Bioethanol Production from Locally Growing Algal Biomass: A Promising and Cost-effective Approach

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Original Research Article

ABSTRACT

Background: Energy production and consumption ratio form the hallmark of the economic prosperity of a country. To keep up with the demand and supply of energy a major switch to biofuels is reasoned but the cost associated with production and the choice of raw material forms two major economical and ethical concerns, especially in the under-developed and developing countries where the food is not sufficiently available to everyone. In this scenario, the use of food sources as raw material becomes unjustified.
Purpose: To address these issues, here we made an effort to obtain bioethanol from a non-edible and easily available resource that requires a modest cost of production i.e., a locally available algal bloom. Also, different methods of pre-treatment were employed and scrutinized for their efficacy. These methods of pre-treatment are very cost-effective and easy to administer.
Materials and Methods: The algal biomass was pre-treated separately in three ways viz., freeze-thawing, mechanical disruption and rotten wood treatment. The algal cake left out after extraction of lipid content for biodiesel production was also used as a fourth sample. After pre-treatment, the supernatant was collected and estimated for reducing sugar content and allowed to ferment using *Saccharomyces cerevisiae*. A distillate was obtained and checked for ethanol percentage through gas chromatography.

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Results: The mechanically disrupted sample yielded the highest percentage of ethanol followed by algal cake, freeze-thawing and rotten wood treatment.
Conclusion: Given present food scarcity, the non-edible algae could be a better alternative for bioethanol production as compared to the use of conventional food crops. Through this study, we have found that a better yield can be achieved if the algal biomass is pre-treated via mechanical disruption.

ABBREVIATIONS

ETBE : Ethyl Tertiary Butyl Ether
LCB   : Lignocellulosic Biomass
DNS   : Dinitro Salicylic Acid
RT   : Retention Time

1. INTRODUCTION

In the milieu of economic and environmental benefits, the interest in biomass-based fuel is increasing. The transformation of agricultural wastes into liquid biofuels is asserted but the process suffers when a commercial supply is desired. Hence, there is still a need for new methods [1]. A variety of fuels like, ethanol, hydrogen, methane, methanol and biodiesel can be produced from a variety of biomass [2,3]. Bioethanol is mainly produced by yeast mediated fermentation of biomass. The physicochemical features of bioethanol are different from gasoline. The bioethanol being a high-octane number fuel serves mostly in the transport sector as octane increaser as in, ethyl tertiary butyl ether (ETBE), a mixture of 45% bioethanol and 55% isobutylene. The annual production of bioethanol is at a constant rise, its demand and supply both are predicted to increase by nearly 134.5 billion liters by 2024 [4]. Depending upon the availability, various kinds of raw materials have been utilized for bioethanol production worldwide. The starch-containing feedstocks form 60% of the global bioethanol production, whereas, sugar cane and sugar beet form about 40% [4]. A major hold back in the bioethanol production is the price associated with raw material which represents 40–75% of the total costs and also the fact that the primary value of these raw materials is a food source, it becomes an economic and ethical issue for its utilization in biofuel production especially in developing countries like India [4]. This demands an urgent need for switching to an alternate raw material that is associated with a low cost of production and is not a food source. To address the food security issue, lignocellulosic biomass (LCB) have gained the attention of biofuel industries [5]. However, its conversion is more challenging because of the complex structure of the cell wall demanding an extra cost of processing. In this situation, the focus of attention has shifted to algae which are non-edible and easy to cultivate with the minimum cost of production [6]. But a prior treatment and hydrolysis is needed for both non-LCB and LCB. There is a well-established hydrolysis process for non-LCB biomass such as corn starch. But the breakdown of LCB faces resistance against chemical and enzymatic treatment [7]. Hence, there is a need to develop an improved and economic technique of pretreatment to cut the associated cost of production and aid commercialization of lignocellulosic ethanol [8]. Pre-treatment is an important step that will disrupts the cell wall and separates its constituents like lignin, cellulose, and hemicellulose [9].

1.1 Algae as a Precursor for Biofuel Production

Algae are the best-explored substrate for the production of biofuel. Firstly, the production of algae involves very little effort, just careful attention is required for a good amount of biomass and it is cost-effective too. The parameters necessary for algal growth are nutrients, sunlight, CO_2, pH of the medium aeration, a good amount of water, water level, the surface area of the water body, temperature. Additionally, the successful cultivation of algae on polluted waters has also provided a means of bioremediation [10]. In addition, the algae can be produced throughout the year and are easy to harvest. These can be cultivated on marginal lands and wastewaters therefore they do not compete with food or other crops. In developing and underdeveloped countries, it is not wise to employ food crops for biofuel production or to involve the agricultural land for the cultivation of non-edible crops. Selecting algae is therefore sensible from both aspects. In this context, the cultivation of algae has gained an utmost

importance in India in recent years. Also, there is an optimal solar radiation, daily sunshine hours, precipitation, rainfall and availability of waste land in India. In Fig. 1, we show various regions in India where algal production and cultivation is on rise [11].

The coloured areas of the map showcased the areas where algae is produced in large numbers. It contains Southeast Indian State of Tamil Nadu's coastline and The Gujarat coast lines. These two areas are the most prominent sites for algae production. In Punjab, along with West Bengal, algae are seen to grow in many water bodies.

Algae are a rich source of lipids, carbohydrates and proteins [12,13]. Certain green algae also contain cellulose and starch in their cell wall [14]. All these attributes of algae can be well exploited for biofuel production. The lipid content can be utilized for the production of biodiesel. The algal cake remaining after the extraction of oil is a rich source of carbohydrates and can be utilized for the production of bio-ethanol [15]. The biomass left after carbohydrate extraction can be used for biogas production [16]. Thus, a single substrate can give us all three forms of biofuels. This is a remarkable feature of algae that can outdo all other substrates. The purpose of the present work is to produce bioethanol from locally available algal biomass and to discover the best pretreatment method for a healthier yield so that it can opt as cost-effective for industrial-scale production.
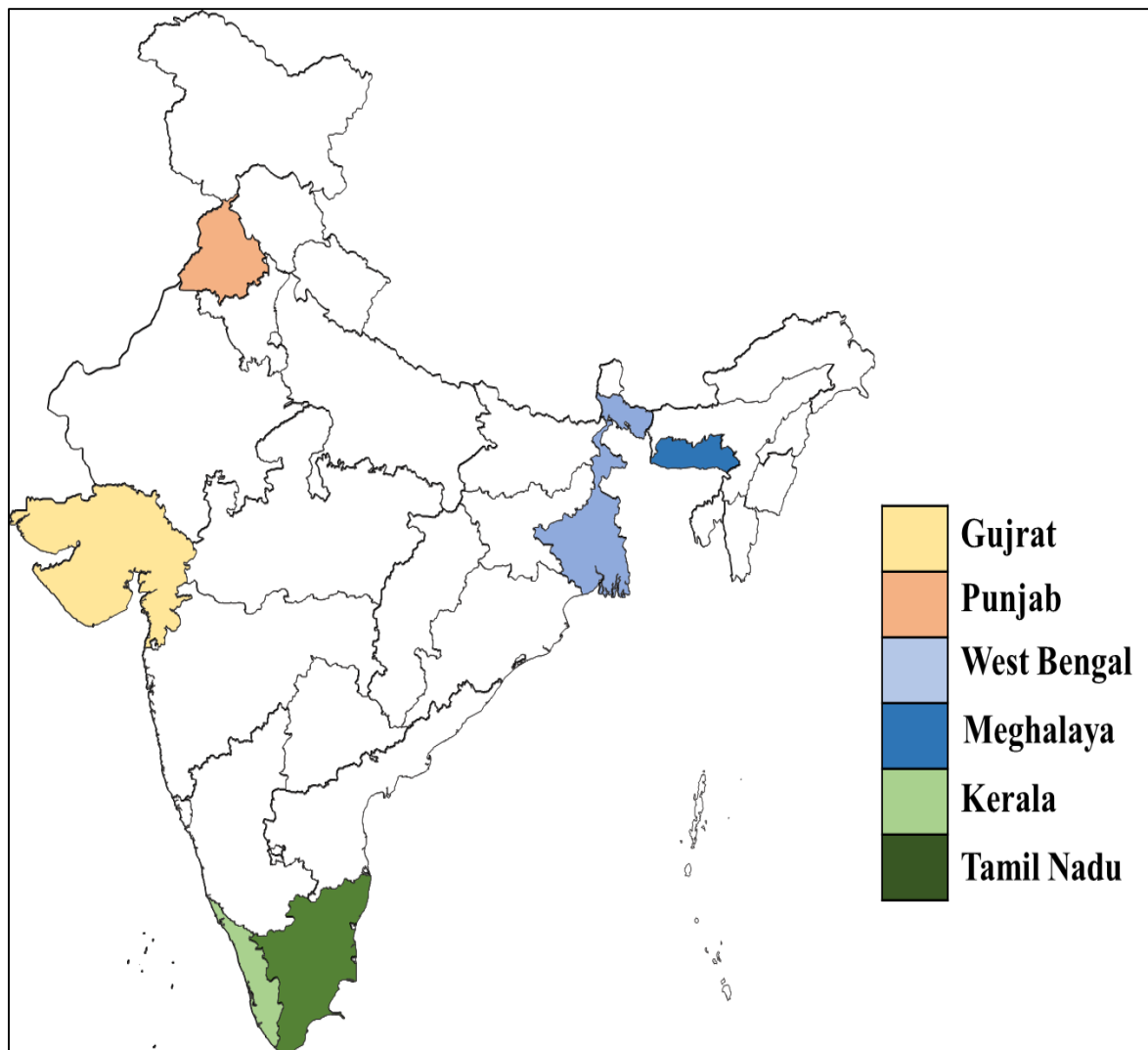

Fig. 1. Algae producing areas of India

2. MATERIALS AND METHODS

2.1 Sample Collection

The dried algal biomass for this experiment was taken from an earlier experiment on "Optimization of growth parameters for indigenous algae" conducted in CSIR-CMERI, CoEFM Ludhiana in 2012. This algal biomass is a combination of different species of algae - *Euglena sp.*, *Closterium sp., Ocillatoria sp., Zygnema sp., Spirogyra sp., Chorococcus sp., Scenedesmus sp., Hydrodictyon colony, Urenoma sp., Navicula sp., Pinullaria sp., Frustulia sp.,* and *Gomphonema sp.*

The algal bloom was cultivated in an open pond under natural sunlight and atmospheric temperature within the premises of CSIR-CMERI, CoEFM Ludhiana. The pH of water was maintained between 8.2-8.7 and regular aeration was administered by using paddle wheels and jet pump. The fully grown algal bloom was harvested, sun-dried and converted to powder form as represented in Fig. 2.

2.2 Sample Preparation and Pre-treatment

Five sterile beakers were taken and 15g of algal powder was placed in each. These samples were pre-treated by different methods. The purpose of pre-treatment is to disrupt the cell to bring out the carbohydrate contents, upon which fermentation can proceed. We have opted for various methods of pre-treatment as depicted in Fig. 3 and compared their effectiveness.

1. The first sample was kept as a control. It was not given any pre-treatment; only distilled water was added to it.
2. The second sample was treated with a powder obtained by crushing rotten wood. About 25g of rotten wood powder and 200ml distilled water was added to it.
3. The third sample was given a thermal shock. It was first frozen and then heated suddenly using a water bath. This cycle was repeated two times. This method of freeze-thawing can be an efficient method of breaking the algal cell wall and is cost-effective too.
4. The fourth sample was mechanically disrupted by using glass beads. Mechanical disruption is an economical method of breaking the cell wall to releases the inner cell matrix.
5. The fifth sample was the algal cake that is left out after the extraction of oil from the algae. The oil extraction was done by using Soxhlet in another experiment to produce biodiesel out of it. We collected the left-over algal cake and utilized it for the production of bioethanol to gain double benefits.

The most efficient method of cell disruption is enzymatic degradation but it is not widely practised in industries due to the high cost of enzymes. Here we attempted an alternate approach, instead of using a pure enzyme, its natural source is used. We treated sample 2 with rotten wood which is supposed to be a substitute for enzyme cellulase. It may act on cellulose, the chief component of the cell wall, and convert it into glucose. This glucose will increase the sugar content of the sample.

2.3 Estimation of Supernatant for Reducing Sugars

After an initial decay and pre-treatment, the supernatant was collected by filtration and Dinitro Salicylic Acid (DNS) test was carried out to quantify the reducing sugar content of the samples. A standard glucose curve was plotted (Fig. 4) through the DNS test and the absorbance of each sample was measured at 575nm. These absorbance values were substituted in the equation: $y = 0.225x + 1.3886$, from the standard curve, and the concentration of glucose was calculated (Table 1). Here y is the absorbance at 575 nm and x is the concentration in mg/ml.
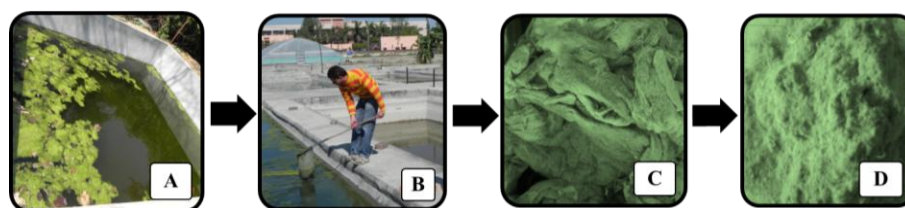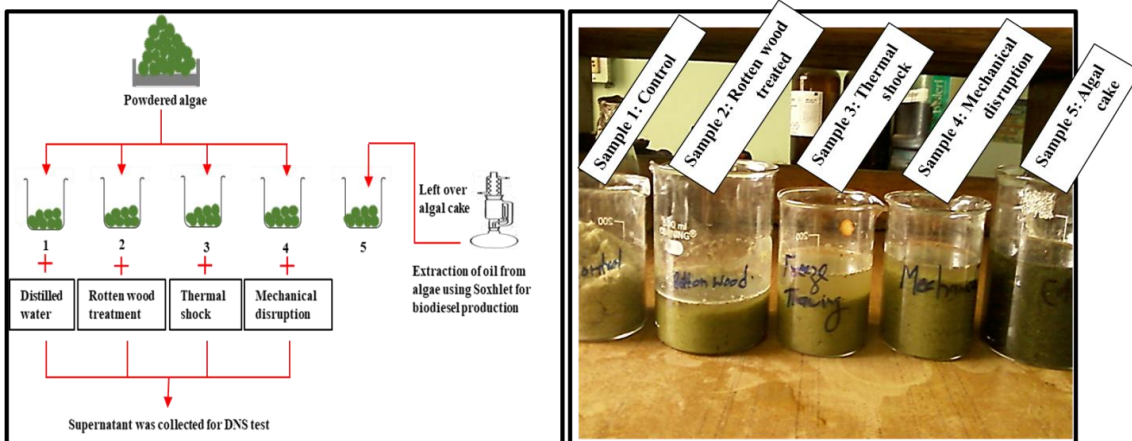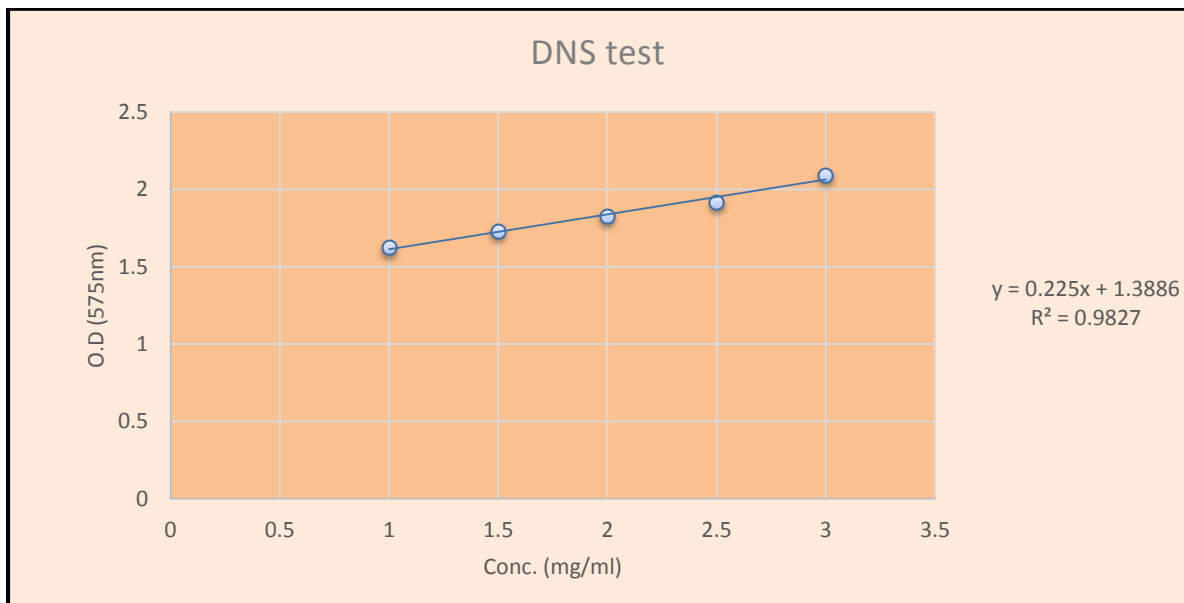

Fig. 2. Algal cultivation and harvesting. (A) Cultivation of algae in an open pond, (B) Harvesting of fully grown algal blooms, (C) Sun dried algal filaments, (D) Powdered algae

Fig. 3. Methods of pre-treatment of algae for the production of bioethanol

Fig. 4. Graph showing the standard curve of glucose obtained through DNS test. Here x-axis represents the absorbance at 575 nm and y-axis represents the concentration of glucose

Table 1. Concentrations of reducing sugar in samples

Samples name and number	Absorbance at 575nm (y)	Concentration in mg/ml (x)
1. Control	0.0023	-
2. Rotten wood treated	0.462	-
3. Thermal shock	0.487	-
4. Mechanical disruption	1.928	2.39733
5. Algal cake	1.626	1.05511

2.4 Fermentation

The samples with considerable sugar content (4 and 5) were fermented using Baker's yeast (*Saccharomyces cerevisiae*). The inoculum was prepared by adding the yeast culture to 25ml of MPYD liquid medium comprising of 0.3% Malt extract, 0.5% Peptone, 0.3% Yeast extract and 2% Dextrose. It was allowed to grow for 48 hours on a rotary incubator maintained at 35^0C and 100 revolutions per minute. 5 ml of this inoculum was added to each sample and allowed to ferment for five days at similar conditions.

2.5 Distillation and Gas Chromatography

After five days distillation was done using the Kjeldahl apparatus. The distillate so obtained was checked for the presence of ethanol with gas chromatography.

3. RESULTS AND DISCUSSION

The present work intends to provide a means to produce bioethanol in a cost-beneficial way. Each step of the process has been chosen to provide for ease of accessibility and reduction in the cost of production. A flow chart depicting each step of this experiment is shown in Fig. 5. The limelight of the present work is the methods of pre-treatment and incorporation of a sample of algal cake which is a left-over product after algal oil extraction in biodiesel production which will deliver double benefit by offering two alternative fuels from same substrate.

The algal sample used in the present work consists of species which co-cultivates and require very little care. These can be easily grown on any stranded water body under normal environmental conditions. Thus, a huge reduction in the cost of cultivation can be attained [17,12,18].

The pre-treatment stage associated with the process of biofuel production constitutes the biggest capital investment [19]. Here we attempted to set out an easy and lucrative method of pre-treatment, wherein the samples were freeze-thawed, mechanically disrupted through glass beads and treated with rotten wood, as an alternative to cut the cost of pure enzymes. The rotten wood forms the habitat for cellulolytic bacteria and thus may exhibit the property of cellulose degradation [20]. The algal cake obtained after lipid extraction for biodiesel was also used. The algal lipid contents have largely been explored for biodiesel production and the carbohydrate left out in algal cake can also be utilized for bioethanol production [21].

The DNS test revealed that the mechanically disrupted sample contains the highest amount of reducing sugars followed by algal cake, freeze-thawing and rotten wood treated sample. The control showed the minimum reducing sugar content because it was not given any pre-treatment. The sample treated with rotten wood showed the second minimum reducing sugar content after control. The failure of rotten wood treated sample to provide a considerable amount of sugar reflect either the presence of inhibitory material or substrate specificity of the cellulolytic bacteria. The pre-treatment of lignocellulosic materials has shown the release of inhibitors and deactivators that influence the enzymatic hydrolysis [9]. A further investigation may be needed in this regard and bacterial strain isolation from its source and examination of its action on the algal cellulosic substrate may provide valuable information. Microbial strain enhancement for cellulosic degradation through genetic engineering can also provide a better tool for cell disruption. A huge possibility has been reported in biological pre-treatments of lignocellulosic biomass [22].
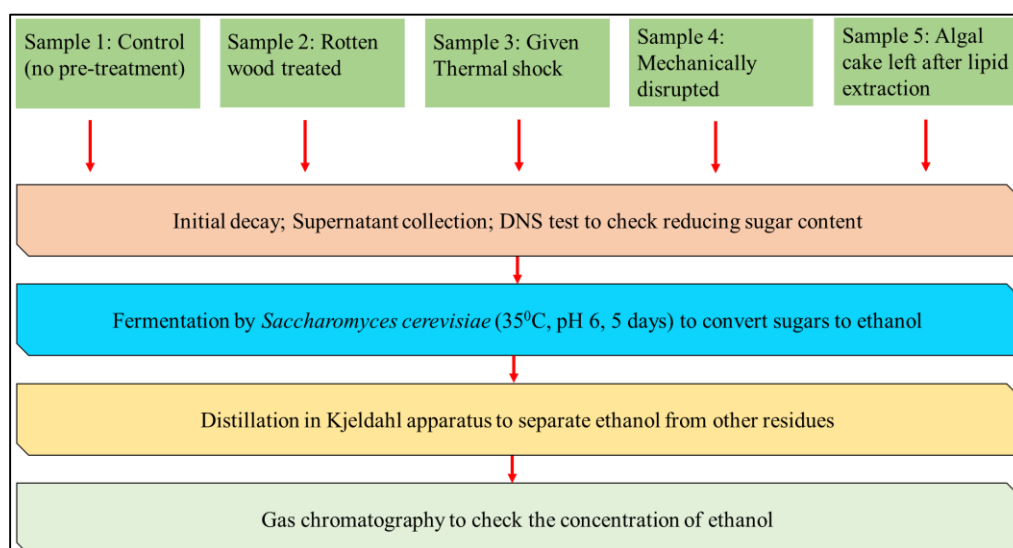

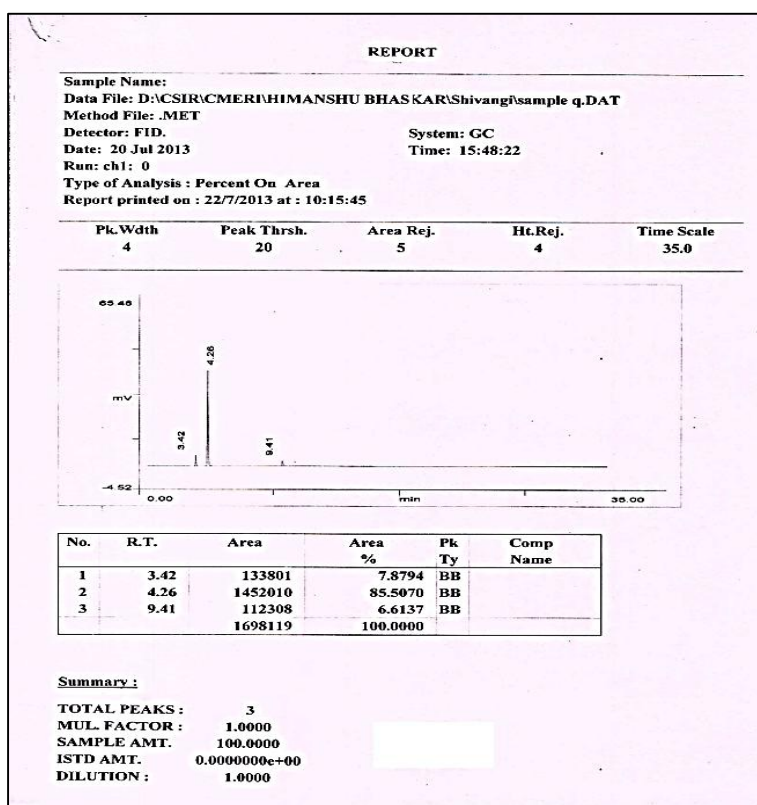**Fig. 5. Schematic representation of bioethanol production from algae**

REPORT

Sample Name:
Data File: D:\CSIR\CMERI\HIMANSHU BHASKAR\Shivangi\sample q.DAT
Method File: .MET
Detector: FID.   System: GC
Date: 20 Jul 2013   Time: 15:48:22
Run: ch1: 0
Type of Analysis : Percent On Area
Report printed on : 22/7/2013 at : 10:15:45

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	20	5	4	35.0

No.	R.T.	Area	Area %	Pk Ty	Comp Name
1	3.42	133801	7.8794	BB	
2	4.26	1452010	85.5070	BB	
3	9.41	112308	6.6137	BB	
		1698119	100.0000		

Summary :

TOTAL PEAKS :   3
MUL. FACTOR :   1.0000
SAMPLE AMT.   100.0000
ISTD AMT.   0.0000000e+00
DILUTION :   1.0000

Fig. 6. Results of gas chromatography of mechanically disrupted sample

REPORT

Sample Name:
Data File: D:\CSIR\CMERI\HIMANSHU BHASKAR\Shivangi\sample q1.DAT
Method File: .MET
Detector: FID.   System: GC
Date: 20 Jul 2013   Time: 16:23:42
Run: ch1: 0
Type of Analysis : Percent On Area
Report printed on : 22/7/2013 at : 10:17:50

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	20	5	4	35.0

No.	R.T.	Area	Area %	Pk Ty	Comp Name
1	3.44	39442	4.4340	BB	
2	4.27	573913	64.5187	BB	
3	9.46	276175	31.0473	BB	
		889530	100.0000		

Summary :

TOTAL PEAKS :   3
MUL. FACTOR :   1.0000
SAMPLE AMT.   100.0000
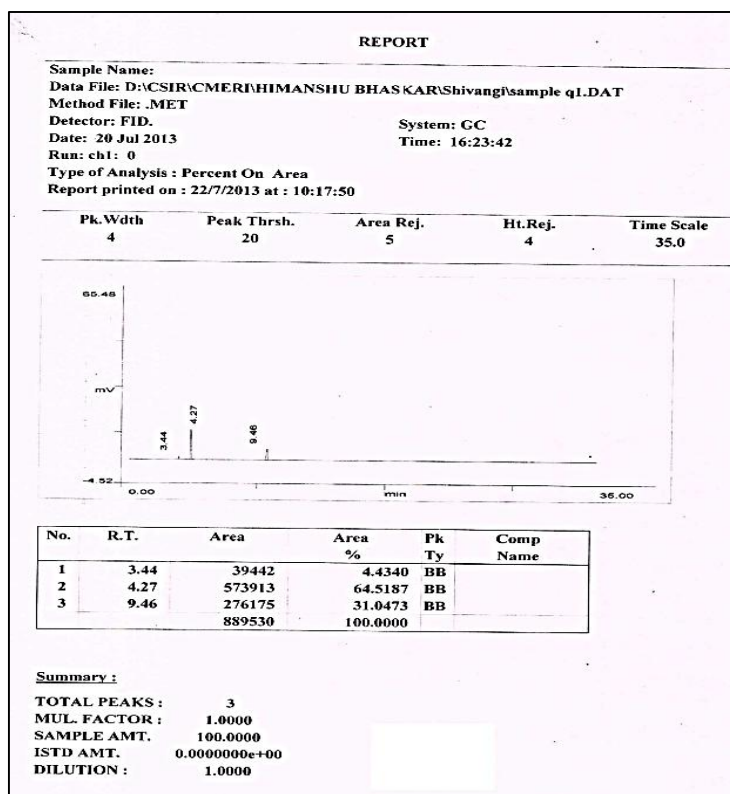ISTD AMT.   0.0000000e+00
DILUTION :   1.0000

Fig. 7. Result of gas chromatography of algal cake left out after extraction of lipids

7

Table 2. Percentage of bioethanol obtained from the Samples 4 and 5

Samples name and number	Percentage of ethanol (%)
4. Mechanical breakdown	85.50
5. Algal cake	64.52

The fermentation of samples 4 and 5 i.e., mechanically disrupted and algal cake produced ethanol and its percentage was checked through gas chromatography. Fig. 6 and Fig. 7 represent the report of gas chromatography. In these two figures, one can see the highest percentage area (85.50% and 64.51% respectively) is covered by the peak corresponding to retention time (RT) of ethanol which is 4.2. These results are further summed up in Table 2.

4. CONCLUSION

The features of algae render it the best feedstock for biofuel production. It can be well exploited in all three ways: biodiesel, bioethanol and biogas production. The present work attempted the production of bioethanol from algae and decode the suitable pretreatment method for high yield. The mechanical breakdown was established as the best way to expose the carbohydrate contents of algae. The algal cake although showed less carbohydrate content, in comparison, but it is having its importance in the case when both the products are desired i.e. biodiesel and bioethanol. The lipid contents can be extracted for biodiesel production and the remaining algal cake can be effectively utilized for bioethanol production.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

ACKNOWLEDGEMENTS

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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