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Bioconversion of Lignocellulosic Waste to Bioethanol as Alternative Fuel using various Microorganisms

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ABSTRACT: This paper gives an outline of the basic technology for the production of bioethanol by lignocellulose. The earlier existing conventional way consist of two major steps. The first step is pretreatment of the lignocellulose for removal of lignin which increases the extent of agents of hydrolysis without affecting the cellulose and hemicellulose chemically. This is followed by the next steps of conversion of the pretreated material to bioethanol conducted by process of hydrolysis and then fermentation. Methods for improvement of conversion of biomass and enhance cost effectiveness is also discussed in brief. The advantages of microorganisms in the mentioned process are also indicated in this paper.

KEYWORDS: fermentation, bioethanol, hydrolysis, lignocellulose, pretreatment

I. INTRODUCTION

Alcohol, is known from ancient history, to be produced from natural products like agricultural materials which has higher sugar or starch content. Some of the popular examples for this are corn, sugarcane, grains and fruits. The carbohydrate matter grows the microorganisms which are used for converting starches and sugars for production of ethanol by metabolism. As informed by Satari, Karimi & Kumar (2019), the polymers are first hydrolyzed. The polysaccharides are polymers that may be of fructose, glucose, single sugar or xylose also called monosaccharides. As mentioned by Pérez, Munoz-Dorado, De, and Martinez (2002), these are fermented by microorganisms after hydrolysis for producing ethanol. Alcoholic mixtures are obtained by such bioprocesses.

Biofuel is harmless to environment and this feature has taken the world's attention. Another feature of biofuel that the countries around the globe have taken huge interest in. As indicated by Hanaki & Portugal-Pereira (2018), this source is carbon neutral and keeps the balance of the content of air in the atmosphere contributing in a big way against global warming. As indicated by Palmqvist, & Hahn-Hägerdal (2000), use of Biofuel is one of the feasible ways to reduce the usage of the traditional resources of fossil fuel. The fast depletion of the crude oil in past two decades has increased the renewable energy demand including biofuels. The two countries who has taken lead in the production of bioethanol are Brazil and the United States of America by using corn and sugarcane. The biofuel production in this way goes against the food security of the world as it competes with food that is consumed widely. Finding an alternative to starch was the next step to continue the production of ethanol without using any food or edible materials.

Lignocellulosic biomass gives that alternate option for bioethanol production as the cellulose in the lignocellulosic biomass, being a polymer of glucose, has similarity to the sugar and starch. The difference in the glycosidic bonds of cellulose and starch indicates the complexity. As mentioned by Xiong, Reyes, Michener, Maness & Chou (2018), the presence of cellulose in plants is in complex form. In plants the cellulose is incorporated with hemicellulose and lignin. Also, it is to be mentioned that lignocellulose is present in abundance around the globe. These are found in the trees and plants in their branches, leaves, bodies, peels, etc. Considering this, it is reasonable that lignocellulose can be taken as an alternative for the production of bioethanol. This is more so suited for the countries with larger agriculture wastes. Along with this, wastes of forestry can also be utilized as raw materials.



II. LIGNOCELLULOSE

As mentioned by Qiu, Yadav & Yin (2017), similar to starch, hemicellulose and cellulose contains sugar. Majority of the cellulose which we found in nature is in lignocellulose form. The structure of lignocellulose which are present in plants are complex. It is found in abundance making it the largely available organic material which is renewable. It can be obtained at a very reasonable price from the waste produced from agriculture, forestry, agro-industrial wastes. Along with this, the residues that are obtained in the form of brewer's residue, saw dust, shells, poplar trees, husks, sugarcane bagasse, leaves, grasses and straws, stems and similar things can be utilized as lignocellulosic material. Along with these, the peels of barley, corn, sorghum and grains can also be considered for this. Presently the lignocellulosic wastes being generated gets accumulated in large quantities causing pollution and other problems to the environment. As reported by Sheehan (2001), this is taking place year after year despite the increase in its usage for the production of various products.

There are three main components of lignocellulose lignin, cellulose and hemicellulose along with traces of minerals and other extracts. As informed by Shavandi, Hosseini, Okoro, Nie, Eghbali Babadi, & Melchels, (2020), the fiber strands of cellulose link to each other by the bonding of hydrogen in the lignocellulose. These structures of polymers are not homogenous. The lignin and hemicellulose surround cellulose fibers as shown in figure 1. The structural complexity provides a protection to polysaccharides creating a hurdle in the step of hydrolysis carried out by chemicals and enzymes. This comes in the way of production of ethanol both chemical conversion and bio-conversion in the formation of ethanol. Hemicellulose is also a very polysaccharide apart from cellulose in the structure of lignocellulose. It is linear in shape at the same time it is a branched polymer which is heterogeneous and is usually made up of five types of different sugar that are D-xylose, L-arabinose, D-mannose, D-galactose and D-glucose. The basic structure of the hemicelluloses' chains can be heteropolymer that is combination of sugars or can be homopolymer. There are few ways which differs hemicellulose from celluloses. They differ in the units of the sugar. Along with this their morphology at the molecular level is amorphous. The short chains branches from main molecular chains. As informed by Sheehan (2001), this chemical characteristic of the hemicellulose is the reason that hemicellulose can be easily hydrolyzed in comparison to cellulose.

As mentioned by McClelland et al. (2017), lignin has higher molecular weight. They coat the fibrils and play a role of adhesive that bunches the fibers in the lignocellulose. Lignin is heteropolymers which are hydrophobic and have a variable and complex structure. These are comprised of phenylpropanoid alcohols. These alcohols are majorly coniferyl, coumaryl and sinapylalcohol with functional groups of carbonyl, methoxyl and hydroxyl. The ratio of the monomers in the lignin between gymnosperms and angiosperms varies.

As indicated by Terrett, & Dupree (2019), lignin cross links the hemicellulose and cellulose. These may be dissolved in alkaline and acidic solutions. This makes lignin hard which resist the agents for hydrolysis. The removal of lignin, therefore, becomes very important. This method is called pre-treatment. As mentioned by Palmqvist, & Hahn-Hägerdal (2000), pretreatment increases the penetration by the agents of hydrolysis making this method an essential step in the conversion process of lignocellulosic biomass to ethanol.

III. CONVERSION OF LIGNOCELLULOSE TO BIOETHANOL- CONVENTIONAL PROCESS

The chemical expression for conversion of carbohydrate into bioethanol can be given as



As mentioned by Packiam, Subburamu, Desikan, Uthandi, Subramanian, & Soundarapandian (2018), the raw materials as carbohydrate can come from variety of sources like wheat, rice, fruit juices, residues soya, peanuts and breweries. Also from grasses, straw and woods. Figure 2 represents the conventional process flow chart.

Pretreatment, as mentioned earlier, is the process that comes first in order during the bioconversion with lignocellulose as raw material. This pretreated material is hydrolyzed followed by the process of fermentation. For the first step of hydrolysis, both chemicals and enzymatic methods can be considered. The hydrolysis by enzymes is more feasible. The hydrolysis by chemicals can lead to higher contents of salts, alkaline or acid. This increased concentration can interfere with the suitable growth of the microorganisms for the process of fermentation.



IV. LIGNOCELLULOSIC BIOMASS PRETREATMENT

Pretreatment is essential before the next step due to many reasons. First and most important of all that this process breaks the cell wall and segregate the lignin, cellulose and hemicellulose for an easier and better yielding hydrolysis. According to Zhang, Cheng, Niu, Fu, Huang & Ye (2019), along with this, it the fiber's porosity is also increased and it enhances the penetration of enzymes and chemicals in the lignocellulosic structure. The three major methods used for the process of pretreatment are chemical, physical and biological.

PHYSICOCHEMICAL METHODS OF PRETREATMENT

THERMOPHYSICAL METHODS: The methods high – pressure steaming, milling, steam exploding and other similar are used as thermophysical methods. This method reduces the cellulose crystallinity and enhances the contact surface, and also breaks the cross-links partially.

Mechanical processes: These processes are significant for not changing the material's chemical properties. They decrease the size of the lignocellulose biomass and with this increases the contact surface. Grinding, cutting, milling and crushing can be done before other processes of pre-treatment to get the benefit of the mechanical processes.

IONIC LIQUID METHODS: Also called as melting organic salts, the ionic liquid has many properties. As indicated by Sorn, Chang, Phitsuwan, Ratanakhanokchai & Dong (2019), these are cellulose dissolvable, non-volatile and highly durable thermally.

CHEMICAL METHODS

As mentioned by Farid, Shaker & El-Diwanly (1983) and supported by Szczodrak, Llczuk, Rogalski, & Leonowicz (1986) chemical methods of pre-treatment by alkaline and acidic solutions are one the result yielding for the production of bioethanol by lignocellulosic as the dissolvability of lignin is very effective. HCl and H₂SO₄ are commonly used for the chemical pretreatment of lignocellulose. Treatment with alkali supports in removing groups of acetyls at the same time it enhances the next step which is hydrolysis. As indicated by Chang, & Holtzapple (2000), an advantage with the pretreatment with alkali is that it reduces the carbohydrates loss that may happen due to hydrolysis. It also inhibits the formation of furfural. Calcium-hydroxide is used for pretreatment with alkali as it costs lesser but is lower in efficiency and precipitation. As reported by Kim & Holtzapple (2006), the chemical sodium-hydroxide, however is the most widely used alkali as it costs low and is high in efficiency.

BIOLOGICAL METHODS

As informed by Rolz, Leon, Arriola & Cabrera, (1986), Mes-Hartree, Yu, Reid & Saddler (1987), Forough, Itoh, Wada, Honda, Kuwahara & Watanabe (2003) and Dzulkefly, Norhafizah, Nazila, & Zamiri (2013), one of the most appealing methods of pre-treatment is by the use of microorganisms as it has many advantages. As mentioned by Zabeed et al. (2019), the most attractive feature of using microorganisms is that it is very cost effective in terms of operative expenses and equipment. Some microorganisms like *Ischnoderma*, *Phlebia*, *Pleurotus*, *Pycnoporus* and others had been developed on lignocellulosic material for utilization of the metabolism of microorganisms for the destruction of crystallinity of the cellulosic material and enhance the removal of lignin.

PRETREATMENT METHODS COMBINATION

The process of pre-treatment can be conducted by employing any one of the methods. As displayed by Deng, Liu, Endo & Yamamura (2018), a combination, however, may provide better yielding outputs. Conventionally, the mechanical process is brought in first which reduces the size of the lignocellulosic biomass material. Crushing, cutting and milling are some of the methods that can be used. As informed by Mes-Hartree, Yu, Reid & Saddler (1987), this is followed by the explosion of steam which is then soaked in acidic or alkaline solutions for the chemical method. As informed by Tran, Vu, Nguyen, Le, Phan, & Mochidzuki (2013), alkaline is



preferred over acid. The pretreated material is then washed and neutralized. As indicated by Nguyen, Le & Tran (2018), the next step after this is puffing the material this makes the material ready for further processes.

V. LIGNOCELLULOSE CONVERSION TO BIOETHANOL

The lignocellulosic biomass which is now pretreated is processed for the next step, conversion to bioethanol. As informed by Wyman (1994), this conversion can be by the action of the microorganisms for direct conversion which involves hydrolysis and fermentation simultaneously. As mentioned by Herrero & Gomez (1980) and supported by Zertuche & Zall (1982), this method takes longer time and the output of the conversion are also comparatively lower along with increased contamination in the yield (34,35). On the other hand, the combination process where the hydrolysis is enzymatic and fermentation is microbial is found to have given better performance. The combination process is more preferred due to this reason.

VI. LIGNOCELLULOSE SACCHARIFICATION

Once the process of pretreatment is effectively completed, the material is enriched with polysaccharide and these are then hydrolyzed with enzymes to produce sugars (pentoses and hexoses). As presented by Willberg-Keyriläinen, Ropponen, Lahtinen & Pere (2019), the enzymes used here are obtained commercially for hydrolysis of hemicellulose and cellulose. These enzymes are mixture of several enzymes which are extracted from various microorganisms. This is called as cellulase. As informed by Beckham et al. (2014), the glycosidic links in the carbohydrates are broken by the enzymes usually by retaining or inverting mechanisms.

Cellulose is comparatively stable with a structure that is crystalline and is also resists the depolymerization in comparison to hemicellulose which is easily hydrolyzed. As mentioned by Hammerer et al. (2018), there are various enzymes that are utilized widely for the process of hydrolysis of cellulose. One of such enzymes is β -D-glucosideglucohydrolase or β -D-Glucosidase (EC 3.2.1.21) for the formation of D-glucose in cellobiose, along with this glucose oligomers. Another such enzymes is 1,4- β -D-glucan 4-glucohydrolases or Endo-1,4- β -glucanases (EG) (EC3.2.1.4). The bonds of 1,4- β -glucan are randomly broken by this enzyme. Apart from these the other enzymes which is used is the Exo-1,4- β -D-glucanases which includes glucohydrolase of 1,4- β -D-glucan (EC 3.2.1.74). This is used to liberate D-glucose from the 1,4- β -D-glucan and then gradually conduct hydrolysis of D-cellobiose. As reported by Chosdu, Hilmy, Erlinda, & Abbas (1993), the enzyme cellobiohydrolase of 1,4- β -D-glucan (EC 3.2.1.91) (CBH) liberates cellobiose from the 1,4- β -glucan.

The enzyme activity of cellulase enzyme is measured by different methods. The standard method for the measured by is described by the National Laboratory of US Department of Energy Office of Energy Efficiency and Renewable Energy (NREL) in its technical report NREL/TP – 510 – 42628. The hydrolysis from enzymes has various advantages, one of which is the growth of the microorganisms in the process of fermentation. This progresses despite the difference in the optimal temperature of the two processes is little.

VII. LIGNOCELLULOSE FERMENTATION

Fermentation of hydrolyzed material is the next step in the conversion of lignocellulosic biomass to ethanol after the processes of pretreatment and hydrolysis. As mentioned by Sklyar & Gerun (2020), microorganisms are brought to have implication on the sugars which have been freed during the process of hydrolysis to metabolize them. There are two ways for the microbial fermentation. As indicated by Maslova, Stepanov, Senko & Efremenko (2019), one is to have the process of hydrolysis completely and the introduce the micro-organisms to the same mixture and for the fermentation of the liberated sugars. This approach is called “Separate hydrolysis and fermentation (SHF)”. This may have some weak areas like contamination of the mixture, inhibitors formation, longer time duration and requirement of some extra equipment.

As informed by Gumel et al. (2018), another approach is to have both the processes of saccharification and fermentation together. This approach is known as “Simultaneous Saccharification and fermentation (SSF)”. Here, the processes of hydrolysis and fermentation by the microorganisms are undertaken same time and in same equipment. The mixture is loaded with both the elements of the enzymes and the microorganisms. As indicated by Kadhum, Mahapatra & Murthy (2019), the output obtained in this approach is considered better than the earlier mentioned SHF. As the time taken to receive the yield is shorted that SHF. Along with that, the SSF approach consumes lesser resources of equipment and additionally, the contamination risk in comparison to SHF is lesser. As indicated by Zaini,



Chatzifragkou&Charalampopoulos (2019), this approach is currently accepted widely and is regarded as optimal for the conversion of lignocellulose material to bioethanol and is reported to give higher yield of the conversion process. Some constrains have been reported in this approach which include the optimization of the temperature. As mentioned by Zhang, Wei, Mo, Zhou, Chen & Ouyang (2018), the required temperature for the hydrolysis by enzymes ranges from forty-five to fifty degree Celsius while the process of fermentation displays best efficiency when taken place at the temperature that ranges from twenty-eight to thirty-five degree Celsius. Along with this, the other recognized constrain is the formation of intermediate products that may provide resistance to the growth of microorganisms. There are various microorganisms, which can be used for increasing the fermentation output. These microorganisms excrete ethanol by metabolizing sugars. *Saccharomyces cerevisiae* is a widely accepted microorganism for fermenting the sugar solution and yield bioethanol. *Saccharomyces cerevisiae* is known to have higher tolerance against the concentration of ethanol and the inhibitors of the materials. This microorganism is familiarly in use traditionally and is most preferred among the other yeasts for the production of bioethanol. The process of fermentation contains an additional step which an addition of a nutrient to the mixture. This nutrient is added to give source of organic nitrogen to the microorganisms to grow. Urea, corn steep liquor (CSL), peptone and sometimes the distilled residue obtained from the process of production of bioethanol is investigated.

As mentioned by Alalwan, Alminshid&Aljaafari (2019), the production of bioethanol from lignocellulosic biomass still has much scope of being more cost-effective. There are few ways to make the complete process more efficient one of which is to use more than one microorganism and use them in consortium. According to Gírio, Fonseca, Carvalheiro, Duarte, Marques, & Bogel-Lukasik (2010), this is effective as both pentose and hexose are consumed for yielding bioethanol outputs. The consortium further added to the mixture of fermentation as a process of simultaneous saccharification and co-fermentation method (SSCF).

VIII. CONCLUSION

The fossil fuel is depleting at a rapid rate resulting in price rise. The alarming levels of the global warming is also a major concern around the globe. This has given rise to look for alternative source of fuel which is environmentally friendly. Fermentation of natural raw materials to make fuel is bioethanol. This conversion from lignocellulosic biomass does not compete with food product unlike sugar cane, maize and similar other yields. Use of lignocellulosic biomass as a raw material require a step of pre-treatment before moving further to saccharification and fermentation. Pre-treatment is required to break the cell wall and free the lignin, cellulose and hemicellulose for effective hydrolysis. Introduction of microorganisms for these processes makes the production more ecofriendly and reduce the cost expenses. There are various studies undertaken by the scientists around the globe for an enhanced production and at a reasonable cost. These studies and further being explored for an expansion at a commercial scale.

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