

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

## **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 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.

**Key words:** *biofuel, bioethanol, biogas, microorganism, sustainable energy.*

## **1. INTRODUCTION**

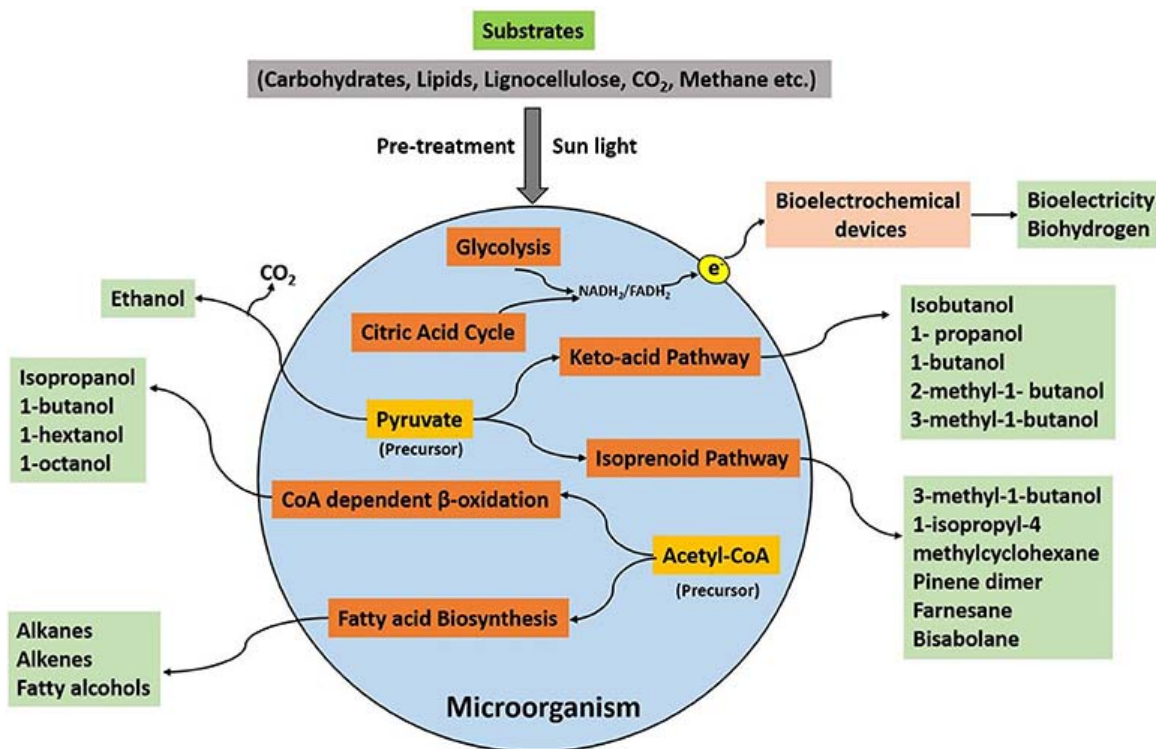
Energy security and global climate change are the two primary concerns globally, as the quest for sustainable energy ranks as one of the most pressing needs of the 21<sup>st</sup> century [1-4]. Proper utilization of energy resources is an interesting debate currently on-going, and it is very essential 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<sub>2</sub> 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 [Figure 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.



**Figure 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 (**Figure 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/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<sub>2</sub>), and hydrogen (H<sub>2</sub>) [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<sub>2</sub>, and CO<sub>2</sub> [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<sub>2</sub>. The H<sub>2</sub> 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<sub>2</sub>; 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]. 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<sub>2</sub>, and CO<sub>2</sub> 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<sub>2</sub>, 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<sub>2</sub> ( $p_{H_2}$ ) is kept low [61]. The elimination of the acidogenic products such as acetate and H<sub>2</sub>/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].

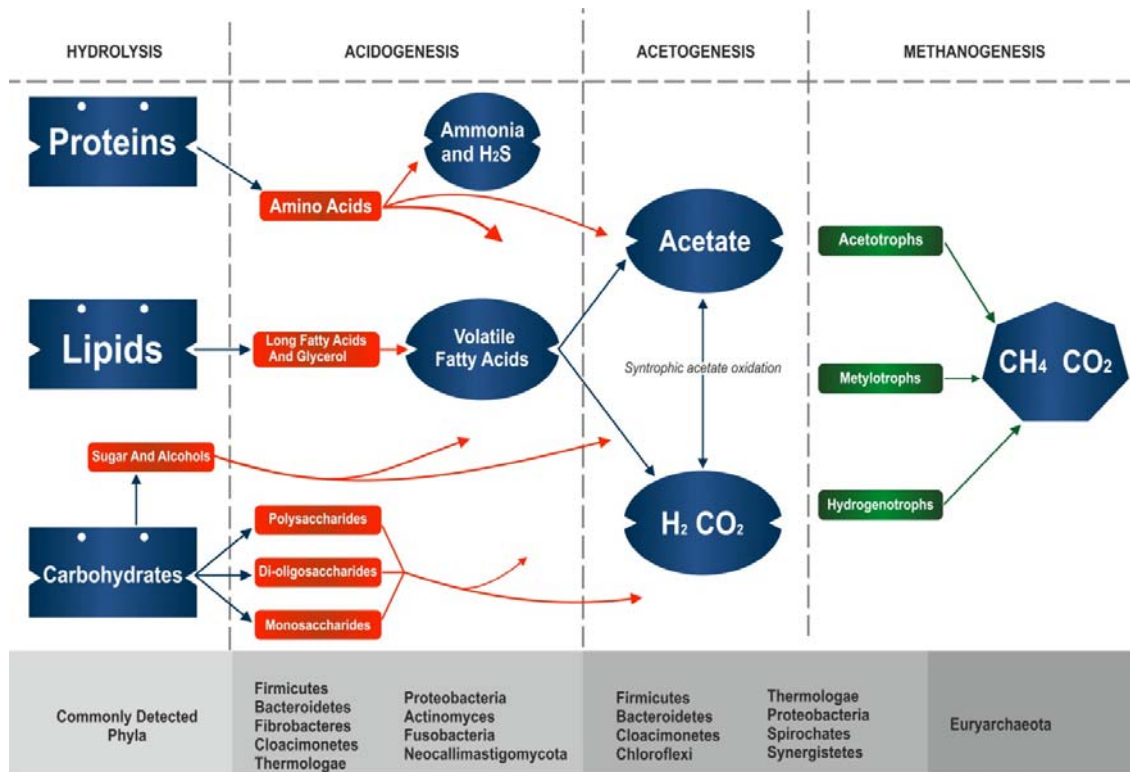
These acetogenic bacteria responsible for the conversion of large LCFAs are obligate hydrogen producers [58] hence, the generation of the acetic and propionic acids during this stage leads to the production of high amount of hydrogen, which brings the pH of the aqueous medium down [48,58,59]. The increased 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<sub>2</sub>, or methylated compounds to produce methane (CH<sub>4</sub>) (**Figure 2**). Acetate is only used by members of the families Methanosarcinaceae and Methanosaetaceae (order Methanosarcinales) [63]. 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]. Methane formation from methylated compounds is performed by members of the Methanomassiliicoccales, Methanobacteriales, and Methanosarcinales [63]. In acetate-utilizing (acetoclastic) methanogenesis, acetate is split into a methyl group and CO<sub>2</sub>, and the methyl group is later reduced to methane using an electron provided by the carboxyl group [33,34]. In CO<sub>2</sub>-utilizing methanogenesis, CO<sub>2</sub> is reduced to methane by hydrogenotrophic methanogens, using H<sub>2</sub> 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<sub>2</sub> [64,65]. Methanogenesis is also considered as the rate controlling portion of the anaerobic process [66].

Only few methanogenic species, which use the acetoclastic pathway (acetate-utilizing), make up the majority of methanogens and are responsible for producing 60-70% of the methane [63-65]. Acetoclastic 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 acetoclastic 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<sub>2</sub> by hydrogenotrophic methanogen, plays a pivotal role in this system's stability as it serves to keep the partial pressure of H<sub>2</sub> 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].



**Figure 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.

### 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 inhibitors.

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;  $\alpha$ -amylase and glucoamylase [81], and *Aspergillus niger* is a vital species in the production of both enzymes [82]. *Bacillus amyloliquefaciens*, *Bacillus subtilis* RM16, *Gliomastixindicus*, *Basillus* sp. MB6, and *Monascussanguineus* also produce  $\alpha$ -amylase from agricultural wastes [83-87]. These enzymes catalyse the breakdown of  $\alpha$ -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 marcescens* 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*, *Acetivibrio* also 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 fermentation [67,92]. *S. cerevisiae* and *Z. mobilis* 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. cerevisiae* 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 lignocellulosic 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 exist in the use of genetically engineered *E. coli* a cost-efficient strategy for commercial production of bioethanol, higher chain alcohols, and biodiesel and biogas. Inclusive, 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, and their networks and functions are in turn influenced by substrate characteristics and operating parameters. 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, 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. 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.

## REFERENCE

1. Ndimba BK, Ndimba RJ, Johnson TS, Waditee-Sirisattha R, Baba M, Sirisattha S. et al. Biofuels as a sustainable energy source: An update of the applications of proteomics in bioenergy crops and algae. *J Prot.* 2013;234-244.
2. Stone R. Devastation in Japan. Fukushima cleanup will be drawn out and costly. *Science.* 2011;331:1507.
3. Shahare VV, Kumar B, Singh, P. Biofuels for Sustainable Development: A Global Perspective. *Green Techno Environ Sustain.* 2007;67-89.
4. González-gonzález LM, Correa DF, Ryan S, Jensen PD, Pratt S. Integrated biodiesel and biogas production from microalgae: towards a sustainable closed loop through nutrient recycling. *Renew. Sust Energ Rev.* 2018;82:1137-1148.
5. Shahzad U. The need for renewable Energy sources. *Int J Inform Techno Elect Eng.* 2015;16-18.
6. Nigam PS, Singh A. Production of liquid biofuels from renewable resources. *Prog Energy Combust Sci.* 2011;37:52-58.

7. Akinsemolu AA. The role of microorganisms in achieving the sustainable development goals. *J Clean Prod.* 2018;182:139-155.
8. Bardhan P, Mandal M, Gupta K. Microbes as Bio-Resource for Sustainable Production of Biofuels and Other Bioenergy Products. *New Future Dev Microbial Biotechnol Bioeng.* 2019;205-222.
9. Li Q, Du W, Liu D. Perspectives of microbial oils for biodiesel production. *Appl Microbiol Biotechnol.* 2008;80(5):749-756.
10. Okamoto K, Nitta Y, Maekawa N, Yanase H. Direct ethanol production from starch, wheat bran and rice straw by the white rot fungus *Trametes hirsuta*. *Enzym. Microb Technol.* 2011;48(3):273-277.
11. Perera F. Pollution from fossil-fuel combustion is the leading environmental threat to global Pediatric health and equity: Solutions exist. *Int J Environ Res Public Health.* 2018;15(1):16.
12. Lelieveld J, Klingmuller K, Pozzer A, Burnett RT, Hainess A, Ramanathan V. Effects of fossil fuel and total anthropogenic emission removal on public health and climate. *Proc Natl Acad Sci U S A.* 2019;116(15):7192-7197.
13. Gullison RE, Frumhoff PC, Canadell JG, Field CB, Nepstad DC, Hayhoe K, et al. Tropical forests and climate policy. *Science* 2007;316:985e6.
14. Janda K, Kristoufek L, Zilberman D. Biofuels: policies and impacts. *Agric Econ Czech.* 2012;58(8):372-386.
15. Delfort B, Durand I, Hillion G, Jaeger-Voirol A, Montagne X. Glycerin for new biodiesel formulation. *Oil Gas Sci Technol e Rev IFP,* 2008;63(4):395e404.
16. Liao JC, Mi L, Pontrelli S, Luo S. Fuelling the future: microbial engineering for the production of sustainable biofuels. *Nat Rev Microbiol.* 2016;14:288–304.
17. Kumar R, and Kumar P. Future Microbial Applications for Bioenergy Production: A Perspective *Front Microbiol.* 2017;8:450.
18. Misau IM, Bugaje IM, Mohammed J, Mohammed, IA, Diyau'deen BH. Production of Bio-Ethanol from Sugarcane: A Pilot Scale Study In Nigeria, *J Eng Res App.* 2012;2(4):1142-1151.
19. Sanchez OJ, Cardona CA. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour Tech.* 2008;99:5270-5295.
20. Nofemele Z, Shukla P, Trussler A, Permaul K, Singh S. Improvement of ethanol production from sugarcane molasses through enhanced nutrient supplementation using *Saccharomyces cerevisiae*. *J Brewing Distilling.* 2012;3:29-35.
21. Park H, Jeong D, Shin M, Kwak S, Oh EJ, Ko JK et al. Xylose utilization in *Saccharomyces cerevisiae* during conversion of hydrothermally pretreated lignocellulosic biomass to ethanol. *Appl Microbiol Biotechnol.* 2020;104:3245-3252.
22. Wagner AO, Lackner N, Mutschlechner M, Prem EM, Markt R, Illmer P. Biological pretreatment strategies for second-generation lignocellulosic resources to enhance biogas production. *Energies.* 2018;11:1797. DOI: 10.3390/en11071797
23. Kovács E, Wirth R, Mároti G, Bagi Z, Rákhely G, Kovács KL. Biogas production from protein-rich biomass: Fed-batch anaerobic fermentation of casein and of pig blood and associated changes in microbial community composition. *PLoS One.* 2013;8:1-18. DOI: 10.1371/journal.pone.0077265

24. Moestedt J, Nilsson Påledal S, Schnürer A, Nordell E. Biogas production from thin stillage on an industrial scale—Experience and optimisation. *Energies*. 2013;6:5642-5655. DOI: 10.3390/en6115642
25. Solli L, Elise Håvelsrud O, Horn SJ, Gunn Rike A. A metagenomic study of the microbial communities in four parallel biogas reactors. *Biotechnol Biofuels*. 2014;7:1-15. DOI: 10.1186/s13068-014-0146-2
26. Rajagopal R, Massé DI, Singh G. A critical review on inhibition of anaerobic digestion process by excess ammonia. *Bioresour Technol*. 2013;143:632-641. DOI: 10.1016/j.biortech.2013.06.030
27. Luo G, Fotidis IA, Angelidaki I. Comparative analysis of taxonomic, functional, and metabolic patterns of microbiomes from 14 full-scale biogas reactors by metagenomic sequencing and radioisotopic analysis. *Biotechnol Biofuels*. 2016;9:1-12. DOI: 10.1186/s13068-016-0465-6
28. Banks CJ, Zhang Y, Jiang Y, Heaven S. Trace elements requirements for stable food waste digestion at elevated ammonia concentrations. *Bioresour Technol* 2012;104:127-135. DOI: 10.1016/j.biortech.2011.10.068
29. Sousa DZ, Smidt H, Alves MM, Stams AJM. Ecophysiology of syntrophic communities that degrade saturated and unsaturated long-chain fatty acids. *FEMS Microbiol Ecology*. 2009;68:257-272. DOI: 10.1111/j.1574-6941.2009.00680.x
30. Luostarinen S, Luste S, Sillanpää M. Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant. *Bioresour Technol*. 2009;100:79-85. DOI: 10.1016/j.biortech.2008.06.029
31. Affes M, Aloui F, Hadrich F, Loukil S, Sayadi S. Effect of bacterial lipase on anaerobic co-digestion of slaughterhouse wastewater and grease in batch condition and continuous fixed-bed reactor. *Lipids Health Dis*. 2017;16:195. DOI: 10.1186/s12944-017-0587-2
32. Kazda M, Langer S, Bengelsdorf FR. Fungi open new possibilities for anaerobic fermentation of organic residues. *Energy Sustain Socie*. 2014;4:6. DOI: 10.1186/2192-0567-4-6
33. Westerholm M, Schnürer A. Microbial Responses to Different Operating Practices for Biogas Production Systems. *Anaerobic Digestion*. 2019 DOI: 10.5772/intechopen.82815
34. Schnürer A. Biogas production: Microbiology and technology. *Adv Biochem Eng Biotechnol*. In: Hatti-Kaul R, Mamo G, Mattiasson B, editors. Switzerland: Springer International Publishing; 2016;156:195-234. DOI: 10.1007/10\_2016\_5
35. Tsavkelova EA, Netrusov AI. Biogas production from cellulose containing substrates: A review. *App Biochem Microbiol*. 2012;48:421-433. DOI: 10.1134/S0003683812050134
36. Azman S, Khadem AF, van Lier JB, Zeeman G, Plugge CM. Presence and role of anaerobic hydrolytic microbes in conversion of lignocellulosic biomass for biogas production. *Crit Rev Env Sci Technol*. 2015;45:2523-2564. DOI: 10.1080/10643389.2015.1053727
37. Lebuhn M, Hanreich A, Klocke M, Schlüter A, Bauer C, Pérez CM. Towards molecular biomarkers for biogas production from lignocellulose-rich substrates. *Anaerobe*. 2014;29:10-21. DOI: 10.1016/j.anaerobe.2014.04.006

38. Krause L, Diaz NN, Edwards RA, Gartemann K, Krömeke H, Neuweiger H, et al. Taxonomic composition and gene content of a methane-producing microbial community isolated from a biogas reactor. *J Biotechnol.* 2008;136:91- 101. DOI: 10.1016/j.jbiotec.2008.06.003
39. Klocke M, Mähnert P, Mundt K, Souidi K, Linke B. Microbial community analysis of a biogas-producing completely stirred tank reactor fed continuously with fodder beet silage as mono-substrate. *System Appl Microbiol.* 2007;30:139-151. DOI: 10.1016/j.syapm.2006.03.007
40. Wang H, Lehtomäki A, Tolvanen K, Puhakka J, Rintala J. Impact of crop species on bacterial community structure during anaerobic co-digestion of crops and cow manure. *Bioresour Technol.* 2009;100:2311-2315. DOI: 10.1016/j.biortech.2008.10.040
41. Liu T, Sun L, Müller B, Schnürer A. Importance of inoculum source and initial community structure for biogas production from agricultural substrates. *Bioresour Technol.* 2017;245:768- 777. DOI: 10.1016/j.biortech.2017.08.213
42. Sun L, Liu T, Müller B, Schnürer A. The microbial community structure in industrial biogas plants influences the degradation rate of straw and cellulose in batch tests. *Biotechnol Biofuels.* 2016;9:128. DOI: 10.1186/s13068-016-0543-9
43. Jia Y, Ng S, Lu H, Cai M, Lee PKH. Genome-centric metatranscriptomes and ecological roles of the active microbial populations during cellulosic biomass anaerobic digestion. *Biotechnol Biofuels.* 2018;11:117. DOI: 10.1186/s13068-018-1121-0
44. Campanaro S, Treu L, Kougias PG, Francisci DD, Valle G, Angelidaki I. Metagenomic analysis and functional characterization of the biogas microbiome using high throughput shotgun sequencing and a novel binning strategy. *Biotechnol Biofuels.* 2016;9:26. DOI: 10.1186/s13068-016-0441-1
45. Koeck DE, Wibberg D, Maus I, Winkler A, Albersmeier A, Zverlov VV, et al. Complete genome sequence of the cellulolytic thermophile *Ruminoclostridium cellulosi* wild-type strain DG5 isolated from a thermophilic biogas plant. *J Biotechnol.* 2014;188:136-137. DOI: 10.1016/j.jbiotec.2014.08.024
46. Liu CM, Wachemo AC, Tong H, Shi SH, Zhang L, Yuan HR, et al. Biogas production and microbial community properties during anaerobic digestion of corn stover at different temperatures. *Bioresour Technol.* 2018;261:93- 103. DOI: 10.1016/j.biortech.2017.12.076
47. Ramsay IR, Pullammanappallil PC. Protein degradation during anaerobic wastewater treatment: Derivation of stoichiometry. *Biodegradation.* 2001;12:247-257. DOI: 10.1023/A:1013116728817
48. Ding S, Liu Y, Zeng Y, Himmel ME, Baker JO, Bayer EA. How does plant cell wall nanoscale architecture correlate with enzymatic digestibility? *Science.* 2012;338:1055-1060. DOI: 10.1126/science.1227491
49. Mendes AA, Oliveira PC, de Castro HF. Properties and biotechnological applications of porcine pancreatic lipase. *J Mol Catal B: Enzym* 2012;78:119-134. DOI: 10.1016/j.molcatb.2012.03.004
50. Stergiou P, Foukis A, Filippou M, Koukouritaki M, Parapouli M, Theodorou LG, et al. Advances in lipase-catalyzed esterification reactions. *Biotechnol Adv.* 2013;31:1846-1859. DOI: 10.1016/j.biotechadv.2013.08.006

51. Mosey FE, Fernandes XA. Patterns of hydrogen in biogas from the anaerobic digestion of milk sugars. *Water Sci Technol*. 1989;21:187–96.
52. Ziels RM, Svensson BH, Sundberg C, Larsson M, Karlsson A, Yekta SS. Microbial rRNA gene expression and co-occurrence profiles associate with biokinetics and elemental composition in full-scale anaerobic digesters. *Microb Biotechnol*. 2018;11:694-709. DOI: 10.1111/1751-7915.13264
53. Weng C, Jeris JS. Biochemical mechanisms in the methane fermentation of glutamic and oleic acids. *Water Res*. 1974;10:9-18.
54. Ma J, Zhao Q-B, Laurens LLM, Jarvis EE, Nagle NJ, Chen S, et al. Mechanism, kinetics and microbiology of inhibition caused by long-chain fatty acids in anaerobic digestion of algal biomass. *Biotechnol Biofuels*. 2015;8:141. DOI: 10.1186/s13068-015-0322-z
55. Kandylis P, Bekatorou A, Pissaridi K, Lappa K, Dima A, Kanellaki M, et al. Acidogenesis of cellulosic hydrolysates for new generation biofuels. *Biomass Bioenerg*. 2016;91:210-216. DOI: 10.1016/j.biombioe.2016.05.006
56. Hwang S, Lee Y, Yang K. Maximisation of acetic acid production in partial acidogenesis of swine wastewater. *Biotechnol Bioeng*. 2001;75:521–9.
57. Kougias PG, Angelidaki I. Biogas and its opportunities—A review. *Frontiers of Environmental Science & Engineering*. 2018;12:14. DOI: 10.1007/s11783-018-1037-8
58. Worm P, Müller N, Plugge CM, Stams AJM, Schink B. Syntrophy in methanogenic degradation. In: Hackstein JHP, editor. (Endo) symbiotic Methanogenic Archaea. Berlin: Springer-Verlag. 2010;143-173
59. St-Pierre B, Wright A-DG. Comparative metagenomic analysis of bacterial populations in three full-scale mesophilic anaerobic manure digesters. *Appl. Microbiol and Biotechnol* 2014;98:2709-2717. DOI: 10.1007/s00253-013-5220-3
60. Ragsdale S, Pierce E. Acetogenesis and the Wood-Ljungdahl pathway of CO<sub>2</sub> fixation. *Biochimica et Biophysica Acta*. 2008;1784:1873-1898. DOI: 10.1016/j.bbapap.2008.08.012
61. Schink B. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiology and Molecular Biology Reviews*. 1997;61:262-280
62. De Vrieze J, Hennebel T, Boon N, Verstraete W. Methanosarcina: The rediscovered methanogen for heavy duty biomethanation. *Bioresour Technol* 2012;112:1-9. DOI: 10.1016/j.biortech.2012.02.079
63. Enzmann F, Mayer F, Rother M, Holtmann D. Methanogens: Biochemical background and biotechnological applications. *AMB Express*. 2018;8:1. DOI: 10.1186/s13568-017-0531-x
64. Liu F, Whitman WB. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annal New York Acad Sci*. 2008;1125:171-189. DOI: 10.1196/annals.1419.019
65. Costa KC, Leigh JA. Metabolic versatility in methanogens. *Curr Opin Biotechnol*. 2014;29:70-75. DOI: 10.1016/j.copbio.2014.02.012
66. Jiraprasertwong A, Maitriwong K, Chavadej S. Production of biogas from cassava wastewater using a three-stage upflow anaerobic sludge blanket (UASB) reactor. *Renewable Energy*. 2019;130:191-205.

67. Pandey A, Twari S, Jadhav SK, Tiwari KL. Efficient microorganisms for bioethanol production from lignocellulosic azolla. *Res J Environ Sci*. 2014;8:350-355.
68. Azhar SHM, Abdulla R, Jambo SA, Marbawi H, Gansau JA, Faik AAM et al. Yeasts in sustainable bioethanol production: A review. *Biochem Biophys Rep*, 2017;10:52-61.
69. Bharathiraja B, Jayamuthunagai J, Praveenkumar R, VinothArulraj J, Vinoshmuthokumar P. Saravananaraj A. Bioethanol production from Lignocellulosic materials- An overview. *Scitech J*. 2014;1(7):27-36.
70. Basic A, Marđetko N, Kundas S, Morzak G, Belskaya H, Santek MI et al. Bioethanol production from renewable raw materials and its separation and purification: a review. *Food Techn Biotechnol*. 2018;56(3):289-311.
71. Paul S, Barman M, Gorain B. Microbial Intervention in Bioethanol Production. *Agrobios Newsletter*, 2017a;26(2):16-17.
72. Gopalakrishnan RM, Manavalan T, Ramesh J, Thangavelu KP, Heese K. (2020). Improvement of Saccharification and Delignification Efficiency of *Trichoderma reesei* Rut-C30 by Gen Bioeng Microorgan. 2020;8:159.
73. Dien BS, Cotta MA, Jefferies TW. Bacteria engineered for fuel ethanol production: Current status. *Applied Microbiol Biotechnol*. 2003;63:258-266.
74. Saini JK, Saini R, Tewari L. Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: Concepts and recent developments. *Biotechnol*. 2015;5:337-353.
75. Pei Y, Li Y, Zhang Y, Yu C, Fu T, Zou J. et al. Lignin and hemicellulosic monosaccharides distinctively affect biomass digestibility in rapeseed. *Bioresour Technol*. 2016;203:325-333.
76. Kim D. (2018). Physico-chemical conversion of lignocellulose: Inhibitor effects and detoxification strategies: A mini review. *Molecules*. 2018;23:309.
77. Tsegaye B, Balomajumder C, Roy P. Bidelignification and hydrolysis of rice straw by novel bacteria isolated from wood feeding termite. *Biotechnol*. 2018;8:447.
78. Hammel KE, Cullen D. Role of fungal peroxidases in biological ligninolysis. *Current Opin Plant Biol*. 2018;11:349-355.
79. Manavalan T, Manavalan A, Heese K. Characterization of lignocellulolytic enzymes from white-rot fungi. *Curr Microbiol*. 2015;70:485-498.
80. Knezevic A, Stajic M, Jovanovic VM, Kovacevic V, Cilerdzic J, Milovanovic I et al. Induction of wheat straw delignification by *Trametes* species. *Science Report*. 2016;6:26529.
81. Butterworth PJ, Warren FJ, Ellis PR. Human  $\alpha$ -amylase and starch digestion: An interesting marriage. *Starch*, 2011;63:395-405. Doi:10.1002/star.201000150.
82. Adefisoye S, Abdulwasii S. Production of Glucoamylase by *Aspergillus niger* in Solid State Fermentation. *Adv Biol Res*, 2018;12(1):7-11.
83. Salman T, Kamal M, Ahmed M, Sidiqa SM, Khan RA, Hassan A. Medium optimization for the production of amylase by *Bacillus subtilis* RM16 in Shake-flask fermentation. *Pakistan J Pharm Sci*. 2016;29(2):439-444.

84. Mishraa SK, Kumarb S, Kumarc S, Singhd RK. Optimization of process parameters for a-amylase production using Artificial Neural Network (ANN) on agricultural wastes. *Curr Trends Biotechnol Pharm.* 2016;10(3):248-60.
85. Paul JS, Lall B, Jadhav S, Tiwari K. Parameter's optimization and kinetics study of a-amylase enzyme of *Bacillus* sp. MB6 isolated from vegetable waste. *Process Biochem* 2017b;52:123-129.
86. Tallapragada P, Dikshit R, Jadhav A, Sarah U. Partial purification and characterization of amylase enzyme under solid state fermentation from *Monascussanguineus*. *J Gen Eng Biotechnol.* 2017;15(1): 95-101.
87. Jujjavarapu SE, Dhagat S. Evolutionary Trends in Industrial Production of a-amylase. *Recent Patents Biotechnol.* 2018;12:1-15.
88. Trono D. Recombinant Enzymes in the Food and Pharmaceutical Industries. *Adv EnzyTechnol.* 2019;349-387. doi:10.1016/B978-0-444-64114-4.00013-3.
89. Sadhu S, Maiti TK. Cellulase production by bacteria: a review. *British Microbiol J.* 2013;3(3):235-258.
90. Rabinovich ML, Melnik MS, Boloboba AV. Microbial cellulases (review). *Appl Biochem Microbiol.* 2002;38(4):305-21.
91. Yotchaisarn M, Saengha W, Karirat T, Deeseenthum S, Maneewan K, Luang V. Identification of cellulose-producing bacteria from soil in Nasinuan Forest, Kantarawaichai District, Mahsarakham Province. *Int Conf Food Agric Biotechnol.* 2018;57-71.
92. Cheon S, Kim HY, Gustavsson M, Lee SYL. Recent trends in metabolic engineering of microorganisms for the production of advanced biofuels 11 *Curr Opin Chem Biol.* 2016;35:10–21
93. Majidiana P, Tabatabaeia M, Zeinolabedinia M, Naghshbandic MP, Chistid Y. Metabolic engineering of microorganisms for biofuel production *Renew Sustain Energy Rev.* 2018;82:863–885.
94. Koppolu V, Vasigala V. Role of *Escherichia coli* in Biofuel Production. *Microbiol insights.* 2016;9:29–35.
95. Lo Y, Saratale GD, Chen W, Bai M, Chang J. Isolation of cellulose-hydrolytic bacteria and applications for cellulosic biohydrogen production. *Enzy Microb Techn.* 2009;44:417–425
96. Underwood SA, Buszko ML, Shanmugam KT, Ingram LO. Lack of protective osmolytes limits final cell density and volumetric productivity of ethanologenic *Escherichia coli* KO11 during xylose fermentation. *Appl Environ Microbiol.* 2004;70(5):2734–2740.
97. Dölle K, Hughes T, Kurzmann DE. From Fossil Fuels to Renewable Biogas Production from Biomass Based Feedstock – A Review of Anaerobic Digester Systems. *J Energ Res Rev.* 2020;5(3):1-37.