ETHANOL PRODUCTION FROM RED ALGAE USING FERMENTATION PROCESS WITH YEAST

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ABSTRACT

Red algae are microscopic photosynthetic organisms found in sea water. They contain high amount of carbohydrates such as starch andcellulose.They also contain lipids and proteins. They have gained importance in the production of alternative source of fossil fuel.Theextracted carbohydrates from marine algae can be used as a source for the production of ethanol. Algae are also the optimal source for second generation bioethanol due to the fact that they are high in carbohydrates/polysaccharides and thin cellulose walls. In our work, we investigated the possible use of Saccharomyces cerevisiaeVITS-M2 for ethanol production using the carbohydrates extracted from marine algae.The algal sample was collected and carbohydrate was extracted. Extracted carbohydrate was used for fermentation using yeast culture and ethanol was produced. Thefermented product, ethanol was distilled by using laboratory model distillation unit and measured qualitatively using G.C (E.C.D) chromatography in comparison with the standard analytical grade ethanol. The overall experimental data provided us the potential of marine algae in the production of ethanol.

Keywords Ethanol Fermentation, Yeast, Marine algae. Gas Chromatography

INTRODUCTION

Ethanol is also known as grain alcohol. It can be made from barley and wheat or from cellulosic biomass such as wood, paper pulp or agricultural wastes (Thomas and Kwong, 2001). Large quantities of ethanol are used as solvent and chemical feed stock in various industries. Most of the industrial ethanol is currently produced from the catalytic conversion of ethylene.(Demirbas, 2010) Now in whole world amount of fossil fuel reduces to low amount due to various uses and becomes low in quantities. Within next 50 years no fossil fuel will be available in earth sothese days considerable research has been focused on fermentation based ethanol production from various renewable biomass sources.(Ladisch and Svarczkopf, 1991;Worleyet al., 1992; Sosulski and Sosulski, 1994;Ingledew et al., 1995; Wang et al., 1997).

Red Algae contains high amount of carbohydrate which is mostly polysachharide. Many valuable products such carrageenan, agar, asthaxanthin and other dozens of valuable products are produced from algae. Incomparision, ethanol is low priced product. Algae biomass would serve as advantageous substrate for production of ethanol due to its ubiquitous nature. The fermentation of carbohydrate present in algae biomass, to ethanol is achieved by Saccharomyces cerevisiae. Algae are considered to be most important source for the production of clean and renewable energy. Some algae such as Sargassum, Glacilaria, Prymnesiumparvum, Euglena gracilisare promising candidates for ethanol production. Yeast metabolizes carbohydrates and produces CO2 and ethanol as metabolic end product in an anaerobic condition. This study was performed to determine the feasibility of using marine algae to produce ethanol.

MATERIALS AND METHODS

Sample Collection

Red algae were obtained from Eritrea located East Africa. The yeast purchased from local chemicals distributer.

Sample Identification

Samples were identified under microscope. One drop of water was added on the slide and then one specimen of algal culture was added and mixed well. Then coverslip was added over the specimen and observed under microscope.

Carbohydrate extraction

50 ml of identified algal culture was taken in a centrifuge tube.1:3 ratio of distilled water was added to the sample and centrifuged at 5000 rpm for 10 mins. Algae species were hydrolyzed in dilute 1ml of 0.70% H_2SO_4 and were heated at 105°C for 6 hrs. Then the samples were neutralized by adding Ba_2CO_3.Samples were again centrifuged at 5000 rpm for 10 mins. Samples were then evaporated in water bath. Filtration process carried out to filter the extract.

Yeast used for Fermentation

Saccharomyces cerevisiae VITS-M2 strain was used for fermentation process. This yeast strain was cultured in 100ml YEPD broth. Compositions of YEPD are as follows:- 10gm of yeast extract, 20gms of peptone, 20gms of dextrose. 5gm of YEPD powder was added to 100ml distilled water. After sterilization, Yeast strain was added to the YEPD broth and incubated for 48hours in shaking incubator at 37°C.

Fermentation Technique

Filtrate samples were added to the Yeast Saccharomyces cerevisiae VITS-M2 culture after 48 hours and again incubated for 24 hours at 37°C for production of bioethanol by Yeast fermentation process.

Confirmation Test of Ethanol production:

Litmus test

Blue colour litmus paper was dipped in to the separated sample.

Iodoform Test

Few ml of separated sample was taken in a test tube and 1% iodine solution was added.Then dilute sodium hydroxide was added as a drop until brown color of iodine was discharged. Tube was then gently warmed on a water bath.

Ester Test

Few ml of sample was taken in a test tube and 1ml of glacial acetic acid was added followed by addition of 2-3 drops of conc. H_2SO_4 was added. Then the mixture was warmed in an water bath for 10 mins. After that cold water was poured on to it.

Analysis of bioethanol from Marine algae

The amount of pretreated marine algae sugar was measured by HPLC Chromatography.The supernatant was separated from the Fermented Yeast culture was analyzed for bioethanol content. The conc. of bioethanol was measured by using HPLC chromatography. Before measuring the supernatant was concentrated 20 fold prior to HPLC chromatography.

Result and Discussion

Identification of Red Algae

The Red algae which was identified under microscope from sample was found to be Chaetomorphasp.Later this identified algal sample was taken and used for ethanol production. (Fig.1)

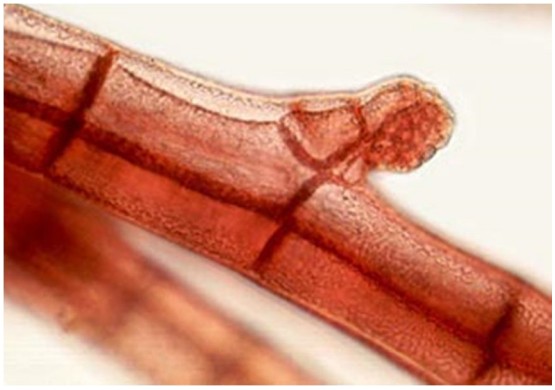

Fig 1. Microscopic views of Chaetomorpha sp.

Extraction of carbohydrate

Dilute acid hydrolysiswas carried out within 100-105ºC for six hours in water bath by adding 0.70% dilute H_2SO_4 in algae sample. After this treatment followed by evaporation and filteration 4.6 gms of carbohydrate were extracted from 50ml of algae sample.

Confirmation Test of Ethanol production

Litmus Test

Blue Litmus paper was changed to red. It indicated the presence of ethanol in the sample

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Iodoform Test

Yellow precipitation was obtained which indicated the presence of ethanol.

Ester Test

Fruity smell indicated presence of ethanol.

Bioethanol Fermentation

Saccharomycescerevisiae VITS-M2 strain was inoculated in 100ml YEPD media and incubated for 48 hours. After 48 hours, Pretreated algae was added to the culture and again incubated for 48 hours.85ml of ethanol was produced after fermentation process.

We compared the unknown sample with standard ethanol. The retention time of both are same. From this we confirmed that bioethanol was being produced from Chaetomorpha sp. by yeast fermentation.

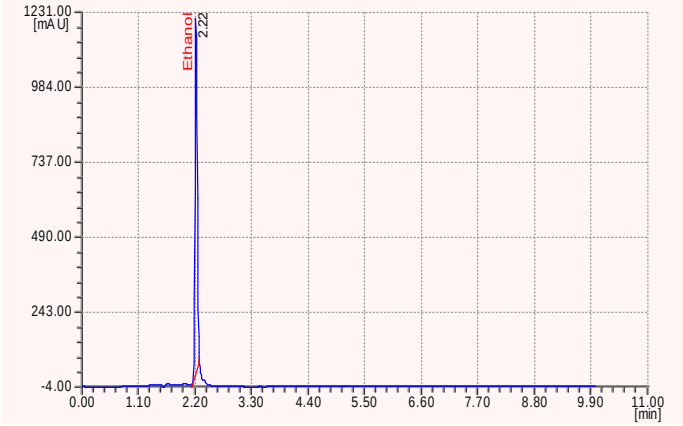

Fig2. G.C analysis of standard Ethanol

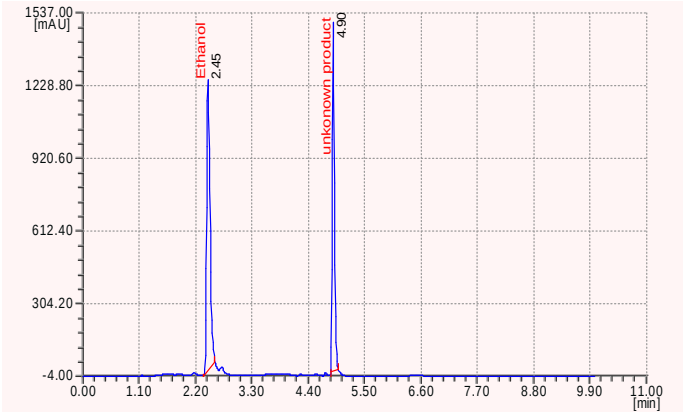

Fig3. G.Canalysis of Ethanol sample

DISCUSSION

In this study by we produced ethanol and unknown organic compound by fermentation of Red algae by using yeast. In previous literature there is ethanol production from different marine plants and algae. In this we got 91% ethanol along with an unknown compound. After chemical and spectral studies we may find out that unknown compound. It is simple and pollution free process. By using this method we may prevent food grains usage in alcohol production.

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