



A REVIEW ON BIOETHANOL AND ALGAL BIOFUEL: SOURCES, CULTIVATION METHODS AND LIMITATIONS

TANYA GARG¹, PAYAL DAHIYA¹, AVADH OZA*

**¹DEPARTMENT OF BIOTECHNOLOGY, MEERUT INSTITUTE OF ENGINEERING AND TECHNOLOGY,
MEERUT, U.P., INDIA**

*corresponding author: oza.avadh@miet.ac.in

Abstract- To gain some outline on the production of Ethanol from different microbial sources. It has been found that *Saccharomyces cerevisiae* cells grown in the presence of ethanol that appear to increase the amount of some fatty acid in lipids. This paper carries out information about different parameters that need to be controlled for the production process. Ethanol is well-known as an inhibitor for growth of micro-organism. Mainly, three types of raw material, that are, Sugar juice, Starchy crops and lignocellulosic materials, are used for the production of ethanol. This review also provides knowledge about various sources of sugar used for Ethanol production such as Rice straw, corn meal, sugarcane and various others. In this review, characteristics and functions of microalgae along with cultivation method are studied and reviewed. Algal biomass has a bright future as a renewable fuel source if it can be grown and harvested optimally per acre of land. Few research studies suggested that algal biomass has 10 to 100 times increased potential to produce biofuels or bioenergy than conventional feed stocks.

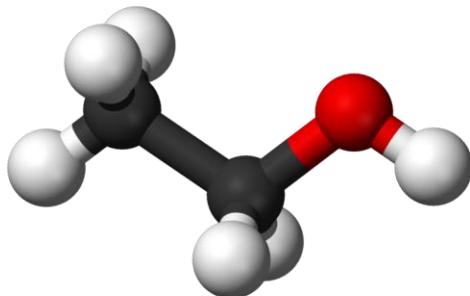
Keywords: Ethanol, *Saccharomyces cerevisiae*, Algal biofuel, Microalgae, Cultivation system

INTRODUCTION

Ethanol

Ethanol is a chemical compound that is highly volatile, flammable, colorless liquid with a slim characteristic odor. Ethanol is naturally produced by the fermentation of sugars by yeasts or petrochemical processes. Some Biosynthetic Pathways are known that can be helpful in production of Ethanol. Ethanol is also used as a clean-burning fuel source. It has many medical applications as an antiseptic and disinfectant. It is widely used as a chemical solvent, either for scientific chemical testing or in synthesis of other organic compounds, and is a vital substance utilized across many different kinds of manufacturing industries.

Chemical Structure: $\text{CH}_3\text{--CH}_2\text{--OH}$ or $\text{C}_2\text{H}_5\text{OH}$ [1]



PRODUCTION OF ETHANOL USING DIFFERENT MICROBIAL SOURCES

There are approximately 58 species of bacteria, 17 species of Yeast/Fungi, 24 species of moulds known till today's date in ethanol production [2]

Zymomonas mobilis

It is capable of producing ethanol from fermenting sugar via a distinct mechanism- The Entner-Duodoroff (ED) Pathway. [3]

The Entner-Duodoroff (ED) Pathway: This pathway describes a series of enzyme catalyzed chemical reaction which are active in bacterial primary metabolism that catabolizes Glucose to Pyruvic acid using various enzymes. The ED Pathway was first reported by Michael Doudoroff and Nathan Entner from Bacteriology Department at University of California, Berkeley in 1952.[4] Recent studies have shown that there is no restriction for this pathway to use on prokaryotes as previously thought.[5]

The advantage of using *Z. mobilis* is that it produce ethanol by the idea that increase in biomass is considered the waste of resources in the context of Biofuel production. The Biggest challenge with using this micro-organism for ethanol production is usually that it only utilizes a few simple monosaccharides/ disaccharides such as Glucose, Sucrose and Fructose. [6]

Escherichia coli

E. coli has been able to avoid accumulation of ethanol and high salt concentration that is usually in the case of using *Saccharomyces cerevisiae*. It metabolizes a wide spectrum of sugars as it lacks the gene that encodes pyruvate decarboxylase but expresses pyruvate formate lyase[7]. Various other organic compounds such as acetic acid and succinic acid are produced with the imbalance of NADH produced inside the cell which causes low ethanol yield.

Saccharomyces cerevisiae

Saccharomyces cerevisiae is a unicellular yeast that is capable of utilizing glucose as energy source this microorganism lacks Xylose reductase enzyme thus does not take up Xylose as energy source. [8] It is the most employed yeast for ethanol production though ethanol can be produced by array of various other yeast, bacteria and fungi. Co-culturing of *S.cerevisiae* with other yeast or microbes is targeted to optimize ethanol production. It also shortens fermentation time and reduces process cost.

Saccharomyces cerevisiae showed high growth rate in glucose containing media rather than media containing fructose or sucrose.

Optimum temperature on which *S. cerevisiae* shows maximum growth is 20-30°C. It gives maximum yield in the pH range of 5-6. [9]

SOURCES USED FOR ETHANOL PRODUCTION

Corn Meal

Corn Meal is obtained by Dry Milling process that was a product of Factory processed corn. In corn meal there are two step enzymatic hydrolysis that are commercially available: α -amylase and gluco-amylase. [10]

Rice Straw

A rice straw is a cellulose utilizing mould which is isolated from rotten rice residues. It is shown that rice straw is a potential raw material that produces high value product of fuel ethanol and biodiesel. It can produce approximately 200 billion liter of ethanol per year in the world. [11]

Sugarcane

Sugarcane is a good source of sugar rich juices used as feedstocks in ethanol production. Its juice contain high amount of free sugars such as sucrose, glucose and fructose that makes sugarcane much more cost effective than other lignocellulosic materials. [12]

Waste Paper and Municipal solid waste

Waste paper is a potential source for producing Bioethanol. From various waste papers such as newspaper, cardboard, and magazine using an enzyme complex (Cellic Ctec 1) was evaluated from an economic stand point. Abundance of waste papers makes it a cost effective raw material. [13]

Impact of Zinc on Ethanol Production

Yeast require a range of metal for optimal growth, metabolism and fermentation processes. In ethanol production, Zinc is of prime importance at 0.3ppm level. It is also necessary for numerous importance biosynthesis and metabolic enzyme include glycolytic enzymes and alcohol dehydrogenase. Zinc play a critical regulatory role in Zn Finger DNA binding protein and affect Yeast-Yeast Flocculation

Zinc can effect on stability and dynamic of cell membrane which lead to Downstream processing effect on cell permeability and signaling system. In brewing fermentation, Zinc is actively assimilated by yeast from malt wort. [14]

Algal Biofuel and its cultivation

The key to algae's potential as a renewable fuel source lies in increasing algal biomass productivity per acre. Some researchers say algae could be 10 or even 100 times more productive than traditional bioenergy feed-stocks. Achieving these high productivities in real-world systems is a key challenge to realizing the promise of sustainable and affordable algal

biofuels. Once harvested, algae can be readily processed into the raw material to make fuels for cars, trucks, trains, and planes.

Algal biomass has a bright future as a renewable fuel source if it can be grown and harvested optimally per acre of land. Few research studies suggested that algal biomass has 10 to 100 times increased potential to produce biofuels or bioenergy than conventional feed stocks. To attain these higher efficiency in an existent system is a key impediment for making it sustainable and affordable algal biofuels [15]

How is Algae Different from Other Advanced Biofuels?

Algae infers green lake filth over water, however this examination utilizes a quickly developing microalgae. Microalgae are equipped for growing 10 times speedier than natural plants for every section of land premise. Oil can make up 30 to 50 percent of algae's weight, contrasted with only 15 or 20 percent oil from a soybean. This implies algae can possibly create more oil than crops generally developed for biodiesel. Despite the fact that ethanol has been effective, it can supplant all oil utilizes totally, for example, stream energizes, yet algae can supplant those employments. The potential there to make significantly more fuel per section of land with algae.

One case of an effective algae oil creation framework incorporates a photograph bioreactor, which is an independent gadget giving a controlled domain to algae to grow. It can be as basic as plastic encased clear tubes, however the framework is exceptionally costly.

Another way to deal with developing algae is in raceway lakes, which are open lakes formed like ovals, that are blended with paddlewheels. In any case, with the raceway lakes being open-not encased, similar to the photograph bioreactor-the algae is presented to nature and can wind up debased from outside components.

Close to issues of suitable frameworks for development, there are different components moderating the progression of algal biofuels.

Difficulties Confronting Algal Biofuel Production

Cost is the greatest obstruction to extending generation of algal biofuels. Not exclusively are costs high to set up frameworks to develop algae, however so is the cost of a barrel of algae oil. In the mid-1990s, scientists assessed algae oil could be made at \$60 to \$80 per barrel. Presently, the evaluated cost per barrel of algae oil is at any rate \$300. The momentum research will bring down that cost, however it might take five years or more to happen. All things considered, algae will speak to a generous commitment to the biofuels blend.

Another test is the way to collect algae and concentrate the oil. As of now, once algae achieve a specific thickness, water must be expelled from it. At that point, once the algae turn into a specific consistency, similar to that of a semi-strong glue, the oil must be extricated. One of the greatest achievements later on will come when the algae really discharge the oil or different biofuels directly into the fluid media. Then, hypothetically, oil could be skimmed from the highest point of the water and changed over, tackling the issue of productively collecting and separating the oil [16]

There are a few diverse natural meanings of algae biomass outlined under the term alga. Right off the bat, algae can be separated into micro and macro algae. From a developmental point of view, macroalgae were arrive based plants that came back to a wet situation. They comprise of branches, leaves and might be enrooted, and can be partitioned into dark colored, red and green writes, which are various types of multicellular eukaryotes with free advancement pathways. By differentiate, microalgae are unicellular and nano-to milli-meter estimated. The phycologists meaning of microalgae is a life form with chlorophyll and a body (thallus) not separated into roots, stem and leaves (thallophyte). This incorporates the two prokaryotes and eukaryotes. In spite of the fact that today blue-green algae are called cyanobacteria in light of the fact that of their bacterial qualities (i.e., cell-divider constituents, ribosome structure and nucleic-corrosive structure)[17]

Biomass energy, together of the representatives of renewable energy, has been developing rapidly in kind of agriculture and cultivation. Biomass energy is typically made by terrestrial crops and marine algae for conversion into biofuel and biogases. Since the 1970s, the United States and Brazil have begun to develop biofuel by using corn to provide bio-ethanol, and achieved significant results.

Biomass energy cannot solely reduce the energy shortage problem, however is additionally much better for the environment than fossil fuel. However, terrestrial crops - the primary and second generation biofuel feedstocks cause new challenges like the occupation of productive land that finish in food crisis so one of the most serious issues relating to sustainability aspects these days is that the need of land to produce food to an increasing population.

Recently, the eyes of world are that specialize in the microalgae biofuel development. Microalgae bioenergy researches are getting down to implement in the world. Several countries within the Americas, Asia, and Europe have their own microalgae

bioenergy research comes to push this renewable energy industry. Therefore, scientist began to pay additional attention to develop microalgae for extracting additional sustainable energy. Microalgae have less complicated structure, quick growth rate and high oil content characteristics. As algae production need less land or will even be cultivated within the shallow ocean directly, it will facilitate North American nation saving productive land to grow food. Therefore, microalgae are additional sustainable than terrestrial crops.

The cultivation of microalgae is the initiative of the total microalgae bioenergy production method, cultivation rely on completely different conditions like climate conditions, water resources, greenhouse gas supply and cultivation methods. These factors can directly result on the microalgae growth. To urge prime quality of microalgae feedstocks, cultivation techniques will be studied. After cultivation, production part are started to research. The production technology of microalgae is one of the foremost difficult and complicated points ought to be resolved, as well as harvesting microalgae, oil extraction and energy conversion elements. Though most of these technological ways can be employed in both algae energy and crops energy extraction, the results have also some variations between marine algae and terrestrial crops, like oil yields, land use and commercial production situations. For achieving giant scale production, the crucial problem is the way to cut back the investment price and cost throughout the total microalgae biofuel production.

Microalgae Cultivation

Algal biomass development is the first step in production of biofuel system. The main obstacle is that how to cultivate algal biomass for feedstock development. In this review, characteristics and functions of microalgae along with cultivation method are studied and reviewed. The most common methods are: Open pond configuration and Close photo-bioreactor system.

The open pond system

This is the traditional and simplest method for harvest microalgae. In general, microalgae samples are kept in an open pond with water containing other nutrients for the growth of algae. With the help of photosynthesis and inorganic nutrients present in water, help algal cells to grow. The raceway design is considered if pond is chosen for the growth of algal cells. Raceway designed pond has paddle also in configuration which helps to mix and cycle the algae cells. After few hours to cultivation, nutrient deprived pond water is replenished with fresh load of nutrients which supports the further growth of algal cells. The nutrients are added near to paddle which helps to circulate all the nutrient in pond during cycling.

Concrete is usually considered to construct raceway. However, occasionally, in order to lessening the investment budget, a pit is digged on the ground by cultivation farms directly. And then a plastic liner is spread inside the pit in order to avert soil eroded by liquid. Most of the open pond systems are superficial ponds. The extent of the raceway is about 15cm-30cm deep for allowing adequate sunlight radiating to the microalgae pond. In the microalgae cultivation method, in order to get the ideal microalgae yields, water, necessary nutrients and CO₂ are necessary conditions to be supplied to the pond. At the same time, O₂ has to be removed as well.

Photo bioreactors System

The close photo-bioreactor system compromises a close atmosphere to culture microalgae with technical equipment. There are diverse kinds of photo-bioreactors: for example, Fermentation tank photo-bioreactor, Tubular photobioreactor, Plate photobioreactor and so on. Among them, tubular photobioreactor system is the utmost appropriate category for the both interior and open-air farming of algal cells. The tubular reactor is constructed by transparent tubes that are made of plastic or glass. Also, exterior lighting situations need to be made to grow microalgae in the tubes that can be arranged steeply, inclined and horizontally. As same as the open pond system, microalgae also need sunlight, water, CO₂ and some nourishments to let microalgae growth. When the close photobioreactors arrangements positioned outside, natural sunlight can be used directly. But if these systems build indoor, non-natural illuminations are needed. Since microalgae cannot absorb CO₂ from the air in the enclosed photobioreactors, CO₂ need to be nourished into the photobioreactors. The photosynthesis of microalgae formed

oxygen. Contrary to the open pond system, oxygen cannot release to the atmosphere from the close photobioreactor system. So the system should periodically remove excess oxygen from the photobioreactors [18]

REFERENCES

1. <https://pubchem.ncbi.nlm.nih.gov/compound/702#section=Top>
2. Vassiliki A. Boumba, and Theodore Vougiouklakis, "Microbial Ethanol Production: Experimental study and Multivariate Evaluation" Forensic Science International, vol. 215, pp. 189-198, February 2012.
3. Yanase H, Nozaki K, and Okamoto, "Ethanol production from cellulosic materials by genetically engineered *Zymomonas mobilis*", Biotechnology letters, vol. 27, pp. 259-263, 2005.
4. Nathan Entner, and Michael Doudoroff, "Glucose and Gluconic acid oxidation of *Pseudomonas Saccharophila*", Journal of Biological Chemistry, vol. 196, pp. 853-862, August 2015.
5. Chen, and Xi, "The Entner-Doudoroff pathway is an overlooked glycolytic route in cyanobacteria and plants", Proceeding of the National Academy of Science, 201521916, 2016.
6. Bai FW, Anderson WA, and Moo-Young M, "Ethanol fermentation technologies from sugar and starch feedstocks", Biotechnology Advances, vol. 26, pp. 89-105, 2008.
7. Dien BS, Cotta MA, and Jeffries TW, "Bacteria engineered for fuel ethanol production: Current status", Applied Microbiology and Biotechnology, vol. 63, pp. 258-266, 2003.
8. Ha S, Galazka JM, Kim SR, Choi J, Yang X, Seo J, Yang X, Seo J, Glass NL, Cate JHD, and Jin, Y, "Engineered *Saccharomyces cerevisiae* capable of simultaneous cellobiose and xylose fermentation", Proceedings of the National Academy of Sciences of the United States of America, vol. 108, pp. 504-509, 2011.
9. F. Noe Arroyo-Lopez, Sandi Orlic, Amparo Querol, and Eladio Barrio, "Effect of Temperature, pH and sugar concentration on the growth parameters of *Saccharomyces cerevisiae*, *S. kudriavzevii* and their interspecific hybrid", International Journal of Food Microbiology, vol. 131, pp. 120-127, January 2009.
10. Ljiljana Mojović, Svetlana Nikolić, Marica Rakin, and Maja Vukasinović, "Production of bioethanol from corn meal hydrolyzates", Elsevier, vol. 85, pp. 1750-1755, September 2006.
11. Chiung-Fang Huang, Thing-Hsiang, Gia-Luen Guo, and Wen Song Hwang, "Enhanced Ethanol production by fermentation of Rice straw hydrolysate without detoxication using a newly adapted strain of *Pichia stipites*", Elsevier, vol. 100, pp. 3914-3920, February 2009.
12. Hossain Zabed, Golam Faruq, Jaya Narayan sahu, Mohd Sofian Azirun, Rosli Hashim, and Amru Nasrulhaq Boyce, "Bioethanol production from fermentable sugar juice", The scientific world journal, vol. 2014, pp 11, 2014.
13. Lei Wang, Mahdi Sharifzadeh, Richard Templer, and Richard J. Murphy, "Bioethanol production from various waste papers: Economic feasibility and sensitivity analysis", Elsevier, vol. 111, pp. 1172-1182, November 2012.
14. De Nicola, R., Anthony, S., Walker, G. and Learmonth. R., "Impact of zinc on yeast membranes and cell physiology during brewing fermentation", Elsevier, pp. 116, October 2005.
15. <https://www.energy.gov/eere/bioenergy/algal-biofuels>
16. M. Kröger and F. Müller-Langer, "Review on possible algal-biofuel production processes," Biofuels, vol. 3, no. 3, pp. 333-349, 2012.
17. https://sdn.unl.edu/algal_biofuels
18. Y. Wang and Y. Wang, "Microalgae as the Third Generation Biofuel Production , Usage , Microalgae as the Third Generation Biofuel Production , Usage , Challenges and Prospects Microalgae as the Third Generation Biofuel Production , Usage , Challenges and Prospects Supervisor ;," Examensarbete, vol. 166, pp. 1-31, 2013.