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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/15109
DOI URL: http://dx.doi.org/10.21474/IJAR01/15109

RESEARCH ARTICLE

BIOCONVERSION OF LIGNOCELLULOSIC BIOMASS TO ETHANOL USING DIFFERENT MICROORGANISMS

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Manuscript Info

Manuscript History
Received: 30 May 2022
Final Accepted: 30 June 2022
Published: July 2022

Key words:-
Fermentative Microorganisms, Lignin, Lignocellulose, Fungi, Lignocellulolytic Enzymes, Biological Pre-Treatment

Abstract

Lignocellulosic material that includes hemicellulose, cellulose and lignin (lignocellulosic complex) is present in the plant cells. The hydrolysis process of the lignocellulose biomass into glucose in the presence of lignocellulytic enzymes is an area of concern in the production process of cellulosic biofuel. Microorganisms like fungi have the ability for degrading the plant cell wall by an enzyme set which acts in coordination. This moves in a direction to release glucose freely. Another challenge is the modification in the plant cell architecture. Along with this, the capacity of microorganisms in degradation by the modification of the genomes is also one of the challenges. The advantage of the biological process of pre-treatment for degradation of the lignocellulosic materials is because of its effective enzymatic system. There are two types of enzymatic systems which is of extracellular nature in fungi. These are hydrolytic and ligninolytic systems. Hydrolases are produced by hydrolytic system which degrades the polysaccharide and produces sugar. The exclusive oxidative advantage and the extracellular ligninolytic system degrades the components of lignin and also opens the rings of phenyl. The reducing sugars are then converted in ethanol production with the use of various fermentative microorganisms. In this paper, the bioconversion of lignocellulosic biomass to ethanol using different microorganisms is discussed along with other relevant aspects.

Introduction:-

Petroleum is a very important natural resource and it is depleting at a rapid rate as its consumption is high around the world (Pop 2018). The development of alternative sources of energy is the area of increasing study as the it may provide options for replacement of fossil fuel used for transportation. (Alper and Stephanopoulos 2009; Gupta et al. 2009). Biofuel has been found to have many advantages like less pollution, low on toxicity, biodegradability. It also produces lesser pollutants which are air-borne than those produced by current options of fuel such as petroleum. Ethanol with the mentioned benefits has an advantage of being easily integrated in the present vehicles (Campbell et al. 2019; McCarthy and Tiemann, 1998). Lignocellulosic biomass denotes a huge amount of renewal resources which are carbon neutral for the Bioethanol production (Ragauskas et al. 2006). Lignin, hemicellulose

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and cellulose are the main components of lignocellulosic biomass (Yu, Paterson, Blamey &Millan 2017; Kuhad et al. 1997). A bioconversion process is important for this which has three major steps. The first step is pre-treatment, followed by hydrolysis and finally fermentation. The process of pre-treatment is required for the removal of lignin and hemicellulose fraction. This increases the porosity of materials and also decreases the crystallinity of cellulose. Biological pre-treatment by microorganisms like fungus (brown, white and soft rot fungi) and bacteria has been conducted by researchers. It has been displayed that this increases the productivity of the hydrolysis and the generation of the inhibitors are low and the utilisation of the energy is also shown low. (Wang et al. 2013; Taha et al. 2015; Hatakka2005). Along with this the biological pre-treatment is also found to be substantially reasonable in terms of cost effectiveness in comparison to other processes of pre-treatment. These biological pre-treatment processes have been comparatively investigated in lesser numbers (Chaturvedi and Verma2013). In the degradation of lignin, white rot fungi for the soft and brown fungi has been the most efficient which attacks cellulose directly among others in the methods of pre-treatment (Kang et al. 2013; Fernandez et al. 2012; Suhara et al. 2012). In addition to these, the white rot fungi also cause lesser damage to the environment and lesser consumption of energy (Narayanswamy et al. 2013; Kumar et al. 2015). Their enzymatic system is very efficient which is the main reason of their ability for biological pre-treatment in the degradation of the lignocellulosic. The effectiveness of the microorganisms along with the white-rot fungi (WRF) is studied in this paper for the process of biological pre-treatment and further in the process of fermentation for the production of bioethanol.

The ethanol biofuel- a brief history

The modern history has witnessed the usage of ethanol as fuel in various ways (Dagle 2020). It is to be mentioned that the fuel used in the invention of ignition engines was bioethanol. Ethanol was also used as illuminators which was used in 1850s. Approximately ninety million gallons of ethanol production were seen in the United States of America. The changes in its status was seen after the imposition of the tax on the ethanol. This was done to provide assistance in the finances during the civil war. Ethanol was replaced as premier illuminant in 1860s by kerosene which became more affordable and cheaper (Rasmussen 2019; Morris1993). Subsequently, in the year 1906, the tax on alcohol was lifted. This brought back the inclination in ethanol. In a breakthrough development, Henry Ford designed an automobile car of Model T which ran on ethanol in the year 1908. The production of ethanol was sufficient by the year 1914 and it reached a supply of approximately ten million gallons but petroleum emerged as a fuel in 1919 putting ethanol at the backseat and consequently the production and usage of ethanol as fuel reduced again. Later, The World Was II brought back the increase in production of ethanol in the years of early 1940s. It was required for making the synthetic rubber. An annual production of about six hundred gallons of ethanol was produced in the United States of America during this time period (Morris1993).The demand of ethanol had decreased by the end of the second world war and it continued to reduce for two more decades. This was mostly due to the petroleum imports which were cheaper. This again witnessed changes from the year 1973 due to the oil embargo by the Arab countries. This resulted in the distinct enhancement in the prices of gasoline (CampbellandLaherrere1998). From the early years of 1970s the shortage and fast depletion of the petroleum has increased the concerns. The crude oil prices have been rising since then. The political instability in different countries around the globe have further added to the concern related to the fuel consumption and prices. With these factors, biofuel is yet again being considered as an alternative biofuel worldwide.

Bioethanol Generations: First and Second

Bioethanol First generation

The bioethanol of first generation was produced by the sugar fermentation like fruit juices, sugarcane juices, sugar beet juices, molasses, and other similar sugar sources. In addition to these, starchy feedstocks like potato, corn and wheat were also utilised for the same (Antonietal.2007; Baig et al. 2019). The methods used for the production of ethanol in the first generation utilized the enzymatic digestion methods for releasing sugars from the stored starches. This was followed by processes of the sugar fermentation, distillation method and finally drying. As indicated by the Energy Information Administration (EIA) 2008, the bioethanol of the first- generation had a substantial part in the formation of the policy drivers and the related infrastructure which was needed for the renewal fuels to support the transport in the global market. The International Energy Agency (IEA) 2008, however has expressed various concerns over the related drawbacks of the bioethanol of first generation. Some of these concerns were, impact on environment, Competition between food vs fuel, Multi-feedstock flexibility and similar other factors.

Bioethanol: Second generations

Itis, now, understoodthatthebioethanol production in the first-generation had an approach which was unsustainable (Branco et al. 2019). Theincreasingcriticisms also brought forward the usage of crops which were nonfoodforsecond-generation bioethanol production. This production was from the lignocellulosic biomass which comprised of theresidual from the parts of the crops which were non-foodparts. The other crops which were notutilisedfor the purposes of foodalong with the industrial, municipal and constructionwaste. It is expected to decrease the net emission of carbon, enhance the efficiency of the energy and also decrease the dependency on energy. It would also help to overcomethelimitations of biofuels from the first-generation (Antizar et al. 2008). Along with these the other benefits can be seen in the long-term sustainability and the nature of ethanol which is renewable. Due to these advantages the switching of existing options to cellulosic ethanol is recommended (IEA,2008). There is scope for muchwork in the areas of improvement of the technology for the second-generation biofuel. Along with this the reduction of the production cost and further improvement in the reliability and performance in theconversion process are also to be looked at, moving ahead further.

Plant Biomass

Plantbiomass is a material that is present in abundance on the planet around the globe. These are available as a naturalresource which arerenewable (Prather et al. 2020). Lignocellulose contains 3 mainpolymers which are lignin,cellulose and hemicellulose (Figure 1). These are together called as lignocellulose.Their chemical properties make them a substratefor extensive usage in ht products related to biotechnological processes(KuhadandSingh,1993; Kuhadet al.1997;Kuhadet al.2007).

Cellulose

Cellulose is a glucan polymer of D-glucopyranoseunits, thatare linked with each otherby β-1,4-glucosidicbonds (Mansora et al. 2019).Thewoodcellulosehasanaveragedegree of polymerization (DP) of at least 9,000–10,000 and possibly as high as 15,000. An average DP of 10,000 corresponds to the linear chain length of approximately 5 µm in wood. An approximate molecular weight for cellulose ranges from about 10,000 to 150,000 Dalton (Goring and Timell1962).

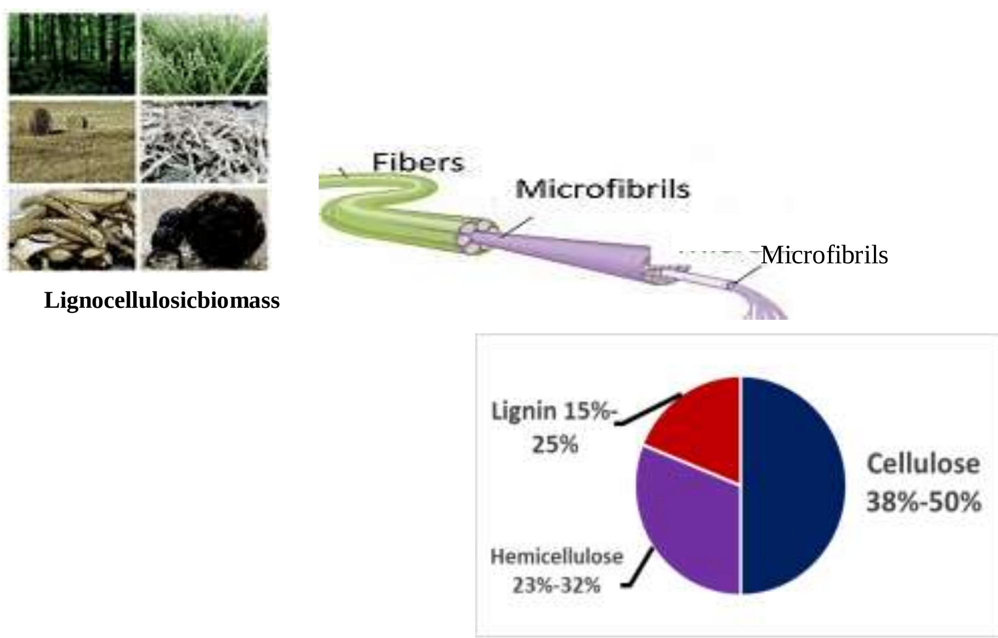

Figure1:- LignocellulosicBiomass components.

Bioconversion – ethanol from lignocellulosicbiomass

The bioconversion to obtain ethanol from lignocelluloses is comprises of 2 major processes (Singh et al. 2018). The first process is the hydrolysisof lignocellulosiccarbohydrate. It converts the lignin to the reducing sugars for further fermentation. The second process is fermentation of the reducedsugarsto the bio-ethanol. The initiation of the process of hydrolysis is generally catalysedby the cellulaseenzymes whereas the process of fermentation isby yeasts or bacteria. Hydrolysis of cellulose can be affected by many factors. Some of these factors may include the crystallinity of cellulose fibre, the porosity (accessible surface are) of the waste material and the content of hemicellulose and lignin(Mosieret al.2005; Kuhadet al.2011a). The access of cellulase enzymes is difficult due to the presence of lignin and hemicellulose in the lignocellulosicmaterials. This reduces the hydrolysis efficiency (Himmelet al.2007). The hydrolysis efficiency is significantly improved due to the process of pre-treatmentoflignocellulosicbiomass carried out before hydrolysis. This is because of the hemicellulose and lignin is removed and the crystallinity is also reduced in the process. Along with this, the porosity is also increased (McMillan,1994;Mosier et al. 2005; Kuhad et al., 2011 a).

Pre-treatmentof lignocellulosic biomass

The advantages of the strategies of pre-treatment process are well received and recommended from a long time (Mosieret al.2005;SanchezandCardona,2008; Kuhadet al.2011a). There are certain requirements which are to be met by the strategies for the pre-treatment (Jung & Kim 2017). One of the requirements for the process of pre-treatment in improving the sugar formation or the capacity to further form the sugars by the enzymatic hydrolysis. Next requirement for the process is avoiding the loss or degradation of the carbohydrate. Similarly, avoiding the hydrolysis formation and the by-products that inhibit fermentation is another requirement. In addition to this, the cost-effectiveness of the process is also required to be looked at. There are many biological, chemical, physio – chemical and physical processes that is being used for the pre-treatment of the lignocellulosic materials.

Bioconversion - Hydrolysis and Fermentation

The process of pre-treatment is necessary to enhance the process of hydrolysis of lignocelluloses and subsequently ethanol production. Biological delignification of the materials from plants has several advantages over the pre-treatment methods conducted by mechanical and chemical methods (Kocher el al. 2017). These advantages include lesser reactor resistance on pressure and corrosion, mild conditions of the reaction, lesser side reactions, fewer energy demand, avoidance of the usage of the toxic and corrosive chemicals and higher product yield. (Lee, 1997; Kuhar et al., 2008; Sanchez, 2009). The delignification from microbes seems to be the most feasible method for an improved output of the depolymerization of cellulose and hemicellulose. One of the most effective microorganisms for the pre-treatment carried out biologically are the white-rot fungi (WRF) due to their property of degrading the lignin in a more extensive and in a rapid way in comparison to the other groups of microorganisms.(Eriksson,1993;Kuhadet al.1997;Kelleret al., 2003;Kuhadet al.2007). Few WRF are reportedto selectively degradelignin. The ability to some WRF have the opportunity to be utilised for the delignification of the plant materials without having much impact on the cellulose (Kuharet al.2008;Gupta et al.2011b). Therefore, it is to be mentioned that the selected WRF that degrades lignin and has relatively lower xylanase and cellulase activities can be of advantage in the effective delignification. This can also have an advantage further in reducing the energy and chemical inputs for enzymatic or chemical hydrolysis of the related material.

There are two types of extracellular enzymatic systems of fungi. These are ligninolytic and hydrolytic systems. Hydrolases are produced by the hydrolytic system (Asif et al. 2017). These are responsible for producing sugar by degrading polysaccharides. This exclusive character of being oxidative and the degrading of lignin component by the extracellular ligninolytic system opens the rings of phenyl (Lundell et al. 2010). The fungi WRF produces one or more of such cellular enzymes. These are important for the lignin degradation. They produce major hydrolytic enzymes like xylanase, amylases, and others. Heme contains manganese peroxidase (MsP), Laccaase contains copper (Lac), lignin peroxidase (LiP), and also aryl alcohol oxidase (AAO) (Rosales et al. 2002; Elisashvili et al. 2006). These significant enzymes of the fungi WRF are very important in the productive and efficient bioconversion of plant residues (Radhika et al. 2013; Songulashvili et al. 2006).

Some studies have mentioned that WRF is effective in the pre-treatment of the plant biomass on the cellulose hydrolysis. As mentioned by Hatakka(1983), the percentage of conversion of wheat straw to reducing sugar after the pretreatment with Pleurotusostreatusfor a time duration of five weeks were found to be thirty-five. Taniguchi et al., (2005)also reported the similar rate of conversionin the material obtained from ricestraw when pre-treatedwith the microorganism P.ostreatusfor a time span of sixty days. Similarly, Keller et al., (2003) also recorded an improvement from three to five times in the digestibility of the enzymatic cellulose in the corn-stover pre-treatment with Coriolusversicolor in a time span over thirty days.The longer incubation time periods have impacted the fungal pre-treatments negatively(Mishra 2018). There is a requirement for more tests on the basidiomycetous fungi to validate for economizing the microbial pre-treatment of the lignocellulosic materials, for improving the hydrolysis of carbohydrates into reducing sugar and for improving the yield of ethanol (Kuhad et al., 2011a).

There are studies conducted in different combinations of other technologies of pre-treatment along with biological method of pre-treatment. (Itoh et al., 2003; Balan et al., 2008).Itoh et al. (2003) indicated ethanol is produced by simultaneous saccharification and fermentation (SSF) from the chips of beech wood after the pre-treatment by bio-organosolvation from the process of ethanolysis with WRF, Coriolusversicolor, Ceriporiopsissubvermispora, Pleurotusostreatusand Dichomitussqualens.

The ethanol yield produced was found to be o.294 g/g pulp of ethanolysis and an amount of 0.176 g/g of wood chips were obtained with the pre-treatment by C. subvermispora for a time period of eight weeks. This output was 1.6 times more in comparison to the amount received in absence of the treatments with fungus. This combination in the processes enabled the separation of hemicelluloses, celluloses and lignin with the usage of WRF, ethanol and water only. The biological methods of pre-treatments curtailed about 15% electricity that was required for the process of ethanlysis. Balan et al., (2008) indicated in their studies that the impact of trearmtent by fungas on rice straw followed by the pre-tretment method of AFEX and hydrolysis by enzymatic process. The study reported that the treatment on the rice straw by WRF, followed by AFEX showed distinctively larger conversions of xylan and glucan. Similarly, it is found that the process of SSF with Lantana camaraand Prosopisjuliflora which is followed by hydrolysis by acid is observed that the fungal treatment distinctly decreases the inhibitors produced and further decreases the detoxifying agent (Gupta et al., 2011b).

The biomass, rich in cellulose, which is pre-treated is followed by the process of hydrolysis. This is conducted with a combination of cellulolytic enzymes which are β-glucosidase, endocellulase and exocellulase. These enzymes act together and hydrolyse the polymers of cellulose to simpler sugars called glucose (Zhangetal. 2006;Kuhadet al.2011a). The process of hydrolysis by enzymatic action has shown enhanced results for the further process of fermentation due to the non-formation of the degradation parts of glucose. The process, however is not cost effective (Sanchez andCardona 2008). Many methods for the better output and other improvements are being tried from past years for decreasing the cost of enzymes, re-usage of the enzymes, increased production od the enzymes and use of genetically engineered advanced systems (Zhanget al. 2006;Kuhadet al.2011a). It is to be mentioned that the need for searching a better and more competent solution still persists.

As lignocellulose has both pentose and hexose sugars, a pre-requisite is there for the efficient fermentation of both the sugars for cost-effectiveness of the bioethanol production. There are many microorganisms which are known for the fermentation of the hexose sugar whereas there are limited numbers of microbes for the fermentation of pentose sugar. Pachysolentannophilus, Pichiastipitis and Candida shehatae are some of the most common microbes for the fermetation of pentose sugar (Abbietal.,1996a, b; Kuhadet al.2011a). The reports suggest that the mentioned yeasts have not been very promising with the output received and there are increased steps in the direction of the utilising all the sugars in the hydrolysates by engaging both the genetic manipulations and also the improvement in the approaches towards the process implementation. At present, the common commercially available cellulases are produced from the micro-organisms Trichodermareesei, Phanerochaetespand Aspergillussp(Adney et al. 2003). These are mostly used for the description of the mixture the cellulolytic enzymes. The coordinated action of which is needed for enhanced breakdown to monomeric units of its substrates.

The actions of endoglucanases (endo-1, 4- β -glucanases, EGs) in combination is involved in the action of cellulases. These can hydrolyse the internal bonds in the regions of cellulose amorphous with the release of newer ends of terminals. This attacks randomly the internal linkages of β1,4-linkages cellobiohydrolase (exo-1, 4 β --glucanases, Cbhs). These act on the endoglucanase which generates the chain ends. Amorphous cellulose is degraded by both the enzymes with few exceptions. The only enzymes which efficiently degrades the crystalline cellulose are Cbhs. Cellobiose molecules are released by Cbhs and EGs. This gives the units of cellobiose from the non-reducing ends of the β 3-glucosidase and glucon. This hydrolyses glucose from cellobiose. Hydrolysis can be carried out on the pre-treated substrate with the microorganism and this can be followed by fermentation to produce ethanol which is utilised as biofuel. The use of biomass in producing the bioethanol has hope in providing an indigenously produced, renewable energy source which may be viable option for the fuels that are based on petroleum.

Conclusion:-

There are lignocellulosic materials in the plant cells and these lignocellulosic materials are made up of lignin, cellulose and hemicellulose The process of pre-treatment of the lignocellulosic biomass before the process of hydrolysis distinctly enhances the hydrolysis efficiency because of the lignin and hemicellulose removal and cellulose reduction. The microorganism degrades the cell walls of the plants by an enzyme set, especially fungi. There are two types of extra-cellular enzymatic systems in fungi. These are hydrolytic and ligninolytic systems. Hydrolases are produced by the hydrolytic system and the lignin component is degraded by the ligninolytic system.

The ligninolytic system also opens the phenyl rings. Thewhite-rotfungi(WRF), among other microorganisms areeffectivefor the pre-treatment done by biological methods. This is due to the reason that thelignin is degraded more rapidly and extensively in comparison to the other related microorganisms. The activities of the lignin-degrading fungi WRF has relatively lower cellulaseand xylanaseactivities. This is advantageousfor an efficient process of delignification. This consequently leads to the reduction of energy and chemical inputs for enzymatic or chemical hydrolysis of the related substrate.

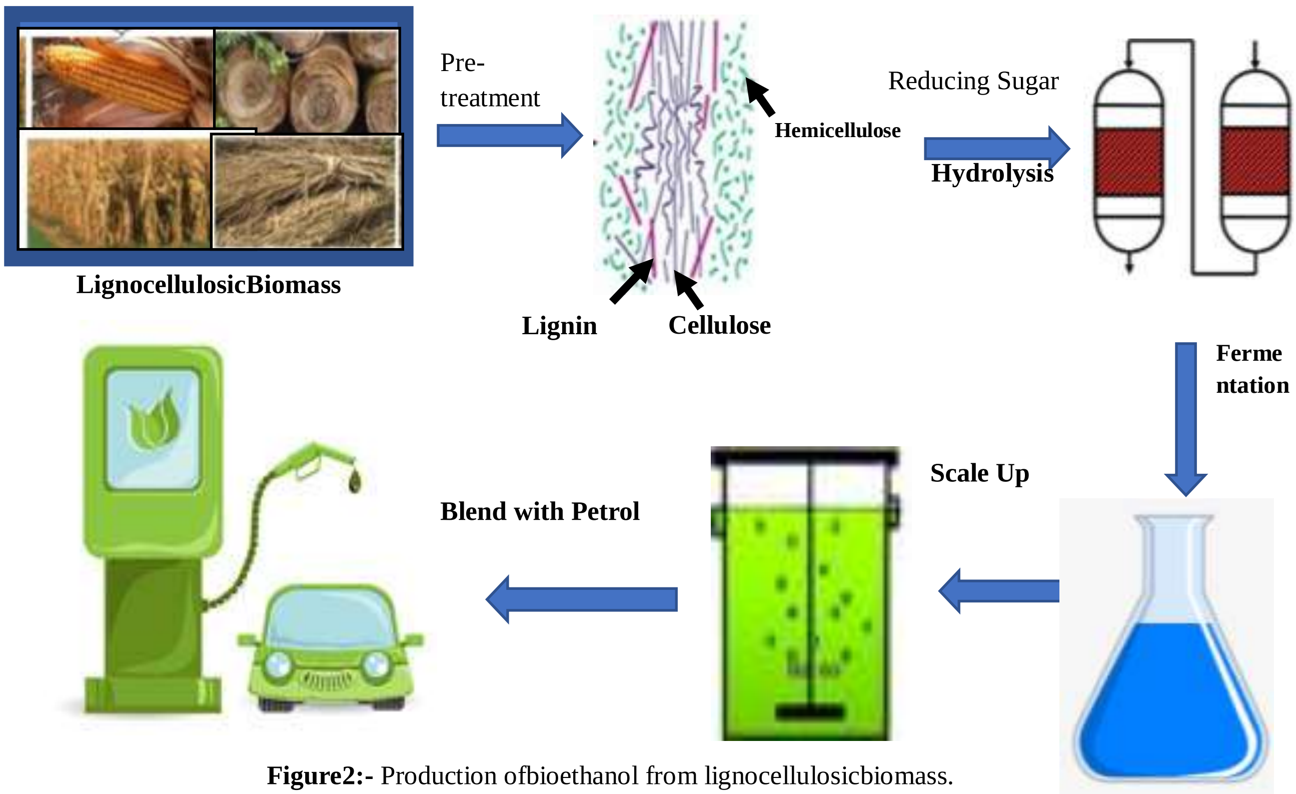

Figure2:- Production ofbioethanol from lignocellulosicbiomass.

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