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BIOETHANOL PRODUCTION FROM INDIGENOUS ALGAE

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Abstract

Enhanced rate of fossil fuel extraction is likely to deplete limited natural resources over short period of time. So search for alternative fuel is only the way to overcome this problem of upcoming energy crisis. In this aspect biofuel is a sustainable option. Agricultural lands cannot be compromised for biofuel production due to the requirement of food for the increasing population. Certain species of algae can produce ethanol during anaerobic fermentation and thus serve as a direct source for bioethanol production. The high content of complex carbohydrates entrapped in the cell wall of the microalgae makes it essential to incorporate a pre-treatment stage to release and convert these complex carbohydrates into simple sugars prior to the fermentation process. There have been researches on production of bioethanol from a particular species of algae, but this work was an attempt to produce bioethanol from easily available indigenous algae. Acid hydrolysis was carried out as pre-treatment. Gas Chromatographic analysis showed that 5 days' fermentation by baker's yeast had yielded 93% pure bioethanol. The fuel characterization of the bioethanol with respect to gasoline showed comparable and quite satisfactory results for its use as an alternative fuel.

Keywords: Indigenous algae, Biofuel, Bioethanol, Fermentation, Acid hydrolysis

Introduction

Population outburst together with increased motorization has led to a rapid increase in the demand for fuel. In search of a suitable fuel alternative to fast depleting fossil fuel and oil reserves and in serious consideration of the environmental issues associated with the extensive use of fuels based on petrochemicals, research work is in progress worldwide. Biofuels refer to renewable and sustainable fuels from biological sources that can be used for heat, electricity and fuel. Many organisms naturally produce compounds that can be considered as advance biofuels and algae are the best candidate to accumulate proportionally high amount of biofuel molecules (Paudel, 2014). The production of fuel from algae provides many advantages when compared to the fuel produced from other sources like agro based raw materials. The fuels obtained from algae are termed as third generation fuels. India has many sources of freshwater such as rivers and lakes, where different types of algae are easily found. Algae have chlorophyll and consist of one or more cells and form colonies. Algae contain organic materials such as polysaccharides, hormones, vitamins, minerals and bioactive compounds. So far, the use of algae as a commodity trading or industrial raw materials is still small compared with the diversity of algae species found in India.

The benefit of using ethanol as a fuel is that it reduces levels of lead, sulphur, carbon monoxide and particulates. In addition, there is the global benefit of reducing CO_2 emissions. With the increasing demand on fossil fuel replacements the USA, Europe and other states are using or considering the substitution of petrol with ethanol (Rupprecht, 2009). Since the 1980s ethanol has been an established alternative to fossil fuels in Brazil. It is produced mainly from sugar and starch (sugar cane, corn) (Amin, 2009). Table 1 shows ethanol yield from different sources.

Table 1: Ethanol yield from different sources

Source	Ethanol yield (gal/acre)	Ethanol yield (1/ha)	References
Corn stover	112-150	1050-1400	Tabak, 2009
Wheat	277	2590	Cheryl, 2008
Cassava	354	3310	Cheryl, 2008
Sweet sorghum	326-435	3050-4070	Lueschen et al., 1991; Hills et al., 1983
Corn	370-430	3450-4020	Tabak, 2009
Sugar beet	536-714	5010-6680	Hills et al., 1983
Sugarcane	662-802	6190-7500	Duallibi, 2008
Microalgae	5000-15,000	46,760-140,290	Cheryl, 2008

Microalgae are a potential source of fermentable substrate since, according to the growing conditions; they have high levels of carbohydrates and proteins that can be used as carbon sources, directly available for fermentation or after pre-treatment (Xuan, 2009). Bioethanol can be obtained from fresh algae or the algal remnants left after oil extraction. Thus a common algal biomass can serve multiple purposes by producing biodiesel and bioethanol. Table 2 shows the amount of carbohydrates and protein measured from different algal species. Bacteria, yeast or fungi are microorganisms used to ferment carbohydrates to produce ethanol under anaerobic conditions. Besides the ethanol as main products, carbon

dioxide and water are also formed as by-products. In general, according to simplified reaction equation below, theoretical maximum yield is 0.51 kg ethanol and 0.49 kg CO_2 per kg of carbon sugar, glucose (Dragone et al., 2010). Theoretically, bioethanol production from algae can be a solution for alternative energy sources.

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

Table 2: Amount of protein and carbohydrates from various species of microalgae on a dry matter basis (%) (Becker, 1994)

Algae strains	Proteins	Carbohydrates
Scenedesmus obliquus	50-56	10-17
Scenedesmus quadricauda	47	-
Scenedesmus dimorphus	8-18	21-52
Chlamydomonas rheinhardtii	48	17
Chlorella vulgaris	51-58	12-17
Chlorella pyrenoidosa	57	26
Spirogyra sp.	6-20	33-64
Dunaliella bioculata	49	4
Dunaliella salina	57	32
Euglena gracilis	39-61	14-18
Pymnesium parvum	28-45	25-33
Tetraselmis maculate	52	15
Porphyridium cruentum	28-39	40-57
Spirulina platensis	46-63	8-14
Spirulina maxima	60-71	13-16
Synechoccus sp.	63	15
Anabaena cylindrical	43-56	25-30

Very less research work has been reported on the fermentation of algae for ethanol production. The principle of ethanol production by microalgae consists in the cultivation of microorganisms, harvesting of cells, preparation of biomass, and fermentation and extraction process of ethanol. The preparation of the biomass can be carried out through mechanical equipment, chemicals or enzymes that break down the cell walls, making the carbohydrates more available, as well as breaking down large molecules of carbohydrates. Most commercial-scale ethanol fermentation is done by yeast, mainly *Saccharomyces cerevisiae* that produce ethanol using glucose, fructose, maltose etc. (Hutkins, 2006). *S. cerevisiae* is also known as bakers' yeast or brewers' yeast that is able to change nearly 90% of glucose to ethanol. When cells are broken down, the yeast is added to the biomass and fermentation begins. In this way, the sugar is converted to ethanol by yeasts. Moen (2008) showed that brown seaweed produced higher bioethanol compared to other algae species. Ueda et al. (1996) patented a detailed system for microalgae fermentation. The ethanol produced from fermentation can be purified to be used as fuel and produced CO_2 was recycled to algae cultivation ponds as a nutrient to grow microalgae, thus reducing the green house gas emissions as well. Bush and Hall (2006) patented fermentation process by adding yeast, *S. cerevisiae* to algae fermentation broth for ethanol production. A study by Hon-Nami (2006) indicated that *Chlamydomonas perigranulata* was fermented to produce ethanol, butane-di-

ol, acetic acid and CO_2. They found that hydrogen recovery from that fermentation was about 139% and carbon recovery at around 105%.

This study exclusively describes the influence of acid hydrolysis as a pre-treatment strategy for bioethanol production from indigenous algae obtained from natural open ponds. Most importantly all of these procedures are easy to be executed in a grass root level which is the main focus of this study.

Materials and Methods

Indigenous algae were collected by a net from an open eutrophic pond near the laboratory of CSIR-CMERI, Ludhiana, India (30°53′ N, 75°51′ E). The species were identified by the Department of Aquaculture, College of Fisheries, Punjab Agricultural University, Ludhiana. It was washed with tap water to remove the dirt and sundried for 2 days. This dried biomass was put into a grinder to make a powder. Then the powdered sample was weighed (Adair Dutt make, MJ 500 series electronic balance of range 0-500 g, having readability of 0.001 g) in a 250 ml Erlenmeyer flask and acid hydrolysis was done to release the carbohydrates from the cell walls of algae. It was done by autoclaving (Hindustan Apparatus Mfg. Company make) algal powder and 2N H_2SO_4 in 1:10 ratio at 121°C and 1.2 bars for 45 minutes (Miranda et al., 2012). The conditions set for Gas Chromatograph for bioethanol estimation is presented in Table 3. After that the hydrolysed sample was filtered by Whatman filter papers (Diameter- 18.5 cm, pore size- 11 µ) and checked for the presence of reducing sugar in Spectrophotometer (ESICO make, model- 30IE, range 340-960 nm) at 540 nm by Dinitrosalicylic acid (DNS) test (Miller, 1959). Bakers' yeast was used for preparing stock culture. It was mixed with Yeast Peptone Dextrose (YPD) media and streaked in YPD agar plate and incubated at 30°C for 48 hours. The media was prepared by adding 0.3% Yeast extract, 0.5% Peptone and 2% Dextrose. After the growth of yeast, a single colony was isolated from the agar plate and inoculated into 5 ml sterile Glucose media containing 10% Glucose, 0.1% KH_2PO_4, and 0.5% $(NH_4)_2SO_4$, 0.05% $MgSO_4.7H_2O$, 0.1% Yeast extract at pH 4.5. The incubation temperature was kept at 30°C for 24 hours. Then the sample, containing reducing sugar was inoculated with the glucose seed culture and kept in an anaerobic condition for 5 days at 30°C. After 5 days, the fermented sample was distilled and the distillate was analysed in Nucon 5765 Gas Chromatograph for the presence of bioethanol and its purity. The properties of bioethanol obtained from indigenous algae were characterized and compared (American Standard for Testing Materials) for its suitability to be used as motor fuel.

Table 3: Conditions set for Gas Chromatograph for bioethanol estimation

Detector	Flame Ionization Detector
Carrier Gas	Nitrogen
Oven Temperature, °C	130-180
Injector Temperature, °C	320
Attenuation	4X

Results and Discussion

The species of algae identified, include *Euglena* sp., *Clostridium* sp., *Chlorella* sp., *Chlamydomonas* sp., *Oscillatoria* sp., *Zygnema* sp., *Spirogyra* sp., *Chroococcus* sp., *Scenedesmus* sp., *Spirulina* sp., *Hydrodictyon* colony, *Navicula* sp., *Pinnularia* sp., *Frustulia* sp., *Gomphonema* sp. (identified by Department of Aquaculture, College of Fisheries, Punjab Agricultural University, Ludhiana). The amount of reducing sugar in the sample was measured from the standard curve of glucose. The sample showed an optical density (O.D) of 0.23 at 540 nm in Spectrophotometer (ESICO make, model- 30IE, range 340-960 nm) which corresponds to 15% sugar as in figure 1.

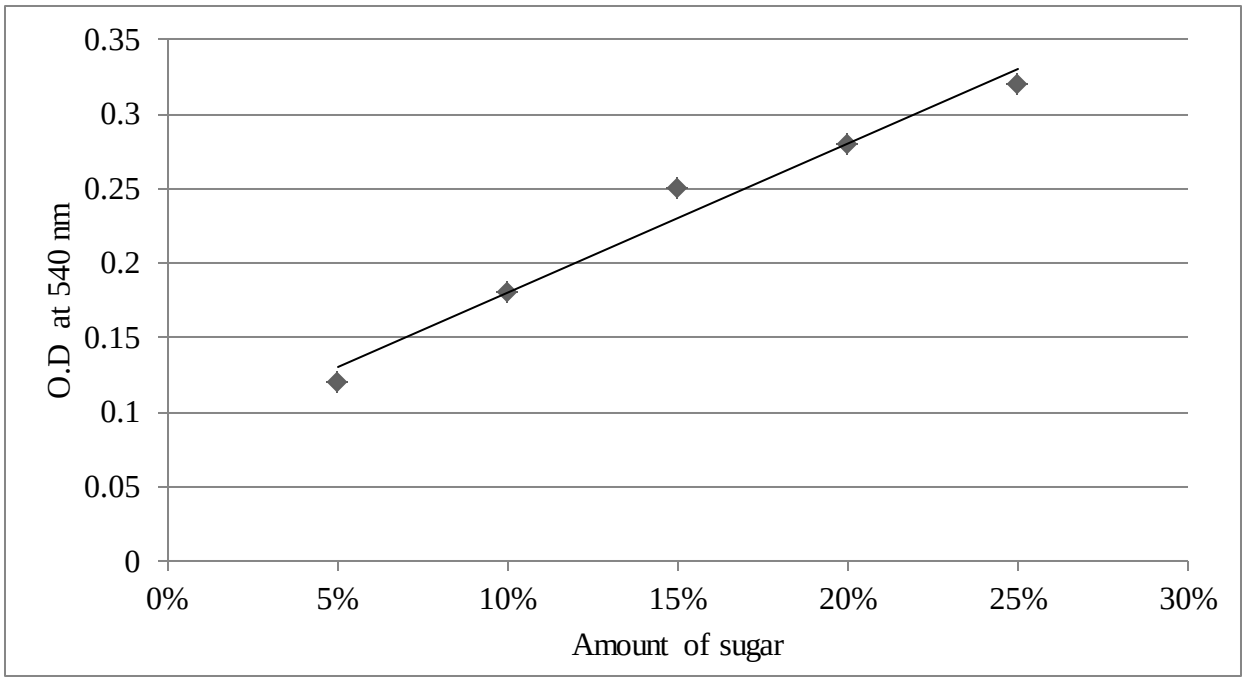


Figure 1: Standard curve of glucose

The distillate was analysed in Nucon 5765 Gas Chromatograph and result showed presence of 93% pure bioethanol.

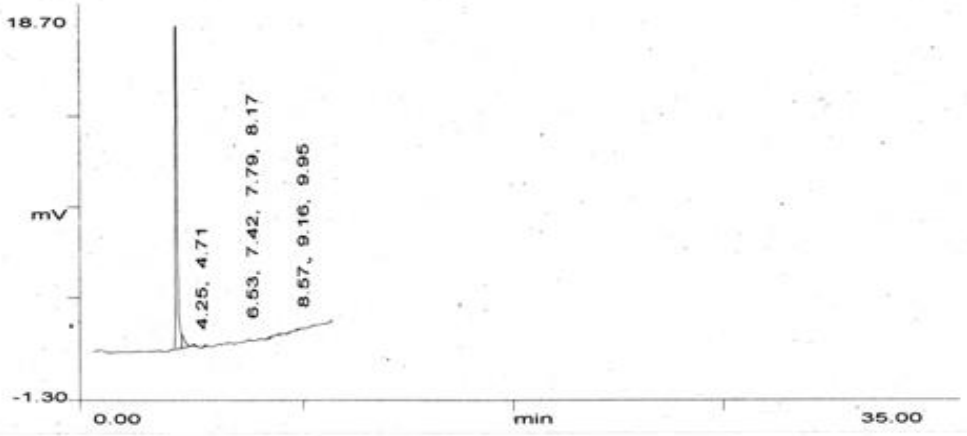
The fuel characterization of bioethanol was determined in accordance with American Standard for Testing Materials' (ASTM) procedures for petroleum products, which showed comparable and quite satisfactory results. The almost neutral pH (6) of bioethanol is suitable for an S.I engine. The boiling point of gasoline is 80°C; whereas the boiling point of the bioethanol was 84°C, 5% higher than the standard. The relative density of petroleum products at 15°C is 0.789 and the experimented result was 0.81 i.e. 2.6% higher than the standard. The API gravity of petroleum has the range of 59.53-55.21; whereas the API gravity of bioethanol was found to be 43.19 i.e. 21% lesser than the standard. The kinematic viscosity of gasoline at 38°C ranges from 1.3-2.4 cS and in case of bioethanol it was found to be 12% higher than the standard, i.e. 2.73 cS. The cloud and pour points of petroleum products were supposed to be 8°C and 3°C respectively; whereas the experimented results for bioethanol were 3°C and -2°C respectively. This 62.5% higher value indicates that this bioethanol can be used in wider range of temperature. The flash and fire points of bioethanol were 87°C and 92°C respectively, which are higher than the standards and is shown in the Table 4. The flash point varies with fuel volatility but is not related to engine performance. Rather, the flash point relates to safety precautions that must be taken when handling a fuel.

The maximum allowable ash content of gasoline at 550°C is 0.01%; whereas the experiments showed there was no ash content after ignition. The carbon residue content of bioethanol was observed 0.04% at 450°C. The calorific value also indicates its suitability as an alternative fuel. Therefore, it can be concluded that this bioethanol will cause very less air pollution and is very safe for our environment. The reports are shown in the figure 2 and 3 below respectively.

REPORT

Sample Name:
Data File: C:\NuChrom\madhuka\ch1def00.DA
Method File: C:\NuChrom\ethenol.MET
Detector: FID. **System: GC**
Date: 13 Mar 2014 **Time: 14:28:22**
Run: ch1: 9
Type of Analysis : Percent On Area

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



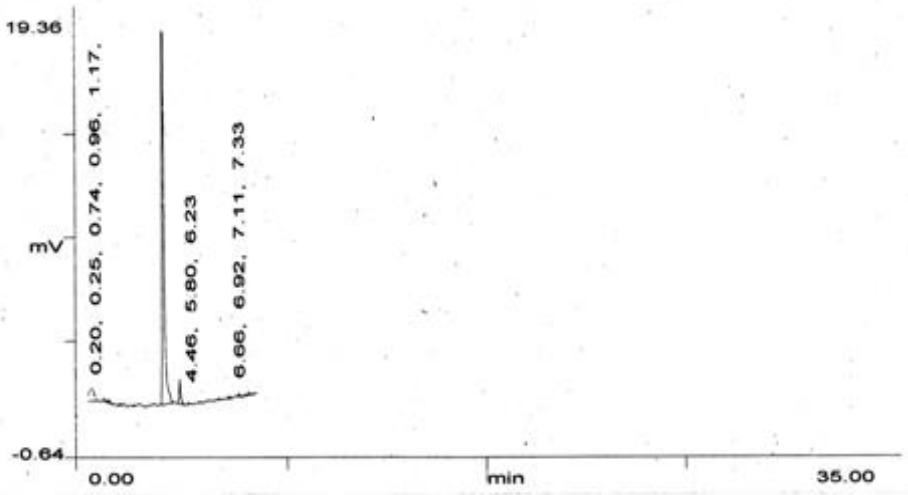
No.	R.T.	Area	Area %	Pk Ty	Comp Name
1	3.52	7106042	98.5844	BV	
2	3.78	74607	1.0350	S	
3	4.25	5582	0.0774	BB	
4	4.71	3592	0.0498	BB	
5	6.53	1694	0.0235	BB	
6	7.42	4284	0.0594	BP	
7	7.79	2729	0.0379	BB	
8	8.17	1975	0.0274	BB	
9	8.57	4648	0.0645	BB	

Figure 2: Gas Chromatograph of absolute ethanol

REPORT

Sample Name:
Data File: C:\NuChrom\madhuka\ch1def00.DA``
Method File: C:\NuChrom\ethenol.MET
Detector: FID. **System: GC**
Date: 13 Mar 2014 **Time: 14:15:30**
Run: ch1: 8
Type of Analysis : Percent On Area

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	0.20	97654	2.5262	BB	
2	0.25	4032	0.1043	TTT	
3	0.74	16314	0.4220	BV	
4	0.96	8823	0.2283	VP	
5	1.45	6972	0.1804	BP	
6	1.93	1411	0.0365	BP	
7	2.09	2666	0.0690	PB	
8	2.97	1595	0.0413	BB	
9	3.34	3628278	93.8593	BB	

Figure 3: Gas Chromatograph of bioethanol

Table 4: Properties of bioethanol sample

Properties	Result
Appearance	Transparent
pH	6
Boiling point	84°C
Relative density at 15°C	0.81
API gravity	43.19
Kinematic viscosity at 38°C	2.73 cS
Cloud point	3°C
Pour point	-2°C
Flash point	87°C
Fire point	92°C
Ash content at 550°C	Nil
Carbon residue content at 450°C	0.04%
Calorific value	3111.75 Kcal/kg

Conclusion

This study demonstrated the feasibility of producing bioethanol from indigenous algae collected from natural resources. Cell wall disruption is essential to release the carbohydrates and it was done by acid hydrolysis with 2N H_2SO_4 at 120°C. *Saccharomyces cerevisiae* fermented the reducing sugar in 5 days and produced 93% pure bioethanol which can be used as an alternative fuel. Though it is too early to comment on this clean energy source at this stage, still it has great potential to be one of the promising alternatives to substitute conventional gasoline in future.

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