PULMONOLOGY

Large-Scale Bioethanol Production from Agricultural Residues Using Pretreatment Technologies: Environmental and Health Impact Assessment

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ABSTRACT:

In this modern era, where renewable sources of energy are in great demand to support sustainable development for posterity. In this pursuit of alternatives, bioethanol comes into the scenario, being the cheapest and the cleanest source of energy till now. Bioethanol can be easily extracted from the rice straw which comes as an agricultural byproduct in the cultivation of rice. Not only availability but also its demand increases manifold when it comes to countries like India, which focuses majorly on the agricultural sector. In comparison with other agricultural waste, rice straw is considered the best alternative for bioethanol production as it contains >50% of fermentable sugars. It makes it one of the most abundant and unused lignocellulosic waste and therefore, making it apt for utilization. Apart from focusing on bioethanol production, this paper also focuses on present trends and future aspects of blending ethanol with gasoline as per the government initiative of blending 20% bioethanol with petrol by 2030. One of the major roadblocks in this path is the cost and that includes the processing cost of various operations involved in refining this useful source. Starting with pretreatment processes like physical, chemical, physiochemical, and biological followed by hydrolysis and fermentation, are central concerns in this review. Considering the future, it demands our utmost attention as it can prove to be useful in eliminating environmental pollution and eventually reduce the over-exploitation of petroleum products.

INTRODUCTION:

As the demand for renewable energy is continuously increasing with the rise in population, there is a need for a new form of a sustainable source of energy for power generation. The main motive behind such technology is waste-to-energy approaches by converting agricultural waste (mainly paddy straw) directly into renewable energy suppliers by providing an innovative biomass recovery process. The main motive behind such cleantech biofuels is that it converts waste biomass to energy. In the present world, the demand for fuels that are renewable in nature and can easily be extracted through available resources has seen a spike. There is a tremendous demand for alternative sources of energy from its renewable forms. In a recent report, it is found out that utilizing surplus food crops for biofuel generation, reduces the loss incurred by farmers in the last few decades. Shortly due to a lack of resources, farmers burn the waste generated through lignocellulosic material. The LCM is a widely available material across the globe. This includes agricultural residue such as rice straw, rice husk, wheat straw, sugarcane bagasse, etc. The most unused lignocellulosic waste is rice straw and one of the most efficient too for bioethanol production. As rice is considered as the main staple food in India, approx. 117.47 MT of rice were produced every year and it was estimated that it generated 360 MT of straws. Rice straw is the most abandoned and easily available biomass for sugar feedstock production. From this sugar feedstock bioethanol is produced commercially by fermenting sugar monomers. (Kang et al. 2019). The lignocellulosic complex contains 32% cellulose, 23% hemicellulose, 14% lignin, ash, and various other compounds at a trace amount. Rice straw contains a high content of cellulose which can be easily hydrolyzed and can be easily fermented to bioethanol, thus, making it an attractive alternative for biofuel production. The lignin component contributes towards the formation of the lignin carbohydrate complex that hinders hydrolysis steps as it decreases the surface area of cellulose for enzymatic action. So, to make the process effective, conversion of the lignocellulosic complex is required. This can be performed by pretreatment, enzymatic hydrolysis followed by fermentation. To increase the overall production of the cellulosic complex, the pretreatment method is the pre-requestee step before hydrolysis and fermentation.

On average 360 MT of rice straw produces approx. 207 billion liters of bioethanol annually. Comparing with data available it will be used as additive fuel with gasoline which decreases alleviating global warming and pollution. According to the study, bioethanol has high octane thus increases the efficiency and performance of the engine and also increases the compression ratio of the engine. Various government initiatives were enacted to increase incentives with advanced biofuel production from lignocellulosic waste.

AVAILABILITY OF RICE IN INDIA:

Rice is considered the main staple food in India to provide protein and energy. The major rice-producing state in India is West Bengal, with a total cultivable land of 50 lack hectares out of which the production stands about 146.05 lakh tons of rice annually, this gives West Bengal an edge of 0.35 tons per hectare over other states, as the avg. being 2.34 tons per hectare. Rice being a semi-aquatic plant, requires suitable climatic conditions which are found in states like West Bengal, Uttar Pradesh, Arunachal Pradesh, Punjab, and Tamil Nadu which are also the top five rice producing states in India. Apart from climatic conditions some of the other factors are pH of the soil (between 5.0 -6.5), a fairly high range of temperature, (20 – 40 degrees), 35 -45 days of bright sunshine, during its ripening period, a high-water retaining capacity of the soil and a high amount of clay with organic matter are some of those factors at the basic level. Due to its importance in food as well as in rituals in Indian tradition, rice has been one of the major constituents at every step of the Indian lifestyle. India is the second-largest producer of rice after China, as the country's production has increased by 3.5 times in the last 60 years, surpassing Thailand and Pakistan. India is also a top exporter in the global rice trade, with more than 11% of the global production share, accounting for more than 20% of the export in the last four years. It caters rice to the Middle East, Africa, U.S., and E.U. The rice production in India was 172.8 million metric tons in 2019 and is projected to register a CAGR of 2.7%, during the forecast period, 2020-2025.

CHARACTERIZATION OF RICE STRAW:

Rice straw is the by-product of rice which is produced while harvesting paddy. According to IRRI, each kg of milled rice roughly produced 0.7-1.4 kg of rice straw depending upon varieties, cutting height of the stubbles, and moisture content during harvest. Rice straw is the lignocellulosic biomass with three components i.e., cellulose, hemicellulose, and lignin.

Cellulose is a fiber material, giving strength and flexibility to the cell wall. It is the most abundant polymer on earth. It is the linear polymer of D-glucose units interlinked with β-1,4 glycosidic linkages. In the polymer state, cellulose molecules are closely associated with each other by inter- and intra-molecular hydrogen bonds which gives it a tightly closed cellulose fibers-like structure. These microfibrils have high crystallinity and are highly resistant to depolymerization.

Hemicellulose is linked with hydrogen bond with cellulose and covalent bond with lignin. It is the second most abundant polymer in biomass. It consists of an amorphous chain of polysaccharides with the highly branched chain of hetero-1,4-β-D-xylan. This branched-chain structure makes it susceptible to enzymatic degradation.

Lignin is the cross-linked aromatic structure that is devoid of any sugar-based structure. This is attached with cellulose and hemicellulose structure making it less susceptible to enzymatic hydrolysis. It decreases the conversion of cellulose and thus yields less product. It also protects cellulose and hemicellulose from invasion against microorganisms.

BIOMASS TO BIOETHANOL CONVERSION:

Bioconversion of lignocellulosic complex requires pretreatment, enzymatic hydrolysis, and fermentation. This lignocellulosic content contains cellulose, hemicellulose, lignin, and some other components in trace amounts. The primary step for bioethanol production is the pretreatment of available rice straw so that it can be used feasibly for subsequent processes. These are simple as well as complex processes, hence the main purpose for this conversion is to efficiently obtain bioethanol. Processes involved in these conversions are described below-

PRETREATMENT:

Pretreatment is the most crucial and expensive step for lignocellulosic conversion. The main purpose of pretreatment is to break the link between polysaccharides and lignin i.e., to isolate cellulose. On removal of lignin, polysaccharides are exposed to saccharifying enzymes by enlarging pore size for further enzymatic hydrolysis.(Sebayang et al. 2016). Therefore, pretreatment is the breakdown of the hemicellulosic compound and cleaving the cellulosic bond for enzymatic hydrolysis. Effective pretreatment is economical, as maximum lignin removal takes place along with less inhibitor formation such as furfurals and acetic acid.(Ebrahimi et al. 2017). After effective pretreatment, biomass is vulnerable to hydrolysis and thus results in more production of fermentable sugars. According to researchers, ethanol yield without the pretreatment process is only 20% while after pretreatment it increases to 90%. An efficient pretreatment method minimizes the time taken in hydrolysis along with cellulase loading and saving a huge amount of money for ethanol production.(Kazemi Shariat Panahi et al. 2020). Changes associated with biomass after pretreatment are the composition of biomass, crystallinity, and microscopic structural changes in treated and pretreated rice straw. These are classified into four methods.

PHYSICAL PRETREATMENT:

Physical pretreatment includes milling, grinding, pyrolysis, and microwave oven, and electron beam irradiation. Both mechanical and extrusion processes are part of physical pretreatment. (Sebayang et al. 2016) The mechanical process reduces the biomass size by breaking its physical structure through milling and grinding of rice straw, as a consequence of which, it is termed as an energy-intensive process. Some other methods included in the physical pretreatment method are wet disk milling, dry milling, and compression milling. The second method is pyrolysis which is performed at high temperatures mainly at >300°C. It is because of this high-temperature half of the total weight of feedstock is lost. Pretreatment by microwave radiations is of immense importance as it is highly heated efficient along with a smooth working principle. It not only requires low energy to operate but also, is a uniform and selective procedure. Along with these qualities, mentioned so far, these provide us the ability to start and stop instantaneously and willingly, it provides us an edge over other pretreatment processes. It also improves the saccharification efficiency and alters the ultrastructure of lignocellulosic materials to a great extent. One of the major advancements of this process is that it increases the susceptibility of the substrate by increasing the activity surface of enzymes so that completion of the process can be taken into account. Earlier studies reveal that microwave radiation alters the super molecular structure of the lignocellulosic material to increase suitability and reactivity, but are slow in changing the structure of biomass because of the low heating rate and heating mode. (Prasad et al. 2020)

CHEMICAL PRETREATMENT:

Chemical pretreatment deals with the structural disruption of biomass using chemical reagents. This chemical pretreatment includes the use of acids, alkali, organic salts, peroxides and recently using ionic liquids.

Acid required for pretreatment are HCl, H_2SO_4, HNO_3, H_3PO_4, etc. but these also release harmful toxic substances. The most tested approach is of H_2SO_4 as it is easily available, highly active, low cost, and is of little concern as far as the environment is concerned. Pretreatment condition at 170○C is considered as the optimum condition for maximum sugar yields.(Singh, Suhag, and Dhaka 2015). It was noted that fewer inhibitors are formed while using maleic and fumaric acid. The main limitation of acid pretreatment is the corrosion of experimental equipment and high capital cost

Alkali pretreatment is considered as most widely used pretreatment method. The effectiveness of such a process is dependent on substrate and treatment conditions. Some of the alkali used for pretreatment are sodium, potassium, calcium, and ammonium hydroxides. It requires lower temperature and pressure conditions. Inhibitor formation in alkali pretreatment is almost negligible and comparatively less sugar degradation. It involves salvation and saponification which increases the internal surface area of biomass and decreases the degree of polymerization. (Prasad et al. 2020)

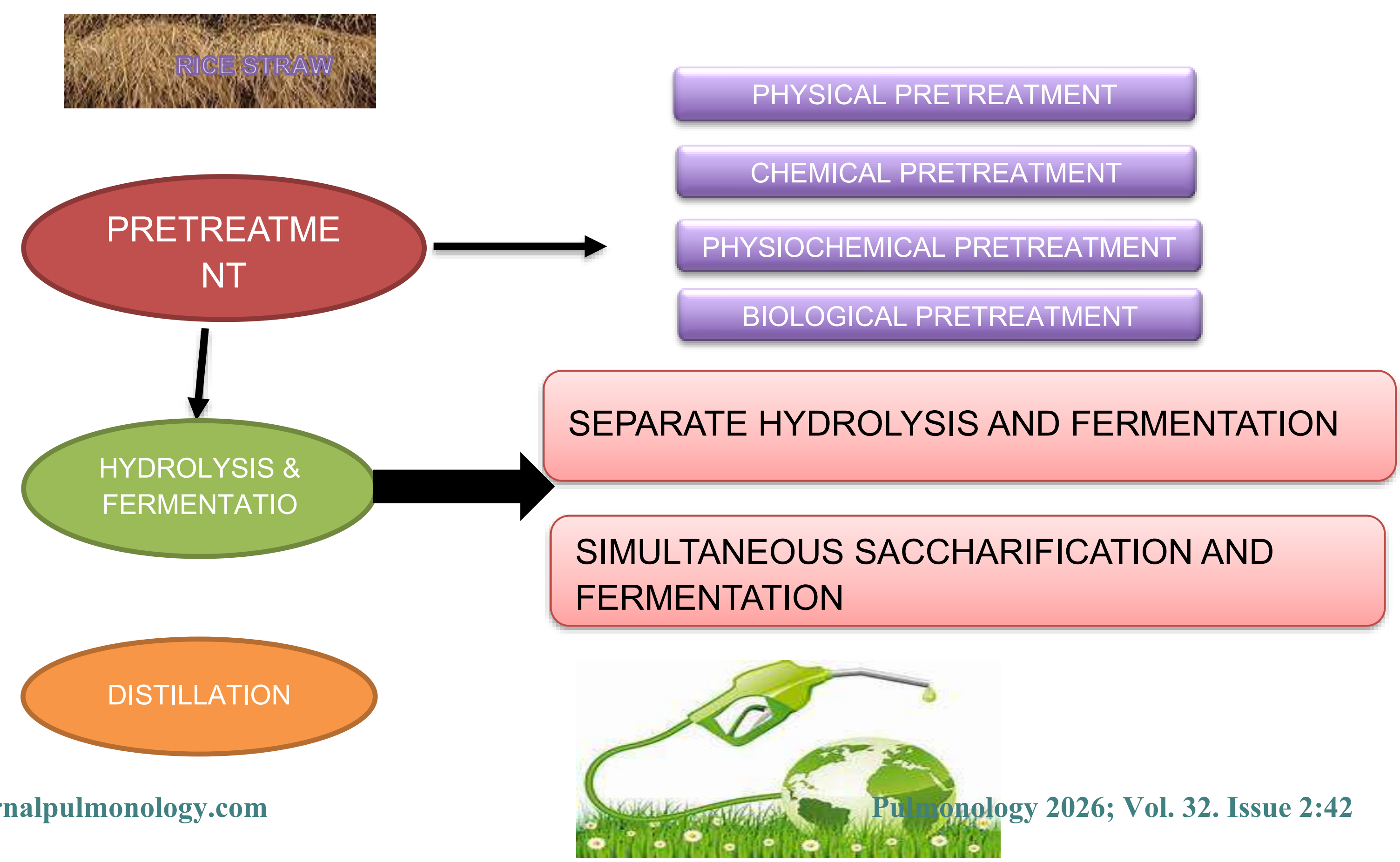
Using ionic liquids is now a major concern as these are termed green solvents due to high thermal stability, ability to dissolve cellulose at normal conditions. (Sorn et al. 2019) Due to the low vapor pressure of ILs, it is assumed that 99% is recoverable and doesn't form any toxic material. Thus, it reduces the cost of solvent usage. ILs have various importance over other chemical pretreatment methods such as high dissolution capacity, low viscosity, high stability as well as low melting point. The temperature required for the dissolution of biomass is 90○C-130○C for 1hr.

PHYSIO-CHEMICAL PRETREATMENT:

This includes steam explosion, ammonium fiber explosion (AFEX), liquid hot water methods, and CO2 explosion. Steam explosion is the most commonly used pretreatment method as it combines both physical and chemical pretreatment methods. It is considered one of the most cost-effective methods for lignocellulosic conversion as well as an environmentally friendly process. It enhances the process for further enzymatic hydrolysis and reduces the dependency on the chemical reagent. It takes place at temperature 200^0C -280^0C, with a residence time of about 2-3 min and recovery yields are up to 65%. Under this condition thermal decomposition of cellulose to sugar takes place. It was assumed that better yields were obtained at low temperature and low residence as the formation of toxic compounds due to hemicellulose degradation is less probable. Additional to SE, LHW pretreatment is a hydrothermal process, which does not require any chemical reagent, this process is almost similar to SE but this water is used at high temperatures i.e., 170-230°C for 15 mins. In this process, a small number of inhibitory products are also formed which inhibits the growth of microorganisms for fermentation but yields more xylose, nearly 85% making this process economically efficient and eco-friendly. (Kazemi Shariat Panahi et al. 2020). Another process is AFEX, in which along with steam explosion, ammonia is also added to the biomass at moderate temperature (70-100°C) and pressure (130-150 psi). Due to the rapid expansion of ammonia gas, it destroys the lignin-carbohydrate complex along with physical disruption of biomass fibers and increases the digestibility of biomass. After completion, this process is terminated by explosive decomposition According to the study, this process is cost-effective, simple, rapid and the optimized recovery of NH_3 makes it economically feasible.(Raud et al. 2019)

BIOLOGICAL PRETREATMENT:

Biological pretreatment is performed by various microorganisms, which mainly include fungi. These fungi include white-rot fungi which belong to the class basidiomycetes. This produces lignolytic enzymes such as manganese peroxidase (MnP), lignin peroxidase (LiP), laccase in presence of Mn (III), and veratryl alcohol (VA). (Singh, Srivastava, and Shukla 2016). These enzymes attack lignin with high delignification efficiency which increases hydrolysis efficiency. These biological methods are cheaper than any other pretreatment methods as no chemical reagent is required and therefore it is a safe and environment-friendly pretreatment process. Low energy is required for the process. The major drawbacks are large time consumption and low hydrolysis rate. And to make biological pretreatment an effective method, it has to be combined with biological pretreatment with any other pretreatment. This will improve the overall yield of bioethanol.



BIOETHANOL

(Flowchart of bioethanol production)

ENZYMATIC HYDROLYSIS:

Enzymatic hydrolysis is one of the crucial steps after pretreatment which converts cellulose complex into glucose for fermentation. Hydrolysis is a mild process and its maintenance cost is comparatively low which enables more glucose yield as there will be no degradation of glucose takes place. Here different types of hydrolytic enzymes are required. Enzyme cocktails are either commercially used or self-prepared. Enzymes such as cellulase (endoglucanase and exoglucanase) and β-glucosidase are commercially used for the efficient conversion of cellulose and hemicellulose into monosaccharides. Endoglucanase activity is mainly shown at low crystalline areas in cellulose fiber and thus cleaves the intramolecular 1,4-glycosidic linkage to form a new chain end and the next two enzymes remove cellobiose from the free ends and hydrolyzed it to glucose. Some of the cellulase-producing bacterias are Acetovibrio, Bacillus, Bacteroides, Clostridium, Streptomyces, Ruminococus, and Thermonospora. (Kazemi Shariat Panahi et al. 2020). The hydrolysis of cellulose yields glucose while hemicellulose yields pentose and hexose. The factors which determine the rate of hydrolysis are cellulose crystallinity and degree of polymerization.(Singh et al. 2016). The enzymes used in enzymatic hydrolysis make it a costly process. These enzymes are loaded with biomass loading at proportionate order usually at 1:10v/v. With the increase in enzyme loading faster will be the hydrolysis. Higher enzyme loading at 20, 25, 30FPU/gm with 0.2M sodium acetate buffer (pH 4.8) with 1 mg/ml sodium azide is the optimum condition for hydrolysis. Hydrolysis is mainly conducted at a high solid concentration which is essential for the conversion of lignocellulosic biomass commercially. (Du et al. 2020). The major concern of this report is to perform enzymatic hydrolysis at low enzyme loading (2.5 & 5 FPU/g). This can be performed by extending the fermentation time which in return increases the ethanol yield. The major limitation with low enzyme loading is enzyme absorption and desorption.(Molaverdi et al. 2019). This is performed at mild condition 45-50°C temperature and pH 4.8 with less formation inhibitory by-product. The enzyme cocktail decides the variability of the process, so to use a self-prepared enzyme by microorganism should be more significant which gives a high level of saccharification. This can be used in a wide range of processes thus reducing the cost of the process. Using various cellulase along with lignocellulose helps in tailoring such cocktails and thus increases sugar yield for fermentation.

FERMENTATION:

Fermentation is the conversion of glucose to bioethanol using microorganisms, which can be either yeast, bacterial, or fungus. The type of yeast which is mainly used is Saccharomyces and Pichia. Bacteria used for this process are Zymomonas, Escherichia, and aspergillus. However, the main purpose to conduct fermentation is to gain better ethanol yield with more production efficiency.

SEPARATE HYDROLYSIS AND FERMENTATION:

When the hydrolysis and fermentation processes are performed sequentially, both the process takes place under optimum operating conditions (pH, temperature, etc.). A solid fraction of pretreated lignocellulosic content undergoes hydrolysis in which cellulose is treated with an enzyme cocktail. After the completion of hydrolysis, the cellulose hydrolysate is fermented into ethanol. This ensures flexibility in the fermentation process by enabling batch and fed-batch processes.(Carrillo-Nieves et al. 2019).

SIMULTANEOUS SACCHARIFICATION AND FERMENTATION:

It is the single-step production of ethanol, in which both enzymes and yeast are added to the same fermenter along with the substrate at optimized enzymatic activity with less accumulation of sugars, this reduces the time consumption. There will be less contamination by microorganisms as the process takes place at the same reactor. According to the researchers, this process is superior to any other process used till now, and to make the process economically feasible, solid loading with sufficient sugar content is required. With the increase in solid loading, there will be chances of the formation of inhibitory compounds such as acetic acid, furfurals, and phenolic lignin which was induced during the pretreatment process. However, due to high solid-loading enzymatic hydrolysis is also affected which also results in less ethanol yield. SSF is considered to be a better process due to end-product inhibition and also because it reduces the processing time. The experimental design of SSF requires optimum temperature at 30°C, pH at 4.5, solid loading

11%(w/v), enzyme loading 0.5%(v/v) for a period of 72hrs. (Akhtar, Goyal, and Goyal 2017). It is the process of using both saccharification and fermentation at the same time, and hence it reduces equipment cost and makes the process feasible. The major limitation in such a process is maintaining optimum conditions for both hydrolysis and fermentation. Hydrolysis is carried out at a high temperature around 50-60°C while fermentation is carried out at a mild temperature around 25-30°C., thus to maintain such conditions, technical efforts are required.(Sebayang et al. 2016).

ECONOMIC ANALYSIS FOR COST-EFFECTIVE PRODUCTION:

The economic analysis mainly depends on improving the conversion technology and the quality of feedstock produced. Conventionally, ethanol production depends on operating cost (labor and maintenance cost), capital cost (depreciation or fixed cost), rice straw credit, ethanol production cost, and these four are interlinked to each other. Capital investment deals with types of feedstock and its availability. Primarily, there is a need to choose optimal facility size, availability of feedstock in an area. This can be measured using the residue to product ratio (RPR=1.5) and the moisture content at 15%.(Diep et al. 2015). So, to reduce the production cost of ethanol, there is a need to improve the rate of utilization of straw resources, there should be the availability of high-value products. As the rice straw contains cellulose, hemicellulose, and lignin along with many bioactive compounds such as phenolic acid, flavonoids, and tannin. These bioactive is considered as a high value-added product during bioconversion. It improves economic competition and reduces the cost of bioethanol.(Shi et al. 2020). The solvent recovery process after pretreatment is one such effort to achieve cost-effectiveness. As the solvent residue after pretreatment is hazardous to the environment, the solvent recovery system must be initiated during the ongoing production process and reusing that solvent for further processes. Further, the most challenging step is enzymatic hydrolysis and it accounts for 25-30% of the operational cost, and thus, the enzyme cocktail is the most challenging step in hydrolysis. For effective bioethanol production, it is needed that both cellulose and hemicellulose are hydrolyzed to sugar completely with minimum sugar loss and effective fermentation of sugar to ethanol. Effective production of bioethanol involves so many steps where there are so many challenges, which includes finding more effective pretreatment, decrease the cost of commercially available cellulase by increasing enzyme efficiency, hydrolysis of both hemicellulose and cellulose for more sugar yield so that more ethanol can be produced with the limited feedstock, using both 6-C and 5-Carbon in the fermentation of sugar. At last residual lignin, unreacted cellulose, hemicellulose, enzyme, yeast, etc. in the anaerobic digestion process can be utilized in the production of biogas. Thus, this reduces dependence of sugar on bioethanol. Such a combined process yields high energy gain with limited stock.(Raud et al. 2019)

BIOETHANOL USAGE AND ITS PRESENT STATUS IN INDIA:

Ethanol is considered more efficient than gasoline in terms of its flame speed, flammability, octane number, and heat of vaporization. In various parts of the country, ethanol blending with gasoline takes place. Maximum gasoline blending takes place in Brazil (24%). (Kazemi Shariat Panahi et al. 2020)

According to the present report, India has set the highest ethanol blending at 7.2% with petrol in the season from December 2018 to December 2019. In 2020 the amount of fuel requires 3300 million liters of ethanol to achieve 10% of ethanol blending by 2022 by the entire country. Since now 84% of India's fuel needs depend on imports, blending can help to reduce this import dependency by margins. Utilizing surplus waste material for biofuel production can ensure better prices for the farmer who otherwise incur losses.

This was first launched in 2003 as EBP (Ethanol Blending with Petroleum) program. This scheme had failed due to various bottlenecks such as lack of consistency, poor supply chain, transaction barriers, lack of technical expertise, and conflicts with agricultural policies. In face of already lacking ethanol policies and associated challenges, the government of India in 2008 launched biofuel policies intending to reduce pollution emission and to raise the price for ethanol biomass for companies to incentivize farmers. Recently, in 2019 government has sanctioned a 766 crore 2G ethanol production plant to IOCL in Panipat, Haryana. The plant operates on lignocellulosic material and is expected to increase farmer's income and promoting green fuel. Based on the above-stated report, it seems that annual targets of 329 crore liters need to be fulfilled. These blending and biofuel policies aimed to focus on sustainable saving and production, price stability, and systematic supply chain management. This can reduce vehicular emissions by promoting clean fuel. As far as pollution is concerned there is a need to increase bioethanol usage in India. There is a huge need for increasing the production of bioethanol through agricultural residues.

CONCLUSION:

Bioethanol satisfies clean technology requirements and fits accordingly in the present energy crisis in India. Its production through lignocellulosic waste material mainly rice straw making it suitable for sustainable development. This paper mainly focuses on a cost-effective method for bioethanol production at a mass scale. Today, despite various government initiatives its production is not up to the mark, so to overcome this limitation, alternatives for that particular method are enacted in the present paper. Approx. 50% of rice straws are burned by the farmer which increases pollution level and hence causes global warming. So, there is an urgent need to develop new technologies that can be used for biofuel production. The different pretreatment methods, the optimum condition for that method, its effect on rice straw, and percentage yield of the cellulosic compound for the further process are described clearly. Along with that, economic analysis is carried out to set up this process at a commercial scale.

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