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Bioethanol: A Sustainable Liquid Fuel as Substitute to Gasoline

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Abstract: Fossil fuel dependence is a growing concern due to its contribution to greenhouse gas emission, climatic change and environmental pollution. This highlights the urgency for alternative source of energy that is renewable, environmental friendly, stability in price, and attractive for sustainable development. Bioethanol, a biofuel has emerged as the most acceptable liquid fuel and as a promising alternative to gasoline. Bioethanol, derived from sugars and starch, has raised sustainability concern as it can lead to competition for land use and potentially driven-up food prices especially in developing countries. Meanwhile, Lignocellulosic biomass, a non-food resources, abundant in cellulose and hemicellulose, present a more sustainable feedstock for bioethanol production. This approach could offer advantages like affordability, environmental friendliness, reduce reliance on traditional fuels and compensate for fuel scarcity. Furthermore, bioconversion technology of lignocellulosic biomass to bioethanol is required to improve its efficiency and cost effectiveness, making it a highly attractive option for a greener energy in the future.

Keywords: Bioethanol, renewable energy, lignocellulosic biomass, biofuels, gasoline.

I. INTRODUCTION

The non-renewable source of energy serves as resources for the global economy which can be harnessed to meet up the challenges of energy demand, Increasing in population leads to utilization of fossil fuel, causing a reduction in this source of energy (Muhammad and Saha, 2022). Fossil fuel is the major source of electricity and is responsible for the generation of greenhouse gases, causing depletion of ozone layers (Muhammad and Saha, 2022). It has environmental, socioeconomical effects against UN SGDs.

In a bid to satisfy the world demand on energy, government are encouraging the use of renewable resources to produce approximately 2000billion tone per year of bioethanol. Lack of knowledge on the importance of these resources, finances, and skills for biofuel production, causes lots of incessant waste disposal and leads to pollution of the environment and health challenges. However, the major disadvantages of biomass is the presence of lignin which makes it not easily degradable. Various pretreatment is required to cause release of fermentable sugars.

The concern on energy security, negative impact of fossil fuels on the environment, has put pressure on society to look for an alternative source of energy. Moreso, in millions of years non-renewable fossil source will be depleted. Hence, the cost of finding fossil fuels deposit will be too expensive. This petroleum crisis has lead to interest in alternative power or fuels produced from biomass. These biofuels are usually in form of bioethanol, biodiesel, biogas and other chemicals (Di Nasso et al., 2021). Energy from renewable substrate has advantages over fossil fuel which is environmental friendly, productive, affordable (Dev et al., 2020).

Bioethanol, an acceptable renewable liquid fuel, very attractive and substitute to gasoline world wide (Efemwenkikie et al., 2019). Fig 2 illustrates the profile between 2015 to 2021 of bioethanol production in various countries (Hoand and Nighian, 2021).

For over ten years, 80% of biomass energy is utilized for heating, cooking and electricity generation in the developing world. In the North America, Africa, ethanol is obtained from corn to reduce use of gasoline. At present, biomass contains a little fraction of carbon which causes its limitation to large scale. In the next 20years, there is going to be a transition to a more complex bio-renewable feedstock such as algae, green plants, municipal waste to replace petrochemical products.

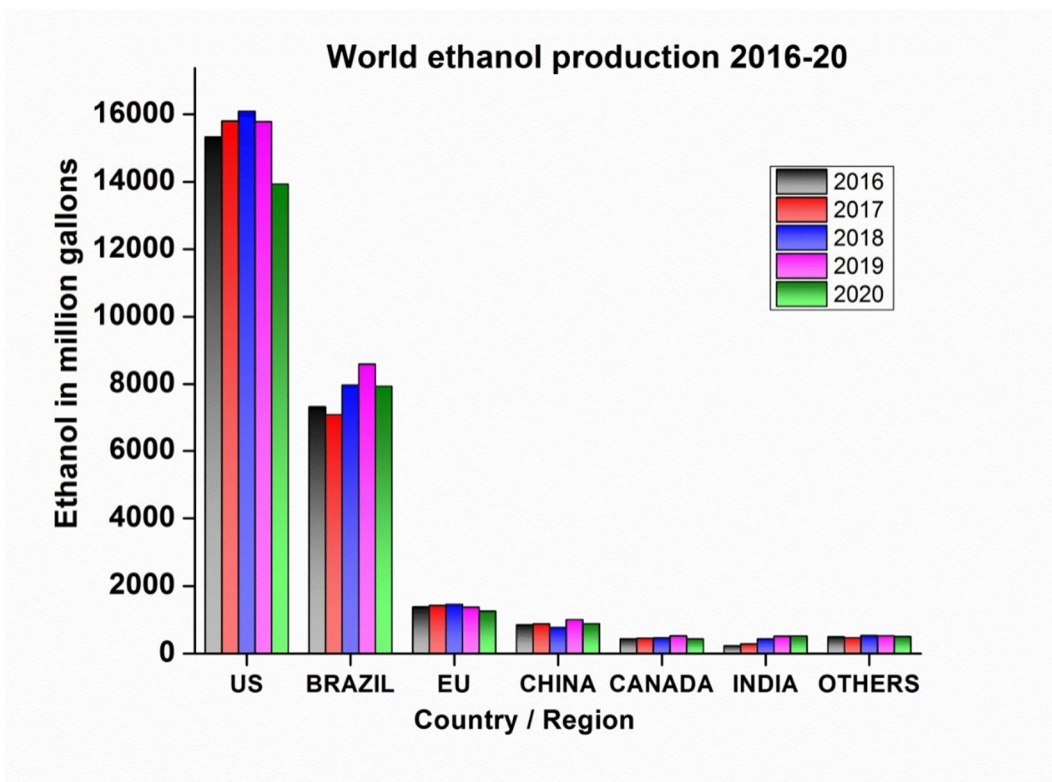


Fig 1: Ethanol Production between 2016-20 in the world

The increase in energy and other factors is depleting the energy reserves of fossil fuel. In the year 2025, the energy demand may increase by almost 50 %. The first generation of bioethanol constitutes over 27 million gallons of fuel produced world as of 2021, with United State of America and Brazil being the indisputable leaders producing almost 85 % of the global output from corn and sugarcane (Chriswardana et al., 2021).

Argentina, and many EU members have already proclaimed commitment to reducing their dependence on fossil fuel. As a result, several countries, including the USA, Brasil, China, Canada, Indian , Thailand, have reduce their dependence on fossil fuels towards developing bioethanol (Robak et al., 2020). However, France and Germany are the leading in bioethanol manufacture in Europe as in Table 1.

Table 1. The ethanol producers in the European Union (in millions of litres per year)

Country/Calendar Year	2014 ^r	2015 ^r	2016 ^r	2017 ^r	2018 ^r	2019 ^e	2020 ^e	2021 ^f
France	1018	1039	987	1000	1138	1299	1049	1095
Germany	920	870	882	810	799	676	875	950
Hungary	456	591	633	633	645	689	639	640
Netherlands	519	563	443	532	563	570	538	570
Spain	454	494	328	377	522	547	487	480
Belgium	557	557	570	620	646	620	380	380
Poland	181	214	241	258	259	286	277	285
Austria	230	223	224	235	251	254	241	255
United Kingdom	329	538	658	684	443	190	127	190
Total	5190	5165	5159	5373	5497	5281	4747	5000

Wheat is a biomass resources in Belgium, France, Germany and U.K. Corn in Central Europe, the Netherlands, and Spain. Sugar beets in France, Germany, the UK and Australia, as well as beat pulp or concentrated juice in Australia and Belgium (European union, 2022). The first generation bioethanol constitutes over 27,000 million gallons (over 102,060 million liters) of fuel produced worldwide (status as of 2021), with the United States of America and Brazil being the indisputable leaders producing almost 85% of the global output mainly from corn and sugarcane, respectively (figure 1) (Sharma et al., 2020).

Over ten decades, the first-generation biomass has paved a way for the utilization of more sustainable feedstock without adverse effects on food supplies and the environment. The existing second-generation bio refineries in United State of America with their total cellulosic ethanol production for 2022 in Brazil is estimated at 55 million litres with an increase of 15 million liters compared to 2021 (Report, 2022).

II. BIOFUELS

The production of liquid fuel from lignocellulosic biomass demand the availability of raw materials. Many countries have functional plants for bioethanol, but no access to raw materials. However, this prone to negative effect on the design of the plant as well as increase the cost of importation. Another aspect of an efficient plant is chemical composition of the raw material which can affect the yield during the pretreatment and fermentation (Robak et al., 2019).

The introduction of biofuel as a substitute to fossil fuel whose emission has resulted destruction of ozone layer (Abo et al., 2019). The alternative energy has become highly interested in the last few decades with the increasing awareness of exhausting primary energy resources, and intensifying research in solar energy and biofuels.

The mixture of bioethanol and gasoline will improve engine efficiency, decrease period of burning and lower combustion. The drawback of bioethanol production includes production cost, inhibitors formation and separation techniques. The production process involves sourcing, transporting, treatment for feedstocks, sugar formation, fermentation process, ethanol separation, purification etc.

Primary biofuel are unprocessed form of biomass materials and pellets used for heating and electricity generation in developing countries and account for 9% of between 2010 - 2016. Common examples are by-products of agriculture, municipal waste etc.

Secondary biofuels are processed materials. It can be solid, liquid or gases used for transportation. They can be classified as 1G, 2G, or 3G biofuel. The first generation biofuel are obtained from seeds, and sugar through fermentation. These compete with food, there need to search for non-edible biomass. Second form biofuel (2G) are got from agricultural biomass which are non-edible and reduces consumption of gasoline and contribute to the change of energy source. The challenge is the recalcitrance of the plant wall to convert to sugar and availability of process equipment.

The third generation (3G) comprises of biomass from algae and sea weeds (Sharma et al., 2022). The fourth generation is a form of fuel from carbon dioxide absorbent feedstock . This is composed of synthetic bioengineering modified microorganism which can increase production and make it much more efficient (Vasic et al., 2021). It has potential of high quality yield. The main drawback is it requires large volume of water to survive even when grown in waste water. Also the product produced is less stable since the biomass contains much oil. The challenge is the cultivation of the biomass and improvement of the microalgae.

However, the use of first generation biomass has been greatly reduced for a more suitable feedstock which does not compete with food. The non-edible feedstock have been identified for its sustainability (Devi et al., 2022). These reduce dependency on gasoline and contribute to change of energy source. However, bioethanol production has solved the problems of energy demand and environmental problem as a good step to a more sustainable sources of energy (Liu et al., 2020).

III. LIGNOCELLULOSE RESOURCES

It is an abundant and renewable resources. It helps to reduce climatic change and solve environmental problem (Jeswani et al., 2022). These resources (fig 2) composed of virgin biomass, energy crops as well as industrial wastes (Arefin et al., 2020).

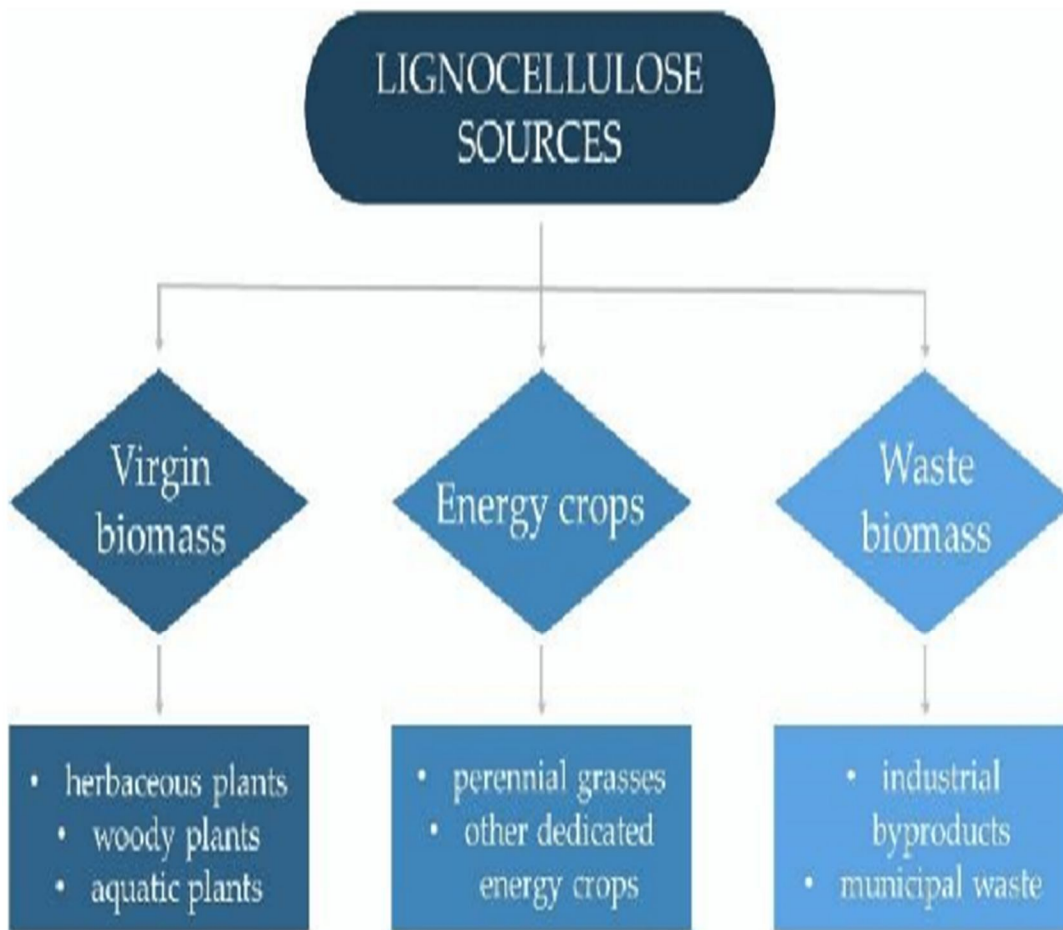


Figure 2 : Resources from biomass (Arrefin et al.,2020)

Biomass is a polysaccharides, varies with percentages (Sharma et al., 2019) along with minor pectin, protein, ash, and extractives. The proportion differs based on the biomass species. Hardwood has more cellulose, while leaves have more hemicelluloses, as a result of the growth, age etc, of the biomass.

Cellulose is a portion of the lignocellulosic biomass constitutes 30 – 50 % with linear chains of glucose linked by $C_1 - C_4$ oxygen bridge with the removal of water (Kumar *et al.*,2017). It is a white crystalline or amorphous form , high strength, as a result of hydrogen bonding between the molecules. It does not dissolves in water except at extreme pH. Cellulose has various properties and used as resources such as fibres, fuels, chemicals etc (Jedvert and Heinze, 2017).

Hemicellulose is about 25 - 40 % made up chains of ylogucan bound by $\beta - (1,4) -$ glycosidic (Sharama *et al.*,2020). A branched polysaccharides with sugar residues carrying different bonds. It readily decomposed into monosaccharides as a result of low degree of polymerization. They are bound with hydrogen and weak forces. They are soluble in alkali solvents.

The lignin constitutes about 15-30% of the biomass , containing p-coumaryl, coniferyl, and sinapyl alcohols (Devi et al., 2021) . An amorphous polymers, rich in aromatic subunits. It bound to hemicelluloses resulting the rigidity of the biomass. It belongs to the polyphenol compound and inhibit the process of hydrolysis. Lignin does not take part in fermentation processes but is used in biorefineries as fuels, in paper production and also to produce value product such as vanillin , benzoquinone and carboxylic acid (Garlpati et al., 2020). The lignin can also be converted to bio-oil, methanol and syngas by thermal depolymerization process. However, reductive ploymerization process produces alkozyphenols, benzyl phenols, catechols and methoxy phenols (Yogalakshmi *et al.*, 2022).

Table 2 :composition of different biomass

LIGNOCELLULOSIC MATERIAL	CELLULOSE (%)	HEMICELLULOSE (%)	LIGNIN (%)
hardwood stems	40-55	24-40	18-25
softwood stems	45-50	25-35	25-35
nut shells	25-30	25-30	30-40
corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Paper	85-99	0	0-15
wheat straw	30	50	15
sorted refuse	60	20	20
Leaves	15-20	80-85	0
cotton seed hairs	80-95	5-20	0
Newspaper	40-55	25-40	18-30
waste papers from	60-70	10-20	5-10

IV. LIGNOCELLULOSIC BIOCONVERSION TECHNOLOGY

This is important production process to produce biofuel from lignocelluloses composition (Ab Rasid *et al.*,2021). It requires various chemicals which produces inhibitory compounds, and affect all the stages of the production and therefore needs detoxification to enhance quality biofuel. During pretreatment, the complex is disrupted and the cellulose exposed (Singh *et al.*, 2017). Hence, it is necessary to reduce the crystallinity of the cellulose, remove lignin and enhance porosity of the materials. An effective pretreatment should be cost effective, produce fewer inhibitors (Sun and cheng, 2021).

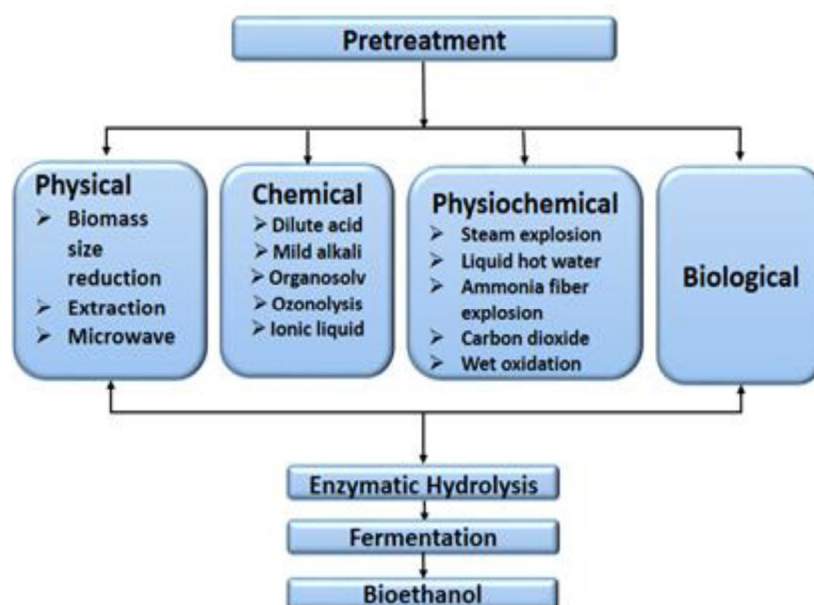


Fig. 3 : Biomass pretreatment techniques

Pretreatment is necessary due to the crystalline nature of the plant cell. It is required for exposing its constituents. Moreso, pretreatment process reduces lignocellulose into small particles to produce high yield (Usmani et al., 2022) as in fig 3. To overcome the challenges in the use of biomass, biological method of pretreatment should be favoured. It improves hydrolysis and fermentation process which results to production of sugars (Awogbemi et al., 2022). The pretreatment process should not be expensive, eco-friendly and producing less inhibitors and resulting in high yield bioethanol. The Pretreatment techniques can be categorized as shown (Table 3). Moreover, pretreatment is based on the types of biomass. Utilization of two different methods could give a satisfactory result. The best method of pretreatment is still on research (Das et al., 2021). Physical pretreatment opens the biomass, reduction in crystals etc (Rajendran et al., 2017). It is effective and expensive (Zhang et al., 2020). Demerit of this process is that it requires powers. These pretreatment require reducing the biomass to small particle sizes, resulting to increase in sugar production prior to hydrolysis (Shen et al., 2020). Milling reduces size particle depending on the category of milling method used (Bai et al., 2018).

It aims at disrupting the cell plant to yield sugars and convert it to biofuel (Oyedemi et al., 2020). Chemical pretreatment includes by acids, alkalis, organosolve, ionic liquids, deep eutectic solvents, oxidative, ozonolysis, SPOR, etc.

Physico-chemical method utilizes both physical and chemical techniques for effective biomass pretreatment. Such are supercritical carbon dioxide explosion, ammonia fibre expansion, steam expansion, liquid hot water, etc.

Biological treatment is the process in which biomass is broken down by using fungi (Rafeenia et al., 2018). Factors affecting pretreatments includes microorganisms, microbes species, their interaction and competition (usmani et al., 2021). Advantages are zero chemicals, less inhibitory compounds, simple operation and environmentally friendly. However, the drawback are large space requirement and slow reaction (Ummalyma et al., 2019).

Combination methods is employed to improve on other techniques for the utilization of the biomass, making it more productive, efficient and eco-friendly (Rezania et al., 2020). These methods requires high temperature (160-290 °C) and pressures and still produce inhibitory compounds. The most combined pretreatment is sodium hydroxide and hydrogen peroxide. The advantage is high biomass penetration, low energy consumption, no inhibitors produced, no need of reactors and eco-friendly. However, the main drawback is high pH of processed biomass and high amount of inhibitors (Dutra et al., 2018).

Pretreatment by Biological-alkaline enhances delignification of biomass, reduces the chemical used, lowering the time and temperature. Thus, reduce the operation cost (Si et al., 2019). This results may lead to high loss of carbohydrate content. Biological-acid pretreatment improves the solubility of the hemicelluloses, decrease toxic compounds. Thus, it increases sugar yield as well as ethanol production (Yan et al., 2018). pretreatment by Biological-oxidative implores using white-fungi and oxidizing reagent e.g hydrogen peroxide on the biomass. This shortens the time and increase biomass delignification with no inhibitors produced. Thus, high sugar yield and very effective (Paudel et al., 2017).

Biological-organosolv pretreatment is basically for wood waste. This enhance increase in sugar production and large amount of biomass component (Xie et al., 2017). Biological-Liquid hot water method enhances high glucose yield owing to enzyme activity (Li et al., 2022) Biological-steam explosion pretreatment increases the sugar yields compared to the steam explosion. This combination raise the energy use, costs of the process and removes the toxic compound in the biomass.

The combined pretreatment methods are very efficient and cost effective (Leonard et al., 2021). However, there are further improvement on step to reduce the limitations of utilization of biomass and making the production of bioethanol more sustainable (Hoet et al., 2019).

V. DETOXIFICATION

Detoxification is to remove fermentation toxic compounds from the hydrolysates, thereby improving the enzyme efficiency and high yield of bioethanol during fermentation (fig 4). Bioethanol yield and productivity could be improved if the following methods are employed for the removal of the inhibitors: adsorption, microbial engineered microorganism etc. This method is employed during pretreatment (da Nogueira et al., 2021). Detoxification breaks down the hemicelluloses resulting in aliphatic compounds, furan aldehydes and furfural. Elumaliai et al., (2018), These inhibitors affecting the quality of biofuel produced and other valuable products.

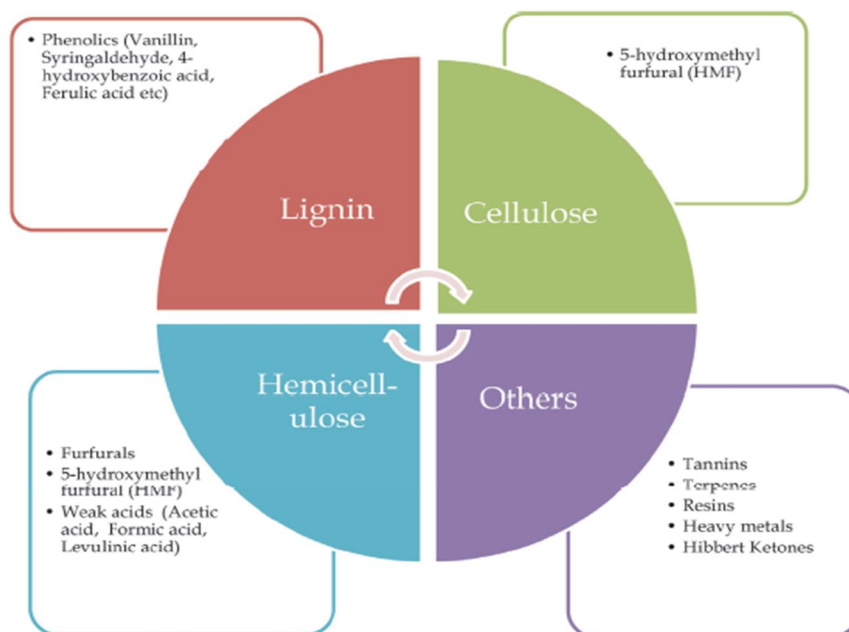


Figure 4 : Detoxification of lignocellulosic biomass

These inhibitors are furfural (furfural), Phenolic compounds, syringaldehyde, corniferl aldehyde (Liao et al., 2021) are formed during pretreatment of biomass. These inhibitors are expected to be removed prior to fermentation.

Inhibitors from lignocellulosic biomass prone a problem in bioethanol production. Cheaper pre-treatment method ensures an economic viable production process but generates high levels of inhibitory compounds. The inhibitory compounds generated affect yeast fermentation rate and yield. Prolonged pre-treatment may result in degradation of formic acid and levulinic acid, furfural breaks down pentose sugars and HMF breaks hexose sugars.

The physical method involves washing the biomass under running water after pretreatment to remove soluble toxic compounds (Xie et al., 2018). Other methods includes evaporation, membrane separation, etc. (Chenetal.,2020). Neutralization method is necessary prior fermentation as a result of the acidic nature of the hydrolyzates. Slake lime is the most preferable base used. It allows the precipitation of inhibitors such as phenolic acid. Addition of excess alkaline to hydrolysate, reduces the inhibitors. Common alkaline detoxifying agents are sodium hydroxide, calcium hydroxide, ammonia solution. This remove toxic compounds giving rise to 10% loss of sugar by adsorption (Zhang et al., 2018). The alkaline adding to the hydrolysate is thereby converted to gypsum. The major draw back is that the yield of sugar is affected, thus lead to reduction of the ethanol yield.

Addition of an adsorbent to precipitate the inhibitors (Liano Astuy et al., 2017). Other adsorbent is polyethyleneimine which has an excellent structure and adsorption performance (Haug et al., 2018). The major setback is that sugar yield is reduced. However, activated charcoal has the strong adsorption ability and is relatively cheap. Adsorption resin and PEI can adsorbed inhibitors such as Furan, Fatty acid, and phenolic acid. The ion-exchange base resins works better at alkaline pH. Though it is very effective and highly expensive. Solvent exchange resin uses extractant to remove the inhibitors from the hydrolysates. The common solvents used are n-hexane, methanol, and ethanol. The extraction could remove high percentage of inhibitors. However, 84% phenolic was removed (Cruz et al., 2018).

Biological method involves detoxification of fermentation inhibitors using microorganism and enzymes to remove the toxicity. Microbial detoxification involves using microorganisms mostly white rot fungi to remove inhibitors by changing their chemical nature. The lignocelluloses hydrolysate may be detoxified using yeast (Larsson et al., 2019). Todhanakasem et al., (2018), removes furfural, 5-HMF with microbial detoxification. The microbial mediated detoxifications is more effective resulting in low sugar consumption.

Enzymatic Detoxification is a biological means of removing toxic compounds using enzymes from white rot fungi *Phenorchete chrysosporium*, *C. Stercorous*. It removes phenolic compound from hydrolysates. Tramontina et al., (2020) completely removed furfural, acetosyringone, and coniferous aldehyde in the lignocellulosic hydrolysates using immobilized laccase. Though, the sugar loss is low.

However, integration of different detoxification increases bioethanol yield. It could be cost effective and gives better yield. Ion-exchange resin with activated charcoal adsorption is known to be the most efficient compare to other methods of removal of inhibitors (Chen et al., 2021). Although biotechnical methods (Moodley et al., 2020) is much reliable compared to other conventional methods.

VI. BIOETHANOL PRODUCTION

Bioethanol is a rapid growing liquid fuel for transportation that reduces dependence of gasoline. Most biorefineries uses the blend E10 and E15 blends fossil and ethanol, respectively. Bioethanol from biorefineries generates various products such as chemicals, fuels via lignocellulosic biomass increase sustainability and reduction of environmental pollution.

Fermentation is the process by which reducing sugars, e.g pentose and hexose are produced and the activities of inhibitors are experienced on the microorganism and the product yield (Zhu et al., 2019). The process is an anaerobic metabolism.

The reaction below shows the glucose and the xylose conversion

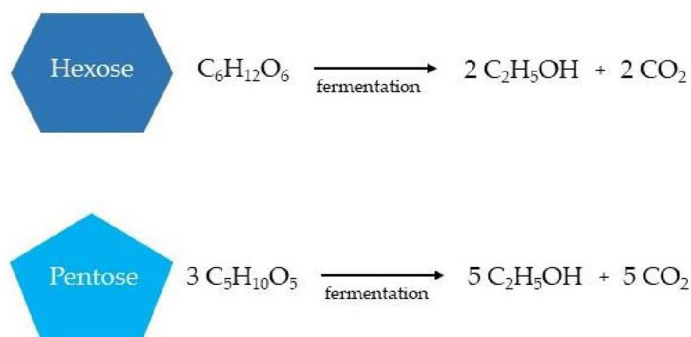
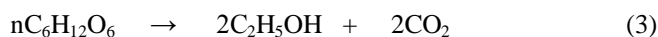
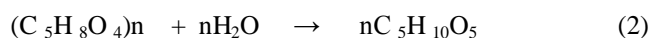
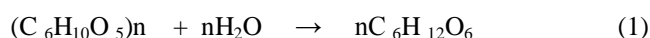


Figure 5: Ethanol production from hexose and pentoses (Zhu et al., 2019)



Presence of microorganism is needed to ferment sugar in the biomass. However, absence of microorganism leads to using metabolic engineering techniques to isolate organism. This is necessary for complete hydrolysis of the carbohydrates (Adegboye et al., 2021). The choice of microorganism depends on the type of sugar present in the hydrolyzates. *Saccharomyces Cerevisiae* is commonly used for glucose fermentation while other microbes are used for xylose and other sugars. Microorganisms. The yield of ethanol depends on the concentration of sugar and the efficiency of the

Fermentation efficiency can also be improved by using microbes cells with carrier which should be non-toxic, biodegradable, and cost-effective. (Soares et al., 2022). Most biorefineries employed different applications to improve the yield and minimise costs (Jahnavi et al., 2017).

VII. FERMENTATION TECHNOLOGY FOR ETHANOL PRODUCTION

- 1) Batch Fermentation: The fermentation occur in separate batch. The process required little energy, low cost of operation, investment, and reduced contamination.
- 2) Semi Continuous Fermentation: In this type, the feed is loaded at intervals and the product removed, though not regularly. This will assist to monitor the growth of the microorganism within a time.
- 3) Continous Fermentation: This process require loading the feed into the fermentation tank continuously at the same time the product removed constantly. The growth of the microorganism can be maintained for a duration and this lead to high yield of the product formed.

A. Fermentation Process

Separate hydrolysis fermentation involves the hydrolysis, and enzymatic fermentation taking place at different time, using two separate reactors. The drawback includes the formation of inhibitors, contaminations, two different equipment required, and it is time consuming (Jin et al., 2020). Separate hydrolysis fermentation neutralizing the toxicity, enzymes and other materials react at the same time. One major advantage of Separate hydrolysis fermentation is that it saves time and quality product is obtained. (Dey *et al.*, 2020).

Bi-saccharification and fermentation techniques involve the sugar and ethanol production occurs in one reactor. The SSF produce quality product, reduce energy input and reduce rate of reaction. It generates less inhibitors with accumulation of sugars produced during hydrolysis which is directly consumed by the fermenting microorganism. The process minimize the production cost since one reactor is needed (Marulanda et al., 2019). However, one major drawback is the difference in condition of operation (quiu et al., 2018).

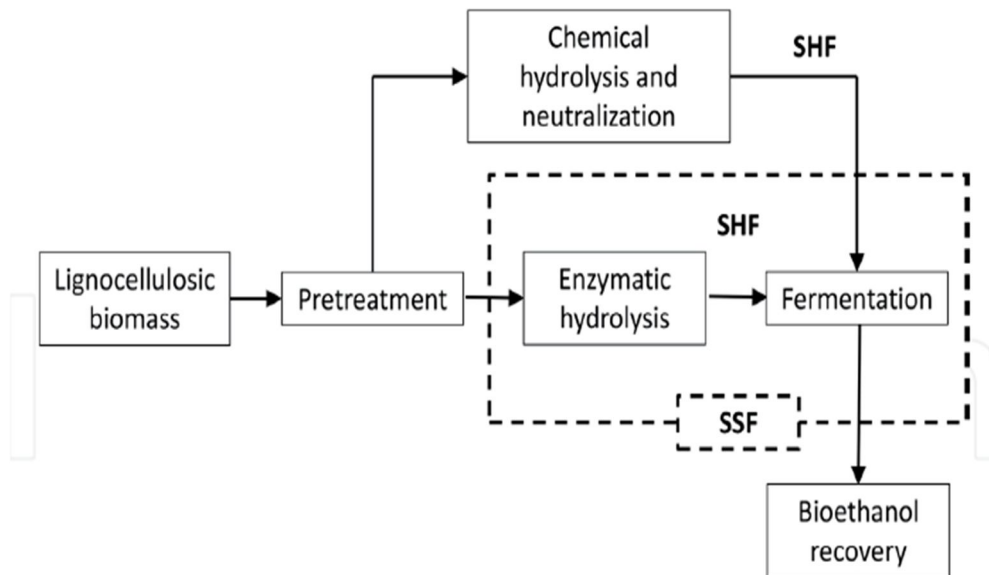


Fig 6: Schematic representation of bioethanol production process (Gaykawad et al., 2018)

B. Bio-Processing of Biomass.

In this process biomass is hydrolyzed with enzymes produced and fermentation the sugar to ethanol occur at a given period (Toor et al., 2020). This is to minimize the cost of bioethanol production and making it cheap. The efficiency of bioethanol produced are influence by inoculum, sugar concentration, pH, agitation rate, oxygen content, and rotation speed.

C. Separation, Recovery and Purification of Bioethanol

This process involves obtaining ethanol from the hydrolyzates. Distillation and dehydration are applied to reduce the water to the standard (ASTM, 2011). The product is 99% concentrated and dehydrated to a high-quality.

The various methods of separation from a fermentation broth are adsorption distillation, azeotropic distillation etc. These methods differ in utilization costs (Li *et al.*, 2018). The most viable and economically suitable for bioethanol production is membrane distillation.

D. Advanced Hybrid- Processes

A high operational costs of distillation-based process results to the development of new design separation processes. Integration of bioethanol separation with fermentation process enables bioethanol at low concentrations by continuous removal. Avoiding yeast inhibition increases ethanol yield and process efficiency, reduce fermentation time and decrease production time. Advanced hybrid process involves in-situ bioethanol which combine fermentation with vacuum fermentation (Huang *et al.*, 2015).

E. Bio-refineries

A bio-refineries is a facility for sustainable biomass processing into feed/food, chemical, materials, and bioenergy in an environmentally sound, socially acceptable and cost effective. The various classification system of biorefineries is based on the source of feedstock, technological implementation and biomass conversion route, and product recovery (IEA, 2021). The most commonly used classification is based on the feedstock. Most of the currently produced biofuel are classified as first generation. The product of biofuel in the second-generation biorefineries relies on non-edible feedstock such as lignocellulosic biomass. The third-generation biorefineries, phototrophically grown alga and micro algae biomass is processes into biofuels and other value-added products (Mat *et al.*, 2020).

VIII. CONCLUSION

The global energy crisis and environmental challenges pose a major problems facing the society and a concern to the researchers. Biofuel produced from renewable and sustainable feedstocks contributes to national development and economic growth. Bioethanol production from lignocellulosic biomass makes the production cost insignificant due to the abundant of the lignocellulosic biomass. The main challenge in bioethanol production include lignocellulosic recalcitrance, high enzyme cost, the inhibition of enzymes and microorganism, low bioethanol yields and high energy demand for bioethanol recovery and dehydration. The pretreatment method is one of the expensive and most intensive step in second generation biorefineries. The physico-chemical is regarded as the most cost effective method of pretreatment but the major disadvantages is that it generates inhibitors that decreases the enzymes activities and microorganism growth rate. The establishment of improved design and sustainable pretreatment methods will lower energy and chemical consumption, and minimize inhibitors generation and improved the carbohydrate recovery.

However, the design of new genetically modified microorganism, enzymes activity, simultaneous assimilation of hexose and pentose sugars and high resistance to inhibitors would promote high yield of bioethanol production and production cost. Distillation method is still in used in recovery of bioethanol from fermentation broth. The application of hybrid processes of azeotrope distillation and dehydration will decrease the energy and operation cost of large scale bioethanol separation.

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