

Bioethanol Production Using Algae Biomass

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Abstract—Algal biomass-based bioethanol offers a viable path towards environmentally responsible and sustainable biofuel production. Algal species, especially microalgae, are perfect third-generation biofuel feed stocks because of their high carbohydrate content, quick growth rates, and lack of competition with food crops. The process of turning algal biomass into bioethanol by enzymatic saccharification and microbial fermentation is examined in this work. In order to break down cell walls and increase enzyme accessibility, the pre-treatment procedure included alkaline treatment and acid hydrolysis using diluted sulphuric acid. Cellulase was used in enzymatic hydrolysis to convert complicated polysaccharides into fermentable sugars, which were then fermented by *Saccharomyces cerevisiae*. The amounts of sugar and ethanol were measured analytically using techniques like the DNS test and potassium dichromate test, respectively. The ethanol content increased steadily, reaching a peak of 0.367 mg/ml after 27 hours, according to the results. The viability of using algal biomass as a substrate for the generation of ethanol is confirmed by this investigation. High processing costs and variations in algal composition, however, continue to be obstacles to process expansion. Process viability can be increased by further research on choosing resilient algae strains, enhancing enzymatic efficiency, and optimising fermentation conditions. According to the research, bioethanol derived from algae has the potential to be a clean substitute for fossil fuels, supporting the objectives of the circular bio economy and sustainable energy.

Index Terms—Bioethanol, Algal biomass, Enzymatic saccharification, Fermentation, *Saccharomyces cerevisiae*, Pretreatment.

I. INTRODUCTION

Fossil fuels like coal, oil, and gas provide the majority of the energy we use today. Although it takes millions of years for these resources to create, humans are rapidly depleting them. These fuels emit

toxic gases as they burn, contributing to pollution and global warming. Governments and scientists are searching for greener, renewable energy sources to address these issues. Bioethanol, a fuel-grade form of alcohol, is one such source. Plants like corn, sugarcane, or algae are used to make bioethanol. It helps cut down on pollutants and can be combined with fuel to power cars.

Traditionally, crops that are also utilised for food are used to make bioethanol. The fact that it competes with food production makes this problematic. In order to circumvent this, scientists are investigating novel approaches for producing bioethanol from non-food sources, such as algae. Algae can take carbon dioxide from the air, grow swiftly in water, and don't require suitable farmland. They are therefore a very promising choice for clean fuel production.

The abundant natural sugars, or carbohydrates, found in algae may be broken down by enzymes and then fermented by yeast to create ethanol. Nevertheless, there are several obstacles, such as the high cost of production and the technological difficulties involved in large-scale algae processing. India is among the numerous nations that are still attempting to advance this technology. Our goal in this project is to use algal biomass to produce bioethanol. We research easy and efficient ways to cultivate, process, and turn algae into ethanol. This strategy lessens the usage of fossil fuels while promoting sustainable energy.

II. LITERARURE REVIEW

The results of numerous studies on the production of bioethanol from algae have improved our understanding of the procedure. These are some significant studies:

1. Making Bioethanol from Microalgae (Harun et al.) Because they contain a lot of carbohydrates, this study concentrated on small algae, such as *Spirulina* and *Chlorella*. In order to liberate sugars, the researchers discussed utilising heat, chemicals, or enzymes to break down the cell walls of algae.

Yeast can then convert these carbohydrates into ethanol. Because they are more specialised and require less energy than chemicals, enzymes are superior. The primary obstacle is the process's high cost and difficulties in scaling up.

2. Production at Pilot Scale Green Algae Utilisation (Hirano et al.) This group employed enzymes to liberate sugars from green algae (*Chlamydomonas*) that they cultivated in specialised tanks. They then produced ethanol using yeast. The amount of ethanol produced was comparable to that produced using conventional methods, according to the data. Additionally, cultivating algae is environmentally friendly because it doesn't require fresh water or cropland.
3. Suganya and Renganathan, two algae-based biorefineries This study examined the production of biodiesel and bioplastics from algae in addition to ethanol. They grew algae using wastewater, which decreased pollution and saved money. According to the study, algae may be used to produce a wide range of beneficial goods in addition to fuel, which increases process profitability and environmental friendliness.
4. Davis et al.'s economic analysis of algae-based ethanol This study examined the expenses associated with turning algae into ethanol. Open ponds and closed tanks were the two growing systems that were compared. If the conversion to ethanol is effective and the algae grows quickly, it suggested, the procedure might be reasonably priced. To cut costs, the study also recommended producing other goods from algae.
5. Present Situation and Prospects (John et al.) The many kinds of algae and their potential applications in the production of ethanol were covered in this review. It examined novel methods for breaking down algae, such as employing microwaves or sound waves. The study also discussed growing algae in wastewater, which lowers costs and protects the environment.

III. MATERIALS AND EXPERIMENTAL METHODS.

1. Raw Material – Algae

- What was used: Dried algae like *Chlorella* or *Spirulina*.
- Why algae? Algae grow fast, don't need farming land, and are full of carbohydrates (sugars) that can be used to make ethanol.

- Where do they grow? Algae can grow in salty water, waste water, or fresh water.
- Other uses: After taking the sugar out, leftover algae can be used for animal feed, fertilizer, or even biodegradable plastic.

2. Composition of Algae

- Mostly made of carbohydrates (20%–60%) – useful for making ethanol.
- Also contain lipids (fats) and proteins – lipids are more useful for biodiesel.
- Fresh algae have lots of water, so drying is needed before processing.

3. Drying and Milling

- Algae were dried using a hot air oven to remove water.
- Then they were crushed into powder to help the enzymes break down the sugars more easily.

4. Equipment Used

- Hot Air Oven: To dry algae.
- Autoclave: To heat algae and break down the structure.
- Water Bath: To maintain a steady temperature during tests.
- Orbital Shaking Incubator: To mix and maintain temperature during fermentation.
- Spectrophotometer: To measure how much sugar or ethanol is present.
- G3 Crucible: For filtering and drying samples during the process.

IV. EXPERIMENTAL METHODOLOGY

1. Pre-treatment (Readiness of Algae) It is difficult to break algae cells. Thus, a mix of alkaline and acid therapy was used.

- The algae were first cooked in an autoclave after being combined with sulphuric acid, a powerful acid. Sugars are released when the cells are broken open, and any potentially dangerous materials are then removed by washing and neutralising the mixture. The algae are now prepared for the following stage.

2. Breaking down sugars by hydrolysis To convert the complex sugars into simple sugars (like glucose), enzymes such as cellulase were introduced. Initially, a fungus (*Aspergillus niger*)-derived enzyme produced in a lab was employed, but the results were poor. Commercial cellulase was later utilised, and it performed better.

3. Ethanol Production via Fermentation The sugar solution was mixed with yeast (*Saccharomyces cerevisiae*).

• After consuming the sugars, the yeast creates carbon dioxide and ethanol. The procedure was run at about 40°C in a shaking incubator. Samples were collected often to measure the amounts of ethanol and sugar.

4. Tests Performed DNS Test: To determine the remaining amount of sugar. Potassium Dichromate Test: To quantify the amount of ethanol generated. Both tests were precisely measured using a spectrophotometer.



Figure.1: grown Algae in Laboratory

5. Current Status and Future Potential (John et al.)

This review discussed different algae types and how they can be used to make ethanol. It looked at new techniques like using sound waves or microwaves to help break down algae. The study also talked about using wastewater to grow algae, which reduces cost and environmental damage.



Figure.2: Algae Biomass used for the Production of Bioethanol

V. RESULTS AND DISCUSSION

The experiment was carried out to find out how much sugar was broken down and how much ethanol was produced from algae.

Test Methods Used:

- DNS Test → measured the amount of reducing sugars (like glucose).

- Potassium Dichromate Test → measured the amount of ethanol made.

Observations:

- In the beginning, sugar levels increased as enzymes broke down the algae.
- After a few hours, ethanol started forming when yeast began fermenting the sugars.
- Ethanol content kept increasing over time as sugar was used up by yeast.

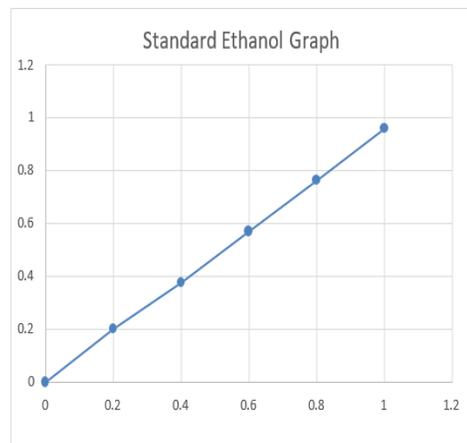
Sample Results:

- At 2 hours → only sugar was seen; no ethanol yet.
- At 20 hours → Small amount of ethanol started forming.
- At 24–27 hours → Ethanol content increased more.
- At 68 hours → Final ethanol and sugar levels were noted.

STANDARD SUGAR GRAPH

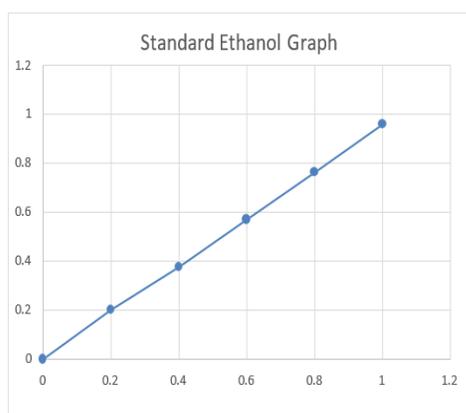
S.no	conc. of glucose (mg/ml)	DNS (ml)	OD values
1	0	3	0
2	0.2	3	0.174
3	0.4	3	0.354
4	0.6	3	0.561
5	0.8	3	0.71
6	1	3	0.913

Table .1: Standard Sugar Graph



S.no	volume of ethanol solution	conc. of ethanol (mg/ml)	K ₂ Cr ₂ O ₇ (ml)	OD values
1	0	0	4	0
2	0.2	0.04	4	0.202
3	0.4	0.08	4	0.377
4	0.6	0.12	4	0.572
5	0.8	0.16	4	0.764
6	1.0	0.2	4	0.961

STANDARD ETHANOL GRAPH



RESULTS IN SAMPLE-1

S.no	Time (hrs)	Sugars		Ethanol	
		O.D (540 nm)	conc. (mg/ml)	OD (620 nm)	conc. (mg/ml)
1	2	0.153	0.182	-	-
2	4	0.177	0.213	-	-
3	6	0.266	0.313	-	-
4	20	0.801	0.927	0.231	0.153
5	22	0.699	0.754	0.269	0.181
6	24	0.625	0.703	0.389	0.249
7	27	0.532	0.598	0.615	0.392
8	68	0.690	0.784	0.371	0.239

UV Visible Spectroscopy Analysis results for Sample 1

RESULTS IN SAMPLE-2

S.no	Time (hrs)	Sugars		Ethanol	
		O.D (540 nm)	conc. (mg/ml)	OD (620 nm)	conc. (mg/ml)
1	2	0.165	0.197	-	-
2	4	0.198	0.229	-	-
3	6	0.280	0.324	-	-
4	20	0.740	0.865	0.216	0.141
5	22	0.615	0.693	0.248	0.175
6	24	0.573	0.645	0.372	0.231
7	27	0.499	0.562	0.594	0.367
8	68	0.654	0.745	0.364	0.241

UV Visible Spectroscopy Analysis results for Sample2

VI. DISCUSSION

1. Pre-treatment Works: Breaking down algae with acid and base both are helped to release sugars from the cells.
2. Enzymes Help Break Sugars: Enzymes like cellulase helped to convert big sugar molecules into small ones (like glucose), which yeast can use.
3. Yeast Makes Ethanol: Saccharomyces cerevisiae was successful in converting sugars into ethanol. The fermentation process was carried out at 40°C. with good yield.
4. Ethanol Yield Was Moderate: The amount of ethanol produced was not very high, it is resulted that yield was obtained adequately from given raw material.

Factors That Affected the Results:

- Type of enzyme: Commercial enzymes worked better than lab-made ones.
- Time: More time allowed more sugar to convert into ethanol.
- Temperature: 40°C was a good balance for both enzyme activity and yeast survival.
- Substrate concentration: More algae could possibly give more ethanol.

VII. CONCLUSION

This project demonstrated that algae can be used to produce bioethanol, a clean and renewable fuel; pretreatment, enzymes, and yeast were used to successfully convert the sugars in the algae into ethanol; commercial cellulase enzymes and Saccharomyces cerevisiae (yeast) produced better

results than lab-made versions; the experiment demonstrated that this method is feasible and environmentally friendly, even though the amount of ethanol produced was not very high; and since algae grow quickly and don't require clean water or farmland, this approach could be a good substitute for fossil fuels.

VIII. FUTURE SCOPE

- Better strains of algae: Using algae that contain more sugar can boost the generation of ethanol.
- Cost reduction: The process can be made less expensive by using wastewater to cultivate algae and improving inexpensive enzymes.
- Large-scale setup: The technique can be expanded for application at fuel stations and industries.
- Integrated systems: In addition to producing ethanol, algae can be utilised to produce biodiesel, animal feed, and bioplastics.
- Environmental advantages: Algae help combat climate change by absorbing carbon dioxide. Algal bioethanol has the potential to play a significant role in green energy in the future with further study and backing.

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