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## Biogas - Turning Waste into Clean Energy

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### Abstract

Expertise in biogas production using anaerobic digestion (AD) can offer many benefits in addition to being an alternative source of energy. This process involves plant digesters and provides an alternative destination for biomass that would eventually go unutilized and deposited in a trash heap. The application of the appropriate plant digester technology can generate energy, and the gas produced can be used for many purposes, such as water and space heating, lighting, and grain drying. In this context, agro residues are one of the most abundant energy sources available world wide. Nevertheless, the bioconversion of organic matter to biogas is a complex process of AD that involves many reactions among several microorganisms living in a stable community. Microorganisms from many diverse genera of obligate anaerobes and facultative anaerobes constitute these steps, and four groups are recognized to be the most frequent in biogas production plants. These groups, in order of substrate hydrolysis, are hydrolytic, acidogenic, and acetogenic bacteria, followed by the core group, the methanogenic archaea. All together, they compose the operation of a systematized activity with synergistic effects that ensure the stability of the process.

**Keywords:** anaerobic digestion, methanogens, methane, hydrogen, waste utilization

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## 1. Introduction

Increased efforts to reduce the utilization of petroleum have encouraged the development of new technologies for the utilization of alternative energy matrices for the production of different compounds such as novel fuels. Among available biofuels, biogas has been produced for over approximately 2000–3000 years for sanitation purposes [1]; however, the first documented

generation of biogas comes from a carefully designed installation from England in 1895. The interest in its usage grew during World War II when France and Germany started to build biogas facilities and used them to fuel vehicles and tractors. After the war, interest in biogas waned, but recovered during the oil crisis of 1973 with improved technology. Nowadays, Germany is by far the world leader in biogas generation.

Biogas is generated from anaerobic digestion (AD) in a bioreactor (also called a digester unit). Its production can be done through a batch or continuous process, in one-, two-, or multiphased steps, and it utilizes mainly organic matter from waste as the substrate. It is considered a carbon-neutral biofuel since it uses carbon dioxide that was recently taken up by plants from the atmosphere and is able to return it through the fermentation of waste residues [2]. This biofuel also protects the environment from pathogens by reducing the waste that would rot in the open air, which would have increased the possibility of attracting disease-carrying vectors. Moreover, it considerably reduces air and water pollution, helps the conservation of forests, and replaces inorganic fertilizer with its digested residues [3]. According to the European Union, biogas has the potential to produce 25% of all clean energy. It can be used to produce electricity, heat, and vehicle fuel, thus substituting conventional sources of energy that produce greenhouse gases.

In recent years, biogas production has increased greatly. This can be evidenced by the rapid construction of biogas plants, which have been built exclusively in Europe. The world's biogas production in 2012 reached 17.2 ktOE/year (the equivalent of millions of tonnes of oil per year) and Europe alone produced 60% (about 10.5 ktOE/year) of this amount. In 2013, European Union production grew to 13.4 ktOE/year, a 27.6% increase, and it is expected to reach 33.0 ktOE/year by 2022. Several European countries face enormous issues related to the excess of organic waste production from industry, agriculture, and households. AD can also contribute to waste minimization by eliminating the accumulation of harmful and persistent wastes while simultaneously lowering prices for waste disposal.

Taking into account the importance of biogas production, this chapter will discuss, in general, the production of this clean energy source. Therefore, the following topics will be addressed: (1) biogas composition; (2) types of substrate used for their production; (3) overview of biogas production; (4) physical and chemical AD; and (5) anaerobic bioreactors. Specifically, greater emphasis will be given to important aspects of fermentation, such as: (1) the microorganisms and the trophic groups involved in each step (hydrolytic bacteria, acidogenic bacteria, acetogenic bacteria, methanogenic groups); (2) factors affecting biogas production efficiency (temperature, pH and chemical aspects of biomass); (3) the biochemical substrates by the population of microorganisms.

The bioreactor types and their strategies for biogas production will be discussed superficially. However, greater emphasis will be given to important aspects of fermentation, such as: (1) the microorganisms involved, and the trophic groups involved in each step (hydrolytic bacteria, acidogenic bacteria, acetogenic bacteria, and methanogenic groups); (2) factors affecting the efficient production of biogas (temperature, pH and chemical aspects of biomass); (3) the biochemical changes in substrates by the microorganism population.

## 2. Biogas composition

The typical composition of biogas is methane ( $\text{CH}_4$ ), carbon dioxide ( $\text{CO}_2$ ), and sulfuric elements ( $\text{H}_2\text{S}$ ). The approximate percentage of biogas components is shown in the **Table 1** [4].

Biogas composition	Typical analysis (%/volume)
Methane	55–65
Carbon dioxide ( $\text{CO}_2$ )	35–45
Nitrogen ( $\text{N}_2$ )	0–3
Hydrogen ( $\text{H}_2$ )	0–1
Hydrogen sulfide ( $\text{H}_2\text{S}$ )	0–1
Oxygen ( $\text{O}_2$ )	0–2
Ammonia ( $\text{NH}_3$ )	0–1

**Table 1.** Approximate percentage of biogas components [4].

The main cause of the high variation in percentages of biogas composition (**Table 1**) is due to the substrate utilized. The fact that methane is present at high concentration makes biogas a very attractive source of energy considering that methane has a heating value of  $8500 \text{ kcal/m}^3$  and that  $\text{CO}_2$  has no energy associated with it. The heating value of biogas is on average  $5000\text{--}7000 \text{ kcal/m}^3$ , approaching nearly  $12,000 \text{ kcal/m}^3$  when in a high degree of purity (65%  $\text{CH}_4$ ). Comparatively, a cubic meter of biogas has the same calorific power as 0.613 L of gasoline, 0.579 L of kerosene, 0.553 L of diesel, 0.454 L of cooking gas, 1.536 kg of wood, and 0.790 L of ethanol and produces the equivalent power of 1.4208 kW.

Typically, 0.2–3% of biogas is composed of gases that enter the digester with air included in the substrate ( $\text{N}_2$  and  $\text{O}_2$ ). Among these, nitrogen and  $\text{CO}_2$  (produced during the digestion process) are included in the inert gases that compose the total biogas mix. On the other hand, the remaining  $\text{NH}_3$ ,  $\text{O}_2$ , and  $\text{H}_2\text{S}$  gases are unwanted gases due to their toxicity to strict anaerobes that are essential for the process. Both  $\text{O}_2$  and  $\text{H}_2\text{S}$  can be removed from biogas through chemical processes such as iron based processes, for example, with the addition of iron chloride, while  $\text{NH}_3$  can be degassed through an  $\text{H}_2\text{SO}_4$  absorber.

Another component, hydrogen sulfide ( $\text{H}_2\text{S}$ ), is normally present in biogas as a by-product from anaerobic digestion. It is considered a major cause of corrosion of metal parts and degradation of engine oil, and during the fermentation process, it can precipitate metal elements. This gas is prevenient to the degradation of sulfur-containing proteins (i.e., cysteine and methionine), and besides being prevenient to normal metabolism of fermentation organisms, it has to be removed from the biogas before utilization.

### 3. Types of substrates

The most utilized residues for biogas production are found in animal manure, agriculture residues, and general organic wastes from food (both vegetable and animal in origin), organic fractions of municipal waste and from catering, sewage sludge and residues from crops dedicated to energy (i.e., biofuels), such as sugar cane and sorghum. These can be classified into various criteria: its origin, organic content, methane yield and dry matter content (**Table 2**). These substrates usually have a high content of sugar, starch, proteins, or fats, which are decomposed through AD. **Table 2** shows several substrates and their classifications according to organic content, carbon:nitrogen ratio, percentage of dry matter, percentage of volatile solids in dry matter, and its biogas yield [5]. It is noticeable how the utilization of different biomasses has a consequence in the biogas yield, for example, it can vary from 0.15 m<sup>3</sup>/kg VS (volatile solids) (utilizing straw) to 0.9 m<sup>3</sup>/kg VS. When the utilized substrate is concentrated whey, a

Biomass type	Organic content	C:N ratio	DM <sup>a</sup> (%)	VS <sup>b</sup> (% of DM)	Biogas (yield m <sup>3</sup> /kg VS)
Pig slurry	Carbohydrates, proteins, lipids	3–10	3–8	70–80	0.25–0.50
Cattle slurry	Carbohydrates, proteins, lipids	6–20	5–12	80	0.20–0.30
Poultry slurry	Carbohydrates, proteins, lipids	3–10	10–30	80	0.35–0.60
Stomach/intestine content	Carbohydrates, proteins, lipids	3–5	15	80	0.40–0.68
Whey	75–80% lactose, 20–25% protein	NR	8–12	90	0.35–0.80
Concentrated whey	75–80% lactose, 20–25% protein	NR	20–25	90	0.80–0.90
Flotation sludge	65–70% proteins, 30–35% lipids	NR	NR	NR	NR
Fermented slops	Carbohydrates	4–10	1–5	80–95	0.35–0.78
Straw	Carbohydrates, lipids	80–100	70–90	80–90	0.15–0.35
Garden wastes	NR	100–150	60–70	90	0.20–0.50
Grass	NR	12–25	20–25	90	0.55
Grass silage	NR	10–25	15–25	90	0.56
Fruit wastes	NR	35	15–20	75	0.25–0.50
Fish oil	30–50% lipids	NR	NR	NR	NR
Soya oil/margarine	90% vegetable oil	NR	NR	NR	NR
Alcohol	40% alcohol	NR	NR	NR	NR
Food remains	NR	NR	10	80	0.50–0.60

<sup>a</sup>Dry matter. <sup>b</sup>Volatile solids. NR, not reported.

**Table 2.** Substrates commonly utilized for biogas production, its composition, and average biogas yield [5].

500% increase in growth can be observed (**Table 2**). Generally, the C:N ratio also affects the production of biogas. As can be seen in **Table 2**, low C:N ratios (between 3 and 20) produce a yield ranging between 0.25 and 0.78 m<sup>3</sup>/kg VS. Higher C:N ratios (above 20, reaching up to 150) do not produce greater yields, since the greater yield obtained is 0.56 m<sup>3</sup>/kg VS, approximately 30% lower than that obtained at lower C:N ratios.

In spite of the numerous advantages of utilizing biogas digesters, there are still challenges that need to be overcome in order to maximize fuel production. Methanogenic archaea, microorganisms that produce methane, have specific requirements such as temperature and pH, and they must be maintained within specific ranges for optimal production, which increases the production cost of biogas [6]. Another challenge is hydraulic retention time (HRT), which is the normal time that the input substrate spends in the digester before it is removed. At tropical temperatures, the HRT is 30–50 days, although in colder atmospheres, it may go up to 100 days without heating, which requires a bigger digester volume and raises costs. While digesters can save energy at small-scale production on farms, finding the right economic balance for large-scale production is yet another challenge.

## 4. Overview of biogas production

Biogas production is an established process in which there is little information available on the microorganisms involved using different wastes. Thus, an understanding of the microorganisms' activity and the factors that can influence biogas composition are crucial in order to maximize fermentation performance and reduce process costs. Therefore, in order to discover which microorganisms are involved in anaerobic digestion, sequencing of 16S rRNA and metagenomics [7] has been performed, as well as the analysis of the methyl-coenzyme M reductase encoding gene, as this is a marker for identification of archaea that are specifically methanogenic [8]. DNA isolated from different bioreactors using different substrates demonstrated a very direct link between reactor type and taxonomic groups. For example, in a stirred digester fed with fodder beet silage, mainly Bacilli, Clostridiales, Deltaproteobacteria, and Bacteroidetes have been found [9], while the microbial population of a thermophilic digester described in another study was particularly rich in Clostridia [10]. Another important relationship is the microorganism present according to the physical location of the digester [11]. The results of several studies inferred that, in the first and second phases of AD, at least 58 species of 18 genera are involved, which categorize biogas production as mixed fermentation.

### 4.1. Microorganisms and the biochemistry of AD

The production of biogas is performed by a microbial consortium through four main reactions: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, where organisms from the bacteria and archaea domains are involved in consortia that lead to substrate conversion into CH<sub>4</sub> and CO<sub>2</sub> among other gases. The microorganism types involved and an overview of the substrate process are illustrated in **Figure 1**.

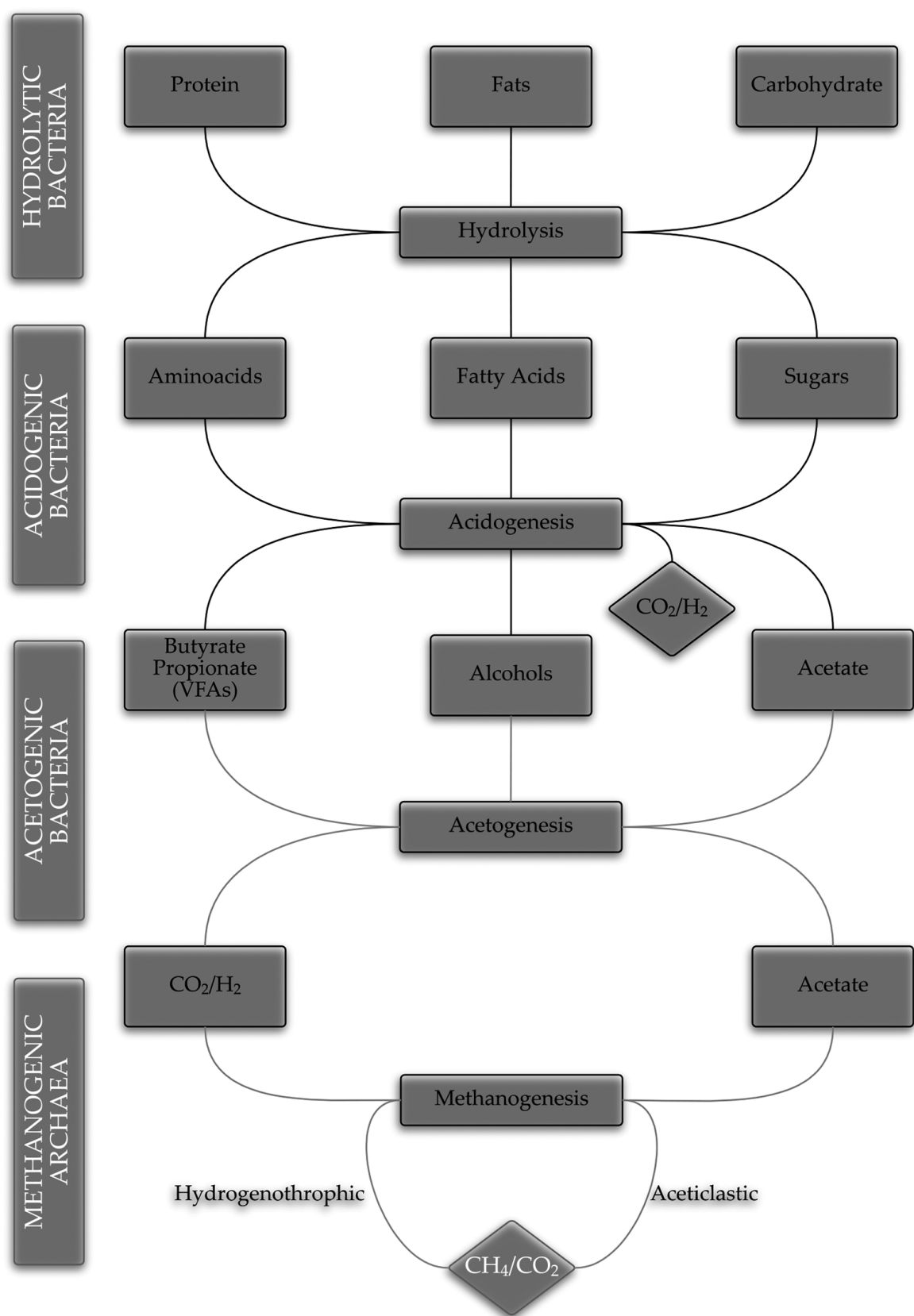


Figure 1. Microorganisms involved in each catabolic step during biogas biosynthesis.



#### 4.1.1. *Hydrolytic bacteria*

Anaerobic digestion starts with the polymer hydrolysis of fats, proteins, and carbohydrates into monomers that are suitable for further digestion. Hydrolytic bacteria, which can be either facultative or strict anaerobes, are capable of hydrolyzing the bonds of these compounds, converting them into oligomers, monomers, amino acids, and unsaturated fats. For example, cellulose  $[(C_6H_{12}O_6)_n]$ , an insoluble substrate commonly found in sludge, is hydrolyzed by bacteria from the genus *Cellulomonas*, resulting in glucose monomers. The hydrolysis of polymers that are difficult to decompose restrains the rate of waste processing, and just half of these compounds experience hydrolysis in a one-stage digester. In some cases, pretreatment involving an aerobic step can be added. The concept of aerobic treatment consists in the knowledge that some aerobic microorganisms can produce hydrolytic enzymes that are able to generate monomers from the polymers present in the biomass. Moreover, inhibitory macromolecules such as lignin may also be transformed, resulting in a less toxic substrate to the microorganisms that start the AD process [12].

Anaerobic digesters that utilize substrates derived from wastewater treatment from industry, such as dairy and agro industries, are usually composed of soluble organic compounds and therefore do not experience this kind of hydrolysis. However, different sugars such as sucrose and lactose must be hydrolyzed despite being soluble, since they are larger than most cells can absorb [13].

#### 4.1.2. *Acidogenic bacteria*

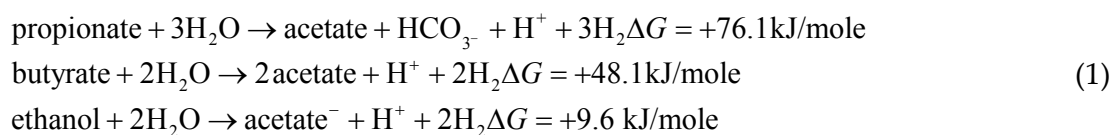
In regard to the second reaction stage, acidogenic bacteria will then convert these molecules into volatile fatty acids (VFAs) with high carbon numbers such as butyrate, propionate, and alcohols in addition to  $CO_2$ ,  $H_2$ , and acetate [14]. These biochemical steps depend on various factors, like pH, enzyme production by bacteria, diffusion, and adsorption of enzymes by the biomass undergoing the process of digestion. This is executed by microorganisms from the group of anaerobic bacteria of genera such as *Streptococcus* and *Enterobacteria*.

However, VFAs produced during this stage may negatively affect the AD process depending on its concentration in the bioreactor. When unstable, the AD process accumulates VFAs inside the reactor, which results in a drop of pH-value and consequently a decrease in methane yield. This is explained by the low tolerance of methanogenic archaea in an acidic environment. It is demonstrated that different digesters can react differently in response to the same amount of VFA, where, in one digester, the concentration may be optimal and, in another, it is a considerable inhibitor to methane production. One conceivable explanation is the microorganism population, which varies from digester to digester. It can also be explained by the buffering capacity of the substrate.

#### 4.1.3. *Acetogenic bacteria*

For the third reaction stage, acetogenic bacteria convert VFAs into acetate. Acetogenic bacteria are obligate proton-reducing bacteria (OPR) and are known for the production of  $H_2$  during acetate production. Some VFA conversions are displayed below in Eq. (1):





In accordance with the examples above, it is important to note that all of them require energy input. However, in the presence of low hydrogen concentrations provided by the digester, the reaction moves to the product side to maintain equilibrium. To this end, they only live in coexistence with a  $\text{H}_2$  utilizing species, which are the methanogenic archaea. A genus such as *Desulfovibrio* oxidizes alcohols and organic acids into acetate and transfers the electrons released to sulfate. Genera such as *Aminobacterium* and *Acidaminococcus* ferment amino acids, trans-aconitate and citrate into acetate,  $\text{CO}_2$  and  $\text{H}_2$ . Sulfate-reducer organisms such as the acetogenic *Desulfovibrio*, which oxidizes organic acids and alcohols to acetate and transfers the released electrons to sulfate resulting in a higher energy yield than fermentation, are deeply involved in compound decomposition by AD. These bacteria form cultures from obligated and facultative anaerobes to ferment available substrates such as lactate and alcohol from the acidogenic step.

#### 4.1.4. Methanogenic group

The last phase of anaerobic digestion is catalyzed by a group of microorganisms from the archaea group. This group is subdivided into two groups: a hydrogenotrophic methanogenic group and acetoclastic methanogenic group. The first group utilizes the  $\text{H}_2$  produced by the OPR group. Their affinity to uptake hydrogen is on the order of parts per million, making them very efficient in maintaining the substrate with a very low hydrogen partial pressure. The acetoclastic methanogenic group consists of only two genera: *Methanosarcina* and *Methanotrix*. These microorganisms can produce methane from acetic acid, and approximately 70% of all methane produced in biogas reactors originates from this conversion. The reactions of the processes are displayed below (Eqs. (2) and (3)).



Methanogenic archaea have, in their metabolism, the enzyme methyl-CoM reductase. This hexamer is a large complex composed by two copies of three different subunits ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) containing a unique coenzyme, the nickel porphyrinoid factor  $\text{F}_{430}$  and with activity deep inside the complex for protection from the surrounding water. This complex catalyzes the release of the  $\text{CH}_4$  from methyl-CoM [15]. The  $\text{F}_{430}$  ring needs a nickel atom that is stabilized in the reactive state, which is an important property of this enzyme because the substrate methyl-coenzyme M is rather inert, which makes the reaction easier.

Acetoclastic archaea are well known for their slow doubling time (1–12 days in thermophilic conditions) because of their relative inefficiency in taking up acetate, but on the other hand, hydrogenotrophic methanogenic bacteria are extremely productive and have moderately quick doubling times (0.5–2 days in thermophilic conditions) [16].

## 5. Physical and chemical AD parameters

The growth and metabolism of anaerobic microorganisms are essentially impacted by physical and chemical conditions such as temperature, pH value, nutrient supply, mixing intensity, and the additional presence of inhibitors.

### 5.1. Temperature

A large portion of reactor cost comes from the energy spent to maintain its temperature stable. Thus, an optimum temperature setting is the most critical factor in temperate countries since more energy is needed to maintain the temperature of AD and consequently methane production. Temperature parameters for AD can take place at different levels: cryophilic (below 25 °C) mesophilic (25–45 °C), and thermophilic (45–70°C). There is an inverse relationship between the temperature range and the HRT, meaning that thermophilic digesters have a shorter retention time than mesophilic and cryophilic ones.

Many facilities operate their biodigesters at the optimum temperature of thermophilic microorganisms because this reduces number of pathogens, favors methanogenic bacteria growth, improves the separation of liquid and solid fractions, and improves degradation of the substrate since there is more metabolic activity. Moreover, the methane production in thermophilic digesters is 25% greater than in mesophilic digesters. Nevertheless, the utilization of thermophilic temperatures also has disadvantages such as a higher degree of imbalance due to an increased production of volatile fatty acids. When dealing with manure, for example, reactors had optimal production in mesophilic reactors with the temperature between 30 and 35 °C, with only a 3% difference in the methane yield between these two temperatures. The same substrate at 25 °C had a decrease in methane yield of 17.4% [17]. In another study, two reactors, a one-stage reactor operated at mesophilic temperatures and a two-stage reactor operated at thermophilic (first stage) and mesophilic (second stage) temperatures, had their volatile solid consumption compared. The results demonstrated that a thermophilic (60 °C) stage was especially effective in degrading sludge waste substrates, with a 35% reduction in VFAs compared to the one-stage mesophilic digester.

### 5.2. pH

The pH value of utilized substrates affects AD by influencing the methanogenic-organisms' doubling time. Moreover, pH also influences the dissociation of some important compounds, such as ammonia, sulfide, and some organic acids. Methane generation takes place in the range of 5.5–8.5 pH, with optimal production in the 7.0–8.5 pH range. Most of the problems in AD can be attributed to acid accumulations and a consequent drop in the pH value. Considering

that CO<sub>2</sub> solubility decreases when the temperature increases, the pH of thermophilic reactors is higher than mesophilic ones and therefore has less carbon dioxide dissolved in carbonic acid form, making it more endurable for methanogenic groups. In a two-phase digester, the hydrolytic-acidogenic and acetogenic phases are separated from methanogenesis, and with this, the pH can be controlled to the optimum range for the first phase (4.0–6.0) and second phase (7.0–8.5). In a single-phase reactor, the pH is usually maintained around the tolerance of the methanogenic group (6.6–8.0) since the other population groups of organisms can tolerate these conditions [18].

### 5.3. Ammonia

Nitrogen in the form of ammonia (NH<sub>4</sub>) is present in the environment of the digester as a gas. It originates from protein degradation and from animal slurry, due to its high ammonia concentration. The precise concentration of free ammonia at which it starts to be toxic remains uncertain, but when dealing with a non-adapted digester (i.e., a digester that has not had enough time to acclimate its methanogenic population to a high ammonia concentration), its inhibition starts at 0.08–0.15 gN/L of free ammonia and 2.5 gN/L of total ammonia. In an adapted digester, it is 0.7–1.1 gN/L of free ammonia and 4–6.5 gN/L of total ammonia [19]. Methanogenic bacteria are very sensitive to the presence of ammonia as its presence can disturb the process in two forms, (1) inhibiting methanogenic enzymes in archaea and (2) entering the archaea cell and causing an unbalance in the electrons and disrupting the process [20].

### 5.4. Micronutrients (trace elements)

The impact of trace elements and changes in its concentration in bioreactors depends on various factors, such as the microbial community structure; population dynamics; individual trophic group metabolism; and meta-community (e.g., the microbial community as a group, incorporating compounds as well as cells). With that in mind, it is hard to fix micronutrient concentrations that are fully satisfactory for the microorganisms' community present in the reactor.

Although nutritional demand for each microorganism species varies, this topic will explore general guidelines of micronutrients, which are limiting for methane-forming archaea. These microorganisms have specific methanogenic enzyme systems with different requirements when compared to other microorganisms. These systems need specific micronutrients that must be incorporated or added to the substrate for its proper degradation and efficiency of CH<sub>4</sub> production.

Cobalt, iron, nickel, and sulfide are obligatory micronutrients, because they are cofactors of the methane pathway enzymes that convert acetate into methane. In some cases, molybdenum, tungsten, and selenium can be obligatory micronutrients as well as barium, calcium, magnesium, and sodium [21].

These micronutrients are usually present in municipal wastewater, although the digester effluent, in some cases, must be analyzed to ensure their presence in enough quantities and

guarantee that these nutrients are in a soluble form since micronutrient deficiency can be mistaken with toxicity from the accumulation of volatile fatty acids.

Simple variations in the amounts of elements can disturb the environment inside the digester by unbalancing the substrate process and then causing inhibition of the whole process. For example, under co-limiting conditions, methanogenic activity was lost within ten days by acidification of a methylotrophic digester. In other study, Zn deprivation affected methane production significantly, which could not be later restored by a continuous supply of Zn [22].

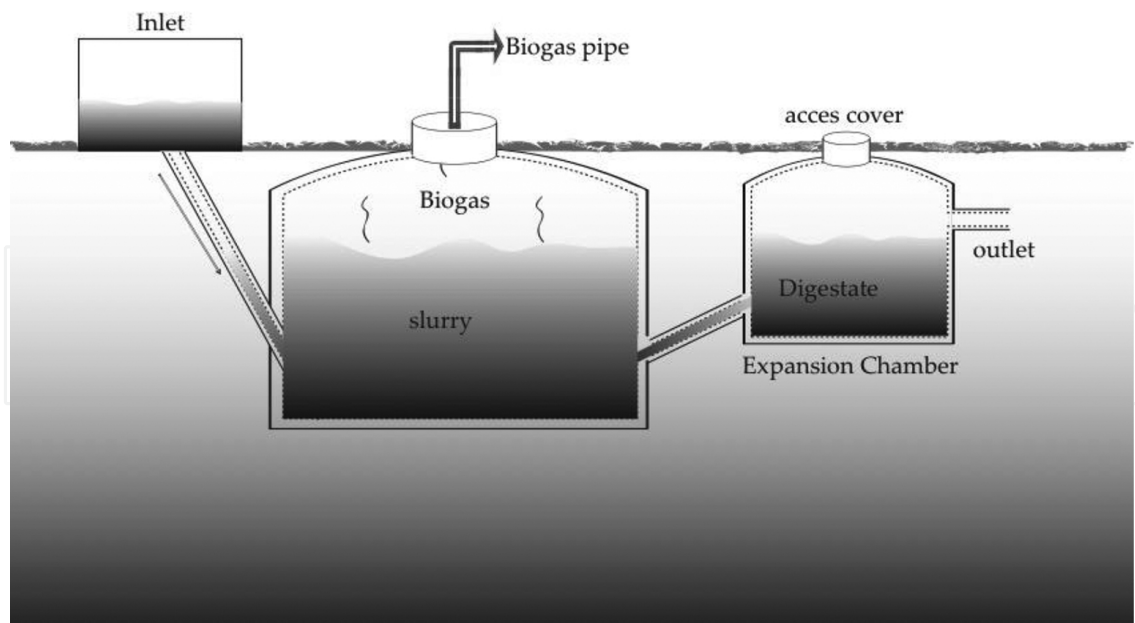
## 6. Anaerobic bioreactors

The biodigester (or anaerobic bioreactors) must guarantee optimal conditions for feedstock transformation to occur, such as the retention of the active biomass and favorable environmental conditions for biomass degradation of organic matter [23]. A report, dating from the 1880s, presents a biodigester, named by its inventor, Donald Cameron (Exeter, England), as a "Septic Tank," which was much more efficient than previous, and more rudimentary tanks since its design promoted microbial growth by adopting an organic material entry and exit system below water level in order to minimize the entry of air and turning of the upper part of the tank [24]. The precursor tank, called the "automatic scavenger," was built by Jean Louis M. Mouras, author of the first reference to the liquefaction of organic matter of wastewater under anaerobic conditions (patented in 1881) [24]. However, it is worth noting that this is not the first AD bioreactor, but one of the first reports in the literature.

The increase in demand of organic matter degradation has allowed for further development of these bioreactors, such as the addition of a heating system [25] and mechanical agitation – Patent US2605220 [26]. Additionally, there are many studies regarding bioreactor design and the way the digestion is conducted, as described in the next Section (6.1).

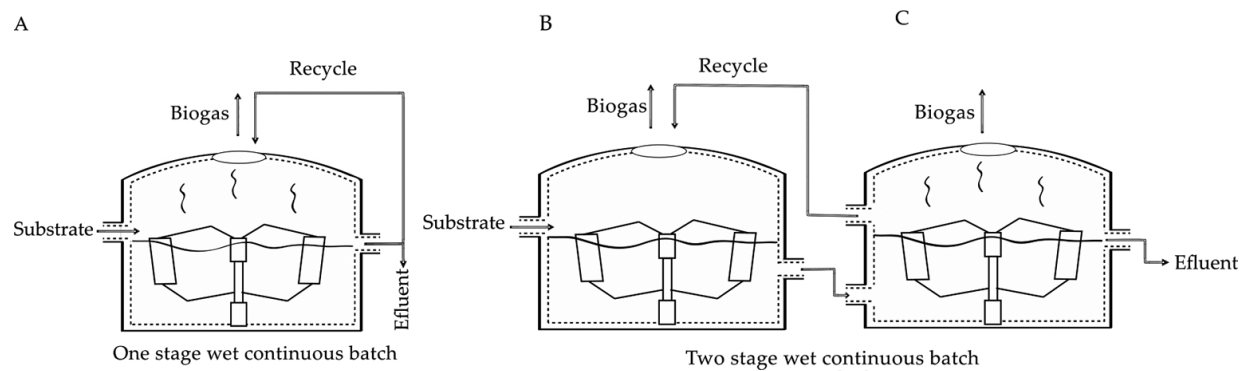
### 6.1. Bioreactors types

The digestion unit is the most important part of a biogas plant; after all, it is where organic matter is reduced into biogas by microorganisms. An anaerobic digester design should allow for a continuously high load rate of organic matter, short hydraulic retention time (to reduce bioreactor volume), and a maximization of methane production. The shape of the bioreactor should take important considerations into account, such as the exchange of heat and the mixture, which is not observed in underground reactors (**Figure 2**). In general, these bioreactors are built from concrete blocks in a rectangular or square shape format that does not benefit the mixture. Furthermore, they have accumulated points (edges) of raw materials that lead to reduction in plant efficiency and require more frequent maintenance and thus idle time [27].



**Figure 2.** Underground reactor.

The choice of bioreactor for biogas production will depend directly on the characteristics of the raw materials utilized such as dry matter content, rate of degradation, and risk of inhibition. Among the main processing technology options available, there are feeding systems, reactor type, temperature reactor, number of phases, and agitation system (**Figure 3**) [28]. Nevertheless, only the most frequently used options of reactor type and number of phases will be described in more detail in this chapter.



**Figure 3.** Fermentation modes utilized for biogas production batch digester—one-stage continuously fed system (A); two- or multistage continuously fed system: first stage (B) and second stage (C).

They may be dry or wet, batch or continuous, one step or multistep, and one phase or multiphase and may operate under different temperature conditions (mesophilic or thermophilic). However, the main bioreactor groups commonly employed are as follows: (1) batch bioreactors (**Figure 3A**); (2) continuous fed system: (a) one stage (**Figure 3B**); and (b) two stage or multistage [29] (**Figure 3C**).



### 6.1.1. Batch bioreactors

In this type of system (**Figure 3A**), a digestion vessel is loaded once with the feedstock then sealed off and left to ferment until gas production decreases. Then, the bioreactor is emptied and filled again with a new batch of feedstock. It is worth noting that part of the digestate should be left in the vessel, which will serve as inoculum for the next batch [30]. This type of bioreactor is generally utilized for feedstock that has a high solid content (between 30 and 40%) and with a high fiber content [31], and it requires little daily attention and it is notable for its simplicity. Moreover, batch reactors may be more suitable when using small amounts of substrate [32].

However, batch bioreactors have some limitations, for example (1) high variation in gas quality and production; thus, a series of batch digesters are employed, which are fed sequentially to generate a reasonably homogenous production of biogas; (2) a considerable time requirement to empty and load the batch digesters; (3) biogas losses during discharging the bioreactors; and (4) limited bioreactor heights [29]. The production of methane may vary from 44.6 to 290 mL/g VS for yard trimmings and rice straw as substrate, respectively [2].

### 6.1.2. Continuously fed system

For continuous digesters, unlike the batch bioreactors, the feedstock is constantly fed mechanically or by flow force by the newly entered feedstock, enabling uninterrupted production of biogas [33]. Among the types of continuous digesters, the multiple tank system (or multistage system) stands out, which will now be described.

#### 6.1.2.1. One-stage, two-stage, or multistage continuous fed system

As previously discussed in this chapter (Section 4.1), there are four biochemical reactions in anaerobic digestion: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. When all of these biochemical reactions take place in one reactor, it is called a one-stage continuously fed system (**Figure 3B**), in contrast, when the biochemical reactions occurs separately in two reactors, it is called a two-stage (or multistage) continuously fed system (**Figure 3C**) [27].

Organic waste treatment systems that use the two-stage system present advantages over one-stage systems, such as high biogas production rates and yields. One study demonstrated a 13% increase in methane production from cellulosic material in a process that used a two-stage process compared to a single phase [34]. A similar increase was obtained using olive mill solid residues as the substrate [35]. Another study [36] compared one- and two-stage digestions for the treatment of thin stillage. It obtained approximately 57% total volatile fatty acids to the total chemical oxygen demand ratio, while the digestion obtained from one stage is only 10%. Additionally, the use of two-stage digestion also increased the production of methane, from 0.26 L CH<sub>4</sub>/g of the chemical oxygen demand added (one stage) to 0.33 L CH<sub>4</sub>/g of the chemical oxygen demand added [36]. This is because the system that performs the separation stages of the biochemical anaerobic digestion benefits the selection and development of different microorganisms for each stage. In addition, the conditions in each respective phase are controlled to generate an optimal environment for the action of each microorganism [37].



Acidogenic bacteria are the prevailing microorganisms in the first stage while the methanogenic group is dominant in the second one. In addition, as previously discussed, the intense production of acids inhibits methane formation in a one-phase system. Hence, the second stage favors bacteria that perform the production of methane gas [28]. The multiple-step system allows a faster, higher performance, and less expensive process than those that use single-stage digester, even though multistage digesters were more expensive to build and maintain [38]. The methane yield from municipal solid waste using a two-stage reactor can be 21% greater than the methane yield obtained from a single-stage process [39].

## 6.2. Microorganisms retention

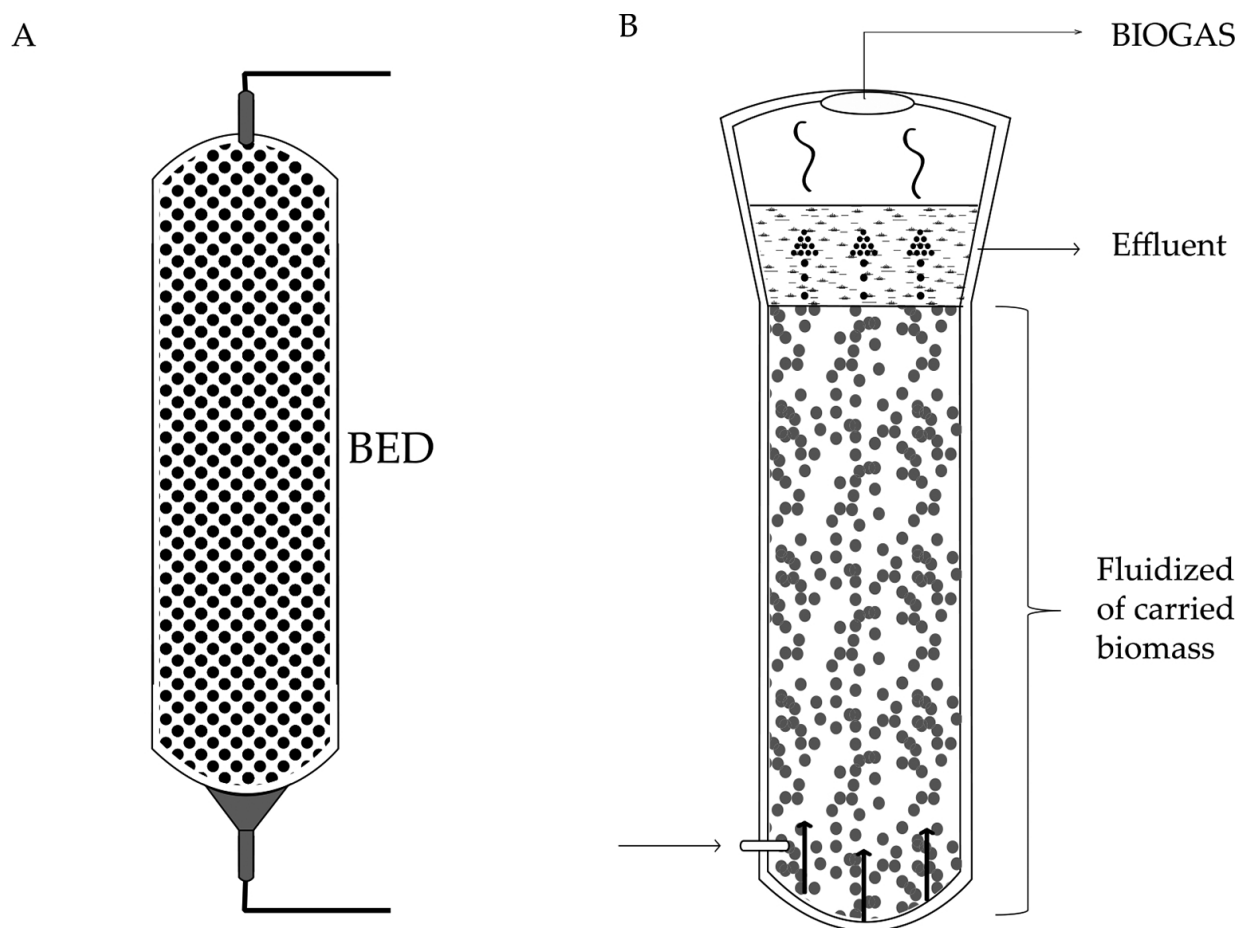
In general, the generation time of hydrolytic and acidogenic bacteria ranges from approximately 1–3 days, whereas methanogenic and acetogenic bacteria range from about 1–4 and 5–12 days, respectively [13]. Due to the slow growth of microorganisms during the process of digestion, a reactor operated in a continuous mode can result in washout. Therefore, the rate of loading and unloading cannot exceed the maximum growth rate of microorganisms. In addition, the calculation of this rate is one of goals of process optimization. Additionally, one other way to prevent this type of accident is to use immobilized cells [19]. The use of microbial consortium retention contributes to increased performance of the anaerobic phase [40]. The use of support material such as toasted coconut shells and wood chips produced 720 and 144 L/kg VS of biogas, respectively, while the use of expanded clay showed nearly no production [40].

Anaerobic filters use inert supporting materials such as clay fibers, polyvinyl-chloride sheets, polyurethane foam, polypropylene membranes, carbon fiber textiles, tire rubber, zeolite filters, glass, and polyethylene fibers [40]. It is practical at this point to highlight that not only is the type of support material directly related to the performance of the anaerobic reactor, but so are other factors such as specific surface area, porosity, surface roughness, pore size, and orientation of the packaging material [40].

Microbial immobilization on the surface and in the pores of the inert material allows a reduction in the hydraulic residence time, which can decrease from 30 days to under a week, and it consequently reduces reactor volume and initial cost and increases the yield [32]. Among the used systems are (1) fixed- or packed-bed reactors and (2) fluidized-bed reactors (**Figure 4**).

### 6.2.1. Fixed-bed reactors

In its initial application, the fixed-bed system was used as biological filters for sewage treatment, so it is also known as an anaerobic filter (similarly called a biofilm reactor or packed bed). In this system, the particles containing the immobilized cells are fixed or packed into the reactor and the liquid flows through the bed. The fixed-bed reactor (**Figure 4A**) allows the application of greater organic loads than those applied in the complete mixture of anaerobic digesters. This system uses one kind of reactor that maintains a high biomass density within the reactor through microorganism retention from biofilms that have developed on the support material [32].



**Figure 4.** Microorganisms' retention reactors: (A) fixed-bed reactor and (B) fluidized-bed reactor.

#### 6.2.2. Fluidized-bed reactors

In fluidized-bed systems (**Figure 4B**), the supporting material particles are maintained in suspension within the reactor due to substrate flow. This allows the particles to become unrestricted, and therefore, its entire external surface is available for interaction with the feedstock. This type of system has an advantage over packed-bed because we could substrate particulate packed beds. Furthermore, control of the temperature and the pH is more effective than the packed beds [32].

The performance of both reactors (fixed bed and fluidized bed) was compared with that of a fixed-bed reactor under similar conditions (feed gas to steam ratios of 1.5 and 0.75 at a reactor temperature of 750 °C, GHSV (gas hourly space velocity) of 300 L/min) [41]. This study showed a conversion of 75% CH<sub>4</sub> in a fixed-bed reactor. On the other hand, when using the fluidized-bed reactor, the production was much greater, reaching up to 90% conversion. The authors of this study reported the low yield of the fixed-bed reactor creates points of temperatures below the optimum process temperatures.

## 7. Conclusions and perspectives

Currently, numerous efforts are being made to reduce energy dependence on oil. This requirement has led to the development of new technologies for the use of other energy sources, such as the production of biogas. This biofuel is an important alternative to ensure the supply of clean and affordable energy and to contribute toward reducing the accumulation of waste, as biomass can be used as raw materials for biogas production. However, obtaining high yields is still a major challenge. One solution is to optimize the process, adjusting some of the physical and chemical parameters, such as temperature and pH. This is because this fermentation process involves several microbial groups and therefore needs to be adjusted to the environment of each of these groups. One way to do this is to include fermentation stages, in which more than one reactor is used, allowing the maintenance of optimum conditions for each microbial group involved in each step. Another challenge is the hydraulic retention time, which is the normal time that the input substrate spends in the digester before it is removed. A solution for this is microorganism retention, where they are imprisoned within inert materials, allowing the microorganisms to remain longer inside the reactor. It is worth noting that a deeper understanding of the physiology of each microbial group participating in the process should be performed in order to be able to more precisely optimize the process parameters. Finally, despite biogas production being an age-old process, little is known about this process. Therefore, further studies on this process are necessary to achieve greater production and thus more amplified outcomes of this process.

## Author details

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