

Bioethanol production from red seaweed using sonication: A review

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ABSTRACT

The most predominate ways for producing bioethanol are from plants, food crops and agricultural wastes. The utilization of such feedstock for bioethanol production leads to critical issues like food deficiency, indirect land use, increased price of food crops. Macroalgae is easily available and carbon-neutral renewable resource that are rich in carbohydrates are suitable for bioethanol production. This study describes the production of bioethanol from red seaweed, *Kappaphycus alvarezii*. The theme of this study is to increase the glucose yield and ethanol productivity through ultrasound assisted saccharification. A comparative study is made for different concentrations of H₂SO₄. The fermentation is performed in a GYP broth by using yeast strain *Saccharomyces cerevisiae* for 48 hours.

Keywords: Bioethanol production, *Kappaphycus alvarezii*, *Saccharomyces cerevisiae*.

1. INTRODUCTION

The increasing demand of an fuel results in various environmental problems, which include global warming, air quality deterioration, oil spills and acid rain. The level of atmospheric CO₂ concentration was reported to be around 350–380 ppm in 2010 and is predicted to increase to 450 ppm by 2020 if no action is taken [1]. The use of biofuel-blended gasoline and diesel has grown in several countries not only to bring environmental

benefits but also to establish cost-competitive domestic energy resources and to generate additional economic development. One of the alternative non-petroleum-based sources of energy being considered worldwide is bioethanol [2]. After the fuel crisis in 1970, worldwide ethanol production was strongly increased. The market of ethanol grew from less than a billion liters in 1975 to more than 39 billion liters in 2006, and is expected to reach 100 billion liters in 2015 [3].

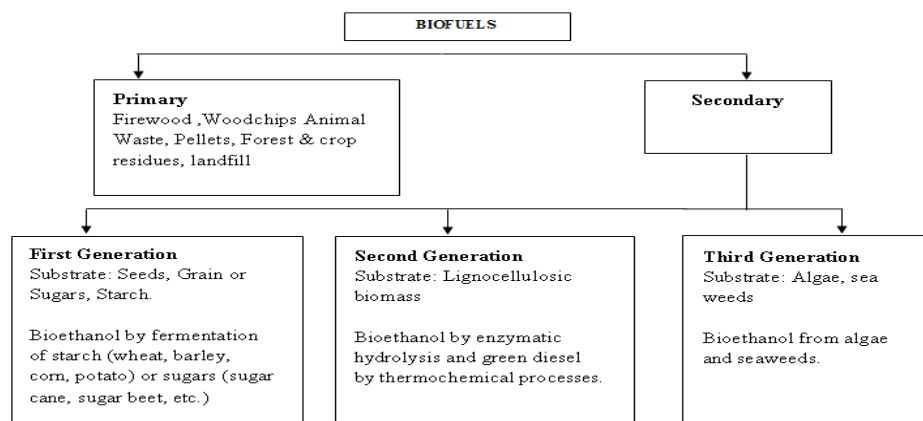


Figure -1: Types of biofuels [7].

The marine ecosystem has vast resources of biomass with high to very high carbohydrate percentage. The marine biomasses like macroalgae having very good potential for bioethanol production [4-6]. They contain high amount of carbohydrates such as starch and cellulose, lipids and proteins. The extracted carbohydrates from marine algae can be used as a source for the production of ethanol [4]. This study has focused on using macroalgae as a feedstock to increase the production of glucose by using ultrasound assisted saccharification which helps in obtaining high yield of bioethanol and to reduce the usage of gasoline.

1.1. Preparation of raw materials

Kappaphycus alvarezii (Figure 2) is collected from Mandapam region, Ramanathapuram district, South east coast of India. The samples are to be washed thoroughly with running water. Using sunlight, the excess water from the sample is drained. The drained sample is dried in an oven at 100°C. The sample is grounded well using ball mill. After size reduction the sample is screened by sieve equipment. The fine particles (Figure 3) retained after sieve analysis is used for the production of glucose which helps in production of ethanol.



Figure - 2: Before pretreatment.



Figure - 3: After pretreatment.

1.3. Estimation of cellulose

1.3.1. Preparation of reagents

Acetic/Nitric Reagent

Mix 150ml of 80% acetic acid and 15mL of concentrated nitric acid.

Anthrone Reagent

Dissolve 200mg anthrone in 100mL of ice-cold 95% sulphuric acid. Prepare fresh and chill for 2hr before use.

67% sulphuric acid

1.3.2. Experimental Procedure

3mL of acetic/nitric reagent is added to a known amount of the sample in a test tube and mix in a vortex mixture. The tube have to be placed in a water bath at 100°C for 30min. The contents of the sample are cooled and are centrifuged for 15-20min. The supernatant is discarded. The residue is washed with distilled water. 10mL of 67% sulphuric acid is added and allowed to stand for 1h. 1mL of the above solution is diluted to 100mL. 10mL of anthrone reagent is added to the 1ml of diluted solution and mix well. The tubes are heated in a boiling water bath for 10min. The tubes are cooled and the intensity of colour is measured by using UV - vis spectrometer. 100mg of carboxymethylcellulose is taken in a test tube and proceed same step after washing residues for standard. Instead of just taking 1mL of the diluted solution, take a series of volumes and develop the color. Cellulose concentration is determined by plotting the graph between concentration and absorbance values and the amount of cellulose in the sample is calculated.

1.3.3. Estimation of glucose

1.3.3.1. Preparation of reagents

Dinitrosalicylic Acid Reagent (DNS Reagent)

1g Dinitrosalicylic acid is dissolved by stirring with 200 mg crystalline phenol and 50 mg sodium sulphite in 100 mL 1% NaOH. It is stored at 4°C.

40% Rochelle salt solution (Potassium sodium tartarate)

40g of potassium sodium tartarate is mixed thoroughly with 50ml distilled water and the volume would make upto 100ml using SMF.

Standard glucose

a) Stock Solution - 100mg is dissolved in 100mL water. b) Working standard - 10mL of stock solution is diluted to 100mL with distilled water.

1.3.3.2. Experimental Procedure

The extract in test tube is pipetted out to 0.5 to 3ml and equalize the volume to 3 ml with water in all the tubes. 3 ml of DNS Reagent is added and the content is heated in a boiling water bath for 5 min. 1 ml of 40% Rochelle salt solution is added. The sample is allowed to cool and the intensity of color is measured by using UV - vis

spectrometer. Run a series of standard using glucose (0-500g). Glucose concentration is determined by plotting the graph between concentration and absorbance values and the amount of glucose in the sample is calculated.

1.3.4. Saccharification

1.3.4.1. Acid hydrolysis and ultrasound assisted Acid hydrolysis of cellulose

Acid hydrolysis of cellulose is performed on preprocessed seaweed using different concentration of sulphuric acid. The process is carried out in a 2.5 L conical flask, 50g dried seaweed is added with 100mL of 0.3 N, 0.6 N, 0.9 N, 1.0 N and 1.2 N sulphuric acid at 100°C in an autoclave for 1 hour. During the process, aliquots of samples are taken for every 30 min. The samples are analyzed for the concentrations of glucose. Ultrasound assisted acid hydrolysis of cellulose is performed on preprocessed seaweed using different concentration of sulphuric acid and are processed in the sono chemical reactor for 2 hours. The same process is repeated and the glucose concentrations are analyzed.

1.3.4.2. Ultrasound assisted saccharification

Ultrasonic exerts its effects by means of generating bubbles in a liquid medium, termed as cavitation. The sound energy created by the ultrasonic probe acted as a source of vibrational wave energy that worked on the molecule within the liquid. This energy produces alternate compressions and stretches towards the liquid medium that produce bubbles. These bubbles are exposed to the same vibrational stresses within the liquid medium, and would grow and eventually collapse violently. This mechanism helps in increased glucose yield [8].

1.3.5. Fermentation

Fermentation process is carried out by using yeast strain *Saccharomyces cerevisiae*. For preparation of inoculum, the yeast strain is added to GYP (glucose 1.0%, yeast extract 0.6%, peptone 0.5%, agar 3.0%,) broth maintaining the pH range 6.4 – 6.8 at 30°C. The inoculated flasks are incubated on a shaker at 150 rpm for 48 hours [9].

2. CONCLUSION

Marine biomass (macroalgae) provides a promising bioethanol feedstock owing to their high biomass yield with superior production relative to various terrestrial crops. Seaweed is one of the most promising biomass materials that can be easily converted to ethanol due to their low concentration of lignin and high biomass yield. The red algae is mainly composed of cellulose, glucan and galactan can serve as an potential feedstock for bioethanol production. Experimental

works are carried out in a sonochemical reactor. Varying concentrations of H₂SO₄, acid hydrolysis is performed with and without the combination of ultrasound. The glucose yields obtained from *Kappaphycus alvarezii* is to be compared with and without sonication.

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