A comparative study of the potential of some Microalgal species as Biofuel energy crop

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Abstract

The shortage of fossil fuels and climate change has led mankind to search for alternative energy sources. Efforts are being made to maximize productivity of biomass to accomplish the future demand of energy. Biofuels play a crucial role in mitigating CO_2 emission and act as a potentially cheap alternative in comparison to conventional energy sources. Hence, the production of algal biomass for biofuels and its application in wastewater treatment is drawing attention. Microalgae are fast-growing and therefore an important biomass source. They can be grown almost anywhere, even on sewage. They can serve as a replacement for oil-based fuels, whose advantages outweigh the disadvantages.

In the present study, an effective and feasible method was employed to extract algal oil from species of microalgae belonging to the genus *Chlorella* and *Scenedesmus*. The microalgae were grown in different media, algal oil was extracted from the dry biomass and the %yield calculated. In BG-11 medium *Scenedesmus dimorphus* showed maximum %yield of 66.67% followed by *Chlorella sorokiniana* (50.00%), *Chlorella regularis* (44.44%), *Chlorella saccharophila* (33.33%), and *Scenedesmus species* (16.67%).In Chu No.10 medium *Scenedesmus dimorphus* showed maximum %yield of 75.00% along with *Chlorella regularis* (75.00%), followed by *Chlorella saccharophila* (66.67%), *Chlorella sorokiniana* (50.00%), and *Scenedesmus species* (50.00%) each. In Chlorella medium, all 3 species of *Chlorella*, i.e. *Chlorella saccharophila*, *Chlorella regularis* and *Chlorella sorokiniana* showed a %yield of 75.00%. This was followed by *Scenedesmus dimorphus* at 50.00% and *Scenedesmus species* provided a meagre yield of 10.00%.

Keywords: Microalgae, Energy, Biofuel, *Chlorella*, *Scenedesmus*

Introduction

Access to affordable and environmentally sustainable fuels and energy sources may be the greatest challenge of this century. As demand continues to grow and new supplies become more expensive to mine, the availability of fossil fuels will inevitably decrease, leading to higher global energy prices. Microalgae are among the most promising renewable alternative feedstocks for non-food crop-based biofuels due to several properties such as non-competition with food and feed crops, high oil content, and fast growth. Microalgae are microscopic photosynthetic organisms found in fresh, brackish and sea water. These organisms use solar energy to create biomass and accumulate triacylglycerides (TAG), which can be converted into biodiesel through a transesterification reaction. The mechanism of photosynthesis in microalgae is similar to that of higher plants, but microalgae have higher photosynthetic efficiency, faster growth, and can synthesize and accumulate larger amounts of lipids. This study focuses on the potential of different species of microalgae prevalent in India as Biofuel energy crop.

Biofuels are obtained from biological material, currently mainly from plants, microorganisms, animals and waste. All biofuels have the same basic and renewable origin. They are based on the "modern" photosynthetic conversion of solar energy into chemical energy, which distinguishes them from fossil fuels, which are based on ancient photosynthesis. In reality, the line between renewable biofuels and non-renewable fossil fuels is sometimes blurred, and only complete life cycle analyzes in the future will reveal which feedstocks are truly renewable for use in biofuel production. Depending on the origin and production technology of biofuels, they are generally called first, second and third generation biofuels (according to EASAC report 2012).

Today, most first-generation biofuels are derived from crops as energy-containing molecules such as sugars, oils, and cellulose. They provide only limited biofuel yields and have a negative impact on food security. Efforts are now needed to accelerate the production of advanced biofuels by identifying and engineering efficient non-food feedstocks, improving the performance of conversion technologies and the quality of biofuels for various transportation sectors, as well as reducing costs (EASAC 2012).

Second-generation biofuels already represent an improvement in the production of biofuels from lignocellulosic non-food feedstocks, which include straw, bagasse, forest residues and purpose-grown energy crops on marginal land. Projects are needed to maximize the amount of renewable carbon and hydrogen that can be converted into "second generation" biomass fuels.

Microalgae are microscopic and found in freshwater and marine ecosystems. They are unicellular species that exist singly or in groups. Microalgae are able to perform photosynthesis using the greenhouse gas CO2 and grow photoautotrophically. They are known to produce almost half of the atmospheric oxygen. The biodiversity of microalgae is huge and they represent an almost untapped resource. Most species of microalgae produce unique products such as carotenoids, antioxidants, peptides, fatty acids, toxins and sterols. All carbons fixed by microalgae are stored in the cell as proteins, starch and lipids. Therefore, microalgae are excellent candidates for biofuel production.

Algal reproduction generally occurs in three ways: vegetatively, sexually, or asexually. Vegetative reproduction occurs only by binary fission. Asexual reproduction is achieved by the formation of different types of spores such as aplanospores or autospores. During sexual reproduction, genetic materials are exchanged to form new combinations. This process is controlled by three basic methods: Isogamy, Anisogamy and Oogamy, which results in the formation of a zygote. Sexual reproduction does not occur in certain classes such as *Cyanophyceae*. The exchange of genetic material can occur through the formation of conjugation tubes. The two different types of conjugation tube formation are scalariform and lateral conjugation.

A significant amount of research is being done worldwide and scientists have found suitable strains of microalgae that can fix CO2 directly from flue gas and produce biofuel from it. Some species of microalgae including *Galdieria partita, Chlorella kessleri, Chlorella sp. T-1, Chlorella KR-1, Chlorella emersonii, Chlorella HA-1, Chlorella ZY-1, Chlorococcum littorale, Synechococcus PCC7942 and Nannochloropsis oculata* (Ono and Cuello 2007; Kurano et al. 1995; Maeda et al. 1995; Sung et al. 1998; Yanagi et al. 1995; Morais and Costa 2007a, b; Iwasaki et al. 1998; Kajiwara et al. 1997; Hsueh et al. 2009) can be used to capture carbon dioxide from power plants. A number of studies have been conducted to study the effect of different CO2 concentrations on biomass and biofuel production among different species of microalgae. Bioethanol is also produced by *Spirogyra* sp. along with *Zymomonas mobilis* and *Saccharomyces cerevisiae* fermentation (Sulfahri et al. 2011). Microalgae species such as *Chlorella sorokiniana* BTA 9031 isolated from rare sampling sites such as coal mines have been used for CO2 capture and biodiesel production from captured CO2 (Mondal et al. 2016a, b).

Third generation biofuels are based on the production of biomass from algae. They are currently undergoing extensive research to improve both metabolic fuel production and separation processes in bio-oil production to remove non-fuel components and further reduce production costs.

First-generation biofuels – direct biofuels from food crops – are likely to be largely banned in the EU. In its rigorous study, EASAC found that "first-generation" biofuels, once all the impacts of biomass cultivation are taken into account, appear to provide little or no greenhouse gas reductions required by the directive, while endangering food, agriculture and natural ecosystems. (EASAC 2012). Although Brazilian sugarcane ethanol is considered by some experts to be the only biofuel currently produced on a commercial scale that meets advanced targets for non-cellulosic renewable fuels, there are many critics, including environmental impact. The EASAC

(2012) report calls for systematic research into the expected improvements of second-generation biofuels derived from inedible plant parts and recommends further development of third-generation biofuels extracted from algae as a suitable means of increasing biofuel production.

These biofuels include the specific production of biodiesel and other algae-based biofuels such as ethanol and biogas (Pandey et al. 2014). Photosynthetic capture of CO2 in algae cultures for biomass production and subsequent extraction of e.g. biodiesel from algae cells has attracted great interest worldwide. The technology is still not economical and sustainable due to low PFCE in biodiesel production. This is partly due to the fact that increased biodiesel production generally occurs under stressful conditions, which in turn reduces biomass growth and production. Recent advances in metabolic engineering of algae to increase lipids without compromising growth are considered an important milestone towards sustainable biodiesel production (Trentacoste et al. 2013). In addition to a number of different engineering approaches to increase algal fuel production (Ho et al. 2014), research on algal biodiversity is also likely to reveal interesting species with high fuel production capacity (Maity et al. 2014). Combining the production of biofuels from algae with the production of high-value chemicals, as well as the use of wastewater and/or seawater as a culture medium and the development of more cost-effective bioreactors, are all technologies that are currently under intense development and will make the production of biofuels from algae more profitable.

In addition, current biorefinery development mostly focuses on the utilization of plant material and carbohydrate-containing waste (lignocellulose, bagasse, straw, industrial waste streams) in the production of fuels, chemicals and materials, while the development of harvesting and processing systems for the production of biofuels from algae culture has so far attracted less attention. It is important to develop and genetically engineer cost-competitive algal fuels to overcome cultivation, harvesting, and processing challenges (Medipally et al. 2015). Real breakthroughs are still waiting for solutions to break down technical barriers and accelerate the development of sustainable and affordable algal biofuels.

Green microalgae produce higher amounts of biofuel compared to blue-green algae. *Chlorella* sp., *Chlorococcum* sp. and *Neochlorosis oleabundans* have been found to be potential raw materials for biodiesel. However, *Haematococcus pluvialis*, a red microalga also appears to be a good choice for biofuel production (Lei et al. 2012). The production of different types of biofuels largely depends on the availability of input raw materials and the technological possibilities that can be implemented. Biofuel production from microalgae is currently limited to laboratories and small industry.

According to many researchers, the use of algal biofuels could reduce greenhouse gas emissions from 101,000 g CO2 equivalent per million British thermal units (BTU) to 55,440 g (Rittmann 2008). According to the United States Environmental Protection Agency Act, biodiesel produced from microalgae has the potential to meet the requirement of the Environment Protection Agency (EPA) Renewable Fuel Standard (RFS 2007). According to the RFS, under the Energy Independence and Security Act of 2007, 15.2 billion gallons of domestic alternative fuels could be produced annually with 50% lower life-cycle greenhouse gas emissions than petroleum-based transportation fuels (Santana et al. 2012; Renewable Regulatory Impact Analysis of the Fuel Standard Program (RFS2) US Environmental Protection Agency 2010 National Algal Biofuels Technology Plan: technology plan resulting from the National Algal Biofuels Workshop (DOE/EE-0332) US Department of Energy 2010). Therefore, microalgae can serve as a raw material for the large-scale production of biodiesel (Li et al. 2008a, b).

Environmental pollution due to the excessive release of nutrients and other chemicals into urban wastewater is increasingly recognized as a major threat to aquatic ecosystems worldwide. A strategy that could counter this threat is the use of algae ponds or bioreactors. This approach is not new (Oswald and Gotaas 1957). Recently, however, it has attracted the interest of many scientists around the world, mainly due to the ability of algae to absorb nutrients and efficiently remove pollutants from wastewater (Hoffman 1998; Sturm and Lamer 2011) and the possibility of producing high-energy biomass from them (Rawat et al. 2011; Park et al. 2011a). For example, García et al. (2006) found that total nitrogen and phosphorus in municipal wastewater could be reduced by 73 and 43%, respectively, using high-algal miniponds in Spain, and in other cases reductions of 90–95% were reported (Hoffman 1998, Ruiz-Marin et al., 2010). The potential of using microalgae to remove nitrogen and phosphorus from wastewater during tertiary treatment has also been extensively evaluated (Pittman et al. 2011). Furthermore, in hectare-scale trials, high-velocity algal ponds fed with primary settled wastewater removed approx. 65% ammonium nitrogen and approx. 19% of dissolved reactive phosphorus (Craggs et al. 2012). Algae have also been cultivated on other types of wastewaters, such as fish and livestock waste streams (Woertz et al. 2009; Riaño et al. 2011).

Among the pollutants that have received a lot of attention recently are pharmaceuticals. These compounds are potent, biologically active chemicals, but little is known about their ecological effects, in contrast to the rich knowledge of their pharmacological and toxicological effects at high concentrations (Santos et al. 2010; Boxall et al. 2012). However, several studies have shown that pharmaceuticals enter waterways, primarily through treated wastewater, and remain biochemically active in aquatic systems (Verlicchi et al. 2012; Hughes et al. 2013). They can also affect aquatic animals at environmentally relevant concentrations (Kidd et al. 2007; Brodin et al. 2013). Furthermore, although municipal wastewater is commonly treated by a combination of mechanical, biological and chemical processes before further discharge into the aquatic environment, this is usually not sufficient to remove pharmaceutical residues (Radjenović et al. 2009; Gros et al. 2010; Verlicchi et al., 2012). Thus, a number of other promising treatments have been proposed to improve removal efficiency, such as ozonation, activated carbon filtration, UV irradiation, H2O2 dosing, and/or retention in constructed surface wetlands with free water

(Joss et al. 2008; Matamoros et al. 2008; Breitholtz et al. 2012). It has also been shown that levels of veterinary antibiotics can be reduced in high-algal ponds fed with synthetic wastewater (de Godos et al. 2012).

Recent studies have shown that *Chlorella sorokiniana* can significantly reduce paracetamol and salicylic acid added to artificial media under laboratory conditions (Escapa et al. 2015) and several other micropollutants from urine and anaerobically digested black water (de Wilt et al. 2016). Moreover, in a study involving the cultivation of algae in municipal wastewater in mini algae ponds during cold and warm seasons in Spain, demonstrated that algae had the ability to remove organic contaminants (Matamoros et al. 2015). Biodegradation and photolysis have been proposed as the main pathways for the removal of micropollutants and emerging organic contaminants including pharmaceuticals (Matamoros et al. 2015; de Wilt et al. 2016). Interestingly, in another study, biosorption, represented by physicochemical adsorption occurring at the cell surface, was shown to be an important mode of biocide removal in both dead and living algal cells (Tam et al. 2002).

The continued increase in atmospheric CO2 levels and the associated demand for green energy sources have highlighted another potential application of algae: their cultivation with CO2 in flue gas to reduce emissions (Hughes and Benemann 1997; Chiu et al. 2011). From an environmental perspective, algae are of particular interest because they can be used simultaneously for wastewater treatment, CO2 reduction (Wang et al. 2008) and production of bioenergy (Craggs et al. 2012). However, few studies have considered the potential utility of algal cultivation for simultaneous treatment of flue gas and municipal wastewater (Kumar et al. 2010).

Materials and Methods

<u>Materials</u>:

The species of Microalgae selected for this study were:

1. *Chlorella sorokiniana*
2. *Chlorella saccharophila*
3. *Chlorella regularis*
4. *Scenedesmusdimorphus*
5. *Scenedesmus species*

The reason why these species were selected is that these species are prevalent in the Indian environment, especially the warm climate of Mumbai.

The three media used for comparison were:

1. BG11 broth
2. Chu's medium no. 10
3. Chlorella medium

(1) BG11 broth: BG11 Broth is a universal medium for the cultivation and maintenance of blue green algae (*Cyanobacteria*).

Composition of BG11 broth:

Components	**Grams / Litre**
Sodium nitrate	1.500
Dipotassium hydrogen phosphate	0.0314
Magnesium sulphate	0.036
Calcium chloride dihydrate	0.0367
Sodium carbonate	0.020
Disodium magnesium EDTA	0.001
Citric acid	0.0056

Ferric ammonium citrate	0.006

Final pH at 25°C = 7.1

Principle: This medium supports growth of photoautotrophic blue green algae. They require light as source of energy. Synthetic nitrogen and carbon sources and other inorganic salts comprise this medium. Exposure to light intensity of 2,000 to 3,000 lux is optimal for cultivation of blue green algae. Neon light source is found to be sufficient to provide this illumination. For maintenance of blue green algae exposure for period of 24 hours a day is optimal. Often the flasks kept for incubation may be covered with grease proof paper. They grow optimally at room temperature between range of 20-25°C. (Allen, M.M, Steiner, R.Y, 1968).

(2) Chu's Medium No.10: Chu's Medium No. 10 is used for culturing blue green algae.

Composition Chu's Medium No.10:

Components	**Milligrams/Litre**
Calcium nitrate	40.000
Magnesium sulphate	25.000
Dipotassium phosphate	5.000
Sodium carbonate	20.000
Sodium silicate	25.000
Iron (II) chloride	8.000

Principle:

Soil algae are ubiquitous in nature wherever moisture and sunlight are available. They are visible to the unaided eye in the form of a green slum on the surfaces of soils. Morphologically, they may be unicellular or filamentous and belong to the families Chlorophyceae (green algae) and Cyanophyceae (blue-green algae).

Cyanobacteria is a phylum (or "division") of bacteria that obtain their energy through photosynthesis. They are often still referred to as blue-green algae, although they are in fact prokaryotes like bacteria. They are a major primary producer of the planetary ocean. They are found in almost every conceivable habitat, from oceans to fresh water to bare rock to soil. Chu's Medium No. 10 is formulated as per Chu for cultivation of blue green algae.

Calcium nitrate serves as inorganic nitrogen source and other inorganic salts supply the necessary growth requirements. (Chu S. P., 1942)

(3) Chlorella Broth: Chlorella Broth Base w/o Dextrose and Citrate is used for cultivation of chlorella.

Composition of Chlorella Medium:

Components	**Grams / Litre**
Cupric sulphate	0.0000078

Sodium molybdate	0.00005
Zinc sulphate	0.00022
Boric acid	0.00028
Manganese sulphate	0.0014
Ferrous sulphate	0.0015
Potassium sulphate	0.217
Magnesium sulphate	2.400
Monopotassium phosphate	2.450
Potassium nitrate	2.500

Final pH (at 25°C) after sterilization = 4.5 (± 0.2)

Principle:

Chlorella is a genus of single-celled green algae, belonging to the phylum Chlorophyta. Chlorella Broth has originally formulated by Shrift and further modified for cultivation and maintenance of *Chlorella*.

All algae utilize inorganic phosphates and sulphates. There is a fairly high requirement of molybdate as a trace metal in nitrogen fixation. Calcium, magnesium, potassium and probably sodium are required by algae. Most algae grow poorly on agar and it is best to let them become established in liquid culture before adapting them to the more rigorous conditions of an agar slant.

Chlorella Broth Base w/o Dextrose and Citrate is the same as Chlorella Broth except that the citrate and dextrose have been omitted from the medium. This media supplies the necessary nutrients for the rapid growth of *Chlorella* species. *Chlorella* being photosynthetic green algae should be cultivated in the presence of light. Bright diffused light, fluorescent light and sunlight are satisfactory sources of light for the growth of *Chlorella*. The inoculated tubes/flasks should be incubated in the presence of light at 25-27°C for a week to permit good growth and pigmentation. at room temperature for 2-3 months without sub-culturing. (Shrift, 1954)

Methods:

Lipid Extraction:

1. 5.0 mL of absolute Ethanol was added to the dried biomass and the flask was placed on a shaker at 200rpm for 24hours to extract lipids.
2. The lipids thus obtained were crude lipids.
3. The crude lipids contain non-polar Tri-Acyl Glycerides (TAGs), other lipids which are polar in nature and are not useful for biodiesel production and photosynthetic pigments.
4. The crude lipids obtained were purified using hexane.
5. To purify the crude lipids, 6.0 mL water and 16.0 mL hexane were added to form a liquid-liquid separation state.

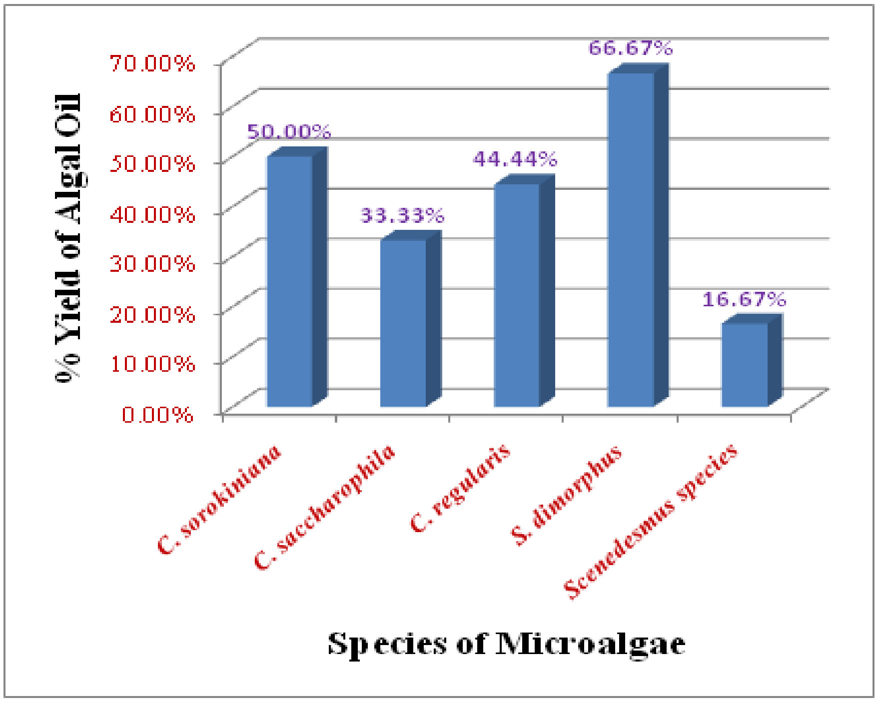
6. Hexane is a very non-polar compound. The lipids necessary for biodiesel production are TAGs which are also non-polar in nature, thus using hexane gives an efficient resolution to the purification process.
7. Ethanol is soluble in water while Hexane isn't and adding water to the mixture helps in the formation of two distinct phases for separation.
8. The upper phase (hexane & some ethanol) was loaded with neutral lipids while the lower phase (most ethanol with water) contained polar lipids and non-lipids.
9. The upper phase containing neutral lipids was transferred to a pre-weighed conical flask and the solvent was allowed to evaporate overnight at room temperature.

The use of Ethanol proved to be more cost effective than using a combination of solvents like methanol and chloroform. Also, ethanol from the hydro alcoholic phase can be efficiently recovered via double distillation. Hexane provides a better resolution of TAGs from a mixture of crude lipids and non-lipids.

Results:

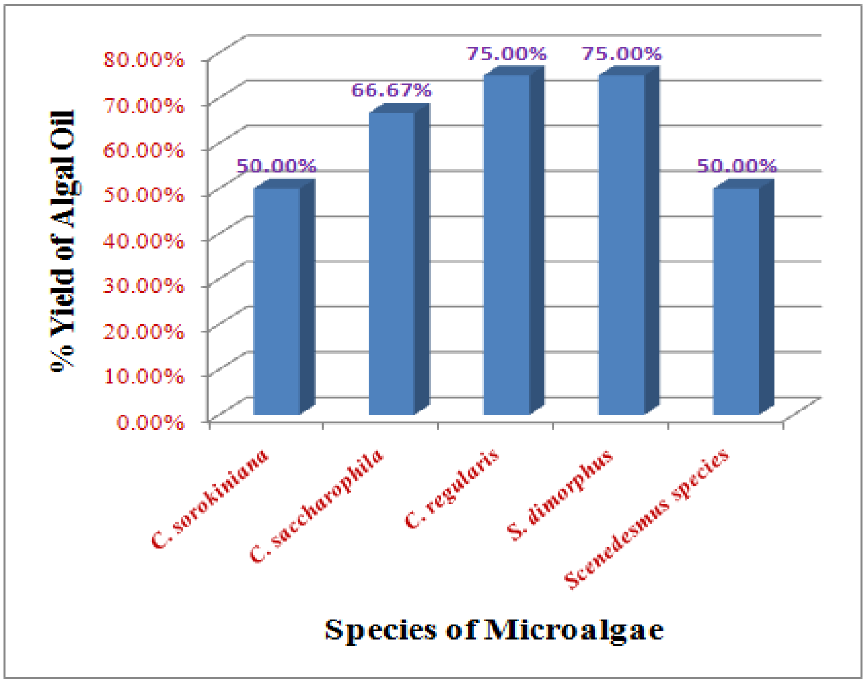
(1) In BG11 Broth:

Species	**Weight of Dry Biomass (in g)**	**Weight of algal-oil (in g)**	**%yield of algal oil**
Chlorella sorokiniana	0.010	0.005	50.00%
Chlorella saccharophila	0.090	0.030	33.33%
Chlorella regularis	0.090	0.040	44.44%
Scenedesmus dimorphus	0.030	0.020	66.67%
Scenedesmus species	0.060	0.010	16.67%



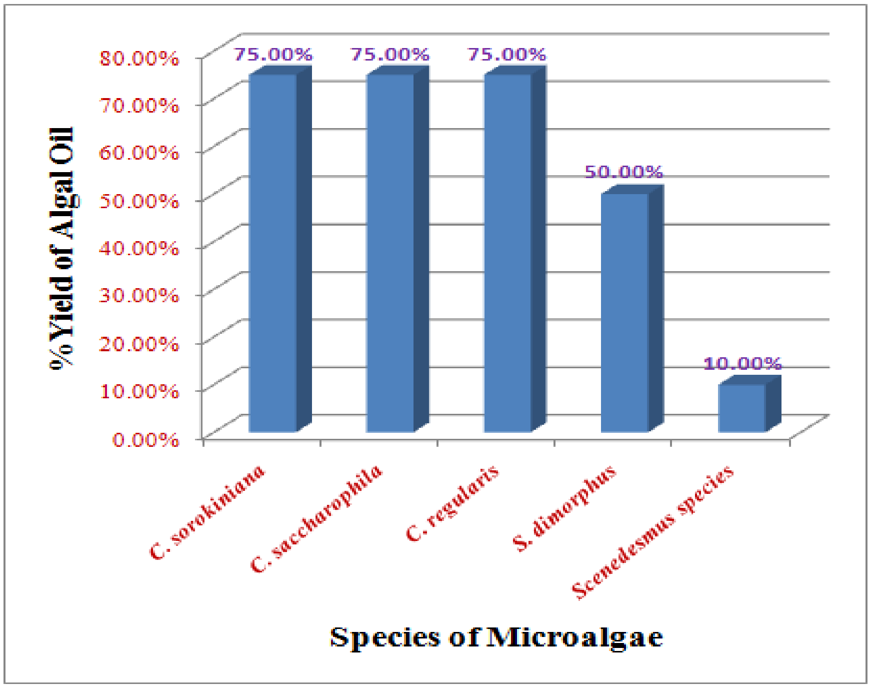
(2) In Chu's medium No.10:

Species	**Weight of Dry Biomass (in g)**	**Weight of algal-oil (in g)**	**%yield of algal oil**
Chlorella sorokiniana	0.020	0.010	50.00%
Chlorella saccharophila	0.030	0.020	66.67%
Chlorella regularis	0.020	0.015	75.00%
Scenedesmus dimorphus	0.020	0.015	75.00%
Scenedesmus species	0.010	0.005	50.00%

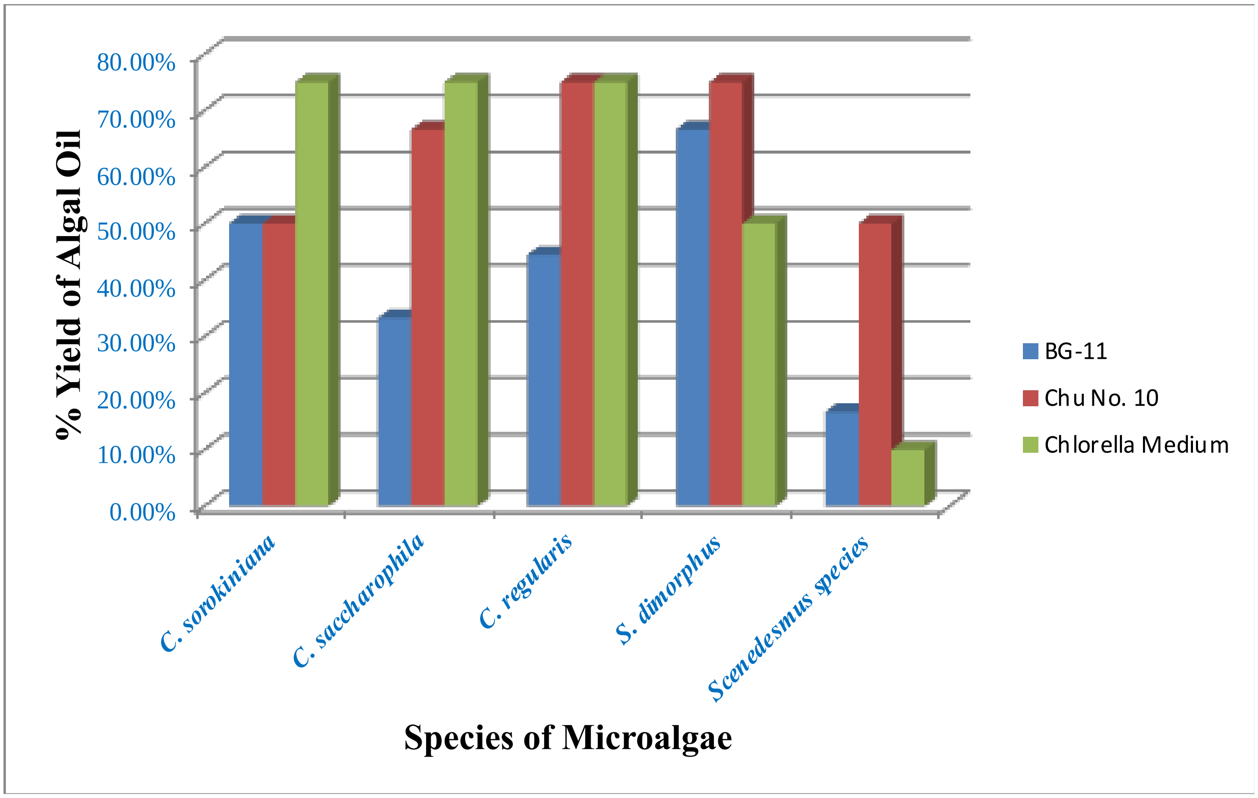


(3) In Chlorella Medium:

Species	Weight of Dry Biomass (in g)	Weight of algal-oil (in g)	%yield of algal oil
Chlorella sorokiniana	0.020	0.015	75.00%
Chlorella saccharophila	0.020	0.015	75.00%
Chlorella regularis	0.020	0.015	75.00%
Scenedesmus dimorphus	0.020	0.010	50.00%
Scenedesmus species	0.010	0.002	10.00%



Results at a Glance:



Discussion:

In BG-11 medium *Scenedesmus dimorphus* showed maximum %yield of 66.67% followed by *Chlorella sorokiniana* (50.00%), *Chlorella regularis* (44.44%), *Chlorella saccharophila* (33.33%), and *Scenedesmus* species (16.67%). This medium supports growth of photoautotrophic blue green algae.

In Chu No.10 medium *Scenedesmus dimorphus* showed maximum %yield of 75.00% along with *Chlorella regularis* (75.00%), followed by *Chlorella saccharophila* (66.67%), *Chlorella sorokiniana* (50.00%), and *Scenedesmus species* (50.00%) each. Algal oil yield was considerably different as compared to a general medium for photoautotrophic blue green algae (BG11). The key component which differentiates Chu's medium No.10 from other media is Iron Chloride. Iron is proven to boost the chlorophyll content in microalgae. This indicates that chlorophyll content is relevant to an algal species' biofuel potential.

In Chlorella medium, all 3 species of *Chlorella*, i.e. *Chlorella saccharophila, Chlorella regularis*and *Chlorella sorokiniana* showed a %yield of 75.00%. This was followed by *Scenedesmus dimorphus* at 50.00% and *Scenedesmus species* provided a meagre yield of 10.00% As expected, this media supplies the necessary nutrients for the rapid growth of *Chlorella* species. *Scenedesmus* species showed a reduced yield in this medium. The reason for this disparity is the pH of the medium which is 4.5 (± 0.2) which is ideal for *Chlorella species* but not for *Scenedesmus species.*

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References:

A.M. Santos, M. Janssen, P.P. Lamers, W.A.C. Evers, R.H. Wijffels. (2012). Growth of oil accumulating microalga *Neochloris oleoabundans* under alkaline–saline conditions. *Bioresource Technology*, Volume 104, 593-599. https://doi.org/10.1016/j.biortech.2011.10.084.

Alejandro Ruiz-Marin, Leopoldo G. Mendoza-Espinosa, Tom Stephenson (2010). Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresource Technology. Volume 101, Issue 1, 58-64. https://doi.org/10.1016/j.biortech.2009.02.076.

Allen, M.M, Steiner, R.Y. 203 (1968). *J.Gen.Microbiol*. 51.

Aro E. M. (2015). From first generation biofuels to advanced solar biofuels. *Ambio*, *45 Suppl1*(Suppl 1), S24-31.

B. Riaño, B. Molinuevo, M.C. García-González (2011). Treatment of fish processing wastewater with microalgae-containing microbiota, Bioresource Technology, Volume 102, Issue 23, 10829-10833, https://doi.org/10.1016/j.biortech.2011.09.022.

B.S.M. Sturm, S.L. Lamer (2011). An energy evaluation of coupling nutrient removal from wastewater with algal biomass production. Appl. Energy, 88, pp. 3499-3506.

Breitholtz M, Naslund M, Strae D, Borg H, Grabic R, Fick J (2012) An evaluation of free water surface wetlands as tertiary sewage water treatment of micro-pollutants. Ecotox Env Safe 78:63–71

Boxall AB, Rudd MA, Brooks BW, Caldwell DJ, Choi K, Hickmann S, Innes E, Ostapyk K, Staveley JP, Verslycke T, Ankley GT, Beazley KF, Belanger SE, Berninger JP, Carriquiriborde P, Coors A, Deleo PC, Dyer SD, Ericson JF, Gagne F, Giesy JP, Gouin T, Hallstrom L, Karlsson MV, Larsson DG, Lazorchak JM, Mastrocco F, McLaughlin A, McMaster ME, Meyerhoff RD, Moore R, Parrott JL, Snape JR, Murray-Smith R, Servos MR, Sibley PK, Straub JO, Szabo ND, Topp E, Tetreault GR, Trudeau VL, Van Der Kraak G (2012) Pharmaceuticals and personal care products in the environment: what are the big questions? *Environ Health Perspect* 120(9):1221–1229. https://doi.org/10.1289/ehp.1104477.

Brodin T, Fick J, Jonsson M, Klaminder J (2013) Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. Science 339:814–815.

Chu S. P. (1942). *J. Ecol.*, 30, 284-325.

Chiu SY, Kao CY, Huang TT, Lin CJ, Ong SC, Chen CD, Chang JS, Lin CS (2011) Microalgae biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and Sulphur dioxide from flue gas using *Chlorella* sp. cultures. Bioresource Technol 102:9135– 9142

Craggs, R., Sutherland, D. & Campbell, H. (2012) Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. *J Appl Phycol* 24, 329–337. https://doi.org/10.1007/s10811-012-9810-8

Dang P. Ho, Huu Hao Ngo, Wenshan Guo (2014). Bioresource Technology. Volume 169, Pages 742-749. https://doi.org/10.1016/j.biortech.2014.07.022

de Godos I, Muñoz R, Guieyssea B (2012) Tetracycline removal during wastewater treatment in high-rate algal ponds. J Hazard Mater 229- 230:446–449.

de Wilt A, Butkovskyi A, Tuantet K, Hernandez Leal L, Fernandes TV, Langenhoff A, Zeeman G (2016) Micropollutant removal in an algal treatment system fed with source separated wastewater streams. J Hazard Mater 304:84–92.

Emily M Trentacoste 1, Roshan P Shrestha, Sarah R Smith, Corine Glé, Aaron C Hartmann, Mark Hildebrand, William H Gerwick (2014). Metabolic engineering of lipid catabolism increases microalgal lipid accumulation without compromising growth. Proc Natl Acad Sci. 110 (49):19748-53. doi: 10.1073/pnas.1309299110.

Escapa C, Coimbra RN, Paniagua S, Garcia AI, Otero M (2015) Nutrients and pharmaceutical removal from wastewater by culture and harvesting of Chlorella sorokiniana. Bioresource Technol 185:276– 284

Greene, R. M., Geider, R. J., Kolber, Z., &Falkowski, P. G. (1992). Iron-induced changes in light harvesting and photochemical energy conversion processes in eukaryotic marine algae. Plant physiology, 100(2), 565-75.

Gros M, Petrović M, Ginebreda A, Barceló D (2010) Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ Int 36:15–26.

Hsueh, H.T., Li, W.J., Chen, H.H. and Chu, H. 2009. Carbon bio-fixation by photosynthesis of Thermosynechococcus sp.CL-1 and Nannochloropsis oculata. J. Photochem. Photobiol Biol. 95:33-39.

Hughes E, Benemann JR (1997) Biological fossil CO2 mitigation. Energ Convers Manage 38:S467–S473.

Hughes SR, Kay P, Brown LE (2013) Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ Sci Technol 47:661–677

I.C. Woertz, T.J. Lundquist, A.S. Feffer, Y.M. Nelson (2009). Lipid productivity of algae grown during treatment of dairy and municipal wastewaters. *J. Environ. Eng.*, 135 (11), pp. 1115-1122.

Ismail Rawat R. Ranjith Kumar Taurai Mutanda Faizal Bux (2011). Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Applied Energy 88(10):3411-3424. DOI: 10.1016/j.apenergy.2010.11.025

Iwasaki, I., Hu, Q., Kurano, N. and Miyachi, S. 1998. Effect of extremely high- CO2 stress on energy distribution between photosystem I and photosystem II in a 'high-CO2 ' tolerant green alga, Chlorococcum littorale and the intolerant green alga Stichococcus bacillaris. J. Photochem. Photobiol Biol. 44:184-190.

J.B.K. Park, R.J. Craggs, A.N. Shilton (2011). Wastewater treatment high rate algal ponds for biofuel production Bioresour. Technol. Volume 102, Issue 1, Pages 35-42. https://doi.org/10.1016/j.biortech.2010.06.158.

J. García, B.F. Green, T. Lundquist, R. Mujeriego, M. Hernández-Mariné, W.J. Oswald. (2006). Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater. Bioresource Technology. Volume 97, Issue 14, 1709-1715. https://doi.org/10.1016/j.biortech.2005.07.019

James P. Hoffmann (1998). WASTEWATER TREATMENT WITH SUSPENDED AND NONSUSPENDED ALGAE. J. Phyol. 34, 757-763. https://doi.org/10.1046/j.1529-8817.1998.340757.x

Jon K. Pittman, Andrew P. Dean, Olumayowa Osundeko. (2011) The potential of sustainable algal biofuel production using wastewater resources, Bioresource Technology, Volume 102, Issue 1, 17-25. https://doi.org/10.1016/j.biortech.2010.06.035.

Joss A, Siegrist H, Ternes TA (2008) Are we about to upgrade wastewater treatment for removing organic micropollutants? Water Sci Technol 57:251–255.

K.D. Sung, J.S. Lee, C.S. Shin, S.C. Park (1998) Isolation of a new highly CO2 tolerant fresh water microalga Chlorella sp. KR-1. Korean J. Chem. Eng., 15 (1998), pp. 449-450. DOI: 10.1007/BF02697138.

Kajiwara, S., Yamada, H., Ohkuni, N. and Ohtaguchi, K. 1997. Design of the bioreactor for carbon dioxide fixation by Synechococcus PCC7942. Energy Convers. Mgmt. 38(suppl):529-532. Khan, M. I., Shin, J. H., & Kim, J. D. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microbial cell factories, 17(1), 36. doi:10.1186/s12934-018-0879-x.

Kidd KA, Blanchfield PJ, Mills KH, Palace VP, Evans RE, Lazorchak JM, Flick RW. (2007) Collapse of a fish population after exposure to a synthetic estrogen. *Proc Natl Acad Sci U S A*. 104(21):8897-901. doi: 10.1073/pnas.0609568104.

Kumar A, Yuan X, Sahu AK, Dewulf J, Ergas SJ, Van Langenhove H (2010) A hollow fiber membrane photo-bioreactor for CO2 sequestration from combustion gas coupled with wastewater treatment, a process engineering approach. J Chem Technol Biot 85:387–394.

Kurano N., Ikemoto H., Miyashita H, Hasegawa T., Hata H., Miyachi S. (1995). Fixation and utilization of carbon dioxide by microalgal photosynthesis. *Energy Conversion and Management*. Volume 36, Issues 6–9, 689-692. https://doi.org/10.1016/0196-8904(95)00099-Y.

Lei A, Chen H, Shen G, Hu Z, Chen L, Wang J (2012) Expression of fatty acid synthesis genes and fatty acid accumulation in *Haematococcus pluvialis* under different stressors. Biotechnol Biofuels 5:18.

Li Q, Du W, Liu D (2008a) Perspectives of microbial oils for biodiesel production. Appl Microbiol Biotechnol 80:749–756.

Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N (2008b) Biofuels from microalgae. Biotech Prog 24(4):815–820.

Maity JP, Bundschuh J, Chen CY, Bhattacharya P (2014) Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives: a mini review. Energy 78:104–113.

Matamoros V, Caselles-Osorio A, Garcia J, Bayona JM (2008) Behaviour of pharmaceutical products and biodegradation intermediates in horizontal subsurface flow constructed wetland. A microcosm experiment. Sci Total Environ 394:171–176

Matamoros V, Gutiérrez R, Ferrer I, García J, Bayona JM (2015) Capability of microalgae-based wastewater treatment systems to remove emerging contaminants: a pilot study. J Hazard Mater 288:34– 42

Medipally SR, Yusoff FM, Banerjee S, Shariff M (2015) Microalgae as sustainable renewable energy feedstock for biofuel production. BioMed Res Int Article ID 519513, p 13.

Mondal M, Ghosh A, Sharma AS, Tiwari ON, Gayen K, Mandal MK, Halder GN (2016a) Mixotrophic cultivation of Chlorella sp. BTA 9031 and Chlamydomonas sp. BTA 9032 isolated from coal field using various carbon sources for biodiesel production. Energy Convers Manag 124:297–304

Mondal M, Khanra S, Tiwari ON, Gayen K, Halder GN (2016a) Role of carbonic anhydrase on the way to biological carbon capture through microalgae—a mini review. doi:10.1002/ep.12394

Morais, M.G. and Costa, J.A.V. 2007a. Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energy Convers. Mgmt. 48:2169-2175.

Morais, M.G. and Costa, J.A.V. 2007b. Carbon dioxide fixation by Chlorella kessleri, C. vulgaris, Scenedesmus obliquus and Spirulina sp.cultivated in flasks and vertical tubular photobioreactors. Biotechnol. Lett. 29:1349- 1352

Ono, E.; Cuello, J.L. Carbon dioxide mitigation using thermophilic cyanobacteria. Biosyst. Eng. 2007, 96, 129–134. DOI: 10.1016/j.biosystemseng.2006.09.010

Pandey A, Lee DJ, Chisti Y, Soccol CR (2014). Biofuels from algae, United States, Elsevier.

Radjenović J, Petrović M, Barceló D (2009) Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res 43:831–841.

Rittmann BE (2008) Opportunities for renewable bioenergy using microorganisms. Biotechnol Bioeng 100(2):203–212.

Schenk, P. M., Thomas-Hall, S. R., Stephens, E., Marx, U. C., Mussgnug, J. H., Posten, C., Kruse, O., et al. (2008). Second generation biofuels: High-efficiency microalgae for biodiesel production. *Bioenergy Research, 1*(1), 20-43. doi:10.1007/s12155-008-9008-8.

Shrift (1954) Am. J. Botany, 41:223.

Sulfahri MS, Sunarto E, Irvansyah MY, Utami RS, Sarwoko SM (2011) Ethanol production from Algae Spirogyra with Fermentation by Zymomonas mobilis and Saccharomyces cerevisiae. J Basic Appl Sci Res 1(7):589–593.

Tam NFY, Chong AMY, Wong YS (2002) Removal of tributyltin (TBT) by live and dead microalgal cells. Mar Pollut Bull 45:362–371

Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban wastewater: 1769 removal, mass load and environmental risk after a secondary treatment—a review. *Sci Total Environ*. 429, 123–55.

Wang B, Li Y, Wu N, Lan CQ (2008) CO2 bio-mitigation using microalgae. Appl Microbiol Biot 79:707–718.

William J. Oswald, H. Gotass (1957). Photosynthesis in Sewage Treatment. Transactions of the American Society of Civil Engineers 122(1) DOI: 10.1061/TACEAT.0007483.

Yanagi, M., Watanabe, Y. and Saiki, H. 1995. CO2 fixation by Chlorella sp. HA-1 and its utilization. Energy Convers. Mgmt. 36(6- 9):713-716.

Yang, F., Xiang, W., Sun, X., Wu, H., Li, T., & Long, L. (2014). A novel lipid extraction method from wet microalga Picochlorum sp. at room temperature. *Marine drugs*, *12*(3), 1258-70. doi:10.3390/md12031258.

Yoshiaki Maeda, Tomoko Yoshino, Tadashi Matsunaga, Mitsufumi Matsumoto, Tsuyoshi Tanaka (2018). Marine microalgae for production of biofuels and chemicals. Volume 50, 111-120. https://doi.org/10.1016/j.copbio.2017.11.018.