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Production of Bioethanol from different Biomasses

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Abstract: *In the current scenario, the overexploitation of fossil fuels and increasing socio-economic challenges force the society to search for the alternative renewable fuel resources, like solar, wind, hydropower and biomass-derived energy. Of these, in context to transport sector, the liquid biofuel sources, such as bioethanol and biodiesel are more preferred, as it represents about 35% of the net worldwide future energy requirement and reduces the emission of greenhouse gases. Among them, bioethanol is the centre of attention in recent bioenergy research that can be used as a co-fuel for petroleum blending to improve the fuel efficiency due to its high octane number, less toxicity and biodegradable nature. When dehydration is used as an extra concentration technique, the procedure entails digesting molasses sugars with microorganisms, followed by the separation and purification of ethanol. This review provides an overview of the production of bioethanol, in different generations. In the article we discussed several biomass sources, such as lignocellulosic, sugar-rich, and starch-rich biomasses, along with the technological unit steps including pretreatment. The purpose of the pretreatment step is to increase the surface area of carbohydrate available for enzymatic saccharification, while minimizing the content of inhibitors. Hence, the article is summarized with new bioethanol byproducts like biobased BDO and PBAT to diversify the sugar production process. Reviewing economic and technological factors, it provides information on the manufacture of bioethanol.*

Keywords: *Biofuels, Bioethanol, fermentation, biomass pretreatment, lignocellulosic biomass*

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1. Introduction

Fuel produced from biomass, such as plant or algal matter or animal faeces, is referred to as biofuel. In contrast to fossil fuels like coal, oil, and natural gas, biofuel is seen as a renewable energy source since its raw material is easily reusable. To reduce emissions of carbon monoxide and other pollutants that contribute to smog, gasoline is blended with ethanol, and alcohol. An efficient pretreatment is needed to clear the way for enzymes to make fermentable sugars from the complex collection of carbohydrates known as lignocell, which are subsequently hydrolysed to produce ethanol [1].

The primary sugar-rich biomasses utilized to produce bioethanol are as follows:

(i) feedstocks containing starch, such as grains like corn, wheat, and root crops like cassava;

(ii) feedstocks containing sugar, such as sugar beet, sugarcane molasses, whey, and sweet sorghum;

(iii) lignocellulosic biomass, which includes straw, agricultural waste, crop, and wood residues [2].

According to data on bioethanol production worldwide, starch containing biomass accounts for 60% of bioethanol production, with sugarcane and sugar beet accounting for the remaining 40% [3]. Sugarcane molasses contains a large quantity of fermentable sugars, such as glucose, fructose, and sucrose, it is regarded as an effective substrate for the synthesis of bioethanol. Due to its high concentration of fermentable sugars (i.e., glucose, fructose, and sucrose), sugarcane molasses is regarded as a good substrate for the synthesis of bioethanol [4,5]. Additionally, molasses is an inexpensive substrate that is widely accessible, noncompetitive with a food stock [6]. In comparison to starchy, cellulosic, and hemicellulose materials, it requires less pretreatment during preparation [7]. Molasses is primarily utilized as an affordable feedstock by the ethanol-producing business. It is a byproduct of the sugarcane industry and comprises the majority of microbial growth factors, including minerals and organic nutrients [8]. Ethiopia produces a massive 542,316 tons of sugarcane molasses yearly, which is used by both state and commercial bioethanol facilities to make bioethanol [9]. Using corn and sugarcane as

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their primary raw materials, Brazil and the USA produced the most bioethanol in 2019, making for 84% of the global output. In the United States, corn starch is used to make 94% of bioethanol, whereas sugarcane is used in Brazil to produce 99% of it [11].

Numerous elements, including carbon, nitrogen, temperature, pH, and oxygen, have an impact on the development and metabolism of strains [12,13]. The concentration of each medium component is directly correlated with the dilution of the natural substrate. High concentrations of carbohydrates result from an overabundance of the natural substrate, which raises osmotic pressure and alters or eliminates the ability of strains to transport and metabolically process each component [14]. Similarly, the global energy requirement will rise significantly despite all further efforts concerning the sensible use of available energy resources (Figure 1). As per the International Energy Agency (US), it is projected that the approximately 50% rise in global energy consumption by 2050. Strains of metabolic pathways vary in settings with varying oxygen contents, the kinds and quantities of products produced by aerobic and anaerobic fermentation will vary [15,16].

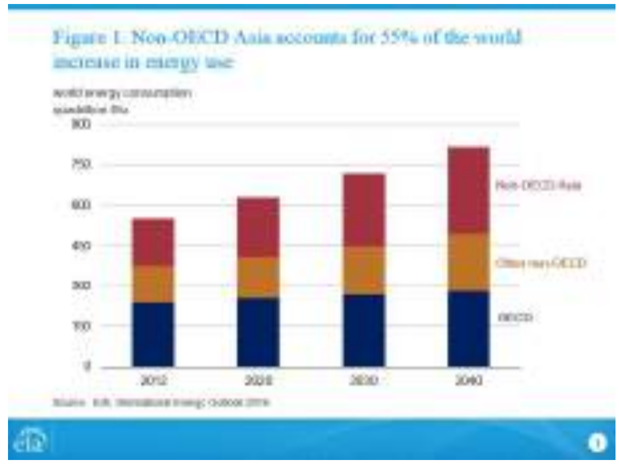

Fig. 1.: Representation of worldwide energy consumption by region (Source: International Energy Agency (US), International Energy Outlook, 2019).

The genetic analysis and verification of the impact of oxygen on the products of the *Lactiplantibacillus casei* strain utilizing food waste substrate were conducted [17]. Various organic acids, including pyruvate, lactic acid, acetic acid, and succinic acid, are synthesized by *L. Casei* [18].

As fermentation proceeds, organic acids that have the potential to raise the fermentation liquid's acidity progressively accumulate. Because high acidity causes metabolically associated components on the cell membrane, including as channel proteins, transport proteins, and signal pathway proteins, to lose their normal function, the drastic shift in pH will impact the way these strains metabolize carbohydrates [19].

BIOMASSES USED IN DIFFERENT GENERATIONS:

There were four different generations in which different feed stocks for biomass was used. Each generation includes different biomass whether it is industrial waste and agricultural waste. Selection of such kind of biomass is environment friendly and is economically viable [20].

First generation Bioethanol (1G)

Approximately 50% of fermentable sugars protein, vitamins, and trace minerals that may be directly digested by microbial fermentation are present in sugarcane molasses, a by-product of the sugar industry [21]. It has been widely used as a low-cost alternative feedstock. Initially starch and sugar-based crops are used to extract sugar by using only fermentation method. For Instance, using barely, wheat and sugarcane crops were previous techniques [22], but they were replaced by sweet sorghum and sugar beet [23]. It can serve as a substrate for the synthesis of butanol, citric acid, succinic acid and many more.

Second generation Bioethanol (2G)

In **Second Generation** or **2G,** by fermenting the fruit, municipal, industrial, and agricultural wastes. The dried carrot pulp has been used as a raw material in this instance, and *Saccharomyces cerevisiae* yeast and beet molasses inoculated at 28 °C for 72 hours have been used to produce bioethanol [24,25].

Third generation Bioethanol (3G)

Microorganisms digest carbohydrates to produce bioethanol, an alcohol that, in its pure form, may be added to gasoline to reduce vehicle emissions and raise octane [26]. Cellulosic biomass, which comes from non-food sources like grasses and trees, may be converted into bioethanol. However, the growth of bioethanol production has been constrained due to the high cost of hydrolysing lignocellulose biomass and lower ethanol titers and yields [27].

Fourth generation Bioethanol (4G)

The **fourth Generation (4G)** strains of metabolic pathways vary in settings with varying oxygen contents, the kinds and quantities of products produced by aerobic and anaerobic fermentation will vary [28,29]. A thorough genetic analysis and verification of the impact of oxygen on the products of the *L. casei* strain utilizing food waste substrate were conducted [30]. Various organic acids,

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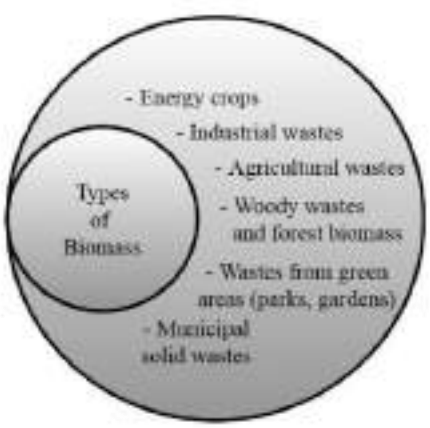
including pyruvate, lactic acid, acetic acid, and succinic acid, are synthesized by *L. casei* [31].

The three pineapple syrups that were produced were referred to as agave juice (thick juices), and they were fermented to produce bioethanol after being kept in a tank. 10% of the generated bagasse and 10 kg of coal were utilized during this extraction stage to generate the necessary energy [32]. Comparison between different parameter values of different biomass, thick juices and yeast strains are discussed in table 1.



Source: Types of Biomass. Data taken from [33]

Types of Pre-treatment

Pretreatment is one of the essential step, which meant to rupture the key cell wall polysaccharides of the macroalgae for releasing reducing sugars using physicochemical or biological methods and relies on the seasonal characteristics of the biomass. Pretreatments can be physical, chemical, physiochemical, or a combination of these to break down lignin and allow cellulose to be hydrolysed for the highest possible sugar content [34]. Physical pretreatments is also known as hydrolysis. Steam explosion is recommended when the biomass is subjected to high pressure steam and quickly depressurized which makes cellulose easily hydrolyse enzymatically. Mechanical milling is recommended with grinding or milling, size of biomass particles has been reduced but increase in surface area accessibility of enzymes during hydrolysis. Chemical pretreatments: Acid Pretreatment: Hemicellodiacetate can be broken down and the digestibility of cellulose increased by acidic solutions like hydrochloric acid or diluted sulfuric acid. Neutralization is frequently done after acid pretreatment. Alkaline Pretreatment: Lignin is helped to be removed by treating cellulose with alkalis such as sodium hydroxide or ammonia, which increases cellulose's accessibility for enzymatic hydrolysis. Organosoly Pretreatment: To separate and extract lignin

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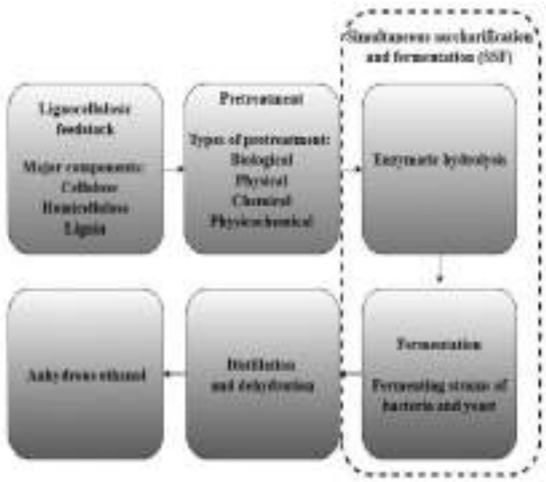
from biomass, organic solvents are frequently used with acid or alkali.



Source: Major steps in bioethanol production. Data taken from [35]

Enzymatic hydrolosis and Fermentation

In order to prevent rivalry with the utilization of arable land for food crops, the availability of under used crops can be employed as feedstocks for biofuels [36,37]. Characteristics compared to molasses, sugar beet thick juice was shown to be more cost-effective, especially throughout the VHG fermentation process [38].

Numerous variables, including temperature, pH, oxygen, nitrogen, carbon, and nitrogen influence the development and metabolism of strains [39]. Alongside this, in the present time the bioethanol production is done through yeast strains by intermixing with molasses to speed up the process and helps in increasing the yield also. Three typical methods used in the manufacturing of ethanol include hydrolysis, fermentation, and pretreatment are used. Temperature, sugar content, pH, length of fermentation, rate of agitation, and size of inoculum are some of the variables that affect the production of bioethanol during fermentation.

By immobilizing the yeast cells, ethanol production and efficiency can be increased. The study focuses on the many strains of yeast, the fermentation process, variables influencing the generation of bioethanol, and the immobilization of yeasts to improve bioethanol production [40]. When compared to free cells, immobilized cells often demonstrated improved fermentative activity, increased ethanol productivity, stability, and cell survival under the same fermentation conditions. The performance and viability of yeast fermentation were impacted by the high concentration of non-sugar components in molasses [41].

Using cane molasses as the substrate medium and the ethanologenic engineering strain L. casei E1 as a starting culture, the viability of *Lactiplantibacillus casei* generating ethanol was assessed. Using a high-performance

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liquid chromatography (HPLC) system, the effects of environmental conditions on the metabolism of *L. casei* E1 were examined.

Table 1: Showing the difference between various parameters values of biomass, thick juices and yeast strains

Parameter value	Biomass	Thick juice	Yeast Strain
pH	7.2	8.6	5-5.5
Colour (EBC)	8.5	0.5	0.20%
Dry matter (%)	85.80%	64.30%	45-55%
Total sugar (%)	53.10%	59.30%	20-25%
Sucrose (%)	50.60%	57.90%	10-16%
Purity quotient (%)	59.00%	90.00%	2-5%
Protein (%)	11.30%	7.30%	3%
Total nitrogen (%)	1.80%	1.20%	15-20%
Free amino nitrogen (%)	0.90%	0.20%	2-8%
Ash (%)	7.90%	2.00%	0.4-0.8%

Quantitative real-time PCR (RT–qPCR) was used to identify the gene expression of important enzymes in the metabolism of carbon sources. The strain could grow well and swiftly ferment sugar in cane molasses, according to the results [42].

New Improvements in Ethanol Production

With the advancement in generations, new by-products has been produced from molasses. This determined if it would be feasible to produce biobased 1,4-butanediol (BDO) or Polybutylene Adipate Terephthalate (PBAT) as a means of diversifying sugar manufacturing facilities [43]. There are two scenarios for biorefinery process. In the first scenario, two rstoic reactors were used to simulate the two-step PBAT polymerization processes (esterification and transesterification) in order to precisely determine the energy need of the polymerization [44].

By diverting towards the economic viability of all the available feedstock (A molasses) from a single sugar mill to a BDO biorefinery in this particular scenario [45]. The production procedure for BDO is comparable to this one. In this case, though, the BDO is marketed as

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the finished product rather than being utilized as a precursor for the creation of PBAT [46].

Both scenarios were profitable, however the sensitivity analysis showed that scenario 1 would continue to be successful whereas scenario 2 would become unprofitable with a 30% increase in total capital investment or a 30% decrease in operating time. The study has shown that it is possible to generate bio-based PBAT from molasses at a cost that is competitive with the costs of the present fossil fuel-based market [47].

Conclusion

To combat the present environmental concerns and increasing fuel prices, the macroalgal biomass has shown greater potential as feedstock for biofuel production. In particular, carbohydrate-rich macroalgae are widely used for bioethanol production. Therefore, the present investigation was mainly focused at the pretreatment optimization and modelling of fermentative bioethanol production from a selected strains with biomass. Various generations of biomass utilization, including molasses, cellulose have advanced the production process, yielding additional valuable byproducts. These innovations underscore the versatility and sustainability of different biomasses as a feedstock for biofuel and biopolymer production. With ongoing research and development, the integration of molasses-based biorefineries into existing sugar manufacturing facilities holds promise for a greener and more diversified energy landscape.

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