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ADVANCEMENTS IN FERMENTATION-BASED BIOETHANOL PRODUCTION: ROLE OF SACCHAROMYCES CEREVISIAE AND NOVEL TECHNIQUES

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Abstract:

Rising energy demand, fossil fuel depletion, and pollution have intensified interest in renewable fuels. Bioethanol is promising for its biodegradability, low toxicity, and infrastructure compatibility. Saccharomyces cerevisiae enables efficient fermentation through high ethanol tolerance, effective glucose metabolism, and robust performance. Conventional ethanol production processes rely primarily on micro-structured yeast and require extended fermentation times, which limit productivity and economic viability. Recent research efforts have therefore focused on improving fermentation efficiency through advanced techniques such as process optimization, genetic and metabolic engineering, immobilization strategies, ultrasound-assisted fermentation, and the development of nano- and hybrid yeast systems. These novel approaches have demonstrated significant improvements in reaction kinetics, ethanol yield, and process stability while reducing fermentation time and energy consumption. This review critically summarizes recent advancements in fermentation-based bioethanol production, highlighting the evolving role of Saccharomyces Cerevisiae and emerging technologies that enhance fermentation performance. The review also discusses current challenges and future perspectives for developing cost-effective, high-efficiency bioethanol production systems to support sustainable energy development.

Keywords: *Saccharomyces Cerevisiae, bioethanol, fermentation, environmental remediation.*

1. Introduction

The increasing demand of fuels for energy generation is a major issue across the world due to the rising of transportation and industrialization (1). Generally, there are three categories of fossil fuels such as solid fuels, liquid fuels, and gaseous fuels (2,3). Coal and charcoal are involved in solid fuels (4) and petrol, kerosene, and diesel are in liquid medium (5). The gaseous fuels involved natural and produced gas which is composed of hydrocarbons, hydrogen, and carbon monoxide (5,6). These energy sources (crude oils and natural gases) are non-renewable energy sources. It takes millions of years to form because it comes from the dead animals and plants that remain underground about 100 to 400 million years ago (7). These conventional fuels are used as energy sources for home, industrial, and transportation purposes due to their combustion nature (8). But these are creating CO, CO_2, NO, CH_4, and NO_2, which create air pollution and are harmful to human health (9). Carbon dioxide is directly affecting on the respiratory and cardiovascular systems (10). The vehicle pollution load was estimated through a deep study conducted by Central Road Research Institute (CRRI). The most polluted cities in India namely Delhi, Mumbai, Kolkata, Chennai, Bangalore, Hyderabad, Kanpur, and Agra are the most affected cities due to vehicle emissions (11). According to the declaration of the National Environmental Engineering Institute of petroleum, Delhi is the most affected city by air pollution among the other mentioned cities (11). Vehicle emission has a damaging effect on human health as well as on the ecology system.

Recently, researchers have developed electrical energy storage sources such as supercapacitors, batteries, and fuel cells which getting greater attention due to the compact management system in vehicle development (12). But it has some limitations due to the use of different organic and inorganic materials to develop the high-energy storage devices which need higher investment to purchase and are not affordable for common people (13). Also for all fuels and energy storage materials, India has to depend on the other country's policies which have a negative impact on the Indian economical system. Moreover, it is a necessity to pay attention to electronic waste. Global electronic waste generation increased from 9.3 million tons in 2005 to 50 million tons in 2017. And currently, it has enhanced up to 20 to 50 million tons per year (14).

To overcome these issues, biofuels are one of the best options which are cost-effective, environmentally friendly, and need natural raw materials for production. Biofuels production enhances energy security and rural employment progress toward more sustainable energy sources which is a preserving option for the future (15,16). Ethanol is one of the interesting biofuels due to its easy production and higher boiling point than the other fuels which will be a better alternative energy fuel for other fuels (17,18). Nowadays, ethanol is used in the vehicle energy source as blended with petrol and diesel. India is the third-largest oil importer country in the world. India spends a yearly 65 billion to import crude oil due that the Indian economy decreasing year by year. To control that Indian government has decided to blend ethanol with petrol which is reducing the cost of fuels and also controlling the foreign investment. In 2003, the government of India launched the ethanol-blended petrol (EBP) program to reduce crude oil importers. The Indian government has increased the blending amount of ethanol from 10% to 20 % in the year 2022. The increasing use of ethanol in the fuels sources will give a positive impact on the Indian economy because ethanol production is derived from agricultural products which are a huge amount available in India (17,18). Bioethanol can be produced from starch- containing raw materials like sugar, corn, potatoes, beetroot, etc. Ethanol is available at an affordable cost as compared to fossil fuels and electronic fuels because its raw materials are available in abundance in nature.

The ethanol production by using agricultural products depends on the fermentation process and glucose content of the base material (19,20). The saccharomyces Cerevisiae yeast plays a catalytic role in the fermentation process and the glucose contents are helping to the growth of the saccharomyces Cerevisiae. Sugar is the most populated raw material in the ethanol production term due to the higher amount of glucose present in the sugarcane. Also after the sugarcane processing for sugar, the industries use the molasses for the production of ethanol which is utilizing the waste sugar from the molasses by conventional ethanol production technique (21–23). The conventional production techniques need 50-70 hours for fermentation. Nowadays, researchers are trying to develop different techniques which could be helpful to improve the reaction time (24–26) Pulidindi et.al has demonstrated the fermentation process by using mild sonication techniques. In this paper, the reaction time of the fermentation process has improved due to the sonication process. Also, he claimed that the sonication process does not affect a negative impact on the yeast life(27). Recently, Junronng and co-workers have prepared the Nano yeast of saccharomyces Cerevisiae and exhibited its application in the SARS-CoV-2 detections (28) . Grewal, Y. S., et al were reported the biomaterial for dengue virus detection. In this study, they developed the single-chain fragments of saccharomyces Cerevisiae nano-yeast. In both works, the nano-based yeast has demonstrated an improvement in the results than the micro-structured yeast (29). The motivation of this review article is to find innovative techniques for developing ethanol production technology. According to the literature, we have understood that all researchers are trying to improve the reaction processing time of the fermentation process. The conventional fermentation processing techniques are using the micro-structured yeast for the production of ethanol and they have improved the reaction processing time by using different techniques which are costly. To control the cost and improvement in the fermentation process, catalytic material development should be required. According to the literature, we are assuming that the nanostructured yeast for the fermentation process will provide a better approach than the conventional ethanol technology.

2. Approaches to synthesis bioethanol

2.1 The fermentation process of sugar to produce Ethanol

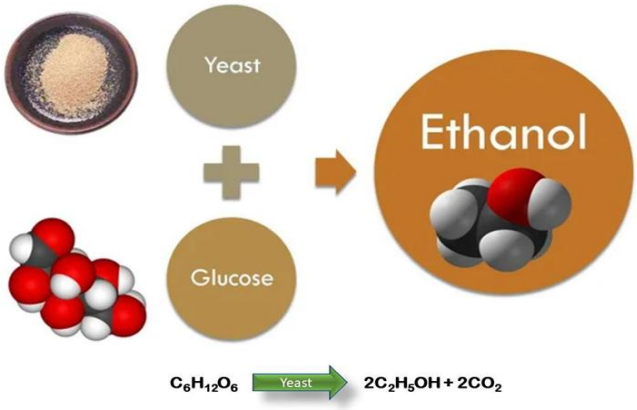

Figure 1: ***Fermentation process to synthesize ethanol from sugar using yeast as catalyst***
(Reference: How-to-Make-Bio-ethanol-From-Regular-Sugar, Instructables)

Molasses is the main by-product of the sugar industry. It can be used to produce bioethanol. The saccharomyces Cerevisiae (baker yeast) is allowed to grow in such molasses for the production of ethanol by the fermentation process. Fermentation is a well-known metabolism process in which the changes take place in organic species by

the action of the enzyme as a well-known catalyst (Figure-1). The saccharomyces Cerevisiae is most common, commercially available, and well known for fermentative behavior.

2.2 Role of Saccharomyces cerevisiae (yeast) in the fermentation process

Yeast belongs to eukaryotic single celled microorganism classified as a member of fungus kingdom. They are available in micro size that is near about 3 to 4 micrometers. The yeast gets their foods from surrounding environment to grow and reproduce itself. So, in fermentation process we use the sugar as a nutrient for yeast. It breaks the carb carbohydrates bonds of sugar and converts in to ethanol and carbon dioxide (CO_2).

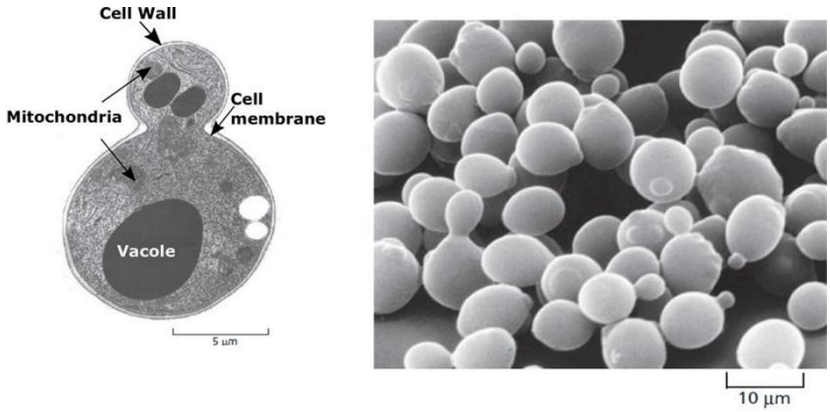

Figure 2: ***Schematic representation of budding in yeast. Ref.*** **Farout, M., 2019.** ***The interaction between metabolism and the plasma membrane potential, and intracellular pH*****. Lancaster University (United Kingdom).**

2.3. Budding in yeast

When we supply the nutrients to yeast it start to budding. In such budding yeast cell are converts in to developed bud and reproduction of bud arises an outer growth of parent body and chain of bunds are formed. Later the nucleus of parent yeast separated in to number of parts. As time increases growth of yeast take place from lag face, log phase, declaration phase up to stationary phase. See Figure 2, which implies the budding in yeast.

2.4 Effect of pH on fermentation of sugar

The pH should be affected on the fermentation process of ethanol. It is a most important regulating factor. The microbial growth of yeast depends on pH value. Yeast is an acidophilic organism that's why it grows better under acidic condition. The optimum pH range for yeast growth can vary from pH 4.0 to 5.0. To optimize such a condition the buffer was used to dilute the enzyme. e.g., phosphate buffer.

2.5. Effect of temperature on fermentation of sugar

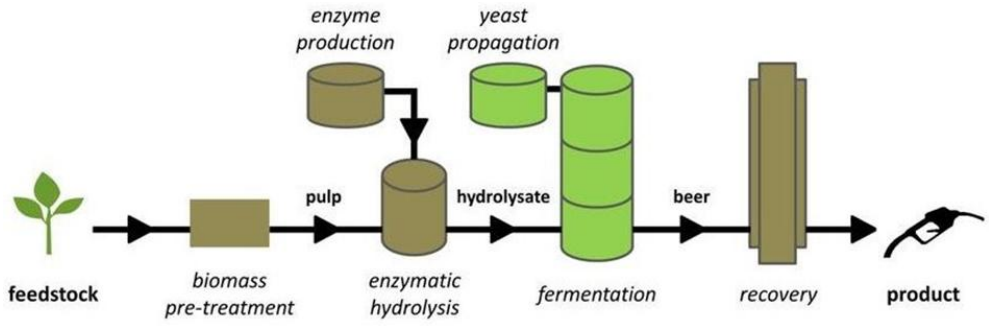

Figure 3: ***Overall fermentation process for the bioethanol production*** **(30)**

At too high temperature the yeast cell is dead and at slow temperature slows down the activity of yeast. So, for getting high amount ethanol yield from sugar the specific temperature rang (35^oC to 40^0C) are required. After 50-70 hours fermentation process was completed, so sample was filtered and separates the unnecessary residue. The pure ethanol can be obtained by distillation method applied on filtrate at 80^0 C temperatures. Schematic representation of overall fermentation process for the bioethanol production is described in above Figure 3.

3. Literature study

All crude oils and natural gases due to its combustion nature contributes polluting emission in environment, especially CO, CO_2, NO, CH_4, NO_2, etc. removed out in environment during this combustion in Table 1.

Table 1: Emission of gases from vehicles

Types of Vehicles	Gases	Percentage
Trucks and Lorries	CO2	28.8%
	NO	39%
	SO2	27.3%
Buses	CO2	96.5%
	NO	2.28%
Two Wheelers	CO_2	86.8%
	CO	7.18%

Bioethanol is one of the most interesting biofuels due to positive impact on the environment and it is renewable energy source, as well as it is used as an organic solvent. Most of the compounds are dissolved in it. The any chemical laboratory is incomplete in the absence of ethanol. Alternative fuels are like ethanol are ecofriendly and unharmful to environment.

Table 2: Present and future plans of blending of ethanol with petrol

Year	Petrol Demand Million litres (ML)	Ethanol blending requirements Million litres (ML)		
		5%	10%	20%
2017-2018	36015	1621	3241	6483
2024-2025	49482	2227	4453	8907
2029-2030	60203	2709	5418	10386

Table 3: Yield of crops and ethanol production in India

Types of crops	Ethanol Yield (liters/tonne)	Crop yield (tonne/ha)	Ethanol Production (liters)
Sugarcane	70	80	5600
Sugar beet	100	50.6	5060
Maize	360	5.9	2133
Sweet Sorghum	26.3	38.5	1013

When ethanol is blend with petrol it decreases the pollution, because it reduces the unharmful emission of gases like CO, CO2, NO, etc. Ethanol fuel was practically blended with gasoline in different proportions like 5%, 10%, 20% ethanol and 95%, 90% and 80% gasoline respectively. It is observed that more ethanol is a blend with petrol there is less emission of gases in environment compare to only petrol-based fuels. Recently, the country decides program

to achieve 10% blending in 2021-2022 and 20% blending up to 2030. Bioethanol can be produced from the starch containing raw materials like sugar, corn, potatoes and beetroot, etc.

Ethanol is available in affordable cost as compare to fossil fuels and electronic fuels because for its production the raw material is available in abundance in nature.

Present state of the arts

Fermentation is a known popular and traditional process for production of ethanol. But the traditional reaction time required to complete the fermentation is big challenge in front of ethanol producing industries. The many research had been developed for decrease the reaction time of such fermentation. It is possible to deploy the foreign moieties to enhance the fermentation rate of sugars. In concern to, Saita et al., and Siva Raman et al. were demonstrating the results using ammonium ions and synthetic zeolites. And these zeolites increased the fermentation rate of sugar that is D-glucose in presence of ammonium ions. The ammonium ion is one of the many substances which may affect the rate of glycolysis in yeast. The vital role of ammonium ion (NH_4^+) was as a source of nitrogen in synthesis of sugar transport proteins. The way in which the presence of nitrogen source stimulates glycolysis important not only from the point of view of metabolic control theory but also in the context of the performance that can be expected of yeast in traditional and new fermentation systems. But the mechanism of ammonium ion in fermentation is not clear. There is a need of further investigation towards establishing the relation between fermentation capacity, protein synthesis and cell growth.

The immobilization of yeast cells within the zeolites had been suggested by Siva Raman as possible colours for the increase the fermentation rate by synthetic zeolites such as silicate (SiO_2). At 200 g per litter total sugar content 85% fermentation take place in 24 hours in presence of 1 g per litter salicylate and while 16 hours were required for the same extent of conversion in presence of 20g per litter salicylate. Among other possible modes of action of zeolites present in fermenting media lowering the product level. Also, some efforts have been made by the scientists in order to enhance the fermentation process and accelerate ethanol production. Sonication may enhance the availability of substances required for fermentation. Also, sonication may produce reactive species in the fermentation medium which may cause of stress on yeast, so increase the fermentation rate. But on other hand sonication may cause oxidation of materials and we know fermentation is anaerobic process. Mohesen Gavahian and Paphawarin Ratchaneesiripap reported that some sonication condition can enhance the ethanol content, but other condition like oxidation of material and yeast inactivation show the negative effects on the fermentation process (31). Pulidindi Indra Neel, Aharon Gedanken, Rakefet Schwarz, and Eleonora sendersky decreased the fermentation time by carried out whole fermentation under the continuous irradiation with mild ultrasonic waves with 40 kHz frequency generated in an ultrasonic bath in water medium with input current 220 – 240 VAC . The reaction rate constant was enhanced by 2.3 + 0.2 and 2.5 +0.2 times as a result of sonication at 300c and 20^0c respectively as compared to stirred reaction and was about 10 times faster than non-stirred fermentation. But on other hand mentioning that bath sonicator could not be operate for more hours continuously because of some mechanical problem.

Conclusion

The present review is to be focused on finding innovative techniques for developing ethanol production technology. This review is summarized as follows:

- The production of the bio fuels is the one of the best options to provide the increasing demand of the fuels. The ethanol production from the biomaterial giving a positive impact in world wide.

- The presence of high amount of the glucose-based sugarcane producing the higher amount of the ethanol which is reducing the water pollution as well as supporting to the rural economy.
- Also, the ethanol blending with petrol or diesel are the reducing the cost of the fuels as well as air pollution.
- The ultrasonic technique reduces the reaction time of the fermentation process but that is taking a higher cost for the process. The development in the catalytic material should require in the fermentation process.

The development of the yeast in nanostructures having the challenging target, but will be the better way for decreasing the reaction time in the low cost. This development in the biofuels sector demonstrating the path toward the sustainable, clean and environmentally friendly fuels technology. Hence, this review will provide innovative idea for decrease the fermentation time of sugar to ethanol production in order to complete the striking demand of sustainable fuels by using affordably available nanocatalysts such as nano yeast.

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