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Anaerobes in the microbiome

Mini review: Update on bioaugmentation in anaerobic processes for biogas production

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ABSTRACT

Anaerobic digestion (AD) is increasingly being used and exploited as a strategy to generate biomethane, which can be used as a renewable and clean energy. AD rests on the biodegradation of organic compounds in anaerobic condition, and these organic compounds are generally agricultural-, industrial- and domestic-wastes. However, problems of AD decrease efficiency, as the result of bioreactor stress, are generally encountered. The primarily cause of this stress is the presence of high concentrations of inhibitory substances such as nitrate, sulfate, heavy metals and oxygen among others. Another cause of AD decrease efficiency is the use of organic compounds that are less amenable to biodegradation such as lignocellulosic compounds. One of the strategies to overcome these limitations is the addition in bioreactors of "stress resistant"- or "efficient biomethane generating"- microorganisms to improve AD process. This strategy, known as bioaugmentation, has been used for the last 15 years to increase biomethane production. In this review, work carried out on this bioaugmentation process has been summarised, and new strategies that could be used or exploited to improve the success of this approach have also been discussed.

1. Introduction

Fossil fuel remains central in the generation of energy for industrial and domestic use. However this fuel is associated with 2 major problems. The first is that it cannot be renewed, thus, will face depletion; the second is that it is associated with a negative impact on the environment because of the emission of greenhouse gases (CO_2, CO), which contributes to global warming. Therefore, to overcome these limitations, alternative energies need to be developed, and these energies need to be renewable and environmental friendly. The process of anaerobic digestion (AD) is one of the options for the generation of renewable and clean energy. AD rests on converting organic compounds-carbohydrate, lipid and proteins-to biogas (consisting of 60–70% of methane and 30–40% carbon dioxide) in presence of a community of anaerobic microorganisms [1–3]. This methane, also known as biomethane since it is generated by microorganisms, can be separated and used as source of energy, heat generation, electricity or transportation gas. Another advantage of AD is that waste products (industrial, agricultural and domestic) are used as main source organic compounds or feedstock. Specifically, agricultural waste products, rich in cellulose, form a cheap and readily available source of feedstock for AD [2,4,5]. Since this feedstock is generated from CO_2 (by conversion through photosynthesis), AD has neutral carbon balance, contributing then to reducing global warming. Many countries in the world are embracing this strategy for energy generation. For instance, European union estimated that at least 25% of its energy need can be met from AD process [1]. Currently, Germany is the country that produces the most biogas in Europe, with an estimated of more than 1000 AD units, which generate an equivalent energy of 39,473 GWh/year, representing 5% of Germany's annual consumption [6]. The developing world is also embracing this strategy as source of alternative energy, although most AD used are small scale. For instance, it was estimated that China had over 20 million biogas plants in 2007, with an output of 10.5 billion m^3 of biogas, and this output had been increasing by 248 billion m^3 (annually) up to, at least, the year 2010 [7].

As will be discussed in Section 2, the metabolic efficiency of microorganisms is central in the success of AD, and different types of microorganisms are required to achieve this success: those involved in hydrolysis, acidogenesis, acetogenesis, H_2-production and methanogenesis (acetate-utilisation and H_2-utilisation). These microorganisms work in synthrophy, one depending upon the

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product of the other, and failure to maintain an appropriate balance between these microorganism groups is the main cause of bioreactor instability, leading to decrease or even inhibition of biomethane generation [8]).

The change in the microorganism balance, due to the growth inhibition of one group of microorganism or outgrowth of another group, is primarily caused by various inhibitory factors in bioreactors. These factors include high level of inorganic toxicants such as ammonium, phosphate, sulphate and metal ions. In addition, parameters such as temperature and pH variation, organic loading rate (OLR), the recalcitrance of feedstock to biodegradation are associated with decrease AD efficiency. The literature is replete with work describing how each of these parameters can reduce the efficiency of AD and the possible strategies that can be employed to minimize their effects. I refer the readers to the following recent excellent reviews on this topic [1,2,9,10]. Among the proposed strategies, are pre-treatment (including ensilage) or dilution of feedstock, use of more than one phase bioreactor, controlling temperature and pH among others. Although some of the strategies are promising, however, they can also be associated with an increase in the time length of AD process, thus an increase in the AD cost [1,9,10].

Since the aforementioned factors leads to a change in the balance of microbial community, therefore, bioaugmentation could be used as alternative strategy to offset these limitations. This bioaugmentation consists of adding specific stress resistant- or efficient-microorganisms into a microbial community in an effort to enhance the ability of this microbial community to generate biomethane. For instance, this approach has been successfully used in the aerobic biodegradation of various contaminants in soil and wastewater, especially, contaminants that are generally recalcitrant to biodegradation [11–15].

For the last 15 years, this approach has been evaluated in the context of anaerobic processes so as to increase the efficacy of biomethane production. In this review, work carried out on this bioaugmentation process has been summarised, with the aim of identifying knowledge gaps for future research. Using the experience of bioaugmentation in aerobic condition, new strategies that could be exploited, including nanotechnology, so as to improve the success of this approach, have also been presented.

2. Basic principles of biogas (biomethane) formation

The anaerobic conversion of organic waste to biomethane consists of interrelated biochemical processes involving different synthrophic microorganisms that have individual substrates specificities. In this syntrophy, an initial organic compound (as pollutant) is used to generate a product by the first group of microorganism, and this product will become the substrate of another group of microorganisms, and this process continue until the generation of biomethane [8]. Overall, the process consists of 4 phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig. 1).

2.1. Hydrolysis

Organic wastes, which are complex carbohydrates, proteins and lipids, are the feedstocks of AD process. Thus, before they can be utilised, they need to be hydrolysed to soluble mono- or dimeric substrates, such as glucose, amino acid, free fatty acids. Lignocellulose, which generally is recalcitrant to hydrolysis, is the most commonly available waste, thus, it is used as the main feedstock or biomass. This complex carbohydrate can be hydrolysed (as the result of the expression of cellulase) by several anaerobic microorganisms including those belonging to the groups of *Cellulomonas, Clostridium, Bacillus, Thermomonospora, Ruminococcus, Baceriodes, Erwinia, Acetovibrio, Microbispora, and Streptomyces* among others [9,16].

2.2. Acidogenesis or fermentation

As part of phase 2, the monomers generated in the first phase will be utilised to produce small and volatile fatty acid (VFA), which are primarily butanoic, propionoic (acetic), ethanoic and lactic acids. Ethanol, H_2 and CO_2 can also be generated during this phase. This 2nd phase, also known as fermentative phase, is generally carried out by bacteria that also mediate hydrolysis.

2.3. Acetogenesis

In the phase 3, VFA will be converted to acetate by acetogenic microorganisms, and in this process, H_2 and CO_2 can also be generated. These microorganisms belong to various genera, including *Clostridium, Acetobacterium, Syntrophomonas, Sporomusa, Syntrophospora, Thermosyntropha, Ruminococcus, and Eubacterium*, among others [9,16]. The production of H_2 and CO_2 can also be the result of the activity of synthrophic acetate oxidising bacteria (SAOB) [Fig. 1], and both microorganism groups, acetogens and, especially SAOB, are inhibited in presence of high H_2 concentration. Thus, these microorganisms thrive in symbiosis with H_2-utilising methanogen, as it will be discussed in the next section [9,16]. Thus, in AD process, a balance needs to be kept between the acetogenic, SAOB and methonogenic populations.

2.4. Methanogenesis

The last phase (phase 4) of the process involves the generation of biomethane by archaea microorganisms, and this takes place through 2 processes. The first involves the conversion of acetate to methane by acetoclastic methanogens or acetate-utilising methanogens, and the most important ones belong to the genus *Methanosaeta* [17]. In the second mechanism, H_2/CO_2 are converted to methane by H_2-utilising microorganisms or hydrogenothropic methonagens. Among the most studied and most efficient hydrogenothrophic archaea are those belonging to the *Methanosarcina* genus [17]. *Methanosarcina* archaea can also convert acetate to methane, making them versatile in the generation of methane. Other hydrogenothropic methonagens belong to *Methanococcus* and *Methanospirullum* genera. Biomethane can also be generated from other methyl compounds such as methanol and methyl amine by archaea of the group M*ethanolobus* and *Methanococcus* [17]. Overall, more than 60% of biomethane is generated by degradation of acetate and about 30% by are H_2/CO_2 oxido-reaction, only a small proportion of biomethane is derived from other methyl compounds [9].

Methanogens are sensitive to changes in environment such as an abrupt variation in pH or temperature, an increase in salt, metal ions or organic matter concentration, an alteration of the loading rate. For instance, if the pace of acetate generation is higher than their utilisation by methanogens, this leads to a decrease in pH, which eventually will inhibit methanogenic growth. Generally, pH between 6.5 and 7.5 are found to be ideal in anaerobic process [9,18,19], and pH change of 0.5 unit or less can be detrimental to the process [19]. Thus, the coupling of acid/acetate generation and their utilisation is critical in the success of anaerobic process. Interestingly, *Methanosarcina* archaea have been found to resist better to changes in environment than any methanogens studied so far, thus, they are known to be the most robust methanogens [19].

Each of the aforementioned phases provides an opportunity to increase the efficiency of AD process by bioaugmentation, a process