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ETHANOL PRODUCTION FROM FERMENTATION OF GOLDEN ALGAE USING YEAST

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ABSTRACT

Golden algae are microscopic photosynthetic organisms found in fresh water. They contain high amount of carbohydrates such as starch and celloulose. The extracted carbohydrates from marine algae can be used as a source for the production of ethanol. Algae are also the optimal source for second generation bio ethanol 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. The fermented product, ethanol was distilled by using laboratory model distillation unit and measured qualitatively using H.P.L.C (P.D.A Detector) 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, Golden algae.

INTRODUCTION

The golden algae or chrysophytes are a large group of algae, found mostly in freshwater.[1] The term "chrysophyceae" should not be confused with the term Chrysophyta, which is more ambiguous. Chrysophytes contain the pigment fucoxanthin. Because of this, they were once considered to be a specialized form of cyanobacteria Because many of these organisms had a silica capsule, they have a relatively complete fossil record, allowing modern biologists to confirm that they are, in fact, not derived from cyanobacteria, but rather an ancestor that did not possess the capability to photosynthesize. Many of the chrysophyta precursor fossils entirely lacked any type of photosynthesis-capable pigment. Most biologists believe that the chrysophytes obtained their ability to photosynthesize from an endosymbiotic relationship with fucoxanthin-containing cyanobacteria.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).

Golden algae contains of carbohydrate in high quantity which is mostly poly sachharide. 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:

Golden algae were purchased from marine products seller form Chennai. The yeast purchased from local chemicals distributer.

Sample Identification:

samples were identified under microscope. One drop of 3D Millipore water was added on the slide and then one specimen of algal culture was added and mixed well. Then cover slip 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% H2SO4 and were heated at 105°C for 6 hrs. Then the samples were neutralized by adding

Ba2CO3.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 bio ethanol 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. H2SO4 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 bio ethanol was measured by using HPLC chromatography with PDA detector. Before measuring the supernatant was concentrated 20 fold prior to HPLC chromatography.

Result and Discussion:

Identification of Golden algae:

The Golden 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 H2SO4 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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Idoform 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 bio ethanol was being produced from Chaetomorpha sp. by yeast fermentation.

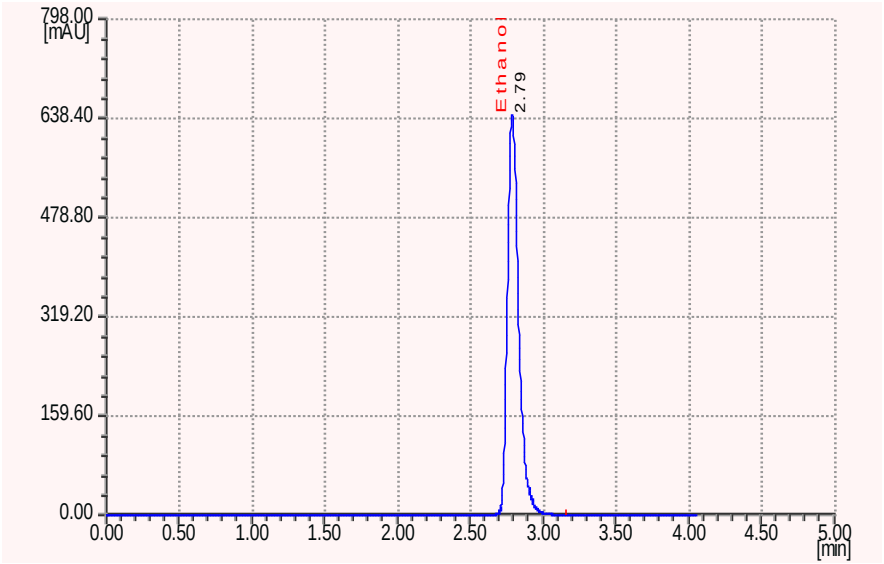

Fig2. G.C analysis of standard Ethanol

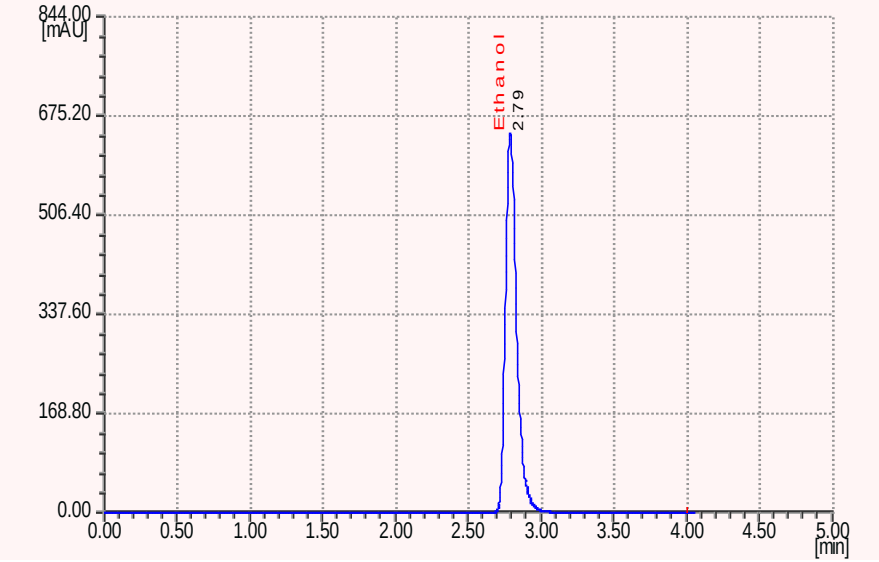

Fig3. G.C analysis of Ethanol sample

DISCUSSION

In this study by we produced ethanol by fermentation of Golden algae by using yeast. In prevoious literature there is ethanol production from different marine plants and algae. In this we got 96% ethanol along with an unknown compound. By using this method we may prevent food grains usage in alcohol production.

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