Available online at www.sciencedirect.com

SciVerse ScienceDirect

Energy Procedia 32 (2013) 242 – 250

Energy Procedia

International Conference on Sustainable Energy Engineering and Application

[ICSEEA 2012]

Microalgae biomass: a renewable source of energy

M.S.Ghayal[a] and M.T.Pandya[a,*]

[a]*Department of Microbiology, Jai Hind College, Mumbai-400 020. India*

Abstract

Species of green and blue algae from water and soil samples were enriched and isolated. The isolated strains were identified as *Chlorella, Nostoc, Chlamydomonas Oscillitoria, Anabaena. Chlorella vulgaris* a variant was cultivated under optimal conditions to produce biomass as laboratory as well as the site in both bioreactor and open pond systems. Dried biomass with 10% moisture was tested for calorific value by standard methods and the same were in a range of 3000-3500 kcal per kg. Biomass was subjected to recovery of oils as well as blended with coal and calorific values were 4200 to 5300 KC/KG. The cultures were grown on large scale at the factory by purging exhaust fumes of boiler containing 9 % C02, 30.6 mg/NM3 S02 and 36.25 mg/ NM3 N0x using wastewater with a yield of 0.242g/lit/day in 30m3-pond volume. Biomass was subjected to HTL and the oil recovered was tested for chemical and physical properties. Oil content was in a range of 11.451% with energy recovery of 20.531%. GCMS and FTIR analysis of oil showed presence of fatty acids, aldehydes, alcohols and amides. With higher heating values, the developed strains are in high potential for generation of energy as well as mitigating the GHG.

Keywords: microalgae; biomass; chlorella; energy; mitigation

1. Introduction

With the increase in shortage of electricity and constant increase in the cost of electricity, we are forced to look for alternative sources of generation of electricity to meet the present demand. Global warming induced by increase in the concentration of GHG in the atmosphere is a matter of great environmental

* Corresponding author. Tel.: +91-932-266-4705.
E-mail address: mukesh_pandya51@hotmail.com.

doi:10.1016/j.egypro.2013.05.031

concern. Annual emissions of CO_2 are estimated to be 2 x 10^{10} tones primarily from combustion of fossil fuels, with an increasing population and industrialization. Attempts have been made to reduce atmospheric CO_2 using physical and chemical treatments to separate and recover CO_2. Micro-algal photosynthesis has increasingly received attention as a means of reducing the emission of CO_2 from atmosphere [1][2][3]. Emissions from power plants, cement-producing factories & other industries can be controlled by growing photosynthetic micro-algae. It is possible to recycle CO_2 to produce microalgae. Microalgal biomass generated can be used as a fuel to supplement coal in generation of electricity [4][5].

Biologists have categorized micro-algae into variety of classes, mainly distinguished by their pigmentation, life cycle and basic cellular structure [6]. The main groups are *Bacillariophyceae, Chlorophyceae, Cyanophyceae, and Chrysophyceae*. Microalgae are primitive organisms with a simple cellular structure and a large surface to volume body ratio which gives them the possibilities of large uptakes of nutrients. They are fast growing and efficient converters of solar energy capable of producing many times the biomass per unit area compared to terrestrial plants. Microalgae are remarkable and efficient biological factories of converting zero energy in the form of CO_2 into a high-density liquid to be used as bio-diesel and heavy biomass [7][8][9][10][11][12].

The studies were aimed at developing mix biomass of micro-algae which can be an alternate to coal used for generating power using wastewater and CO_2 generated at the industrial site.

2. Material and methods

2.1. Enrichment, isolation and identification

Water samples from freshwater bodies, cooling water deposits, soil and sludge were enriched in CHU no-3 medium. Flasks were incubated at ambient temperature for 10 days in chamber with artificial light (white) source. Four serial subcultures were made in the fresh medium and algal biomass developed was cultivated under same conditions. Biomass after serial subculture was isolated on CHU-no-3 solid media plates and incubated for 7 days in chamber with artificial light sources. Selected microalgal species were cultivated in various culture media like Beneck's liquid medium, CHU no 10, Kuhl and Lorenzen medium, Erddekokt and Salze, Pringsheim medium and Czurda medium [13][14]. Strains were identified using up to genus level using microscopic techniques and Stain C-1 was identified using molecular methods using genetic markers by outside laboratory.

2.2. Cultivation in bioreactor and analysis of biomass

Chlorella species were grown in flasks containing enrichment culture medium for 7 days and the biomass separated was inoculated in 1 liter and 5 liter capacity rectangular open bioreactor and incubated at ambient temperature in chamber with light sources. Developed biomass was then cultivated in larger volumes in photobioreactor as well as open ponding system at thermocol molding factory using exhaust fume using same conditions.

Biomass was allowed to settle and the sediment collected was centrifuged and dried at 105^{o}C. Dried biomass was analysed for TOC (Dichromate reflux method) Protein (colorimetric method), Lipid contents (Soxhlet extraction) and Calorific value (using Bomb calorimeter) as given in Standard Methods for Water and Waste Water Analysis 1989 [15].

Blending with coal was carried out to prepare mixture with 25 and 50% coal and calorific values were determined. Sample processing for determination of calorific value of *Chlorella* sp. was done by drying them at 8O ^{0}C for 24 hours and recording their dry weight. The dried samples were then ground into a fine powder using a mortar and pastel, and placed in desiccators. The calorific content of dried samples was

measured using a Phillipson Microbomb Calorimeter. Small pellets of dried sample (15-30 mg) were placed in the bomb chamber, pressurised to 425 psi with pure oxygen, combusted and amount of heat generated recorded. The calorimeter was calibrated against benzoic acid standards [16]. Dried biomass of *Chlorella* was subjected to hydrothermal liquefaction to recover oil and the same was analysed for oil contents along with GCMS and FTIR analysis to determine the chemical and physical properties of oil.

3. Results and discussion

The energy crisis of the 1970s was followed by increased concern for the environment, with particular emphasis on protection of the global ecosystem. The RAPAD established in Japan investigated the development of technologies for biomass conversion and utilization, in particular, the production of ethanol from cellulosic biomass. Various forms of biomass resources exist. Among these, sugar and starch crops are inappropriate for use as energy sources since they are primary food sources, and are unstable from the viewpoints of long-term supply and cost. Cellulosic resources, on the other hand, represent the most abundant global source of biomass, and have been largely unutilized [17].

Development of technologies that efficiently produce biomass and convert it to more convenient forms of energy is therefore very important [18]. Microalgae have been investigated for the production of a number of different biofuels including bio-diesel, bio-oil, bio-syngas, and bio-hydrogen. The production of these bio-fuels can be coupled with flue gas CO_2 mitigation, wastewater treatment, and the production of high-value chemicals. Developments in micro-algal cultivation and downstream processing (e.g., harvesting, drying, and thermo-chemical processing) are expected to further enhance the cost-effectiveness of the bio-fuel from micro-algae strategy [19]. Biofuel is a renewable energy source produced from biomass, which can be used as a substitute for petroleum fuels [20]. Biofuels are expected to play an increasingly important role throughout 21st century [21][22]. Algae based biofuels are considered as a viable option as the oil productivity of many algae exceeds that of oil crops.

Table 1. Comparison of potential oil yields of algae and other oil seeds [23]

Crop	Oil yield gallon/acre
Corn	18
Cotton	35
Soyabean	48
Mustard seed	61
Sunflower	102
Rapeseed/Canola	127
Jatropha	202
Oil palm	635
Algae	
10g/m²/day at 15% Triglycerides	1,200
50g/m²/day at 50% Triglycerides	10,000

Table 2. Identification of isolated algal cultures

Site where the sample was collected	Abbreviation	Taxon identified.
Puddle at Churchgate	C1	Chlorella vulgaris
Darjeeling soil sample	D2	Chlorella
Toxic effluent from Industry	Al	Chlamydomonas
Sea water isolate	SWI	Dunaliella
Darjeeling tree bark	D4	Chlorella
Gangtok tree bark	U4	Chlorella
Powai lake sample	PL	Oscillatoria
Borivali National park water sample	NP	Nostoc
United States soil sample	USA 1	Rivularia
Goregaon Soil sample	GS	Spirulina
Rain water pool sample	U K	Lyngbya
Bandra wall scraping sample	BW	Anabaena
Upvan lake sample	UL	Microcystis
Darjeeling tree bark sample	D4	Chlorella
Cement factory	Ultra1	Oscillatoria
Cement factory Green deposit	Ultra2	Spirulina
Cement factory	Ultra3	Spirogyra
Cement factory Air condition duct	Ultra4	Chlorella
Cement factory soil sample	Ultra5	Scendesmus
Cement factory effluent sample	Ultra6	Closterium
Thermacol factory-1	KK1	Chlorella
Thermacol factory-2	KK2	Ulothrix
Thermacol factory-3	KK3	Oscillatoria

In the present studies microalgae strains were enriched and isolated from various samples using cultivation techniques used for mineral salt enrichment culture. After serial subculture in CHU-3 medium algal strains were isolated on solid media and identified up to genus level. Table 2 shows the results. Most of these were green and blue green algae species. These strains showed excellent growth in mineral salt media supplemented with carbonates as source of carbon. Cooling water deposits showed wide variety of algae species. All the selected media supported heavy growth of both green and blue green algae. The organisms isolated were identified by microscopic methods as species of *Diatoms, Chlorella, Chlamydomonas, Nostoc Oscillitoria and Anabaena*. Growth of *Diatoms* and *Chlorella* was more as compared to other strains. Both *Nostoc* and *Anabaena* showed ability to fix nitrogen and grew in media without nitrogen source. Nitrogen and Phosphorus concentrations influenced the composition of cellular biomass. At the limiting levels of nitrogen the lipid contents increased.

Isolate C-1 was identified using genetic markers and identified using sequence matching with standards as available on website www.scigenom.com. as *Cholrella vulgaris* shows significant similarity with *Chlorella vulgaris* based on nucleotide homology and phylogenetic analysis. Blast reports showed E value to be zero. Zero E value indicated there was no random matching. Blast results indicated 97 % identity with Chlorella vulgaris which means this particular strain C1 may be a mutated strain. This strain showed

a significant growth and the growth rate was excellent. This strain along with other was used for different studies.

Microalgal biomass generated can be used as a fuel to supplement coal in generation of electricity [24][25]. Preliminary results have shown that the co-firing of 7 % biomass, on a heat input basis, with crushed, pulverized coal can lower NO_x and CO_2. Micro-algae offers have excellent lipid contents and thus are good source for bio-diesel. The use of microalgae as sources of liquid fuels is an attractive proposition from the point of view that microalgae are photosynthetic renewable resources, are of high lipid content, have faster growth rates than plant cells, and are capable of growth in saline waters which are unsuitable for agriculture.

Four strains of Chlorella C-1, D-2, D-4 and U-4 were used for large scale cultivation at the site using Boiler exhaust fume. Growth in photobioreactor as well as open ponding system was excellent and gave promising results. Table 3 shows results of Biomass of Chlorella species.

C-1 grown in the presence of chimney fumes and CO_2 in a photobioreactor showed better growth as compared to other strains including chimney fumes. Conditions of cultivation were same for both photobioreactor as well as ponding system. Increase in biomass indicated these cultures could grow in the presence of 9 % CO_2, 2.66 kg/d of SO_2 and 36.25 mg/nm^3 of N0x. This was average concentrations of chimney gases at the site. Growth rate was quite good in the modified medium and with changes in cultivation conditions it improved well. C-1 was with higher growth rate compared to other strains.

Table 4 shows the biomass of C-1 strain produced in open pond and this cultivation was carried out in ponds with 10Kl to 30KL capacity and rate of growth was determined. Similar results were obtained in photobioreactor with 10lit capacity.

Table 3. Biomass of chlorella species

Purging	C1 mg/l	D2 mg/l	D4 mg/l	U4 mg/l
CO2	1100	860	950	750
Chimney fumes	1020	670	780	700

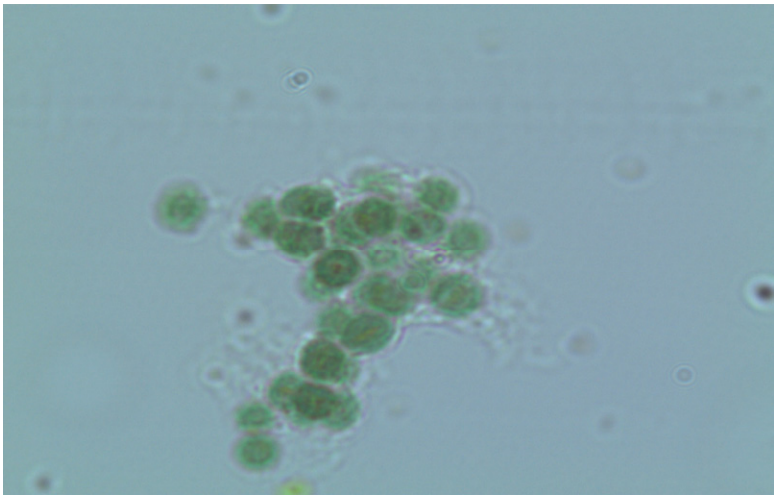

Fig 1. The photograph shows morphology of C-1 (chlorella vulgaris) strain.

Table 4. Biomass in ponding system

	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5
Biomass g /250 l	418	426	435	410	425
Biomass g / l	1.672	1.7	1.74	1.64	1.7
Biomass g / l /d	0.238	0.242	0.248	0.234	0.242

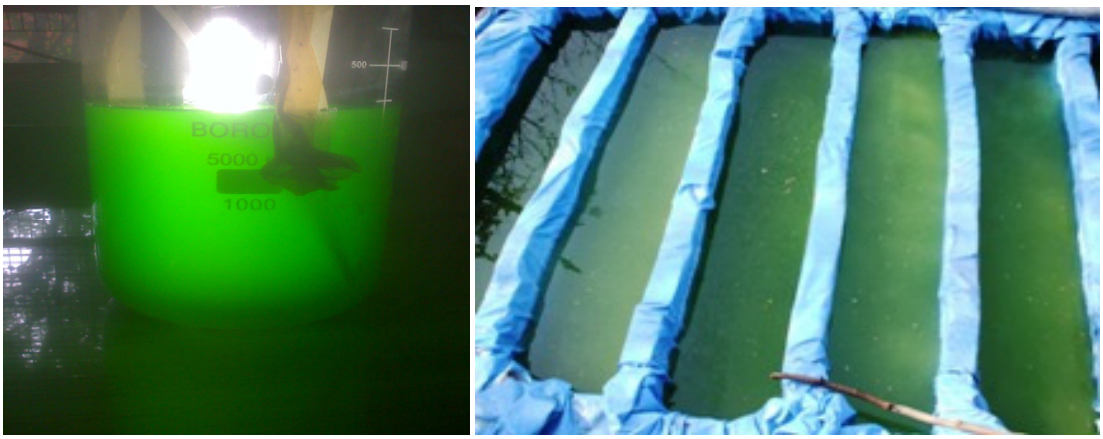

Fig 2. Photograph of photobioreactor and ponding system.

3.1. Biomass analysis

Strains were grown on large scale in bioreactor with 10lit capacity. The biomass was allowed to settle and the collected sediment was centrifuged and dried in oven at 105^{o}C. In a period of 7days the growth yields were in a range of 5-10gm/lit. The Protein content of these strains was in a range of 40-60% TOC 40-55% and lipid contents 12-18%.

3.2. Hydrothermal liquefaction

While the lipid content of microalgae, on a dry cellular weight basis varies between 20 and 40 %, lipid contents as high as 85 % have been reported for certain micro-algal strains. *Botryococcus braunii,* is a unique micro-algal strain, having a long-chain hydrocarbon content of between 30 and 40% (dry weight basis), which is directly extractable to yield crude oil substitutes. Both physical and chemical processes are applicable in the production of liquid fuels from algal strains of high lipid content. These processes include direct lipid extraction in the production of diesel-oil substitutes, trans-esterification in the formation of ester fuels, and hydrogenation in the production of hydrocarbons. Oily substances are also produced via liquefaction of micro-algal biomass through thermo-chemical reactions under conditions of high pressure and temperature.

Algal biomass collected during cultivation of Chlorella in open pond was subjected to hydrothermal liquefaction to get bio oil or bio crude. The hydrothermal liquefaction of Chlorella was performed using a hydrothermal reaction system which consisted of a 1.8 L batch stirred reactor (PARR Instruments Co.USA). HTL was done in stainless steel autoclave with mechanical mixing.

The products of liquefaction include gaseous, aqueous and solid phases [26]. Viscous tar-like matter attached to the cooling pipe and the inner wall of the autoclave, and some floated on the aqueous surface. Total amount of bio oil extracted from Chlorella C1 cells after HTL was 11.45 %.

3.3. Energy balance of the liquefaction process

Energy recovery (%) = HHV of biocrude X mass of biocrude / HHV of feed X mass of feed. X 100
= (34720 X 1.44/3386 X50) X 100 = 29.531% (1)

This does not compensate for any processing energy used in the liquefaction reaction (Biller et al 2011) The GCMS results indicated presence of C17,C19 hydrocarbon (alkane and alkenes) alicyclic and aromatics acids, amines and carboxylic acids. The presence of alkanes and alkenes were confirmed by FTIR. FTIR results showed presence of alkane, alkene, carboxylic acids, amino acids, primary and secondary alcohol, aliphatic amine and aromatic compounds. The heating value of oil was higher as compared to the reported [27], when compared with the various properties of algal oil recovered during this study.

3.4. Calorific values of Chlorella strains

Table 5 shows the results of Calorific values of Chlorella strain and the same was quite good comparable with commonly used substrates for energy generation. As the strains were cultivated on exhaust fumes it showed one method for mitigating problems due to GHG emission effects Chlorella species were grown in medium with purging CO_2 as well as chimney fumes for 7 days. Cell mass of each species was harvested, dried, and then subjected to analysis for Calorific values as given below. The biomass was analysed for its calorific value. The dried biomass with about 10% moisture had calorific values in a range of 3000-3500Kcal/kg. Attempts were made to grow micro-algae on large scale economically. One kilogram of dry algal biomass utilizes up to 1.7 KG CO_2

Table 6 shows calorific values of Chlorella biomass mixed with coal in 25 and 50% levels. It can be seen that biomass blending can be one alternative to reduce coal usage as the even at 25% coal blending Calorific values are quite comparable although not as high as coal itself. It can be also blended with other renewable sources of energy.

Table 5 Calorific values of various chlorella strains

Chlorella strains	Open tray KC/KG		Photobioreactor KC/KG	
	Purging CO2	Purging chimney fumes	Purging CO2	Purging chimney fumes
C1	2540	2380	3280	3105
D2	1600	1575	2300	2692
D4	1630	1600	2584	1700
U4	2500	2708	3300	3832
C1+D2+D4	2670	2800	3400	3190
C1+D2+D4+U4	3160	3008	3290	3465

Table 6. Calorific values of chlorella strains blended with coal

Biomass +Coal powder	KC/KG	Ash (%)	KJ/KG
C1	4205	30.5	17605
C1 + Coal powder(50+50)	5300	22.3	22190
C1+Coal powder(75+25)	4500	32.83	18840
Coal	6200	13.38	25958

4. Conclusion

Different green algae, blue green bacteria and diatoms are isolated from various samples Concentration of CO_2, which is a major greenhouse gas, can be reduced by generation of biomass for production of electricity. The algal species *Diatoms, Chlorella, Chlamydomonas, Nostoc and Anabaena* had good calorific values and lipid contents. Extensive studies carried out using C-1 strain which was identified as variant of *Chlorella vulgaris* showed excellent results and it has added advantage of separation making downstream process very easy. The oil contents were 11.45% with energy recovery of 29.531% with higher heating values as compared to other strains. C-1 could be easily blended with coal as solid fuel since the bled had calorific values comparable. The growth rate of C-1 was quite good in both photobioreactor as well as open ponding system and it could be easily cultivated using chimney fumes from the plant. Using these strains can develop biomass on large scale in photobioreactor as well as open ponding system which can be dried and used as a supplement for coal. Microalgae development can give tailor made solutions for industries with uninterrupted power supply at reasonable cost.

References

[1] Kodama M, Ikemoto H, Miyachi S. *J Mar Biotech* 1993; **1**:21-25.

[2] Yang SS, Chang EH, Lee JY, Hong YY, Lan CR, *Biol Fertil Soils* 1997;**25**:245-251.

[3] Yang SS, Chang EH, Lee JY, Hong YY, Lan CR. Month. *J Taipower's Eng* 2000;**624**:65-82.

[4] Danielo, Olivier. An Algae based fuel. *Biofutur* 2005;No.255.

[5] Schulz, Thomas. The Economics of micro-algae production and processing into biodiesel. Farming Systems, Department of Agriculture and Food, Western Australia; 2006.

[6] Mauseth DJ. *Botany an introduction to plant biology*. Jones and Bartlett Publishers; 2003.

[7] Sawayama S, Inoue S, Dote YD, Yokoyama SY. *Energy Convers Mgmt* 1995;**36**: 729-731.

[8] KuranoN, Ikemoto H, Miyashita H, Hasegawa T, Miyachi S. *J Mar Biotech* 1995;**3**:108-110.

[9] Kurano N, Ikemoto H, Miyashita H, Hasegawa T, Hata H, Miyachi S. Energy Consers Mgmt 1995;**36**: 689-692.

[10] Chang, Ed-Haun, Yang, Shang-Shyng. *Bot Bull Acad Sin* 2003;**44**:53-52.

[11] Yue L, Chen W. *Energy Conservation & Management* 2005;**46**(11-12):1868-1876.

[12] Moris MG, Costa JAV. *Jour Bact* 2007;**129**(3):439-445.

[13] Sheldon A. *Experimental microbial ecology*. Academic Press; 1970.

[14] Vonshak A . Laroratory techniques for the cultivation of microalgae. In: Richmond A, editor. *Handbook of Micro-algal mass cultures*, FL: CRC Press; 1986, p. 117-199.

[15] APHA. *Standard Methods for the Examination of Water and Wastewater*. 17th Edn, American Public Health Association, Washington, D.C.; 1989.

[16] Lamare DM, Wing RS. Calorific content of New Zealand marine macrophytes. *New Zealand Journal of Marine and Fresh Water Research* 2001;**35**:335-341.

[17] Hall DO, House JI. *Biomass and Bioenergy* 1994;**6**:11-30

[18] Borowitzka MA. In: Borowitzka MA, Borowitzka LJ, editors. *Micro-algal biotechnology*, Cambridge: Cambridge University Press; 1988, p. 257-287

[19] Li Y, Horsman M, Wu N, Lan CQ, Calero ND. *Biotechnol Prog* 2008; Article 10.1021/ 371-2.

[20] Demirbas A. Use of algae as biofuel sources. *Energy conversion and management* 2010;**51**:2738-2749.

[21] Hoekman K. Biofuels in U.S.-challenges and opportunities. *Renewable Energy* 2009;**34**:14-22.

[22] Atadashi IM, Aroua MK , Abdul Aziz A. High quality biodiesel and its diesel engine application: a review. *Renewable and Sustainable Energy Reviews* 2010;**14**:1999-2008.

[23] Pienkos PT. *Potential for biofuels from algae.* NREL/PR-510-42414. National Renewable Energy Laboratory (NREL), Golden, CO; 2007.

[24] Zeiler KG , Heacox DA, Toon ST, Kadam KL, Brown LM. *Energy Convers Mgmt* 1995;**36**:707-712.

[25] Kadam KL, Sheehen JJ. Microalgae technology for remedation power plant flue gas. *World Resource Review* 1996;**8**(4):493-504.

[26] Biller P, Ross AB. Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. *Bioresource Technology* 2011;**102**:215-225.

[27] Amin S. Review on biofuel oil and gas production processes from microalgae. *Energy Conversion and Management* 2009;**50**:1834-1840.