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Production of Biobutanol as an Alternative Biofuel by Bacterial Fermentation of *Clostridia sp.* Using Agricultural Waste Substrate

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Abstract:
Background*: These days, the need for fuel for people around the world is increasing, while fossil fuel reserves are running low. So it is necessary to carry out trials and search for alternative renewable fuels as a substitute for fossil fuels, especially petroleum.One of these alternative energy sources is biofuel. Biofuels are often called green energy because of their environmentally friendly origins and emissions and do not cause a significant increase in global warming. Various wastes from processing agricultural materials have great potential to be processed into alternative biofuels to substitute coal and fuel oil. These alternative fuels include biobutanol, bioethanol, biodiesel, biogas, and biobriquette. Biofuel whose application is an alternative to petroleum fuels and is known as the best biofuel for vehicles to replace gasoline, is butanol. Butanol has better properties than other bioalcohols when used as fuel, due to its higher octane number, lower corrosivity, higher energy content, and lower solubility in water. Biobutanol can be produced by hydrolysis and fermentation of Clostridia sp.*
Materials and Methods*: The preparation of this review article was carried out through literature studies by searching for international journals literature sources related to biobutanol production through fermentation of the bacteria Clostridia sp. using agricultural waste in the last 10 years (2010-2020). In preparing this review article, data search was carried out using online media with the keyword fermentation of agricultural waste by the bacteria Clostridia sp. which produces the biobutanol as an alternative fuel.*
Results*: Clostridia sp. used consists of three types of species, namely Clostridium acetobutylicum, Clostridium beijerinckii and Clostridium saccharoperbutylacetonicum. Agricultural waste used as a substrate for fermentation Clostridium acetobutylicum is de-oiled rice bran, wheat starch liquid waste, rice straw, mango (Mangifera indica L.) peels waste and cauliflower waste with the biobutanol yields were 6.87 g / L, 12.70 g / L, 7.10 g / L, 10.5 g / L and 2.99 g / L; fermentation of Clostridium beijerinckii is corn fiber, wood pulp, brown seaweed (Laminaria digitata) and pineapple waste juice with the resulting biobutanol were 7.9 g / L, 13.46 g / L, 7.16 g / L and 3.14 g / L; and fermentation Clostridium saccharoperbutylacetonicum using a palm oil mill waste and crude sugarcane bagasse produced biobutanol as much as 0.9 g / L and 10.0 g / L.*
Conclusions*:From this article, the study of substrate after fermentation with Clostridia sp. produced biobutanol on each substrate. The largest biobutanol produced using the bacterial fermentation of Clostridium beijerinckii was found on the wood pulp substrate which was 13.46 g / L, pretreatment which was carried out through alkaline extraction followed by dilute acid hydrolysis and fermentation conditions at 37°C with resin adsorption detoxification method and gas stripping as a separation method.*
Keywords*: biobutanol; bacteria, fermentation; Clostridia sp.; agricultural; waste*

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I. Introduction

The demand for fuel around the world continues to increase from time to time because it is estimated that the availability of fossil fuels in particular fuels produced through processing petroleum as an energy source, is estimated to decline[1]. The decline in the availability of fossil fuels is due to the fact that the main natural resources that produce fossil fuels cannot be renewed in a fast time, however, it requires years of natural processes. In addition to providing benefits for the sustainability of life in the world, fossil fuels also have a large impact on environmental damage, several environmental problems related to the use of these fuels will leave residues that have an impact on environmental pollution and increase in earth temperature.

The use of fossil fuels accounts for 76% of greenhouse gas (CO_2) emissions, which consists of 65% industrial processes. The use of gasoline and diesel fuel in motorized vehicles causes pollution caused by CO_2 gas. The increase in the number of motorized vehicles is directly proportional to the increase in CO_2 emissions[2].

In the search, development and exploration of alternative energy sources, the main factors must be considered, which are energy, economy and ecology. In other words, the system being developed must be able to produce large amounts of energy at low cost and have minimal impact on the environment. One alternative that might meet these criteria is the use of vegetable oil as a biofuel. Therefore, alternative renewable fuels that can replace petroleum and are environmentally friendly are needed[1].

The Indonesian government has issued two policies related to alternative energy. This policy is stated in Presidential Regulation No.5/2006 concerning the National Energy Policy and Presidential Instruction No.1/2006 concerning the Provision and Utilization of Biofuels. This policy describes instructions for the provision and use of biofuel as a substitute for fuel oil. Biofuels are one of the easiest examples of renewable energy to be realized or implemented. Meanwhile, renewable energy according to Presidential Decree No.5/2006 is a source of energy that is produced and a source of natural energy that will not run out and can be sustainable if it is managed properly[3].

Bio-alcohol is a part of alternative energy biofuel as a substitute for oil fuel[3]. Bioalcohols including acetone, butanol and ethanol have received wide attention among academia and industry. Acetone, butanol and ethanol can be applied as multipurpose chemical raw materials. In addition, as the best biofuel for alternative petroleum fuels, butanol and ethanol are considered as the next generation biofuels for vehicles[4]. One of the best liquid biofuels that can replace gasoline is biobutanol, which has similar properties to gasoline[1]. Butanol has many properties that are better than ethanol when used as a fuel, such as a higher octane number, higher energy content, lower corrosivity and lower solubility in water[4].

The processing of agricultural waste has great potential which can be used as a source of raw materials in an effort to provide alternative fuels to meet the energy needs of the community. Such as bagasse waste, corn cobs, straw, coconut shell and dregs, market waste consisting of fruit peels such as pineapple peels and banana peels, decaying vegetables and fruits, and many other agricultural product processing wastes. Which generally becomes garbage and has the potential to pollute the environment. This waste has the potential to be used as a source of raw material in the effort to produce alternative energy sources to replace or substitute fuel oil that comes from the bowels of the earth. Utilization of agricultural waste as a source of raw material for alternative energy production will be able to increase the added value of these materials from less valuable to high value products.

Thus, the concept of zero waste in agro-industry is easier to materialize. This means that in the processing of agricultural products there is no such thing as garbage or waste. All remaining components of agricultural products will be products of high economic value[5].

Lignocellulosic biomass is composed of the main components such as hemicellulose, cellulose and lignin, whose amounts vary depending on the type of plant. Examples of variations of the three components are found in several types of plants that have been published in various scientific journals. Then an important step in the process of converting lignocellulose biomass into alcohol is breaking polysaccharide, cellulose and hemicellulose molecules, then converting cellulose or hemicellulose which is insoluble in water into monosaccharides in the form of soluble sugar compounds such as glucose, silose or others. Finally, through the fermentation process sugar compounds are converted into alcohol[3].

In the production process of biobutanol, it is generally carried out by hydrolysis and microbial fermentation. Hydrolysis is a chemical reaction between water and other substance that produces new or more substances and also the decomposition of a solution using water, acids, bases, or enzymes.

Meanwhile, fermentation is an anaerobic or partial anaerobic oxidation process of carbohydrates and produces alcohol and some acids with the help of certain microbes. In most studies, biobutanol uses anaerobic microorganisms from *Clostridium sp.* which can degrade cellulose and hemicellulose[3].

II. Materials and Methods

In the preparation of this review article, it was carried out through literature studies with literature sources in the form of international journals related to the production of biobutanol as an alternative fuel through fermentation of the bacteria *Clostridia sp.* using agricultural waste in the last 10 years (2010-2020).

In preparing this review article, data search was carried out using online media with the keyword fermentation of agricultural waste by the bacteria *Clostridia sp.* which produces the bioproduct butanol as an alternative fuel.

III. Results

According to the following international standard scientific journals, several examples of agricultural waste can be used as a substrate in the biobutanol production process. This review will provide information on various types of agricultural waste that are suitable for use as substrates with the pretreatment process studied in the last 10 years that can be used to increase the production of biobutanol as an alternative fuel.

Table1. Microorganisms, substrates and fermentation conditions used in the production of biobutanol

Micro-organisms	Substrate	Pretreatment / Hydrolysis	Fermentation Conditions	Total ABE (g/L)	Butanol Production (g/L)	References
Clostridium acetobutylicum	De-oiled Rice Bran	Dilute acid hydrolysis(H2SO4)	72 hours Continuous fermentation at 30°C	12.42 g/L	6.87 g/L	1
Clostridiumacet obutylicum	Wheat starch waste water	No pretreatment	72 hours of ABE fermentation with temperature at 37°C	21.51 g/L	12.70 g/L	4
Clostridium acetobutylicum	Rice straw	- Organosolv - Enzyme hydrolysis	72 hours of ABE fermentation with temperature at 37 °C	10.05 g/L	7.10 g/L	6
Clostridium acetobutylicum	Mango (*Mangifera indicaL.*) Peel waste	Enzyme hydrolysis	Batch fermentation for 7 days at 37 °C	20.40 g/L	10.5 g/L	7
Clostridium acetobutylicum	Cauliflower waste	Acid pretreatment and detoxification	96 hours Batch fermentation with temperature at 80 °C	5.29 g/L	2.99 g/L	8
Clostridium beijerinckii	Corn fiber	Dilute acid hydrolysis(H2SO4)	72 hours of Fed-batch fermentation with temperature at 37 °C	12.9 g/L	7.9 g/L	9
Clostridium beijerinckii	Wood pulp	Alkaline extraction followed by dilute acid hydrolysis	ABE fermentation at 37 °C with resin adsorption detoxification method and gas stripping as a separation method	17.73 g/L	13.46 g/L	10
Clostridium beijerinckii	Brown seaweed (*Laminaria digitata)*	Enzyme hydrolysis	ABE fermentation with temperature at 36.5 °C	NA	7.16 g/L	11
Clostridium beijerinckii	Pineapple waste juice	No pretreatment	ABE fermentation with temperature at 37°C	4.17 g/L	3.14 g/L	12
Clostridium saccharoperbut ylacetonicum	Palm oil mill effluent	No pretreatment	ABE fermentation with temperature at 30 °C	2.09 g/L	0.9 g/L	13
Clostridium saccharoperbut ylacetonicum	Crude sugarcane bagasse	Dilute acid hydrolysis(H2SO4)	72 hours Fed-batch fermentation with temperature at 30 °C	NA	10.0 g/L	14

*NA- Data not available.

IV. Discussions

Clostridium sp., butanol-producing bacteria were first discovered by Louis Pasteur in 1861 (microscopic form as shown in Figure 1) [15, 16]. Then it was produced on an industrial scale by a scientist named Chaim Weizmann in the 20th century. Chaim Weizmann used anaerobic spore-forming bacteria from the genera *Clostridium* for the production of butanol. *Clostridium sp.* bacteria are an anaerobic Gram positive bacteria that can produce organic compounds, acids, alcohols and other solvents, by fermenting large amounts of carbohydrates. However, from the saccharolytic and mesophilic species *Clostridium sp.* capable of producing butyric acid, only a few species are capable of producing butanol with high fermentation yields: *C. acetobutylicum, C. aurantibutylicum, C. beijerinckii* and *C. tetanomorphum*[15].

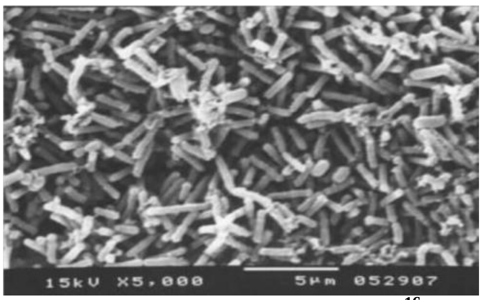

Fig. 1. *Bacteria Clostridium sp.*[16]

The raw material preservation process is required to break down the lignocellulosic matrix and optimize the overall conversion of biomass to fuel[17]. The pretreatment method aims to break down and remove lignin and hemicellulose by damaging the crystalline structure of cellulose. Damage to the structure of the hemicellulose crystals can facilitate the disintegration of cellulose into glucose and break down hemicellulose into simple sugars. This simple sugar compounds can then be fermented with the help of microorganisms to produce biobutanol[18]. The pretreatment methods are distinguished based on processes with heat mechanics, acid treatment, alkaline treatment, and treatment using organic solutions[19]. The pretreatment process can be carried out by physical treatment (microwave irradiation, pyrolysis, gamma irradiation), physico-chemical (steam explosion, ammonia fiber exploration, hot water liquid), chemically (O_2 or H_2O_2 oxidation agent), and biologically (using microorganisms or enzymes that can break down cellulose and lignin)[17].

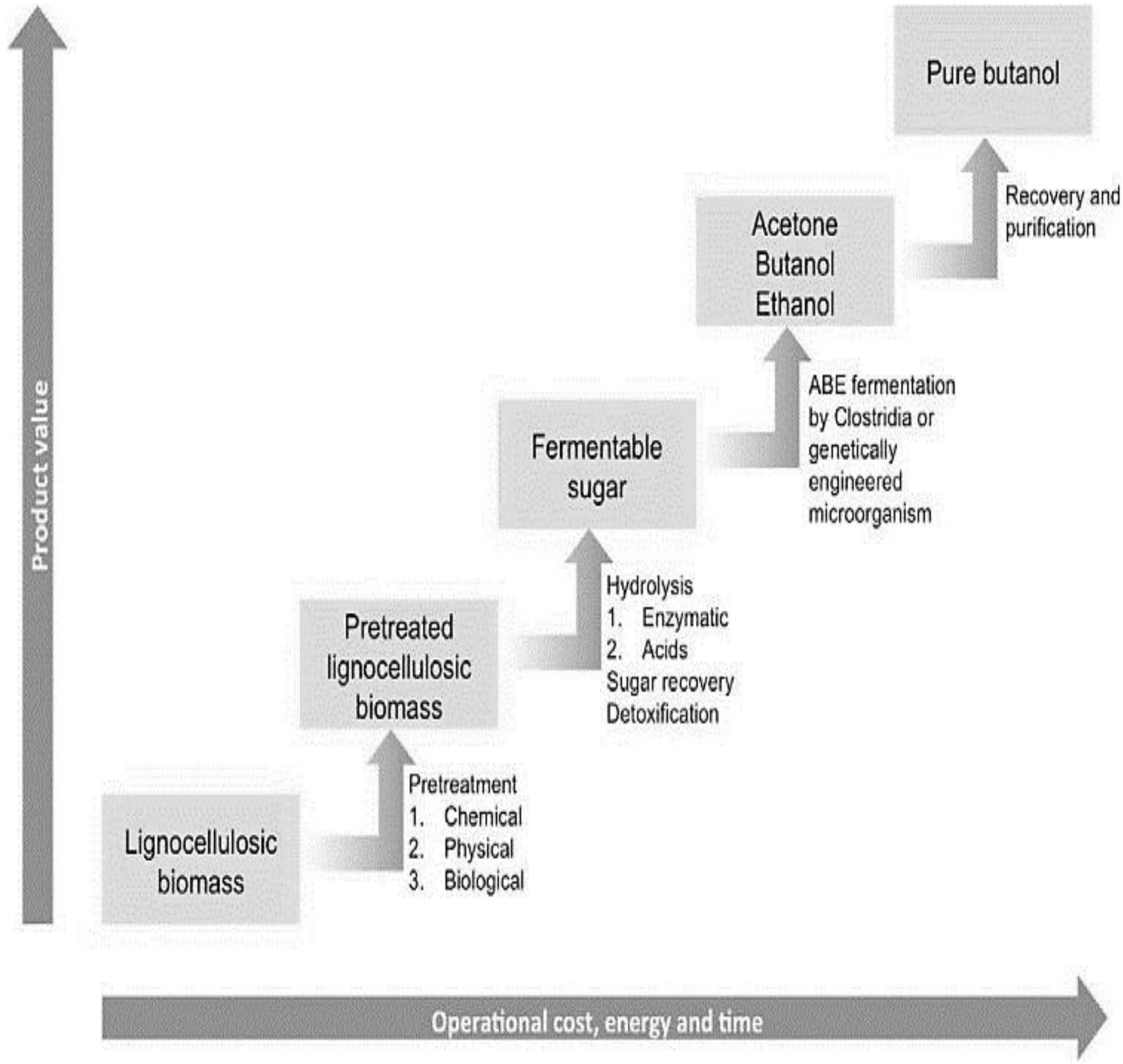

Fig. 2. Biological conversion process of lignocellulosic biomass to butanol[20]

Physical pretreatment techniques are generally used as pretreatment raw material before proceeding to the next procedure or other pretreatment. Physical pretreatment is referred to as a dry process which increases the surface area of the raw material by reducing its particle size[21]. Meanwhile, pretreatment by enzymatic hydrolysis is usually performed optionally for algal biomass, where to increase the release of sugar several times with the use of cellulase enzymes which significantly increase ABE production up to eight times.

There are several factors that affect the efficiency of hydrolysis such as the size of the biomass particles, the crystallinity and degree of polymerization of cellulose, the percentage of lignin and its distribution, the heterogeneity of the reaction, the binding of the enzyme to the surface and the thermal inactivation of the enzyme[22]. Meanwhile, chemical pretreatment of biomass uses several chemical compounds including acids, bases, peroxides, organo solvent. Chemical pretreatment aims for biomass, which has a high lignin content so that it can increase the hydrolysis process[23].

- Acid pretreatment, efficient because it reduces hemicellulose to xylose; part of the lignocellulosic biomass which is rich in xylan content. It can be used either at high temperatures with low solid loads or at low temperatures with high loads. Acidic substances such as H_2SO_4 and HCl[24].
- Alkaline pretreatment facilitates delignification and dissolution of large amounts of hemicellulose but takes longer to release sufficient amounts of sugar. Acid pretreatment is only suitable for low lignin-containing biomass such as hardwood, whereas alkaline pretreatment is more suitable for softwood[25]. Basic substances such as NaOH, KOH, Na_2CO_3, and aqueous ammonia. However, NaOH is more efficient in increasing the internal surface area of cellulose, breaking down lignin and reducing the degree of polymerization and crystallinity of the biomass structure[26].
- The organo solvent process uses organic solvents with inorganic compounds (HCl, H_2SO_4, NaOH) to assist in delignification. The effectiveness of the subsequent processing was significantly increased after the organo solvent pretreatment. However, this is not economically attractive because of the high costs involved in the solvent and detoxification process[27].

Biobutanol can be produced by hydrolysis and microbial fermentation. Hydrolysis is the process of breaking down complex substances into simpler substances by using water to separate chemical bonds from these substance. The hydrolysis process is influenced by several factors, such as: enzymes, heating, particle size, temperature, pH, hydrolysis time, ratio of liquid to raw material (substrate volume), and stirring. The particle size can affect the water solubility rate. The smaller particle size will increase the surface area of the contact area, causing the hydrolysis reaction speed to increase rapidly and increase the reaction conversion. The temperature factor is related to the reaction rate, the higher the hydrolysis temperature, the faster the hydrolysis. This is because the reaction rate constant increases with increasing operating temperature. Enzymes can be isolated from animals, plants and microorganisms. Most agricultural waste containing lignocellulose can be degraded by cellulase enzymes in the hydrolysis process into the simpler compounds, namely glucose[3].

Fermentation is a process of breaking down organic compounds carried out aerobically or anaerobically into simpler organic components, where the resulting product involves microbes with controlled microbial activity, here oxidation and reduction reactions occur using energy sources and sources of carbon, nitrogen and others. While the principle of fermentation, increases the growth and metabolism of microbes and inhibits microbial growthwhich can interfere with the fermentation process[3]. ABE fermentation is anaerobic fermentation that utilizes a carbon source to produce solvents (acetone, butanol and ethanol) and by-products including organic acids (butyric and acetic acids) and gases (hydrogen and carbon dioxide) through anaerobic assistance, generally the metabolic pathway of Clostridia consists of two the different characteristic phases which are acidogenesis (conversion of sugars to organic acids) and solventogenesis (solvent production).

The acidogenic process occurs in the early growth phase (usually within the first 24 hours) while the solventogenic process occurs in the microbial growth phase (usually after 48 hours). During the acidogenic phase, the rapid growth of microorganisms produces cells, biohydrogen, carbon dioxide, butyric acid and acetic acid. This stage is characterized by a decrease in pH due to the rapid secretion of butyric acid and acetic acid into the medium. This acid is mainly produced within 24 hours after fermentation. When the pH decreases further, the metabolism shifts from the acidogenic phase to the solventogenic phase.

During the solventogenic phase, the acid generated from the assimilation phase returns to the cell and further increases the pH value. The carbon source is continuously consumed and converted to acetone, butanol and ethanol[20].

There are some factors that affect the fermentation process which are: fermentation substrate / media which functions to provide the nutrients needed by microbes to obtain energy, a place to growth, helps in cell formation and the biosynthesis of metabolic products; the number of microbes (many microorganisms are inoculated into the fermentation medium). The number of microbes (inoculum / starter) added ranged from 3-10% of the volume of the fermentation medium; water content; pH of the fermentation medium; as well as fermentation temperature. In glucose fermentation by the bacteria *Clostridium sp.*, glucose is converted into a solution of acetone-butanol-ethanol (ABE), remaining glucose, CO_2 gas, and H_2O. In most studies, biobutanol uses anaerobic microorganisms from *Clostridium sp.* which can degrade cellulose and hemicellulose[3].

From table 1 it can be seen that the largest butanol produced is found in the wood pulp substrate. This is because the wood pulp substrate has a high lignin content, which can produce high levels of phenolic compounds formed during pretreatment as well as the presence of a resin adsorption detoxification method which results in reduced inhibitor compounds (furfural, HMF, acetic acid, formic acid, phenolic and levulinic acid) in the butanol fermentation reaction[11].

V. Conclusion

In this article, biobutanol has been discussed as a potential future bio-based alternative transportation fuel. Biobutanol is produced by hydrolysis and fermentation of *Clostridia sp.* The substrate was tested by fermentation with several pretreatments before incubation with predetermined fermentation conditions. The study of substrate after fermentation with *Clostridia sp.* produced biobutanol on each substrate. Where the largest biobutanol produced was found in wood pulp substrate at 13.46 g /L, pretreatment was carried out through alkaline extraction followed by dilute acid hydrolysis and fermentation conditions at 37 °C with resin adsorption detoxification method and gas stripping as a separation method.

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