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Cellulosic biobutanol by Clostridia: Challenges and improvements

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ABSTRACT

The gradual shift of transportation fuels from oil based fuels to alternative fuel resources and worldwide demand for energy has been the impetus for research to produce alcohol biofuels from renewable resources which focus on utilizing simple sugars from lignocellulosic biomass, the largest known renewable carbohydrate source as an alternative. Currently, the usage of bioethanol and biodiesel do not cover an increasing demand for biofuels. Hence, there is an extensive need for advanced biofuels with superior fuel properties. Biobutanol is regarded to be an excellent biofuel compared to bioethanol in terms of energy density and hygroscopicity, could be produced through acetone-butanol-ethanol (ABE) fermentation process. Even though the ABE fermentation is one of the oldest large-scale fermentation processes, biobutanol yield by anaerobic fermentation remains sub-optimal. For sustainable industrial scale of biobutanol production, a number of obstacles need to be addressed including choice of feedstock, low product yield, product toxicity to strain, multiple end-products and downstream processing of alcohol mixtures plus the metabolic engineering for improvement of fermentation process and products. Studies on the kinetic and physiological models for fermentation using lignocellulosic biomass provide useful information for process optimization. Simultaneous saccharification and fermentation (SSF) with *in-situ* product removal techniques have been developed to improve production economics due to the lower biobutanol yield in the fermentation broth. The present review is attempting to provide an overall outlook on the discoveries and strategies that are being developed for biobutanol production from lignocellulosic biomass.

1. Introduction

Global warming, climate change, unstability of petrol price, depletion of petroleum reservoir and severe environmental pollution due to consumption of fossil fuel for energy generation are among the recent world crisis [1]. These situations happened due to the increase in the number of human population that subsequently contribute to the increase in energy demand for industrial activities, transportations and households. Utilisation of fossil fuel has been reported as the major contributor to the increment of carbon dioxide percentage in the atmosphere [2] that brings to the rise of the global mean temperature and cause many environmental problems [3]. Due to the rapid increase of human population, there is an urgent need for scientists to find alternative energy source to solve our energy problems. The alternative energy should be renewable, clean, environmental friendly and has potential for future energy development.

Biofuel produced from biomass is one of the potential alternative energy. At present, the largest commercial biofuel produced worldwide is bioethanol, particularly in Brazil. Since the 1970s, Brazil has implemented a bioethanol fuel program which has allowed the country to become the world's second largest producer of bioethanol (after the United States) and the world's largest exporter [4]. This bioethanol produced from corn and sugar cane, which create concern on worlds food supply. Therefore, in recent studies the production of bioethanol has been switched from food source to non-food source like lignocellulosic biomass. Lignocellulosic biomass is a plant material composed of lignin, cellulose and hemicellulose in its cell wall structure. The cellulose and hemicellulose can be digested into sugar monomers [5], which can be subsequently used as substrate for fermentation. Besides, lignocellulosic biomass also abundantly available, mainly generated from agricultural and forestry activities, considered as a waste and need to be treated before discharge to environment [6]. Due to these advantages, research on utilisation of lignocellulosic biomass as fermentation feedstock for bioethanol has grown rapidly over the years.

Abbreviations: **ABE**, acetone-butanol-ethanol; **HMF**, Hydroxymethyl furfural; **PTS**, Phosphoenolpyruvate (PEP)-dependent phosphotransferase system; **SHF**, Separate hydrolysis and fermentation; **SHFR**, Separate hydrolysis and fermentation with *in-situ* recovery; **SSF**,, Simultaneous saccharification and fermentation; **SSFR**, Simultaneous saccharification and fermentation with *in-situ* recovery; **CBP**, Consolidated bioprocessing; **SF**, Severity factor; **PKC**, Palm kernel cake

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Unfortunately, bioethanol has lower energy content, more volatile and more corrosive as compared to gasoline. Thus, bioethanol cannot be directly used in cars engine and distribution system without modification. Therefore, alternative biofuel that is better than bioethanol has been searched. Out of many types of biofuels available (biodiesel, biohydrogen, biomethanol and bioethanol), biobutanol has almost similar characteristic to gasoline, overcome major problems posses in other type of biofuel [7,8]. Biobutanol can be produced through fermentation route by a microorganism, usually *Clostridium* spp. The process known as acetone-butanol-ethanol (ABE) fermentation since this microorganism produces acetone, butanol and ethanol as a solvent in its metabolic pathway [9]. Besides solvent, *Clostridium* sp. also produces acids (acetic and butyric acid) and gases (hydrogen and carbon dioxide) [10].

Although biobutanol production has been studied for many years ago, it is still unviable to compete with fossil fuel due to high production cost and impractical process for commercial production in industrial sector [11]. The cost could be reduced by employing free raw material like lignocellulosic biomass, however, there are still several major drawbacks that contribute to the limitation of cellulosic biobutanol production such as (1) multiple processing steps (from pretreatment until recovery process), (2) low sugar concentration produced from lignocellulosic biomass, (3) presence of inhibitors, (4) strain capability and (5) multiple end products that lead to low biobutanol concentration, yield and productivity. These limitations subsequently contribute to inefficient biobutanol recovery and make the whole process yet unviable for commercial production. Thus, this review paper will discuss on the ABE fermentation current progress by Clostridia that focus on the challenges and improvements of this process to produce cellulosic biobutanol as biofuel.

2. Biobutanol characteristics and applications

The production of biobutanol has been started during the late 19th century. It is the second largest fermentation in the world after bioethanol production during World War II. In 1945, it was reported that two-third of the industrially used biobutanol was produced through fermentation in the United States. However, the production of biobutanol has become non-competitive in the 1960s due to the increase of feedstock and cheaper petrochemical products [9]. At present, the biobutanol production through biological process is back to tackle the industrial attention due to the depleting resources of the non-renewable fossil fuel.

Butanol (CAS No: 71-36-3) is a primary alcohol with a structure of $C_4H_{10}O$. It is also known as butyl alcohol, *n*-butanol and butan-1-ol, a straight-chain isomer ending with an alcohol functional group –OH. The molecular weight of butanol is 74.12 g/mol. Butanol is a colourless liquid with a distinct odour and it is completely miscible with organic solvents and partly miscible with water [9]. Butanol properties include a boiling point of 177.7 °C, a melting point of −89.3 °C, an ignition temperature of 35 °C, a flash point of 365 °C, a density of 0.8098 g/mL at 20 °C, critical pressure of 48.8 hPa and critical temperature of 287 °C.

Biobutanol has a great potential in the global market to replace bioethanol as the leader in the biofuel market. The global market demand for biobutanol accounted around USD 7.0–8.4 billion with the expansion over 3% per year [12]. It has been estimated about 4.5–5.4 million tonnes of petrol-butanol are produced every year through the chemical synthesis from petroleum [13]. As a fuel blend stock, biobutanol has the potential to meet a demand of 122 million tonnes per year by 2020. The biofuel blend opportunity for biobutanol alone exceeds USD 80 billion and the overall biofuel opportunity for biobutanol is USD 700 billion. The current butanol price is around USD 4.00 per gallon equivalent to about USD 1.05 per litre estimated in 2013 [14].

Chemically synthesis butanol is widely used for manufacturing variety of products in many industries, where half of the butanol production is used in the latex industry [12]. The primary use of butanol is as a chemical intermediate in the production of other chemicals such as butyl acrylate, methacrylate and plastics. It is also being used as a solvent in the production of glycol ethers and butyl acetate for manufacturing of paint, lacquer, dyes, vegetable oil and waxes [8]. Besides, it is also being used as a solvent in the production of hormones, antibiotics and vitamins. Butanol also acts as a swelling agent for coating fabric in the textile industry and as an ingredient in eye make-up, lipstick and foundations in cosmetics industry. Recently, butanol also shows as a potential alternative fuel for transportation [7].

Biobutanol is a better option as biofuel compared to bioethanol due to its physico-chemical properties. Biobutanol receiving renewed interest because it can directly replace the use of gasoline or can be used as a fuel additive [9]. It has almost similar characteristics to the gasoline that make it a better candidate as our future energy supply. Compared to other alcoholic biofuels (bioethanol and biomethanol), biobutanol has a higher heating value, higher energy content which is 110 kBtu/gal and 25% more energy than bioethanol. These properties can reduce the fuel consumption and better mileage could be obtained. Biobutanol allegedly can be burned directly in existing gasoline engines. It is also can be used either in its pure form or in a mixture with gasoline at any concentration while bioethanol can be mixed only up to 85% [11]. This is due to the fact that the oxygen content in biobutanol is lower than in bioethanol. In addition, biobutanol can also decrease particle number concentration and emissions compared to when using gasoline only [15]. Biobutanol is feasible to be used directly, hence will not require any modifications to the existing engine system [7,8], and can be shipped and distributed through existing pipelines and filling stations [7].

With higher prices of petrol fuel and concern on the environmental problems, several groups are attempting to increase the biobutanol yield by improving the process involved in order to improve its competitiveness. Two large companies (British Petroleum and DuPont) developed several plans to convert an existing bioethanol plant for biobutanol production as soon as the technology is available [16]. There are a number of companies that are working obstinately towards the production of biobutanol from lignocellulosic biomass [17]. Since the fermentation substrate is an important factor that influencing the cost of biobutanol production, it is necessarily relevant to use inexpensive agricultural residues and wastes as fermentation feedstock [18].

3. Lignocellulosic biomass

Lignocellulosic biomass is a plant-based material composed of lignin, cellulose and hemicellulose. This class of biomass includes wood and fibrous materials from organic sources, agricultural wastes, organic municipal wastes and organic industrial wastes [19]. On average, lignocellulosic biomass composed of 38–50% of cellulose, 23–32% of hemicellulose and 15–25% of lignin. Cellulose is physically associated with hemicellulose, and physically and chemically associated with lignin [20]. The individual cellulose molecules are linked together to form elementary microfibrils, in which aggregated by intermolecular hydrogen bonding into larger subunits called fibrils. The microfibrils contain alternating phases of highly ordered (crystalline) and randomly oriented (amorphous) cellulose embedded in a matrix of hemicellulose. The cellulose and hemicellulose fractions are covered in an amorphous layer of lignin [21]. The presence of lignin and hemicellulose makes the access of cellulase enzymes to cellulose becomes difficult, thus reducing the efficiency of the hydrolysis process. The ratio of cellulose, hemicellulose and lignin within the polymer varies between different plants, wood tissues and cell wall layers [5].

The major factor to be considered in utilizing lignocellulosic biomass for biofuel production is the yield of sugar that could be obtained from the hydrolysis process. Sugar yield depends on the type