33]
Dates processing	410	-	471	-	-	37	-	-	6.1	[33]
Tomato processing	294	-	322	-	-	21	-	-	5.3	[33]
Beetroot processing	501	-	630	-	-	43	-	-	6.2	[33]
Butadiene and styrene	800–1500	4000–8000 (BOD ₅)	-	200–500	-	-	-	-	-	[34]
Acrylates	2000–3200	1000–2000 (BOD ₅)	-	50–100	-	-	-	-	-	[34]
Acetaldehyde	40,000–60,000	15,000–25,000 (BOD ₅)	-	150–300	-	-	-	-	-	[34]
Ketones	20,000–40,000	10,000–20,000 (BOD ₅)	-	50–100	-	-	-	-	-	[34]
Methyl acrylate acid	7000–12,000	-	-	6000–12,000	-	-	-	-	-	[34]
Organic acids	5000–15,000	300–600 (BOD ₅)	-	100–200	-	-	-	-	-	[34]
Raw materials for the pigment industry	1000–2000	200–400	-	80–200	-	-	-	-	-	[34]

BOD—biochemical oxygen demand; COD—chemical oxygen demand; TS—total solids; SS—suspended solids; VSS—volatile suspended solids; TN—total nitrogen; TP—total phosphorus.

3. Anaerobic Wastewater Treatment

3.1. Aerobic Versus Anaerobic Wastewater Treatment

Today, aerobic biological wastewater treatment processes are increasingly used in connection with the anaerobic sewage sludge stabilization in large and medium wastewater treatment plants. In the last decades, anaerobic digestion has grown more and more into an attractive technology for wastewater treatment due to its low capital and operation costs compared to the other technologies available: physicochemical and aerobic biological treatments. Here, practice has shown that anaerobic sludge and wastewater treatment as biological treatment has recently increasingly appeared to be a more environmentally friendly and economical process, both in terms of the formation of excess sludge and the conversion of the substrate to biogas [35]. Substantial savings, reaching 90% in operational costs as no energy is required for aeration and a 40–60% reduction in investment cost, are reported. Produced CH₄ can be used for energy recovery or electricity production, excess sludge production is low, and sludge is well stabilized and easily dewatered, so extensive post treatment is not required [36]. When aerobic and anaerobic treatment is compared, some advantages which bring appropriate application of anaerobic technology can be found (Table 5). Anaerobic treatment produces up to ten times less biomass, which is related to very low requirements for nutrients and energy in the form of biogas. Excess sludge does not need to be further stabilized, which is a necessary part of aerobic treatment [37].

Table 5. Basic comparison of aerobic and anaerobic treatment [36,38].

	Aerobic Treatment	Anaerobic Treatment
Transformation of input substrate	50% microbial biomass 50% CO ₂	5% microbial biomass 95% biogas
Energy balance	60% microbial biomass 40% reaction heat	90% biogas 5–7% microbial biomass 3–5% reaction heat

From the bioreactor and the overall cleaning process point of view, anaerobic wastewater purification is an alternative to aerobic technologies. The anaerobic process has mainly been used for sewage sludge stabilization. However, research [39] has showed that industrial wastes can also be treated by anaerobic processes. Young and McCarty [40] found that low-strength soluble organic wastes can also be efficiently treated anaerobically.

In anaerobic reactors, a high concentration of biomass is still maintained, which is related to the ability to treat wastewater with a very high material load. On the other hand, anaerobic microorganisms grow very slowly, which is why it is still necessary to maintain their high concentration and leave the substrate in the anaerobic reactor for a long time >15 days [11,41]. However, one of the biggest advantages of this is that the high sludge retention time in the reactor promotes the growth of microorganisms with a long generation time, which are able to degrade pollutants that cannot be degraded in aerobic conditions or are toxic to aerobic microorganisms. Finally, we cannot omit the price of operation, which is significantly lower for anaerobic technologies [37].

It should be noted that anaerobic treatment also has its disadvantages. The total loss of organic matter is still not achieved in anaerobic processes and it is often necessary to treat the wastewater aerobically. Another major disadvantage is the high sensitivity of anaerobic organisms, especially methanogenic archaea, and their long generation time, which is associated with a long time to start the operation of reactors [36].

3.2. Anaerobic Digestion

Anaerobic digestion is a promising biotechnology for wastewater that is highly organically polluted and therefore contains high concentrations of biodegradable substances [36]. The digestion

of substrate in anaerobic reactors results in the significant reduction in the volatile solids content as well as the volume and weight of the substrate. Figure 2 shows the fate of volatile solids during anaerobic digestion.

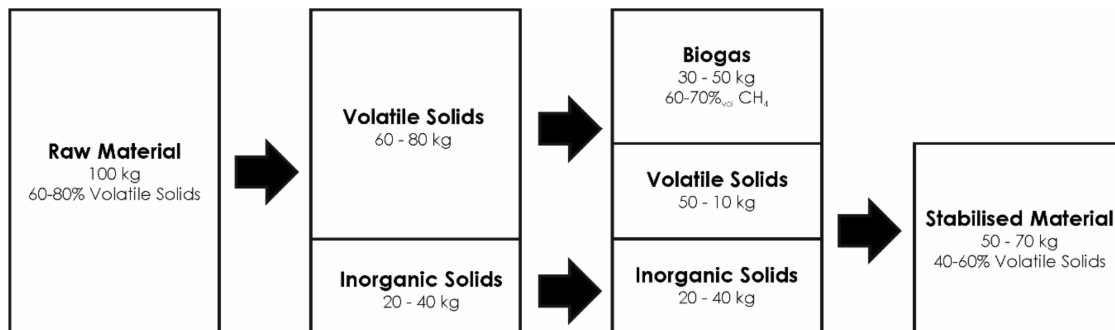


Figure 2. Volatile solids balance during anaerobic digestion.

Anaerobic digestion is a complex process (Figure 3) that consists of a number of biochemical processes and is mediated by systematically interconnected microorganisms mainly from the *Bacteria* and *Archaea* domains [42] and also to a lesser extent by Eukaryotes and a very small percentage of viruses [43]. By means of individual biochemical transformations, complex organic compounds are decomposed into as many oxidized and reduced forms of carbon as possible, i.e., carbon dioxide and methane [44].

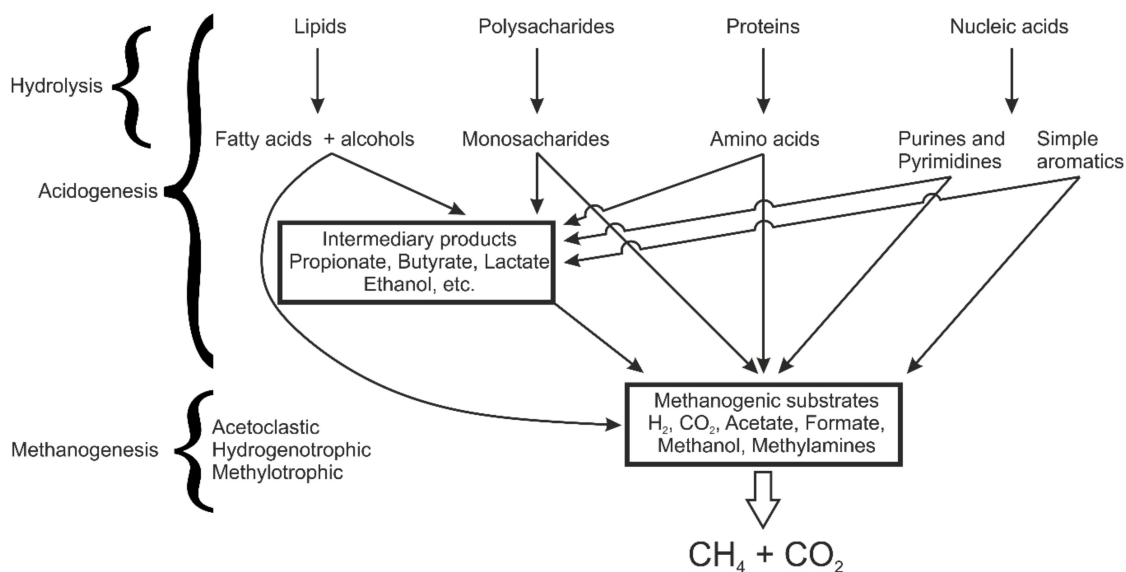


Figure 3. Scheme of reactions during anaerobic decomposition of polymeric materials [45], edited by authors.

During hydrolysis, the complex biopolymers are cleaved by extracellular enzymes of fermentative bacteria into smaller parts, monomers, which are further able to process the bacteria directly in the cell. Fermentative bacteria convert monomers mainly into volatile fatty acids such as butyric, acetic and propionic acids or alcohols, lactic acid and molecular hydrogen during acidogenesis. Most of these bacteria are obligate anaerobes, but some facultative anaerobes may be present. During acidogenesis, acetate, carbon dioxide and molecular hydrogen are formed from the already mentioned volatile fatty acids. Hungate [46] was the first to show that hydrogen production and utilization can influence the anaerobic digestion. In addition, acetate can also be formed by homoacetogenesis, i.e., from carbon dioxide and hydrogen. Bryant [47] pointed out that the products of the hydrolysis other than

acetate, H₂ and CO₂, i.e., alcohols, propionate and longer-chain fatty acids and aromatic acids are anaerobically oxidized to acetate or acetate and CO₂ by a group of H₂-producing acetogenic bacteria. During methanogenesis, methane is formed by three major pathways—acetoclastic, hydrogenotrophic and methylotrophic—by methanogenic archaea [36,48]. All processes in the anaerobic bioreactor run in parallel after the equilibrium has been established.

The anaerobic process usually takes place in mesophilic (35–50 °C) or thermophilic (50–60 °C) cultures of microorganisms, and, in terms of biogas yield, thermophilic cultures show higher biogas production. The biogas produced consists mainly of carbon dioxide and methane. Typical methane content in biogas ranged from 50 to 85%vol. Other gases (H₂S, NH₃ and water vapor) are usually present in biogas in the hundreds or thousands of ppm.

In addition to methanogenic communities, bioreactors can also contain bacteria that can compete with methanogens for the available substrate and thus negatively affect biogas production. These are facultatively anaerobic bacteria that can use molecular oxygen as an electron acceptor, denitrifying bacteria that use nitrates, sulfate-reducing bacteria (SRB) using sulfides and sulfates, or bacteria that reduce iron ions [36]. Recent research demonstrates strong competition between methanogenic archaea and SRB for molecular hydrogen. These are mainly hydrogenotrophic methanogens, which use hydrogen to reduce the methyl group to methane. Reducing the metabolic activity of SRB could contribute to improving the quality of biogas. Hydrogen sulfide is known to corrode the engines of cogeneration units for electricity producing [49].

3.3. Anaerobic Bioreactors

Anaerobic processes for the treatment of wastewaters and sludges are well over 100 years old [50]. In the last 50 years, anaerobic reactor technology evolved from localized lab-scale trials to worldwide successful implementations at a variety of industries [51]. The occurrence of the new environmental laws within the EU, tightening limits for discharged water, increased prices for energy and final sludge disposal, and a high amount of highly strengthened wastewater from industry accelerated development in anaerobic reactor technology. Since the 1970s, when Lettinga et al. [52] first described the Upflow Anaerobic Sludge Blanket (UASB) process, a lot of progress has been made in the anaerobic wastewater treatment. The development of the high-rate generation of anaerobic reactors took place in the 1980s, with the aim to decrease the hydraulic retention time; this was the main weakness of anaerobic reactors in that time. Since the 1990s, the development and application of anaerobic reactors for simultaneous treatment and methane production have found considerable success [31]. Today, different types of high-rate anaerobic reactors characterized by short hydraulic retention time, high pollutant removal efficiency and high applicable volumetric loading rates are commonly used across the industry worldwide. A total of 2360 full-scale installations were in operation in 2019 based on author surveys of the following renowned vendors: PAQUES (Biopaq); VEOLIA (Biothane); WATERLEAU (Biotim); GLOBAL WATER & ENERGY (ANUBIX and ANAFIX); EVOQUA (ADI); KURITA; SUEZ (Degremont); ENVIROCHEMIE (Biomar); HYDROTHANE (HydroThane). Such vendors are mainly in the food and beverage industry (Figure 4). The data also showed that UASB was the most common type of reactor followed by Internal Circulation (IC) and Expanded Granular Sludge Bed (EGSB). These types of reactors represent in total 89% of all reactors installed (Figure 5).

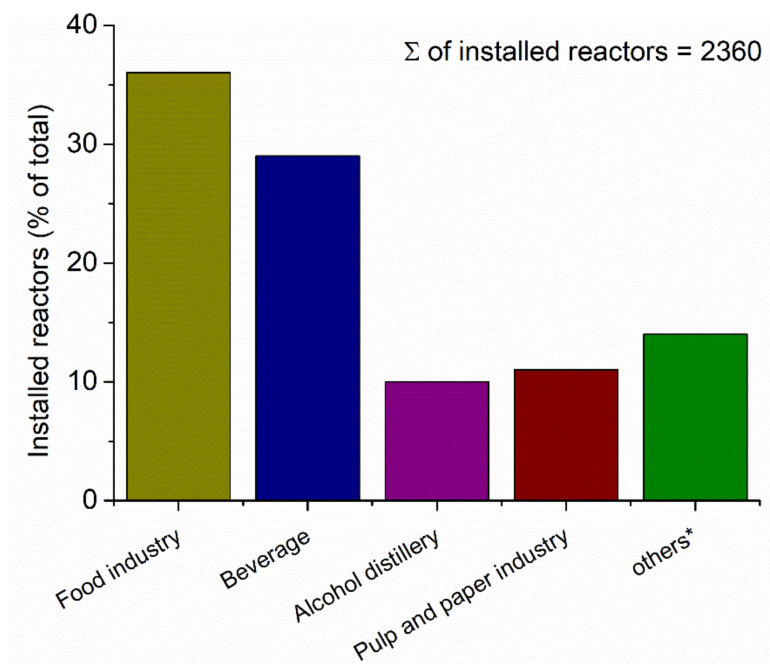


Figure 4. Application of anaerobic technology to industrial wastewater. Total number of registered installed reactors = 2360; * chemical, pharmaceutical, sludge liquor, landfill leachate, acid mine water, municipal sewage; adopted from [53] and updated and edited by the authors.

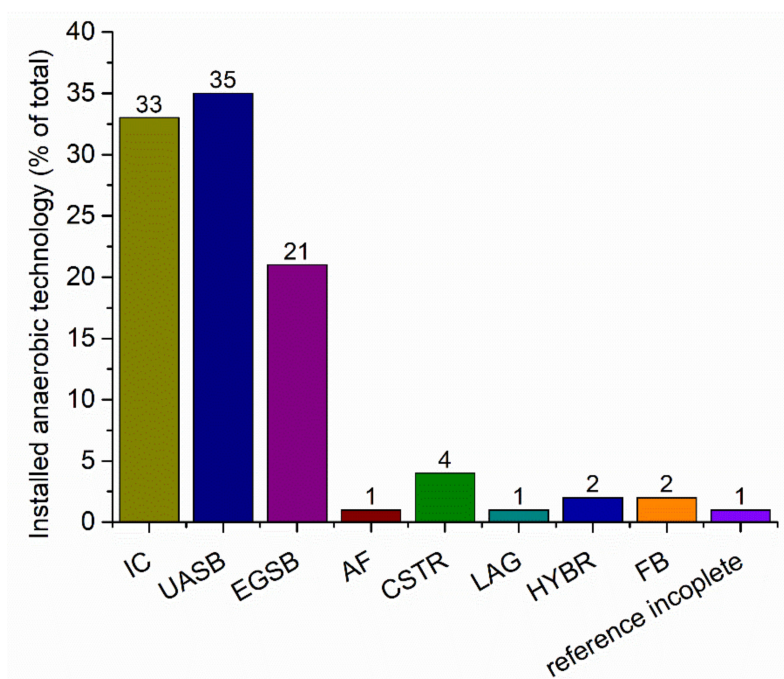


Figure 5. Implemented anaerobic technologies for industrial wastewater pictured for the period 2002–2019. IC: Internal Circulation; UASB: Upflow Anaerobic Sludge Blanket; EGSB: Expanded Granular Sludge Bed; AF: Anaerobic Filter; CSTR: Continuously Stirred Tank Reactor; LAG: anaerobic lagoon; HYBR: combined system with sludge bed at the bottom section and a filter in top; FB: fluidized bed reactor; adopted from [36], updated and edited by authors.

Anaerobic bioreactors initial drawbacks (biomass slow growth rate, susceptibility to toxic compounds, huge reactors volumes, etc.) were overcome. The new generation of reactors with a short hydraulic retention time (HRT) (2 h to 48 h) and the ability to process high organic loading

rates (4 to 40 kg COD/m³ reactor per day) was developed (Table 6). In these reactors, the problem of slow growth rate was turned by capturing the biomass in the form of biofilms on static or moving supports but also by selecting well settling flocculating biomass. This development resulted in much smaller reactors volume but also in a much more stable operation than before.

Table 6. Typical processes and performance data of anaerobic technologies used for industrial wastewater treatment [39,54].

Process	Inflow COD	Hydraulic Retention Time	Organic Loading Rate	COD Removal Efficiency
	(mg/L)	(h)	(kg _{COD} /m ³ per day)	(%)
Anaerobic lagoons	N.A.	24–1200	0.04–1	30–50
Anaerobic contact process	1500–5000	2–14	0.5–5.3	75–90
Fixed Bed reactor	10,000–70,000	24–48	1–15	75–85
Upflow Anaerobic Sludge Blanket (UASB) reactor	5000–90,000	4–12	4–12	75–85
Expanded Granular Sludge Bed (EGSB) reactor	1000–90,000	5–10	5–30	80–85
Internal Circulation (IC) reactor	5000–90,000	3–25	5–40	80–87

Anaerobic bioreactors for wastewater treatment can be divided in terms of the biomass cultivation of microorganisms. Biomass can be cultured in suspension or it can form the biofilm. Anaerobic reactors with suspension culture are particularly suitable for the treatment of wastewater with a high proportion of suspended solids. By constant homogenization of the sludge, a good access of microorganisms to the substrate is achieved. Reactors with biomass cultivation in biofilm can be further divided into reactors with fixed and moving charge. The two technologies differ only in the carrier on which the biomass forming the biofilm is captured, namely the solid and the fluid or expanded carrier. Biofilm anaerobic reactors are then modified in terms of wastewater flow or rotation. Most widespread technologies for anaerobic wastewater treatment reactors are upflow Anaerobic Sludge Blanket Process (UASB), Internal Circulation (IC) and Expanded Granular Sludge Bed (EGSB) (Figure 6A–C). The UASB reactor is a suspended-growth reactor that maintains a very high concentration of microbial biomass by promoting granulation. The anaerobic granules are 1–3 mm in diameter and dense enough to settle down in the reactor. The biomass concentration in the UASB reactor reaches 50 g/L or higher and thus maintains a very long sludge retention time >15 days irrespective of the short hydraulic retention time of 4–12 h. Upflow velocity typically ranges from 0.5 to 1.0 m/h, 4.5 to 6.5 m high [41]. The EGSB reactor was developed from UASB reactors. It has a high recycle ratio, and the upflow of this reactor is typically maintained higher than 6 m/h, 12 to 16 m high [41]. The IC reactor is a new concept being mostly used for the treatment of industrial effluents of high strength. The higher OLR of the IC reactor is mainly due to its internal circulation, which allows for an improved contact between the biomass and the influent [41].

In the case of granular biomass reactors, the microorganisms combine to form granules of different color, density and size. The granules are present in different proportions and the microorganisms that are part of them can play various roles in anaerobic processes. It is also important to note that, across the types of these granules, the methanogenic archaea forms a core, more or less active, and bacteria form the outer layers of the granules [55].

Many reactors operate despite the same names on the same or similar principles. Due to the evolving technology, only specific modifications are made to the basic structures due to several design companies. The potential of anaerobic reactors in wastewater treatment is now great but will increase as the parameters are unified and the technology is universalized.

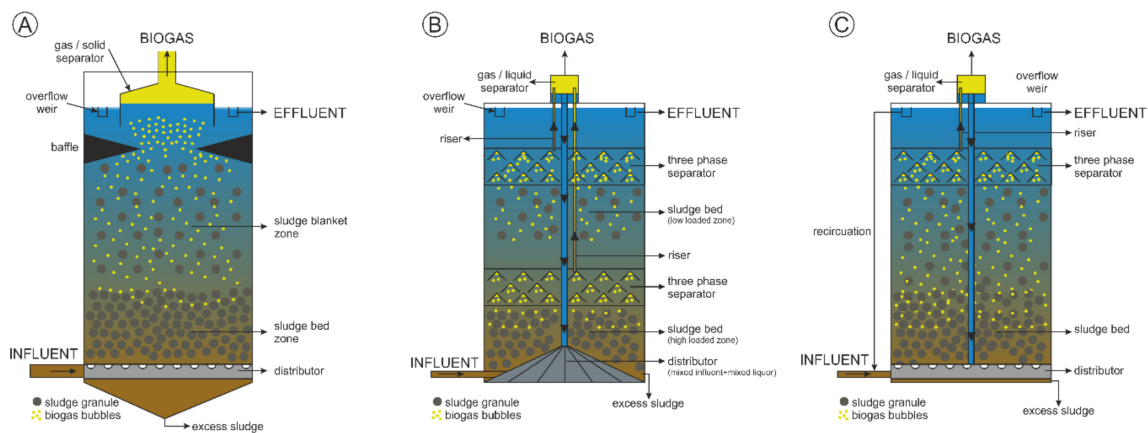


Figure 6. Scheme of an Upflow Anaerobic Sludge Blanket (UASB) reactor (A), an Internal Circulation (IC) reactor (B) and an Expanded Granular Sludge Blanket (EGSB) reactor (C).

4. Methanogenic Microorganisms in Industrial Wastewaters

The composition of microorganisms in anaerobic bioreactors will be affected by many factors. These are reactor design, temperature, pH, C:N ratio, wastewater composition, organic loading rate, hydraulic retention time, and agitation [11]. A very important fact is that anaerobic bioreactors during start up are usually seeded with inoculum from other biotechnology. A wide range of anaerobic bacteria are reported inhabiting anaerobic fermenters. These belong mainly to the *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Spirochaetes*, *Chloroflexi*, *Planctomycetes* and *Synergistes* [56–61]. Archaea represents approximately 5–6% of the total microbial population in anaerobic fermenters. Most studies examining the presence of archaea in anaerobic degradation and methanogenesis mention a representative of the *Euryarchaeota* phylum. Furthermore, their results point to the fact that the most abundant microorganisms from the *Euryarchaeota* phylum in anaerobic reactors are closely related to the genus *Methanotrix* [55,60,62,63]. Representatives of the acetoclastic order *Methanosarcinales*, especially *Methanosarcinaceae* and less often *Methanosaetaceae*, are also often detected in anaerobic reactors [64–68]. Moreover, genera of the order *Methanomicrobiales*, such as *Methanoculleus* sp. and *Methanospirillum* sp., can be found during anaerobic wastewater treatment. Species isolated from anaerobic biotechnology are, for example, *Methanospirillum hungatei*, *Methanoculleus bourgensis* or *Methanolinea tarda* [69–71]. Under thermophilic conditions, the diversity of microorganisms is not so large. *Methanobacteriales* is becoming the dominant order [72], especially the genus *Methanobacterium*. This hydrogenotrophic genus includes species adapted to higher temperatures and isolation of its species from anaerobic sludges is common. Examples are *Methanobacterium subterraneum*, *Methanothermobacter thermoautotrophicus*, and *Methanothermobacter wolfei* [73–76]. Within the order *Methanosarcinales*, mesophilic species are replaced by thermophilic species [77,78].

4.1. Inhibitors and Methanogenic Activity

Anaerobic digestion is particularly susceptible to the strict control of the environmental conditions, as the process requires an interaction between fermentative and methanogenic organisms [11]. Anaerobic biodegradability and methanogenic toxicity are strongly dependent on wastewater characteristics. Toxic substances present in wastewater can interfere with the metabolism of readily biodegradable substrates [79]. There is a lot of industrial wastewaters amenable to anaerobic biotechnology. Nevertheless, these effluents may have properties that can cause process inhibition. The main influencing properties are temperature, pH, alkalinity, volatile acids concentration, redox potential, salinity, macro and micro nutrient deficiency, presence of specific cations (Ca^{2+} , Na^+ , K^+ , Mg^{2+} , NH_4^+), hydrogen, sulphide, heavy metals, bleaching and dyeing agents, antibiotics, etc. [12,45,48,51]. The temperature is influencing the enzymatic reaction rates and substrate diffusion rates. Changes in pH, alkalinity and volatile acid concentration may affect enzyme activity and increase the toxicity of

a number of compounds [11]. Ammonia inhibition on methanogenic process is poorly understood. The research shows that high ammonia concentration would result in a shift in methanogenic acetate utilization from direct acetate cleavage toward syntrophic acetate oxidation [80]. Change in the intracellular pH, increase of maintenance energy requirement, and inhibition of a specific enzyme reaction was also reported [81]. The analysis of natural ^{13}C abundances of CH_4 and CO_2 indicated that the acetoclastic methanogenesis was more sensitive than hydrogenotrophic methanogenesis [82]. The toxic effect of heavy metals is attributed to the disruption of enzyme function and structure by binding the metals with thiol and other groups on protein molecules or by replacing naturally occurring metals in enzyme prosthetic groups [81,83]. Cations Ca^{2+} , Na^+ , K^+ , Mg^{2+} were found to be antagonistic to ammonia inhibition [84]. Sulphide causes native protein denaturation through the formation of sulfide and disulfide cross-links between polypeptide chains [12]. High-salinity wastewater causes bacterial cells to dehydrate due to osmotic pressure [12]. The relative toxicity of chlorophenols has been investigated by many researchers. The results show that chlorophenols with greater hydrophobicity accumulate more efficiently in membranes, causing a greater disturbance to the membrane structure [85]. Halogenated aliphatics are strong inhibitors of methanogenesis. Brominated compounds were more inhibitory to methanogens than their chlorinated analogs [86]. Long-chain fatty acids (LCFAs) show acute toxicity by adsorption onto the cell wall/membrane and/or interference with the transport or protective function [87]. Lignin derivatives with aldehyde groups or apolar substituents are highly toxic to methanogens [81]. Effluent containing chlorine-bleaching agents, surfactants and antibiotics is problematic for anaerobic wastewater treatment due to its high toxicity for methanogenic archaea. Many of the above-described principles causing inhibition thus decrease in treatment efficiency [11,81].

4.2. Slaughterhouse Wastewater

In the slaughter processes according to Massé and Masse (2000) [88], there is 90 to 140 L of produced wastewater per slaughtered pig. However, in terms of the consumption of “clean” water, these values are up to 30% higher since there is some water loss between the inflow and outflow from the slaughterhouse. In this case, however, these are still relatively low values due to efficient water management. Slaughterhouses, including the processing of meat into meat products, use about seven to eleven times more “clean” water per slaughtered pig [88]. However, wastewater volumes vary over time and on specific slaughterhouses and their facilities [13]. It can also be said that, in general, slaughter of poultry consumes many times more water than the slaughter of pigs or cows (Mittal, 2004). Slaughterhouse effluents may contain inedible residues such as skin, offal, blood, manure, fat, undigested stomach contents, and intestinal contents, etc. The effluent is accumulated in a retention tank, which is usually located under the slaughterhouse floor. Water is used here for many processes from hairlessing to cooling to rinsing tools. The composition of slaughterhouse wastewater depends mainly on the type of animal and the processes that take place in the slaughterhouse [13]. Wastewater from poultry slaughter may contain a certain amount of blood and offal, but after the separation of these residues, the content of the organic component is relatively small [89]. According to Massé and Masse (2000) [88], wastewater from pig slaughterhouses contains high concentrations of biodegradable organic substances and is very suitable for anaerobic treatment in terms of biochemical oxygen demand (BOD) values, amounts of nutrients and micronutrients. Generally, the wastewater of slaughterhouses has a high pollution load. The high lipid and protein content of slaughterhouse wastewater makes it a challenging material for anaerobic treatment. Lipids and protein hydrolysis may cause inhibition caused by volatile fatty acids (VFAs) and ammonia accumulation [80,90]. Uneven wastewater inflow and organic load during the day can also cause problems during anaerobic digestion of these wastewater type.

Relatively permanent representatives of methanogenic archaea in anaerobic bioreactors are representatives of the genus *Methanothrix* [91–93]. In laboratory or pilot anaerobic reactors, which were filled with various substrates such as breeding wastewater, slaughterhouse wastewater or a

mixture of different residues from meat processing and livestock farming, representatives of the genus *Methanothrix* prevailed in methanogenic community in bioreactors [43,63,92,94–96]. In addition to the genus *Methanothrix*, similarities to effluents from alcoholic beverage and food processing can be found primarily in the presence of the genera *Methanosarcina*, *Methanospirillum*, *Methanobacterium* and *Methanoculleus* across these reactors [43,63,92,94–96]. In a study by Senés-Guerrera et al. (2019) [43], the percentage of *Methanoculleus* sp. increases with increasing bioreactor operation, while the proportion of *Methanothrix* sp. decreases. *Methanoculleus* sp. is also associated in this study with the period of the highest methane production in the bioreactor. These are species representatives of *M. marisnigri* and *M. horonobensis*. Representatives of *M. marisnigri* use molecular hydrogen and carbon dioxide, formate, and sometimes secondary alcohols to produce energy while producing methane. Their natural habitats are marine sediments or anaerobic bioreactors [97]. *M. horonobensis* also uses hydrogen and carbon dioxide or formate as starting materials for methane formation [98].

Studies of methanogenic communities arising in the reactor after a continuous inflow of pig and slurry effluent highlight the presence of representatives of the genera *Methanocorpusculum* and *Methanobrevibacter* [63,91,92]. These authors relate members of the *Methanogenium* genus to samples from anaerobic reactors that process wastewater from pig farming. Senés-Guerrero et al. (2019) [43] in their study samples from the laboratory bioreactor filled with biomass, which is to represent residues from beef breeding and processing. The composition of the methanogenic microbial community in this case differed from all those mentioned. The most prevalent were two genera, *Methanoregula* sp. and *Methanofollis* sp. Han et al. (2019) [92] also by studying samples from the reactors directly at the place of pig breeding, found genera representatives who were not mentioned in the previously mentioned studies, the genera *Methanimicrococcus* and *Methanosphaera* representatives.

4.3. Brewery Wastewater

The average water consumption to produce one liter of beer is five to six liters, but the consumption can increase up to eleven liters of water per liter of beer. Most of the water is consumed in beer production processes and approximately one third of the water is consumed for washing equipment and premises [99]. Brewery high-strength effluents with a temperature > 35 °C are generally well biodegradable. Brewery wastewater has a favorable COD/BOD ratio. Organic compounds present in brewery wastewater consist of sugars, soluble starch, ethanol, volatile fatty acids, etc. therefore represent a suitable substrate for anaerobic digestion [22]. However, the composition of wastewater can vary quite a bit depending on the part of the day or even the season. This water will vary significantly over time in temperature, pH, insoluble matter content, and organic and inorganic content [100]. Fluctuations in pH values in brewery wastewater can be somewhat problematic to an anaerobic treatment. Wastewater as a result of various brewing processes acquires both acidic and basic values (Table 4). For final neutralization before biological treatment processes, all these waters are accumulated in a buffer tank, where they are subsequently treated with hydrochloric acid, sodium hydroxide or phosphate buffers [101]. The use of chemical pH equalization is another step that increases the cost of wastewater treatment. An alternative to such methods may be gases generated during anaerobic treatment. Carbon dioxide has already been used to neutralize alkaline wastewater from the brewery in the study but is not yet used as an acidifying agent in buffer tanks before anaerobic treatment [22]. Most common methanogens present in the reactors processing brewery wastewater belonged to the orders *Methanobacteriales*, *Methanococcales* and *Methanomicrobiales*. *Methanothrix* sp. (formerly *Methanosaeta*), acetoclastic methanogen was found to be the dominant genus in reactor treating brewery wastewater [55,102–107]. This could be explained by the favorable concentration of acetate in brewery wastewater [27]. Other methanogens found in the reactors processing brewery wastewater were *Methanococcus* sp., *Methanosarcina* sp. and *Methanospirillum* sp. also present.

4.4. Distilleries Wastewater

Alcohol distilleries generate large volumes of high-strength wastewater. Its characteristics are highly variable and depends on the input material used and various aspects of the production process. Generally, the distillery wastewater is characterized by its low pH, dark brown color, high temperature 50–100 °C, low dissolved oxygen content, high BOD and COD content (Table 4). Distillery effluent also contains significant amount of phenols, chlorides, sulphates, nitrates, phosphates, heavy metals and organic compounds, such as polysaccharides, reduced sugars, lignin, proteins, waxes, melanoidin, etc. [23]. The dark brown color of the effluent is mainly caused by melanoidin formation during a non-enzymatic browning reaction called Maillard reaction [108]. The high protein content of distillery wastewater can lead to a high concentration of ammonia released during the anaerobic digestion process. This can lead to process inhibition, biogas yield reduction, malodor and low methane content [80]. Heavy metals present in wastewater can be stimulatory, inhibitory, or toxic for microorganisms during anaerobic digestion, depending on their concentrations [109]. Melanoidins are highly recalcitrant and have antioxidant properties which make them toxic to many microorganisms. During the thermophilic anaerobic co-digestion of Sherry-wine, distillery wastewater increase in the organic load caused a reduction of the *Methanosarcina* sp. and an increase in *Methanotherix* sp., while, in the case of thermophilic reactor, it favored the increasing of *Methanothermobacter* sp. and *Methanoculleus* sp. [110]. In thermophilic anaerobic hybrid reactors treating energy cane stillage, *Methanothermobacter* was abundant. Methanogenic genera correlated to the stabilization and perturbation stages were methanogenic *Methanothermobacter* and *Methanosarcina* [111]. Most dominant in microbial community in anaerobic digesters processing wheat-based fuel ethanol waste streams were genera *Methanothermobacter marburgensis* and *Methanosarcina barkeri* [112].

4.5. Pulp and Paper Industry Wastewater

The pulp and paper industry supplies an essential product—paper—to over 7 billion people worldwide. The pulp and paper industry generates specific wastewater that contains compounds from pulp production and chemicals added during the processing. The pulp and paper industry effluent contains high values of BOD, COD and hundreds of chlorinated chemicals termed as absorbable organic halides (AOX). Cellulose, hemicellulose and lignin are the main components of wood and therefore have a decisive influence on the composition of wastewater from this production process. Lignin is degraded to a variety of high-, medium-, and low-molecular-weight chlorinated and non-chlorinated fractions. Lignin and its derivatives are highly toxic compounds responsible for the high BOD and COD values of effluents as well as the dark brown color of pulp effluents formed during pulping [113]. Lignocellulosic materials present in this type of wastewater can slow down the hydrolysis step of the anaerobic digestion process. The lignin is converted to alkaline-soluble compounds by treatment with bleaching agents (chlorine, chlorine dioxide and hypochlorite), and then washed out with sodium hydroxide. During this process, a number of highly toxic compounds, such as chlorinated lignosulfonic acids, chlorinated resin acids, chlorinated phenols, guaiacols, catechols, benzaldehydes, vanillins, syringo-vanillins, and chloropropioguaiacols, are formed [114]. Bleaching agents can cause anaerobic process instability due to the presence of toxic substances that affect methanogens. Bleachery effluents mainly contain degradation products of lignin. Smaller amount of polysaccharide and wood-extractive degradation products are generated. Methanol and various hemicelluloses are dominant organic compounds (over 90%) in bleaching liquors. A vast variety of organochlorines are created. Non-chlorinated compounds present in wastewater are resin acids, fatty acids, sterols, diterpene alcohols, and tannins [115]. The toxicity of tannins to methanogens, which depends on the degree of polymerization, has been reported. Effluent dissolved compounds from alkaline peroxide bleaching (5–20 kg/t pulp) consist of carbohydrates (60%), acetic acid, formic acid and methanol (40%) [116]. Very important is the anaerobic reactor configuration. Operational and environmental conditions such as the reactors' operating temperature and pH play a crucial role in relation to HRT. Microbial communities of the sludge samples from anaerobic bioreactors operated with wastewater

from pulp and paper industry were investigated in Thailand [117]. Predominant methanogenic archaea are in this process hydrogenotrophic methanogens *Methanobacterium* sp. and acetoclastic *Methanothrix* sp. These archaea in anaerobic bioreactors coexist with sulfate-reducing bacteria (SRB). For SRB, sulfate or sulfite in a bioreactor environment is very important. They use those compounds in the anaerobic oxidation process for the formation of hydrogen sulfide. Hydrogen sulfide is a gas that can reduce the quality and quantity of produced biogas. In paper industry sulfates represent the principal wastewater component. Jantharadej [117] and Roest [118] have identified uncultured *Desulfobulbus* sp., *Syntrophobacter* sp. and *Desulfovibrio* sp. In bioreactor sludge samples. SRB are a polyphyletic group of bacteria which are physiologically versatile. They are playing not only the role of sulfate reducers, but they have also suitable enzymes for the decomposition of propionate and butyrate as the key processes during organic matter degradation and methane production.

SRB can probably combine the properties of syntrophic growth and sulfate reduction. These bacteria have butyrate oxidizing capabilities in syntrophy with methanogenic archaea [118].

We can conclude that the wastewater originating from the pulp and paper industry is characterized by a typical microbial composition. The members of the family *Cellulomonadaceae* are irreplaceable in hydrolyzing cellulose and carbohydrates. A very important role also involves propionate producers, for example, members of the genus *Propionibacterium*. On the metabolic products of the previous groups follow short-chain fatty acid oxidizers and sulfate reducers which are very common in paper industry wastewaters. Together with methanogenic archaea members, *Methanobacterium* sp. and *Methanothrix* sp. represent an efficient consortium of microorganisms with the aim of high methane production.

4.6. Food and Drink Processing (Dairy Products, Vegetable Oil, Fruits and Vegetables, Canneries, Juice Making, etc.) Wastewater

4.6.1. Dairy Industry Wastewater

The dairy industry generates approximately 0.5–2 m³ of effluent per m³ of processed milk [119]. In the dairy industry, wastewater quantity and quality are very problematic because of great fluctuation. Typically, the dairy industry wastewater has an elevated temperature (30–40 °C) and large variations in pH, total suspended solids (TSS), BOD, COD, total nitrogen (TN), total phosphorus (TP) and fat, oil and grease. Wastewater with the highest concentration of pollution is generated in terms of daily production at the end of the day during the washing and sanitizing of the equipment and treatment facilities. In terms of the seasonal production of milk and dairy products, wastewater changes mainly in the volumes and concentrations of organic pollution [20,21]. Major constituents of dairy wastewater are lactose, soluble proteins, lipids, mineral salts and detergents while low concentrations of some heavy metals have been reported [20]. As one of the main components in dairy wastewater, we can find milk fat (4–22% of DM), proteins and hydrocarbons in various forms, which are associated with milk [20]. Lactose is also an important dairy industry wastewater component. Lactose is converted through the Emden–Meyerhof–Parnas pathway to an intermediate product (acetate, lactate, ethanol, and formate, propionate and valerate), of which acetate represents 70% [120]. Proteins are hydrolyzed by extracellular proteases into peptides. Peptides are metabolized by peptidases to amino acids, which are degraded to end products (volatile acids, ammonia, H₂ and CO₂, and sulfur-containing compounds) [121]. Ammonia, known for its toxicity, can be a major problem during the anaerobic fermentation of dairy wastewater if generated in high concentrations [80]. Dairy wastewater is characterized by very low alkalinity; thus, volatile fatty acids could rapidly accumulate within the anaerobic digester and cause a drop in the pH. Additionally, lack of sufficient buffering capacity could lead to failure in bioreactor operation, and low pH may inhibit methanogenic activity [41]. The high salinity of dairy wastewaters is associated with the addition of NaCl, KCl and calcium salts during production process. Sodium toxicity is a common problem which inhibits the methane-producing consortia during the anaerobic treatment of dairy wastewater [122]. Dairy wastewater components, which are relatively readily biodegradable, cause the BOD of wastewater from the dairy industry to be quite high and are therefore also a suitable substrate for biological treatment. However, wastewater

may contain other components, such as cleaning agents [19] causing inhibition. Most of the used chemicals (NaOH, HCl, HNO₃ and H₃PO₄) are very toxic to microorganisms. The cleaning solutions are usually high in temperature 60–80 °C. Strong oxidants or bleaches (NaOCl and ClO₂) are applied for sanitizing installations. NaOH, the main cleaning agent, causes the pH of dairy wastewater to often be around highly basic values [20]. *Methanothrix* sp. was found to be the predominant active methanogen throughout the trial at low-temperature anaerobic treatment of dairy wastewater [123]. During synthetic wastewater treatment, most abundant microbial taxa in the reactor belonged to the *Methanobacteria* and *Methanomicrobia* classes, which solely consisted of the hydrogenotrophic genus *Methanobacterium* and the acetoclastic genus *Methanothrix* [124]. *Methanomicrobium*, *Methanobrevibacter*, *Methanocalculus*, *Methanosarcina*, *Methanothrix*, *Methanoculleus* and *Methanofollis* genera were reported in reactor processing wastewater that originated from the dairy industry [120].

4.6.2. Vegetable Oil Industry Wastewater

Palm, soybean, olive, cottonseed, and sunflower oils are essential in human diet over the world. In 2019, the annual production of vegetable oils was 203.91 million metric tons. The most utilized vegetable oil is palm oil with 37% of the vegetable oil production [125]. The production of edible oil is associated with the generation of various wastes. Outside of organic solid wastes (seeds, husks) and inorganic residues a big amount of wastewater is generated. During the palm oil production, 0.5–0.75 tons of palm oil mill effluent per one ton of fresh fruits is generated on average [126]. The olive oil mill wastewater represents 8 million tons generated annually worldwide [127]. On average, 1.2–1.8 m³ wastewater is generated per one ton of olives because of the oil extraction process. Wastewater generated in the vegetable oil industry is typical high in COD, BOD, TDS and TSS content. It is also common for this wastewater to be in high phosphate and sulfate content. Important is a high concentration of lipids, more than 100 mg/L, and it is acidic in nature with a pH of approximately 4.5. Lipid-containing material is rich in long-chain fatty acids (LCFAs), which are degraded anaerobically via the β -oxidation pathway to acetate and H₂. Some publications reported an LCFA toxicity effect on methanogenic *Archaea*, caused by a not well described mechanism, probably by the surfactant effect of the LCFAs [128]. The amounts of cellulose, hemicellulose and lignin in palm oil mill effluent (POME) are 11%, 7% and 42%, respectively [129]. In the oil industry, fluidized bed reactors, upflow anaerobic sludge blanket reactors and continuous stirred-tank reactors are widely used for the treatment of wastewater. The very-cost-effective anaerobic treatment method represents anaerobic ponds and lagoons [126]. Disadvantage of ponds and lagoons treatment is the high retention time of wastewater. Comparing aerobic and anaerobic treatment methods for oil wastewater treatment brings clear results. The anaerobic technology shows a higher removal efficiency and 20 times lower sludge formation [130]. Palm oil mill effluent can be very efficiently converted to methane, with a maximum methane yield of 33.2 CH₄/t POME [129]. In literature, we can find only little information about microorganisms involved in the POME methanization process. Miller (2015) [131] described a relatively high abundance of hydrogenotrophic methanogens from the *Methanobacteriales* order (*Methanosphaera* sp.). MacIlroy et al. (2017) [132] mentioned the presence of hydrogenotrophic genera *Methanobrevibacter* and *Methanothermobacter* in mesophilic temperature and acetoclastic *Methanothrix* sp. and *Methanosarcina* sp. present only at 50 °C process temperature.

4.6.3. Fruit and Vegetable Processing Industry Wastewater

Water consumption in fruit and vegetable processing industries is high (5–50 m³/t), and most of the process water became wastewater. Fruit and vegetable process plant wastewater typically contains discarded fruits and vegetables, soil particles, fruit and veg pulp and fibers, cleaning agents, blanching agents, salts and residues of pesticides. Wastewater streams are characterized by lower COD and BOD loads compared to other food sectors, including meat processing industries and olive oil processing plants [29]. Fruit and vegetable waste contains low cellulose content, and the C:N ratio of the wastes may accelerate ammonia release, resulting in the inhibition of methanogenesis in anaerobic reactors. The low

pH of these wastewaters can cause inhibition of anaerobic digestion when dilution of wastewater is not applied. The accumulation of VFAs was also reported as a possible reason for process inhibition. Predominant methanogens genera during the anaerobic co-digestion of fruit and vegetable waste were *Methanoculleus*, *Methanothrix* and *Methanosarcina* [133]. During the operation of a lab-scale UASB reactor treating fruit and vegetable waste, the *Methanothrix* spp. was dominant. After acclimation, the percentages of hydrogenotrophic methanogens including *Methanolinea*, *Methanospirillum* and *Methanobacterium* genera obviously increased; however, acetoclastic methanogen *Methanothrix* sp. and methylotrophic methanogens *Methanomethylovorans* sp. and *Methanomassiliicoccus* sp. decreased in the study [134]. During the digestion of the syrup wastewater produced while canning fruit, *Methanothrix* spp. was dominant when the methane gas vigorously evolved. Other methanogens found during anaerobic digestion were *Methanobacterium* sp. and *Methanosarcina* sp. [135].

Large volumes of wastewater are also produced in citrus-processing industries. About 120 million tons of citrus fruits are produced annually in the world and 20% of citrus fruits are industrially processed [136]. There are two types of residues emerging during technological processing, peel with 50% of wet fruit mass and wastewater. The most citrus-processing wastewater arises during fruit washing and device cleaning. The physicochemical characteristics of this type of wastewater determines many constraints for its disposal. Wastewater is typically low pH, low concentration of nutrients with a high content of organic compounds and essential oils. Commonly used wastewater treatment processes include aerated filter systems, aerobic granular sludge sequencing batch reactors or microfiltration membranes [137]. Anaerobic wastewater treatment in lagoons represents an economically acceptable solution. Wastewater is treated for a long time (months) in large volumes in lagoons. During this time, adaptation of degrading microorganisms occurs not only to high substance load but also to low pH values typical for this type of wastewater. Anaerobic lagoons do not require energy and show 90% removal capacity of organic loading. Anaerobic digestion of citrus-processing wastewater is not practiced due to the inhibitory effect of inhibiting compounds as limonen [138].

4.7. Chemical Industry Wastewater

The chemical industry produces a wide range of organic chemicals. The number of organic chemicals produced is increasing rapidly and the toxicity limits for the environment is far from being known for all the chemicals manufactured [34]. Chemical industrial wastewaters usually contain organic and inorganic contaminants in varying concentrations. Many materials used in the chemical industry are toxic, mutagenic, carcinogenic or simply almost non-biodegradable aromatic compounds [139]. Thus, it is very difficult to summarize general facts about the composition of chemical industry wastewater. Each company produces wastewaters possessing different types of contaminants under various operational parameters. The contamination of chemically polluted wastewaters depends mostly on the company production lines and formulations [34].

Phenol derivatives are often found in the wastewaters from industry. Phenol is known as biocide and disinfectant, so it is often associated with being inhibitory to microorganisms. Fang et al. conducted an experiment to prove that phenol can be effectively degraded (97%) at UASB reactor. The results of archaeal community analysis showed the presence of two hydrogenotrophic representants, *Methanospirillum* sp. and *Methanobrevibacter* sp., and one acetoclastic representant, *Methanotrix* sp. [140]. Muñoz et al. [141] found that *Methanosarcinales* (46.86%) was the dominant archaea order in the UASB and *Methanobacteriales* (26.22%) and *Methanosarcinales* (23.99%) orders in the anaerobic membrane bioreactors (AnMBR). At the end of the experiment, *Methanotrix* sp. dominated in AnMBR. During glycerol containing wastewater anaerobic treatment, two main groups, *Methanobacterium* sp. and *Methanosarcina* sp., have been found [142]. Another study [143] reported that *Methanosarcina*, *Methanosarcinales*, and *Methanobrevibacter* were dominant genera during residual glycerol anaerobic digestion. In sulfate-rich chemical wastewater, the largest group of methanogens was *Methanotrix* sp., which accounted for 42.5% in the library. Among them, the species closely related to *Methanotrix concilii* (similarity 99%, relative abundance 40.0%) and *Methanotrix harundinacea* (similarity 99%, relative abundance

2.5%) used acetate for growth and methane production as a carbon source. *Methanobacterium* sp. and *Methanoregula* sp. show the second and third largest relative abundance of the genera, respectively [144]. During acetone–butanol–ethanol wastewater treatment, *Methanocorpusculum* sp. and *Methanoculleus* sp. were found as dominant methanogens [145]. The ability of *Methanotrix* sp. for the degradation of acetone in enrichment culture was proved in this study [146]. During anaerobic solvent degradation in the low-temperature occurrence, *Methanotrix* sp. and *Methanosarcina* sp. were confirmed as the most abundant species [147]. In the UASB reactor for iso-propyl alcohol (2-propanol) wastewater treatment, *Methanospirillum* sp. (61%) was dominated. Clones from the genera *Methanolinea* sp. (36%) and *Methanomicrobium* sp. (3%) were also detected [148].

5. Conclusions

Anaerobic wastewater treatment is highlighted in many sources as a very efficient and ecological alternative to aerobic technologies. Today, this technology is already used in many industries, especially in the food industry. Technically, it is a relatively simple technology. However, anaerobic sludge, which is essential for anaerobic decomposition, remains a little-explored and complex part of the technology. Anaerobic digestion is the process in which macromolecules, such as polysaccharides, lipids, and proteins, are broken down by an anaerobic community of microorganisms into simple components from which the methanogenic archaea forms methane as a component of biogas. Representatives of the methanogenic communities of the *Archaea* domain then belong to the *Euryarchaeota* phylum (Table 7). Based on the literature review, it is possible to conclude at least that a relatively permanent part of the community of methanogenic archaea in anaerobic sludge, with a few exceptions, are genera *Methanotrix* and *Methanosarcina*, acetoclastic representatives of methanogens. The diversity of methanogens is reflected in the different growth conditions, temperature, pH and osmolarity. Most of the methanogens grow optimally around neutral pH. Higher wastewater pH can be tolerated by halotolerant strains of methanogens. For example, in dairy wastewater, *Methanocalculus* sp. was reported as part of the methanogenic community. On the other hand, moderately acidic environments can be inhabited by methanogens as, for example, *Methanoregula* sp. *Methanosarcina* sp. and *Methanococcus* sp. Salt concentration may also be an important physiological parameter for methanogens. In high-salinity brewery, paper and dairy wastewater representants, *Methanobacterium* sp., *Methanosarcina* sp. and *Methanotrix* sp. can be found. We can prove the theories that *Methanotrix* sp. plays a key role in granulation and in the core of the granules' function as nucleation centers that initiate granule development. From Table 7, it is clear that hydrogenotrophic methanogens diversity richness is higher in most anaerobic bioreactors.

Table 7. Methanogens prevailed in anaerobic bioreactors treating industrial wastewater.

Genera of Methanogens	Methanogenic Metabolic Pathway	Industry							
		Slaughterhouse	Brewery	Distillery	Paper	Dairy	Vegetable Oil	Fruit and Vegetable	Chemical
<i>Methanothrix</i>	acetoclastic	•	•	•	•	•	•	•	•
	acetoclastic								
<i>Methanosarcina</i>	hydrogenotrophic	•	•	•		•	•	•	•
	methylotrophic								
<i>Methanomicrobium</i>	hydrogenotrophic					•			•
<i>Methanobrevibacter</i>	hydrogenotrophic	•				•			•
<i>Methanocalculus</i>	hydrogenotrophic					•			
<i>Methanoculleus</i>	hydrogenotrophic	•		•		•		•	•
<i>Methanofollis</i>	hydrogenotrophic	•				•			
<i>Methanobacterium</i>	hydrogenotrophic	•			•	•		•	•
<i>Methanoregula</i>	hydrogenotrophic	•							•
<i>Methanococcus</i>	hydrogenotrophic		•						
<i>Methanospirillum</i>	hydrogenotrophic	•	•					•	•
<i>Methanocorpusculum</i>	hydrogenotrophic	•							•
<i>Methanogenium</i>	hydrogenotrophic	•							
<i>Methanimicrococcus</i>	methylotrophic	•							
<i>Methanosphaera</i>	hydrogenotrophic	•					•		
<i>Methanothermobacter</i>	hydrogenotrophic			•					
<i>Methanolinea</i>	hydrogenotrophic							•	•
<i>Methanomethylovorans</i>	methylotrophic							•	
<i>Methanomassiliicoccus</i>	methanol + H ₂							•	

• means methanogen presence in anaerobic bioreactor.

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