Towards Sustainable Energy: The Requisite Role of Microorganisms in the Production of Biogas and Bioethanol

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Authors' contributions

This work was carried out in collaboration among all authors. Author OCE conceptualized and designed the review article. Authors OCE and ESO reviewed the literature and wrote the first draft of the manuscript. Author OVN managed literature searches and provided the images and illustrations. Authors JCA and AGN reviewed the manuscript and helped in shaping it. All authors read and approved the final manuscript.

Article Information

Review Article

ABSTRACT

Increased energy consumption coupled with the ongoing climate change are urging us to develop more sustainable energy alternatives, including biofuels produced from renewable biomass. Our heavy reliance on fossil-derived fuels has not only gained intense public attention in recent years, but has also prompted us to intensively study the production of sustainable biofuels from renewable energy sources via microbial fermentation. Owing to the recent advances and availability of state-of-the-art molecular tools, our knowledge about anaerobic microorganisms and their direct and indirect contributions in the production of different biofuels have increased tremendously. Anaerobic microorganisms are mainly utilized for commercial production of biofuels such as; biogas and fuel alcohols from renewable organic matter, while photosynthetic microorganisms convert inorganic carbon and water to potential fuels (e.g. fuel alcohols) and fuel precursors (e.g. biomass, starch, lipids). Although metabolically engineered microorganisms, programmed to redirect renewable carbon sources into desired fuel products, are contemplated as best choices to obtain high

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volumetric productivity and yield, however, native populations of anaerobic microorganisms are still considered the primary choice for the production of biogas and bioethanol. These anaerobic microorganisms responsible for different degradation pathways and their functions in anaerobic digesters are continuously being updated. In this review, we discuss the essential role of anaerobic microbes in biogas and bioethanol production via consolidated anaerobic process. Additionally, key enzymatic reactions and microbiota involved in the degradation steps and in the production pathways are specifically highlighted. We also discussed the challenges that still exist for biofuel production from native populations of anaerobic microorganisms and their possible solutions.

1. INTRODUCTION

Energy security and global climate change are the two primary concerns globally, as the quest for sustainable energy ranks as one of the most pressing needs of the 21st century [1-4]. Proper utilization of energy resources is an interesting debate currently on-going, and it is very essential to choose which source of energy must be used and why. Factors such as cleanliness, stability, cost, efficiency and environmental impacts must be evaluated [5]. Rapid industrialization and overall boom in world population size has led to a steep rise in the demand for petroleum based fuels [6-8]. Many countries around the world still depend on fossil fuels for power generation. There is no doubt that these fuels are effective sources of power generation, however, their use pose greater threats to our environment on the long run.

The dependence on conventional fossil fuels, mainly petroleum, gas and coal has a pronounced effect on the depletion of existing global supplies of these non-renewable energy sources [9]. However, the over-reliance on these fuels have greatly contributed towards global warming and climate change due to the continuous emission of green-house gases [10-12], and is a cause of many ecological hazards [5,11]. The burning of fossil fuels produces sulphur dioxide, nitrogen dioxide, and carbon monoxide gases, which directly gives birth to air pollution [5]. The negative effects also include receding of glaciers, loss of biodiversity, rise in sea level, etc. [6,13]. These problems have led to continuous search for alternative sources of energy that are environmentally friendly. Thus, researchers are working assiduously towards alternative energy sources that can be applied profoundly towards sustainable energy production. In this regard, harnessing the immense potential of microorganisms for the production of renewable energy from biomass and agro-industrial by-products is a very promising option that can solve these problems to a very large extent. Renewable energy sources have infinite supply. They are hygienic, and with lesser negative environmental impacts [5].

Biofuels are a good representative of the renewable energy family. They are derived from biomass and include a wide range of fuels such as solid biomass, liquid fuels, and biogases [14]. Wood, saw dust, and waste bagasse are solid; ethanol, methanol, propanol, butanol and plants oil are liquid; while methane gas and syngas are examples of gaseous biofuels [3]. Biofuels have emerged as one of the most strategically significant sustainable fuel sources and are considered an important means of progress for limiting greenhouse gas emissions, improving air quality and finding new energetic resources [15].

Interests have grown towards production of various biofuels using microorganisms in the recent years [16]. This is particularly as a result of the metabolic diversity of different microorganisms that enables the production of biofuels from various substrates [17]. Microorganisms in their process of feeding, consume organic substrates, which are further utilized in metabolic processes, and consequently generate essential products that can serve as alternative source of biofuels [18]. For example, majority of bacteria can easily convert sugars into ethanol, and cellulolytic microbes can utilize plant-driven substrates. On the other hand, cyanobacteria and microalgae possess the potential to photosynthetically reduce the atmospheric CO_2 into biofuels, and methanotrophs can use methane to produce methanol [16,17]. Below is an illustration of the microbial pathways for the production of different biofuels [Fig. 1]. To effectively produce biofuels at optimal level, the selection of microbes, substrates, and the production processes are pivotal. It is also important to fully understand better ways to manipulate the microbial species, substrates or the processes to improve yield. This review highlights the significant roles of specific microorganisms in the stages involved in the production of biogas and bioethanol.
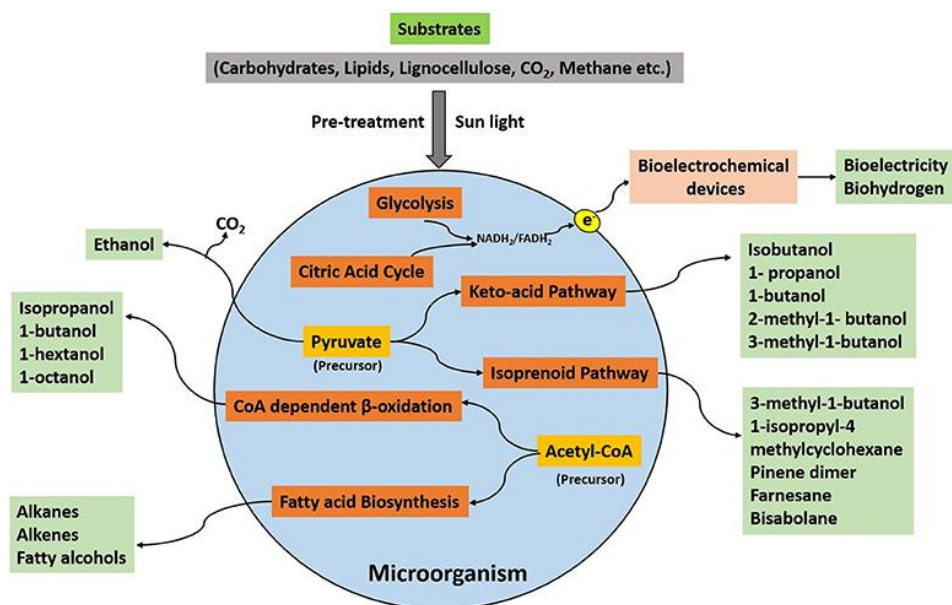
Fig. 1. An overview of microbial metabolic pathways for biofuel production [17]

2. BIOGAS

The production of Biogas demands a group of diverse microbial population that function in a coherent and closely interacting manner. Feedstocks for biogas production include lignocellulosic materials, protein rich materials and feedstocks with high fat contents. Lignocellulosic materials such as straw (wheat, rice, corn, barley) and sugarcane bagasse are the most abundant renewable biomass and have high potential to contribute to the expansion of worldwide biogas production [19-22]. Protein-rich materials for biogas production include wastes from animal rearing (slaughterhouse, dairy, animal manure, aquaculture sludge), ethanol fermentation (distiller's waste), food industry, and households [19-28]. On the other hand, slaughterhouse wastes, food wastes, and grease-separation sludge are materials with a high fat content [29-31]. The role of microbes in the biogas production is linked with the main degradation steps i) hydrolysis, ii) acidogenesis, iii) acetogenesis, and iv) methanogenesis (Fig. 2), and this process has to be efficient and balanced in order to obtain successful anaerobic digestion. A simplified diagram of the main degradation steps is shown in Fig 2. This diagram shows the feedstock origin, the intermediate products, the biogas end product of the four reaction stages, and the types of microorganisms involved in each degradation step.

2.1 Hydrolysis and Liquefaction

Hydrolytic bacteria, and possibly also fungi perform the initial step – hydrolysis – involving the conversion of polymers (polysaccharides, lipids, proteins, etc.) into soluble monomers (long-chain fatty acids LCFAs, glycerol, amino acids, sugars, etc.) [32,33]. The extracellular enzymes secreted by bacteria to the bulk solution and/or attached to their cell wall mediate the first step of hydrolysis [34]. Cellulose is hydrolyzed to cellobiose and glucose, while hemicelluloses are degraded to monomeric sugars and acetic acid by bacteria that often have several different enzymes combined into so-called cellulosomes situated on their cell wall [22,34,35]. These cellulosomes, present in the cell wall of cellulose and starch-degrading bacteria, contain proteins that have the ability to bind to cellulose, which makes the degradation more efficient because the enzymes can work directly "on-site" [34]. The cellulose and starch-degrading bacteria are found within the genera Acetivibrio, Butyrivibrio, Caldanaerobacter, Caldicellulosiruptor, Clostridium, Eubacterium, Halocella, Ruminoclostridium and Ruminococcus (phylum Firmicutes), Bacteroides and Paludibacter (phylum Bacteroidetes), Fibrobacter (phylum Fibrobacteres), Spirochaetes (phylum Spirochaeta), and Fervidobacterium and Thermotoga (phylum Thermotogae) [36-46]. Fungal cellulases, however, use a different mechanism and not only bind to the surface of

the cellulose, but also to penetrate inside the complex biomass materials (e.g., plant cell walls) [47]. Protease, an important extracellular enzyme, mediates the hydrolysis of proteins into amino acids, which are eventually degraded in the Stickland reaction or through uncoupled oxidation. In the Stickland reaction, volatile carboxylic acid which is one carbon atom shorter than the original amino acid is produced from oxidation process, where one amino acid acts as an electron donor and the other as an electron acceptor [34]. For example, alanine with its three-carbon chain is converted to acetate [48]. Lipases are produced by hydrolytic bacteria and catalyze the hydrolysis of lipids at the water-lipid interface [49], forming saturated or unsaturated LCFA and glycerol [50].

2.2 Acidogenesis

Acidogenesis is usually the fastest reaction in the anaerobic conversion of complex organic matter in liquid phase digestion [50]. During acidification of sugars, long chain fatty acids and amino acids resulting from hydrolysis are transported through microbial cell membranes of acetogenic bacteria, where the LCFAs are converted to acetate via beta-oxidation by families of Syntrophomonadaceae and Syntrophaceae [29,51], to acetate, carbon dioxide (CO_2), and hydrogen (H_2) [52,53]. Depending on the anaerobic microbial species present and the bioreactor conditions, the soluble monomers produced in the hydrolytic and acidogenic steps are further degraded to intermediate products. These mainly comprise volatile fatty acids (e.g., acetate, propionate, butyrate, lactate, valerate, and caproate), alcohols, formate, H_2, and CO_2 [54,55]. The concentration and proportion of individual Volatile Fatty Acids (VFAs) produced in the acidogenic stage are important in the overall performance of the anaerobic digestion system, since acetic and butyric acids are the preferred precursors for methane formation [56].

In this stage, amino acids undergo deammonification in groups via the Stickland reaction, [45,50]. First, one amino acid is anaerobically oxidized via deammonification to form ammonia, VFA(s), and H_2. The H_2 produced during this conversion, by the families of Syntrophomonadaceae and Syntrophaceae, is utilized for reductive deammonification of other amino acid(s) [29,45,51]. The reductive deammonification reaction also produces ammonia, VFA(s), and H_2; the ammonia molecules produced from these coupled reactions accept free protons, which help to control pH drop within the bioreactor [15]. Increased concentration of ammonia within the bioreactor may impede optimal functionality of the anaerobic digestion process [55].
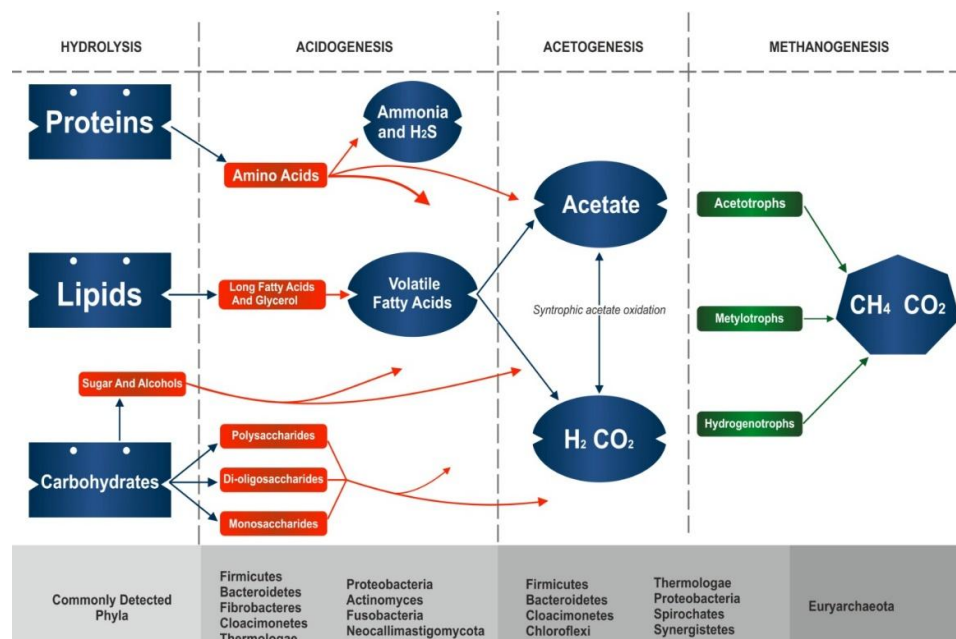

Fig. 2. Anaerobic degradation of carbohydrates, lipids, and proteins leading to biogas production, and the microbial phyla commonly reported to be involved in the different steps

Acidogenesis is called the acidifying stage, since sugars, proteins and other neutral compounds are converted into carbonic acid and VFAs in this step by genera Acetivibrio, Butyrivibrio, Caldanaerobacter, Caldicellulosiruptor, Clostridium, Eubacterium, Halocella, Ruminoclostridium and Ruminococcus (phylum Firmicutes), Bacteroides and Paludibacter (phylum Bacteroidetes), Fibrobacter (phylum Fibrobacteres), Spirochaetes (phylum Spirochaeta), and Fervidobacterium and Thermotoga (phylum Thermotogae) [36-46]. Hence, fermentative anaerobic microorganisms are also considered to be acidogenic or acidifying [42]. Also, rapid acidogenesis can inhibit methanogenesis due to low pH generated from the accumulation of VFAs [33].

2.3 Acetogenesis

During acetogenesis, a group of bacteria called acetogens converts the products formed in hydrolysis/acidogenesis to acetate, H_2, and CO_2 as main products. Acetogenesis is often performed by bacteria belonging to the genera Clostridium and Acetobacterium (phylum Firmicutes), but have also been grouped to the phylum Proteobacteria [36,57-59]. In acetogenesis, CO_2, nitrate, sulfate, and protons can be used as electron acceptors; however, proton is the most important in the biogas process [60]. As a result of thermodynamic, many reactions such as oxidation of organic acids and LCFA, performed by acetogens, can only proceed if the partial pressure of H_2 (pH_2) is kept low [61]. The elimination of the acidogenic products such as acetate and H_2/formate and some methylated compounds primarily proceeds through consumption by methanogens. The energetic situation for the methanogens is comparatively more favourable than acetogens, and thus combining these reactions allows both organisms to obtain energy for growth [34]. This symbiotic relationship, in which neither organism can function optimally without the other, but together they perform metabolic activities that they could not accomplish on their own, is called syntrophy [58,61].

These acetogenic bacteria responsible for the conversion of large LCFAs are obligate hydrogen producers [58] hence, the generation of the acetic and propionic acids during this stage leads to the production of high amount of hydrogen, which brings the pH of the aqueous medium down [48,58,59]. The increased amount of this hydrogen can impede the ability of acetogenic bacteria to metabolize the large LCFAs; thus, to curb this limitation, it is always preferable to decrease the amount of hydrogen present in the bioreactors in order to optimize acetogenesis [58]. The hydrogen produced during the acetogenic step can either be consumed by the hydrogen-utilizing methanogens, which generate methane from hydrogen and carbon dioxide in the methanogenesis step.

2.4 Methanogenesis

In the last step, methanogenic archaea utilize acetate, CO_2, or methylated compounds to produce methane (CH_4) (Fig. 2). Acetate is only used by members of the families Methanosarcinaceae and Methanosaetaceae (order Methanosarcinales) [62]. In comparison, members of the Methanosarcinaceae are more versatile, because of their reported ability to utilize different substrates, such as acetate, hydrogen, and methanol; while members of the Methanosaetaceae use only acetate [62,63]. Methane formation from methylated compounds is performed by members of the Methanomassiliicoccales, Methanobacteriales, and Methanosarcinales [63]. In acetate-utilizing (aceticlastic) methanogenesis, acetate is split into a methyl group and CO_2, and the methyl group is later reduced to methane using an electron provided by the carboxyl group [33,34]. In CO_2-utilizing methanogenesis, CO_2 is reduced to methane by hydrogenotrophic methanogens, using H_2 or formate as primary electron donors [33,34]. In methanogenesis from methylated compounds such as methanol, methylamines, and methylsulfides, the methyl group is reduced to methane [64-68]. Most methylotrophic methanogens then obtain the electrons they require for reduction from oxidation of additional methyl groups to CO_2 [64,65]. Methanogenesis is also considered as the rate controlling portion of the anaerobic process [66].

Only few methanogenic species, which use the aceticlastic pathway (acetate-utilizing), make up the majority of methanogens and are responsible for producing 60-70% of the methane [63-65]. Aceticlastic methanogens are slow growers and require several days to reach a double population [66]. The other 30-40% of the methane generated during methanogenesis is mainly derived through the hydrogenotrophic pathway [34,66]. The hydrogenotrophic pathway has been reported as the most metabolically efficient pathway among microorganisms; because it has the most efficient carbon fixation

mechanism and thus yields the most energy [63-66]. Comparatively, reports from studies have shown that hydrogenotrophic methanogens have a better growth rate than aceticlastic methanogens, and require only 4 to 12 hours to reach double in population [55]. The impressive stability of anaerobic high rate reactors, witnessed during anaerobic digestion, even under varying conditions, is attributed to the hydrogenotrophic methanogen's high growth rate [66]. The consumption of H_2 by hydrogenotrophic methanogen, plays a pivotal role in this system's stability as it serves to keep the partial pressure of H_2 low in the surrounding environment, allowing for the continued production of oxidized soluble products including acetic acid [55,64,66]. Interestingly, almost all methanogenic species have the ability to produce methane from hydrogen and carbon dioxide [43].

3. BIOETHANOL

Bioethanol is a suitable alternative energy source and also the potential solution to all the problems related to the environment and energy crisis [67]. The biological production of bioethanol by microorganisms is based on fermentation [68]. Generally, three types of feedstock are used: lignocellulose, starch from corn and cereals, and simple sugars from beet and cane. Since human demand for food has not been met, interest has been shifted to the use of lignocellulosic materials to solve both the energy and food problems [69]. Lignocellulosic raw materials represent low-cost feedstocks that do not compete with the food and food chain [67,70]. Lignocellulosic materials such as agricultural waste and crop residue, including corn straw, rice straw, wheat straw, cassava stem, saw dust, and cotton seed hair, among others could be used as alternative resources to generate bioethanol in an environmentally free manner [71,72]. To promote the production of bioethanol and its use, not only do we need to rely on cheap feed-stocks, but also to obtain suitable microorganisms with sufficient fermentation yield [67,72]. According to Dien et al. [73], microorganisms for bioethanol production must fulfil the following traits:

i)   Ethanol yield must be greater than 90% theoretically
ii) Ethanol tolerance should be greater than 40 g/L
iii) Ethanol productivity rate must be 1 g/L/h
iv) Simple growth requirement and robust grower

v)   Culture conditions retard contaminants
vi) Able to grow in undiluted hydrolysates-resistance to inhibibitors.

In processing lignocellulosic materials into bioethanol, three major operations are involved:

(i)   Delignification to release cellulose and hemicellulose,
(ii) Hydrolysis of the cellulose and hemicellulose to yield fermentable sugars, and
(iii) Fermentation of reducing sugars [70,72].

3.1 Delignification

The Biological pre-treatment uses microorganisms that have the capability to degrade lignin and polysaccharides in the substrate [69]. The presence of lignin hinders the digestive enzymes from accessing the cellulose and hemicellulose which serve as substrates for bioethanol production [74]. Therefore, the breakdown of lignin is very essential in gaining access to the full carbohydrate in efficient bioethanol production [75,76]. The bacterium Ochrobactrumoryzae degrades lignin to release cellulose and hemicellulose [77]. Data from studies have also reported that microbes such as Nocardia, Rhodococcus, Athrobacter, Streptomyces and Thermomospora spp. are capable of Lignin degradation [77]. However, white-rot fungi are the most efficient members of microorganisms in degrading lignin with notable species including Lentinulaedodes, Phlebia radiate, Ganodermaspp. and Pleorotus spp. [72]. The white-rot fungi are considered efficient based on their ability to produce various classes of lignin modifying enzymes such as manganese peroxidase (MnP), Lignin peroxidase (LiP), Laccase (Lac) and versatile peroxidase [78-80].

3.2 Hydrolysis

Microorganisms cannot directly utilize polysaccharides to produce ethanol; hence, they are further hydrolysed into simple sugars by saccharification. Hydrolysis of polysaccharides requires two essential enzymes; α-amylase and glucoamylase [81], and Aspergillus niger is a vital species in the production of both enzymes [82]. Bacillus amyloliquefaciens, Bacillus subtillis RM16, Gliomastixindicus, Basillus sp. MB6, and Monascussanguineus also produce α-amylase from agricultural wastes [83-87]. These enzymes catalyse the breakdown of α-1,4-glycosidic

bonds of long chain polysaccharides to produce glucose [87,88]. A. niger also produces cellulase enzyme which makes them a better candidate in the hydrolysis of cellulose and hemicellulose [67]. Cellulose degrading bacteria, Serratia marcesceens and Bacillus cereus, have been isolated from wood feeding termites [77]. Cellulose is hydrolysed to glucose while hemicellulose is converted to several pentoses and hexoses [71]. Several species of Clostridium, Streptomyces, Thermonospora, Bacteroides, Cellulomonas, Bacillus, Ruminococcus, Erwinia, Microbispora, Acetivibrioalso produce cellulase enzyme [71,89]. Many fungi such as Penicillium, Fusarium, Trichoderma, Humicola, Phanerochaete, Schizophyllum sp, also have been reported for cellulase production [71, 90,91].

3.3 Fermentation

During fermentation, the simple sugars produced from hydrolysis are converted into ethanol and other organic acids. Saccharomyces cerevisiae, Zymomonas mobilis, Escherichia coli, Pachysolentannophilus, Pichiastipitis, Candida shehatae, Candida brassicae, Mucorindicus etc. are wild type microorganisms used in fermentration [67,92]. S. cerevisiae and Z. mobilisare are the most commonly used microbes utilized in bioethanol production [67,92,93]. Genetic modifications of microorganisms can also improve fermentation yield. For instance, S. cerevisiae ferments glucose to produce ethanol but lacks xylose reductase, thus cannot ferment xylose [19,94]. This drawback in the use of S. cerevisiae for bioethanol production can be averted through genetic engineering [94]. To overcome this challenge, the incorporation of genes XYL1 and XYL2 that encode for xylose reductase and xylitol dehydrogenase respectively, enables the utilization of xylose by S. cerevisiaevia via the pentose phosphate pathway [67]. Some examples of these genetically modified microorganisms include: S. cerevisiae ATCC 26603, P. Stipitis NRRLY-7124, recombinant E. Coli KO11, C. Shehatae NCL-3501, and P. stipitis BCC15191 [71]. These metabolically engineered microbes programmed to redirect renewable carbon sources into desired fuel products, are considered as best choices to obtain high volumetric productivity and yield.

4. OPPORTUNITIES AND CHALLENGES IN THE PRODUCTION OF BIOFUELS

The success of using any microorganism for industrial production of fuels depends on its ability to quickly convert renewable raw material into fuel with high productivity at a low cost, without being toxic to the organism itself. Even though the production of biofuel from renewable energy source such as lignocellulosic biomass is considered as both sustainable and environmentally friendly, however, this process still has various biological and technological barriers which should be addressed. One of such barriers is the development of methods to enhance the biodegradation of lignocellulosic biomass, so as to make the substrate more bioavailable for microorganisms [95,96]. Developing methods to curb these barriers will not only diminish production cost, but will also increase the volumetric yield of biofuels. Diverse chemical, physical, and biological pretreatments have been employed; however, only biological pretreatment generates fewer inhibitory by-products [95]. The conventional pretreatment processes include more than one pretreatment to mineralize lignocelluslosic biomass, hence increasing the overall cost of biofuel production [94,97]. Interestingly, bioaugmentation has been considered an attractive technology, since it has the potential to simplify the biogas production process and it can allow the development of more economical processes without the need for pre-treatment [95].

Additionally, the development and availability of genetic and molecular tools has designated E. coli as the microorganism of best choice in order to produce biofuels from renewable energy sources [95]. These state-of-the-art tools, used to engineer existing native pathways or to create a synthetic new pathway have made the difference in recent years. Although significant work has been done, some challenges still exists in the use of genetically engineered E. coli a cost-efficient strategy for commercial production of bioethanol, higher chain alcohols, and biodiesel and biogas. Inclusively, many other strategies can also be applied, such as:

- Developing potent technologies for cultivation and harvesting of the biomass
- Searching for new feedstock that are more biodegradable and bioavailable to microorganisms and do not compete with the food supply

- Utilizing the degradation potentials of microorganisms through bioaugmentation, to metabolize lignocellulose and reduce the number of steps in the process, thereby making the process cheaper and;
- Developing or modifying processes that allow obtaining the maximum possible biomass products, such as methane, hydrogen, biocell (electricity), fertilizers, among others.

5. CONCLUSION

Biogas and bioethanol production through anaerobic digestion enables recovery of renewable energy and of nutrients from various organic waste materials and is thus highly important for the transition to a more sustainable society. The performance and stability of the biodigestion process is highly dependent on an array of different microbial groups [33,34], and their networks and functions are in turn influenced by substrate characteristics and operating parameters [19,74,97]. With recent advances in molecular techniques, knowledge about anaerobic microorganisms and their response to various operating conditions have increased tremendously. This quantum leap in knowledge has enormously helped in the development of more controlled management and monitoring systems, thus enabling high process efficiency and stability. On the other hand, the noticeable increase in our knowledge about the microbiology of biofuel processes has made it clear that, the microbiota involved is even more complicated and difficult to monitor than initially contemplated [42,46,71], since many members within a specific genus are capable of degrading compounds with different chemical composition. Furthermore, many microorganisms involved in biofuel production are still unknown and are yet to be isolated and characterized for deep understanding on their specific role in biofuel yield [34,38]. Hence, it is absolutely important to improve our current understanding about microorganisms, their respective functions and the interplay between microbial community structure and operation parameters and performance.

ACKNOWLEDGEMENT

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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