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A Review on Biofuel Production from Biomass by Microorganisms

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The increased demands for energy coupled with growing emphasis on environmental conservation have turned the attention of people towards renewable energy. Biomass-derived fuels have received great attention in recent years because of high energy content, biodegradability, regeneration. This paper presents a comprehensive review on biofuel production through microbial conversion technologies. Five main clean energy carriers and the microorganism used in the process of biofuel production are discussed, respectively. Biomass-derived fuels will be a vital source of renewable energy with the development of microbial conversion technologies.

Key word: Biomass; Microorganism; Biogas; bio-ethanol; Bio-hydrogen; biodiesel; MFC (microbial fuel cell).

The world's energy requirements are increasing sharply due to the growth of the population and economic development. However the current energy consumption is heavy reliance on fuels primarily including coal, oil, gas, which with limited reserves are decreasing. The unbalanced distribution of conventional energy resources also led to energy security of many countries. Meanwhile, the use of fossil fuels causes serious environmental problems. These concerns have turned the attention of the scientists and governments toward alternative energy such as solar, wind, unclear, hydro and biomass. Based on its cleaning, regeneration and environmentally friendliness, biomass appears to be the most promising sources of renewable energy in the world (Stephanopoulos, 2007; Atsumi *et al*., 2008; Fortman *et al*., 2008). Biomass-derived fuels have been recognized as a world renewable energy due to its near-carbon neutrality and the ample availability of various sources of biomass(Ni *et al*., 2006). It is composed of carbohydrate compounds, which are determined as the elements of carbon, hydrogen and oxygen and posses a high energy content (Tsai *et al*., 2006; Kim *et al*., 2010). Moreover, the utilization of biofuels will foster socioeconomic developments for many rural communities in developing nations and reduce carbon dioxide emission and replace fossil fuels directly(Zhang and Long, 2010; Srirangan *et al*., 2012). Biofuels are made from organic matter resulting from forestry, agriculture or municipal wastes while providing an efficient method to deal with waste. It can also greatly mitigate current energy security of many countries. In the utilization of biomass-derived energy, it can be directly burned to obtain energy and also serve as a feedstock to be converted to various liquid or gas fuels for practical applications (Srirangan *et al*., 2012). Given all the benefits, biomass will probably be the major energy source among renewable energy sources in the future. Among the conversion technologies, microbial conversion is considered to be more effective because minimum byproducts and pollution. The rest of this paper discusses the five main energy carriers and the utilization of microorganisms in the conversion of biomass into biofuels.

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Biofuel production from biomass by microorganisms

Biogas

Biogas, with methane and carbon dioxide as the major component, is produced from a variety of biological wastes via anaerobic digestion. Biogas is generated by a mixed community of microbes which could use almost any organic waste residues as a substrate. The other benefits offered by the use of biogas are as;(p!) it help in alleviation of waste disposal problems and reducing the pollution by the agricultural wastes, (q!) it will benefit the people living in rural areas lack of household energy, (r!) biogas after upgraded to refined biomethane is a good substitute of natural gas which can be used in automobiles or other power equipment. The methane fermentation is a complex biological process, and each phase is carried out by various groups of microorganisms. Firstly, the conversion of carbohydrates into simple sugars, fats into fatty acids and proteins into amino acids are performed by hydrolytic bacteria (facultative anaerobes and anaerobes); secondly, the simple substrates (simple sugars, fatty acids and amino acids) are convert by different facultative and obligatorily anaerobic bacteria, which are degraded into short-chain organic acids, C_1-C_2 molecules (e.g. butyric acid, propionic acid, acetate and acetic acid), alcohols, hydrogen and carbon dioxide; thirdly, methanogenic bacteria(e.g. *Methanobacterium, Methanosarcina, Methanoccus*) utilize hydrogen with carbon dioxide, formate, methanol, and acetate as substrates for methanogenesis(He *et al*., 2011; Chandra *et al*., 2012). Although the economical production of biogas is limited by lower conversion efficiency, the technology has been experiencing significant development.

Bio-ethanol

The fermentation technology of ethanol production, which has been used to make food such as soy sauce, rice wine since the ancient time, is one of the earliest industrial applications of microbes in the long history of mankind. However, it was only until late nineteen century when ethanol was used as an energy source(Song *et al*., 2010). Currently, ethanol has been mainly utilized as a gasoline extender and an octane enhancer.

The traditional feedstock for ethanol production is the first-generation feedstock derived from starch crops and sugar. Currently, most of the ethanol produced comes from grain (predominantly corn). The three primary operating stages which used in industrial production of bio-ethanol from the first-generation feedstock are as: (p!) mono- and disaccharides are released through either chemical or enzymatic hydrolysis, (q!) ethanol fermentation using microbial cell factories such as *Saccharomyces cerevisiae* and other yeast, fungi or bacteria, (r!), distillation for ethanol separation and concentration (Srirangan *et al*., 2012). The ethanologenic bacterium *Zymomonas mobilis* is a promising microorganism which can be used in the industrial production of ethanol. It has several appealing properties, including high tolerance to ethanol (0^ÿ120g/L), minimal byproduct formation, higher ethanol yield in comparison to the traditional yeast-based microbial platform, it lacks the ability to ferment pentose sugars(Lin and Tanaka, 2006). On the other hand, the *Escherichia coli* and certain types of yeast (e.g. *Pachysolen tannophilus* and *pichia stipites*) are candidates of metabolizing pentose sugars. However, pentose-fermenting yeasts are not suitable for industrial scale because of the organisms' low ethanol yield, heightened sensitivity to ethanol (40g/L), inability to fermenting xylose in acidic environments, and strict requirement for microaerophilic conditions (Zaldivar *et al*., 2001; Lin and Tanaka, 2006). The commercialization of ethanol production requires the microorganism with the ability to metabolize different fermentable sugars. As no naturally existing microorganisms have the ability to satisfy all the requirements such as high yield, high productivity, wide-substrate fermenting capacity, ethanol tolerance, metabolic engineering and genetic engineering may play a vital role in improving the technology of ethanol production (Song *et al*., 2010).

Although the technology of the first-generation feedstock is a relatively mature process and has been used in industrial production for a long time, the direct competition of biofuels with edible food leads to price increase of these crops. Moreover, the crops need arable crop land and other farming input and food supply is a problem in some developing countries. With the increases of the food resulting from the bio-ethanol production of edible food, the use of crop waste residues and lignocellulosic biomass is receiving great interest. The utilization of organic residue can help to reduce the current disposal problems as well as providing a source of fuel. In comparison with the first-generation feedstock, the non-food materials are considered ideal due to its economic and environmental benefits. The lignocellulosic ethanol production needs the pretreatment (e.g. physical, physico-chemical, chemical and biological) of the feedstock. The objective of pretreatment is to degrade the structure of the lignocelluloses by removing lignin and hemicelluloses, providing a surface area for enzymatic hydrolysis through exposing of the cellulose, and making cellulose accessible for the production of pentose and hexose sugars(Puri *et al*., 2012). Due to the technical difficulties in pretreatment process and high cost of enzyme, the current fuel grade ethanol produced is still not economically feasible. Each raw material requires a different processing and pretreatment strategy, thus understanding and overcoming the barriers for enzymatic hydrolysis of different raw material is essential for the development of economically competitive processes based on enzymatic treatments(Menon and Rao, 2012). The research of direct microbial conversion, which can directly ferment untreated lignocellulosic substrates by anaerobic thermophilic bacteria (*Clostridium thermocellum and C. thermosaccharolyticum*), are receiving great attention in recent years because of simple technological process.

Bio-hydrogen

Hydrogen, with water instead of greenhouse gas upon burning, is an excellent and clean energy. Meanwhile, the high combustion value and good quality make hydrogen to be an ideal fuel for new energy vehicle. Biotechnology might be the most important way of hydrogen production in the future for its characteristics of low costs, regeneration and low pollution(Wu and Chang, 2007). Bio-hydrogen can be generated via biophotolysis (in green algae and cyanobacteria), photo-fermentation (in purple non-sulfur bacteria), and dark fermentation (in anaerobic bacteria)(Navarro *et al*., 2009). The green algae and cyanobacteria can use sunlight and carbon dioxide as the sole sources to generate molecular hydrogen and oxygen(Kýrtay, 2011). The photo-synthetic bacteria (non-sulfur bacteria, *Rhodospirillum, Rhodobacter, Rhodopseudomonas, Thiocapsa*) can use organic compounds and light energy to evolve molecular hydrogen(Deng, 2010). The bacteria without generating oxygen in the processÿalso have the ability to trap energy under a wide range of the light spectrum and the conversion efficiency of light energy is high. The dark fermentation is carried out by converting organic compounds into bio-hydrogen in anaerobic bacteria, grown in carbohydrate-rich substrates, and the anaerobic bacteria can produce hydrogen continuously without the need for light energy (Ren *et al*., 2006; O-Thong *et al*., 2008).

Biodiesel

Biodiesel is defined as mixture of alkyl esters of long chain fatty acids, which are synthesized through esterification and transesterification of free fatty acids and triglycerides, and the major feedstock available for biodiesel are edible oil and organic wastes (Borugadda and Goud, 2012). In comparison with conventional diesel, biodiesels have many advantages such as environmentally friendly, safety in storage and transportation, completely combustion, easily biodegradable and low content of sulfur. The carbon monoxide emission from the combustion of diesel fuel is 10% of conventional diesel fuel, toxic organic matter 10%, and particulate matter 20% (Shen *et al*., 2006). Meanwhile, the biodegradation rate of biodiesel is around 95% and the ignition point is 150ºC. Based on these properties, biodiesel has driven the research interest in the world. Europe has been the leader in the production of biodiesel, the output accounts for 80% of all biofuels, the feedstock are mainly rapeseed oil, sunflower oil, and soybean oil. Although biodiesel production from the first-generation feedstock (predominantly plant oil) is technically feasible, the cost of raw materials accounts for about 60%-80% of the total cost and the use of first-generation feedstock may cause the competition with the food (Borugadda and Goud, 2012). Thus, choosing a right feedstock is very important. The conversion of biomass feedstock (organic wastes and residues) to biodiesel is considered to be an attractive way as the biomass feedstock is distributive variety and large quantity available. Among the conversion technologies, microbial conversion has many advantages, such as low pollution, low energy consumption, and the production can be together with sewage and waste treatment. Although people had discovered the microorganisms in seventeen century, it was until recently scientists began to consider the microbial lipid as a potential bio-energy source(Hillen *et al*., 1982).

In recent years, a number of researches into the biodiesel production from a variety of microorganisms have been attempted. The fermentation of oil wastewater, using selected bacteria, yeast and mildew respectively, was studied and the single cell oil can be at high levels up to 39.6%(Qin and Liang, 2007). The biomass production of *Rhodotorula glutinis*(*strain Rh8*), grown in monosodium glutamate wastewater, was 15.6 g/L, oil production rate was 29.61% in a 250mL shaking flask and COD reduction rate reached 45.1% (Xing *et al*., 2010). The biomass and lipid content of *Phanerochaete chrysosporium,* using cornstalk dilute acid hydrolysate after detoxification under optimized conditions, were 21.2g/L and 50.9%, respectively(Feng *et al*., 2011). The bioconversion of partially detoxified acid hydrolysate of Spartina anglica to lipid by *Trichosporon cutaneum* was studied, the lipid was produced up to 5. 9 g/L (Shen *et al*., 2007).

Some microorganisms, like microalgae capable of photosynthesis can convert light energy into chemical energy in the form of lipids. In some microalgae the oil content can exceed 80% by weight of dry biomass and oil levels of 20-50% are common in any microalgae. The production of biodiesel by microalgae is particularly attractive due to high efficiency, short growth cycle, low requirement of environmental conditions. The microalgae such as Bacillariophyta, Chlorophyta under osmotic stress (light stress and nutrition stress) can produce more than 40% of oil in the biomass(Hu *et al*., 2008). The microalgae can be cultivated in either open pond or photobioreactor. Open pond is a simulation of growth environment of a natural lake environment, which style is a constructed open-type and advantageous are economical to build and operate. Currently, the race way open pond is widely used. However, the lower productivity, the risk of contamination by other bacteria, the difficulty in the recovery process and the large area occupied are primary problems in open pond. For instance, it took American energy department twenty to research approximately five thousands of microalgae and they didn't found any microalgae which can be suitable for cultivation in open pond and simultaneously have a high lipid content(You *et al*., 2011). On the other hand, photobioreactor is expensive to establish and operate, though it can achieve sterile culture, high productivity, the recovery cost is low and the culture environment is satisfactory. Vertical bioreactors, tubular bioreactors and flat plate bioreactors are three main types of photobioreactors in recent year (Ugwu *et al*., 2008). An open tank containing transparent rectangular chambers (TRCs), made of transparent acrylic, was developed to improve the photosynthetic efficiency of microalgae cultivation, and the TRCs conducted light deep into the photobioreactor (Hsieh and Wu, 2009). The total biomass obtained was 56% more than that of similar culture systems without TRCs. Although photobioreactor is not economically feasible because of high cost, combining the fuel production with high-value byproducts obtained by microalgae bio-refinery technology may be an effective in the future.

MFC (Microbial fuel cell)

A microbial fuel cell is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms(Allen and Bennetto, 1993). The link between electricity and metabolic processes in living organisms was first studied in the eighteenth century, when Luigi Galvani observed electricity production in the legs of a frog and first established his theory of 'animal electricity'(Leropoulos *et al*., 2005).

A typical microbial fuel cell consists of anode and cathode compartments separated by a caution (positively charged ion) specific membrane: in the anode compartment, fuel is oxidized by microorganisms, generating electrons and protons; electrons are transferred to the cathode compartment through an external electric circuit, while protons are transferred to the cathode compartment through the membrane; finally, electrons and protons are consumed in the cathode compartment, combining with oxygen to form water(Wikipedia, 2011). In this area, some of the research focused on the material and method used in MFC, and some other researches concentrated on the utilization of MFC together with a certain field such as wastewater treatment (Daniel *et al*., 2009; Wang *et al*., 2009; Oh *et al*., 2010; Zhang *et al*., 2010). Currently, the bacteria which can be used in MFC are *Desulfovibriodesulfuricans, Escherichia Coli, Shewanella purefaciens, Geobacteraceae sulferreducens, Clostridium bytyricum, Rhodoferax ferrireducens, Pseudomonas aeruginosa, Alcaligenesfaceallis, Enterococcus gallinanm* (Ieropoulos *et al*., 2005; He *et al*., 2011). However, the system with microalgae is receiving increased attention because MFC with microalgae cathode can perform various functions such as carbon dioxide capture, wastewater treatment, obtaining microalgae biomass. For instance, the output power density of MFC, which was constructed using separated *Chlorella vulgaris*, was up to 11.82 mV/m^2, and the removal rate of COD reached 40%(He *et al*., 2009).

CONCLUSION

The depletion of fossil reserves and environmental concerns associated with the conventional fuel have allowed the production of biofuel to rise significantly during the past decade. The utilization of biomass to produce biofuel acquires its impetus from reducing the pollution caused by the organic wastes, the low cost, regeneration and availability of the feedstock. In comparison with the traditional conversion (e.g. thermochemical, physical), microbial conversion is a more effective way because of minimum byproducts, low pollution and other functions such as the production can be together with waste treatment. Although the microbial conversion technologies have been greatly improved recently, the current technology cannot be competitive with the existing fossil fuel technologies because of high costs. A comprehensive understanding of different raw material, as well as the metabolism and physiology of microbial cell are considered to be an effective way to make the conversion process economical.

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