British Journal of Engineering and Technology
URL: http://www.bjet.baar.org.uk /current-issue.html
ISSN: XXXX-XXXX
Vol. 1, No. 1, pp 251 – 264, **October 2012**

BIODIESEL PRODUCTION POTENTIAL OF WASTEWATER MICROALGAE *CHLORELLA* SP. UNDER PHOTOAUTOTROPHIC AND HETEROTROPHIC GROWTH CONDITIONS

Buddolla Viswanath
(Corresponding Author)
Applied Microbiology Laboratory, Department of Virology, Sri Venkateswara University, Tirupati – 517 502, INDIA

Email: buddolla@gmail.com

Faizal Bux

Center for water and wastewater Technology, Durban University of Technology, Durban – 4000, South Africa.

ABSTRACT

In the present study, a microalgae (*Chlorella* sp.) isolated from wastewater pond has been studied in both photoautotrophic and heterotrophic growth conditions in bioreactor to evaluate the cell growth rate and lipid content for biodiesel production. Maximum amount of biomass was recovered from the bioreactor of *Chlorella* sp. grown under heterotrophic growth conditions with 8.90 gL^{-1} compared to photoautotrophic growth conditions, which was almost 3.6, fold lesser than the former. Heterotrophic growth of *Chlorella* sp. resulted in the accumulation of high lipid content in cells compared autotrophic growth by enhancing lipid production by 4.4 fold. The results suggested that heterotrophic growth of microalgae is an efficient method for the production of biomass and high lipid content in the cells, which can reduce the cost of microalgal biomass production and microalgal oil production. The quality of the oil produced from the cells of heterotrophic growth is also superior compared the oil from photoautotrophic growth.

Keywords: *Chlorella* sp. Bioreactor. Glucose. Biomass. Protein. Lipids.

Introduction

The use of fossil fuels is not infinite and it has been accepted that fossil fuels are not sustainable due to depleting oil reserves and an increase in the greenhouse gas emissions in the atmosphere [1-3]. We are currently experiencing what is the termed the 'Peak Oil' period and the demand for energy is still increasing [4]. This has lead to the development of renewable alternate fuel sources. These sources primarily include bioethanol and biodiesel. Bioethanol is well established as a transport fuel [5] and is primarily manufactured from the fermentation of sugarcane [6]. Sugarcane is a food crop the increased pressure on the crop to supply more ethanol can have a negative impact on the food security. This had lead to other fuels being investigated such as biodiesel. Biodiesel typically comprises of alkyl fatty acid esters of short chain alcohols with carbon varying from C_{14}-C_{22} predominantly in the form of methanol or ethanol and it contributes no sulphur or net carbon dioxide into the atmosphere and releases a lower amount of pollutants than ordinary diesel fuel releases [7,8]. The current sources of biodiesel include soybean oil, rapeseed oil, corn oil and vegetable fat. It is not possible to upscale using these sources as the extra demand would be displacing food crops [9]. Current research has shown a huge potential for microalgae biomass to be used as a lipid source for biodiesel production.

Microalgae are unique photosynthetic organisms given that they accumulate storage lipids in substantial quantities. The presence of lipids in microalgae has been shown to vary between 1% and 70% under specific lipid favoring conditions [10]. To promote the lipid productivity, culture conditions such as nitrogen concentrations, [11,12] heterotrophic culture conditions, [13-15] light limitations, [16] temperature [14,17] and cell concentration [13] have been investigated. Nevertheless, under mixotrophic conditions some microalgae are known to grow rapidly and to have a higher growth rate than under photoautotrophic conditions [13,14].

Microalgae are able to grow photoautotrophically or heterotrophically depending on the available conditions [18]. Photoautotrophic growth conditions require the microalgae to use carbon dioxide as a carbon source and solar energy obtained from the sun. The systems for photoautotrophic include open raceway ponds, closed photobioreactors using natural sunlight or photobioreactors using artificial light [18]. Microalgae grown under heterotrophic conditions utilize organic carbon sources such as sugars or organic acids as the sole energy source therefore light and carbon dioxide is not a requirement [18]. Heterotrophic growth of microalgae has previously been used for efficient production of biomass and lipids [19]. Under optimal conditions, microalgal populations are capable of doubling within hours and achieving high cell densities, corresponding to as much as 60g of heterotrophic biomass per liter and 5 g of photoautotrophic biomass per liter [19]. *Chlorella protothecoides* grown under heterotrophic conditions has shown to increase lipid production from 14.5% under autotrophic conditions to 55.2% [14]. For optimum lipid production, heterotrophic conditions may seem advisable as they produce larger quantities of lipids than microalgae grown under autotrophic conditions. Recent developments in heterotrohic cultivation and low-cost photobioreactors have provided additional economic advantages for the growth of microalgae [7,20]. The present study focused on the adaptation of *Chlorella* sp. isolated from wastewater ponds to heterotrophic growth conditions and their effect on the growth and lipid production.

Materials and methods

Chemicals

The media components and chemicals used in the present study were from Sigma-Aldrich, Germany and all chemicals used in this study were of analytical grade.

Microalgae

Chlorella sp. used in this study was isolated from wastewater treatment works i.e., maturation pond in Kwa-Zulu Natal, South Africa. Identification of this strain up to Genus level was based primarily on morphological characteristics using selected reference material [21] and carried out using Nikon Eclipse 80i microscope equipped with a Nikon Digital Sight DS-U1 camera (Nikon, Japan).

Media and culture conditions

Two weeks old culture of *Chlorella* sp. grown in Bristol medium [22] was used as inoculum at 20% (v/v). The isolated *Chlorella* sp. was grown autotrophically and axenically in bioreactors containing 10 l of Bristol medium (pH 7.5) under 28^0C (±1) temperature and 80µmol m^{-2} s^{-1} light intensity (16:8h light dark cycle). Aeration was provided by bubbling air at regular presser. For the heterotrophic growth of isolated *Chlorella* sp., 10gL^{-1} glucose was added to the Bristol medium under 28^0C (±1) temperature without any light source. All experiments were carried out in triplicates.

Measurement of Growth and Biomass Estimation

18 days old cultures of *Chlorella* sp. were harvested by centrifugation at 3500rpm and the cells were washed twice with distilled water. Then the pellet was dried in oven at 70^0C. The dry weight of microalgal biomass was determined gravimetrically and growth was expressed in terms of dry weight (gL^{-1}).

Qualitative estimation of lipids

Qualitative estimation of lipids was done using Nile Red Staining as per the protocol described by Greenspan *et al.* [23]. Microscopic examination was done using a Zeiss microscope (Ziess, Germany) equipped with a color CCD digital camera (AxioCam MRc, Ziess, Germany) using a 450–490-nm excitation filter, a 510-nm diachronic mirror and a 515-nm barrier filter with x40 or x100 objective lens under immersion oil to see the oil droplets in the cells of isolated microalgae.

Lipid extraction and quantification

Lipid extraction was performed and quantified as per the slightly modified protocol described by Fajardo *et al.* [24]. Figure1 illustrates the protocol used in the present study for the extraction and quantification of lipids from microalgae.

Estimation of proteins, carbohydrates and Chlorophyll

Calibration curves were constructed to estimate cellular protein and carbohydrate concentrations using: bovine serum albumin (BSA) as the standard for proteins and D-glucose for carbohydrates. Proteins were extracted following the procedure of Weis *et al.* [25] and quantified using the Lowry *et al.* method [26]. Carbohydrates were quantified as glucose by phenol-sulphuric acid method [27]. To estimate chlorophyll, a known volume of *Chlorella* sp. culture was centrifuged and the residue was extracted with methanol repeatedly. The chlorophyll content in the pooled extract was estimated spectrophotometrically by

recording absorbance at 652 and 665nm and quantified using the method of Lichtenthaler [28].

Chemical conversion of algal lipids into biodiesel

Chemical conversion of algal lipids into biodiesel is a sequence of operations, performed approximately in 4 h. At first the alkali agent is dissolved in methanol (25g KOH in 1 L methanol) in a temperature near 50°C permitting the formation of the basic catalysis active species. 50 mL of this solution and 100mL of algal oil, under vigorous and constant agitation, are introduced in a simple reactor equipped with reflux device. The molar ratio of alcohol/oil is 10 and catalyst/alcohol is $1.78.10^{-2}$. The temperature is increased until 60°C, near the boiling point of the alcohol. The system stays in this condition for approximately 1h. After this period the system is cooled down to approximately 25°C. The second main step of the procedure consists of the addition of 60 mL methanol and 1.5mL sulfuric acid 18 mol/L in the reactions mixture followed by soft heating. The reaction mixture is submitted to constant agitation and the temperature is elevated until 60°C by using the reflux equipment. The heating stabilizes and the system remains in this condition for 1 h. After this period the system is cooled slowly to approximately 25°C. In the end of this step the formation of two phases occurs. The two phases are separated and processed further. The biodiesel phase is washed with cool-water and the residual alcohol is removed by evaporation under vacuum. The lower phase presents pH approximately 6 and is used for recuperation of the methanol excess, the glycerol, as well as, other secondary products

Properties of Biodiesel

Densities were measured in an Anton Paar DMA 60 digital vibrating tube densimeter, with a DMA 602 measuring cell. Air and sterile distilled water were used for the calibration of the densimeter. Viscosities were obtained with a Haake Falling Ball Viscosimeter (Hoppler design), calibrated with sterile distilled water. The electronic digital stopwatch, with the uncertainty ±0.01 s, was used to measure the falling time of the ball. In all the measurements, the temperature maintenance and control were performed using the Haake D8-G thermostatic water bath, which has a temperature precision of ±0.01 K. In the case of density measurements, the Pt resistance thermometer was placed inside the vibrating tube densimeter to find the actual temperature of the measurements.

Statistical analysis

Average values of the results of three independent experiments (with two analytical replications in each) and their SD are presented.

Results and Discussion

Microalga which was isolated from wastewater treatment works *i.e.,* maturation pond in Kwa-Zulu Natal, South Africa, was identified as *Chlorella* sp. based on morphological characteristics using selected reference material [21]. Microscopic observation revealed that the isolated microalgae are single-celled green algae, belonging to the phylum Chlorophyta. They are spherical in shape, about 2 to 10 µm in diameter, and are without flagella (Figure 1). Microalgal biomass was harvested from the both photoautotrophic and heterotrophic bioreactors after 18 days of incubation. As shown in Figure 2, heterotrophic growth of *Chlorella* sp. resulted in the high density of the cell. In addition, heterotrophic growth of *Chlorella* sp. resulted in the partial disappearance of chlorophyll in cells. After Nile Red

staining, the heterotrophic cells of *Chlorella* sp. were full of lipid vesicles, which could be easily observed under Zeiss microscope (Figure 2). Maximum amount of biomass was recovered from the bioreactor of *Chlorella* sp. grown under heterotrophic growth conditions with 8.90 gL^{-1} compared to photoautotrophic growth conditions which was almost 3.6 fold lesser than the former (Table 1).

In the present study, procedure used for the extraction of lipids from *Chlorella* sp. yielded maximum lipids from their cells. Lipid content in the heterotrophically grown *Chlorella* sp. reached as high as 59.80%, which was almost 4.4 fold more than the cells of autotrophically grown *Chlorella* sp. (Table 1). The heterotrophic cells were full of lipid vesicles, which could be easily observed under fluorescence microscopy after Nile red staining (Figure 2). Other major cell components such as proteins, carbohydrates and chlorophyll were low in heterotrophically grown cells than that of autotrophically grown cells (Table 1). Protein content was significantly lower in the cells of heterotrophically grown *Chlorella* sp. and it was 3.8 fold lesser than that of the proteins extracted from the cells of photoautotrophically grown (Table 1).

Viscosity and density are the very important parameters required by biodiesel and diesel fuel standards because of being key fuel properties for diesel engines. After chemical conversion of algal lipids into biodiesel through a process called transesterification, density and viscosity were measured. In the present study both viscosity and density of the oil extracted from the cells of *Chlorella* sp. grown under heterotrophic conditions were comparatively lower than that of the cells of *Chlorella* sp. grown under photoautotrophic conditions (Table 2). Acid value also slightly higher in the oil extracted from heterotrophically grown *Chlorella* cells (Table 2). The other parameters like solidifying point and heating value of oils are same in the oil extracted from both autotrophically and heterotrophically grown *Chlorella* sp (Table 2). Properties like viscosity, density, solidifying point, acid value and heating value meet the terms with the limits established by ASTM related to biodiesel quality [23]. The rigorous culture of photosynthetic microalgae in ponds or photobioreactors does not require cultivable land and associated farming costs and materials. Such cultures can consume CO_2 and biomass wastes as nutrients [24,25]. The ultimate energy yield limit of autotrophic production is governed by the light energy input in the photosynthetic spectrum and the biochemical conversion efficiency, assuming no other nutrient or process limitation. To avoid these limitations of photosynthesis, heterotrophic culture of the microalga *Chlorella protothecoides* has been studied for algal lipid/FFA biodiesel precursor production [13,14]. If suitable reduced carbon food can be synthesized abiotically from CO_2 or waste water, agricultural resource consumption will be minimized, with reduced net carbon emissions as a result.

In the present study, *Chlorella* sp. isolated from wastewater pond was screened for its efficiency in lipid production by cultivating in both photoautotrophic and heterotrophic conditions. Biomass and lipid content were higher in the microalgae grown under heterotrophic conditions compared to photoautotrophically grown microalgae (Table 1). For microalgae that are able to survive heterotrophically, exogenous carbon sources offer prefabricated chemical energy, which the cells often store as lipid droplets [26]. In the study by Xu *et al*. [12], heterotrophically cultivated *Chlorella protothecoides* has been shown to

accumulate as such as 55% of its dry weight as oil, compared to only 14% in cells grown photoautotrophically. Carbon sources are necessary to provide the energy and carbon skeletons for cell. Heterotrophic microalgae are capable of growing in darkness; therefore, they must derive energy from at least one organic carbon source which is often provided in the form of glucose [27]. Other carbon sources include mono-, di- and polysaccharides such as fructose, sucrose, lactose and starch. Vegetable oils such as linseed, corn and canola oils may promote growth and/or polyunsaturated fatty acid production, depending on the microalgal species used [18]. Generally, a C/N ratio may influence cellular lipid content by controlling the switch between protein and lipid synthesis [28]. May be owing to this reason, protein content in the cells of *Chlorella* sp. which were grown heterotrophically were shown drastically less protein content compared to autotrophically grown *Chlorella* sp. (Table 1). A high C/N ratio favours lipid accumulation, which triggered by nitrogen depletion in the culture [26]. In the heterotrophic cultivation of green microalgae *Chlorella sorokiniana*, a C/N ratio of 20 was found to indicate a change from carbon to nitrogen limitation [29]. Cellular lipid content was at a minimum at this value and increased at both higher and lower C/N rations [29]. A C/N ratio also affects fatty acid composition. Chen and Johns [29] found that a low C/N ratio favored a high proportion of unsaturated fatty acids.

In the present study, procedure used for the extraction of lipids from *Chlorella* sp. yielded maximum lipids from their cells. The process of extraction of lipids comprise of two steps (Figure 3). First, ethanol (96% vol/vol) was used to extract the lipids from the lyophilized biomass. Second, a biphasic system was formed by adding water and hexane to the extracted crude oil. In this way, most of the lipids were transferred to the hexanic phase while most impurities remained in the hydroalcoholic phase. The process used in this study is an alternative to the traditional methods of lipid extraction, which uses less toxic solvents and reduces the total amount of solvents used. The obtained results in the present study suggested that the processes used for the extraction of lipids from microalgae and chemical conversion of algal lipids into biodiesel could be feasible and effective methods than the methods used by Miao and Wu [14] and Xu *et al.* [13] for the production of high quality biodiesel from heterotrophic microalgal oil. Properties tested in the present study to measure the quality of biodiesel comply with the limits established by ASTM related to biodiesel quality [23].

Conclusions

This work demonstrated that heterotrophic growth of microalgae is an efficient method for the production of biomass and high lipid content in the cells, which can reduce the cost of microalgal biomass production and microalgal oil production. High cell density cultivation of microalgae via heterotrophic growth mechanism could effectively address the issues of low productivity and operational constraints presently affecting the solar driven biodiesel production. However, investigations of heterotrophic biodiesel production from microalgae are still in its infancy. Although the system employed in this work was rather small, the results positively suggested that the upscale investigation for this particular system was highly attractive. Further research needs to focus on exploring new heterotrophic microalgal strains. In addition, an in-depth understanding of the factors that affect biodiesel production is needed in order to develop high biodiesel production strategies.

Acknowledgements

We are thankful to the National Research Foundation (NRF), South Africa and Durban Ethekwini Municipality and Durban University of Technology for their encouragement and financial support.

References

Schenk, P.M., S.R. Thomas-Hall, E. Stephens, U.C. Marx, J.H. Mussgnug, C. Posten, O. Kruse and B. Hankamer, 2008. Second generation biofuels: High-efficiency microalgae for biodiesel production. Bioene. Res., 1(1): 20-43.

Rodolfi, L., G.C. Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini and M.R. Tredici, 2009. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor, Biotech. Bioeng., 102 (1): 100–112.

Vasudevan, V., R.W. Stratton, M.N. Pearlson, G.R. Jersey, A.G. Beyene, J.C. Weissman, M. Rubino and J.I. Hileman, 2012. Environmental performance of algal biofuel technology options., Env. Sci. Tech., 46 (4): 2451-2459.

Chen, C.Y., K.L. Yeh, R. Aisyah, D.J. Lee and J.S. Chang, 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Biores. Tech. 102 (1): 71-81.

Grey, K.A., 2006. Bioethanol., Curr. Opi. Chem. Biol., **1**0(2): 141-146.

Nigam P.S and A. Singh, 2011. Production of liquid biofuels from renewable resources. Prog. Ene. Comb. Sci., 37(1): 52-68.

Lang, X., A.K. Dalai and N.N. Bakhshi, 2001. Preparation and characterization of biodiesel from various bio-oils. Biores. Tech., 4(1): 59-64.

Chisti, Y, 2007. Biodiesel from microalgae. Biotech. Adv., 25: 294-306.

Buddolla, V., T. Mutanda, S. White and F. Bux, 2010. The Microalgae-A Future Source of Biodiesel. Dyn. Biochem. Proc. Biotech. Mol. Biol., 4(1): 37-47.

Meng, X., J. Yang, X. Xu, L. Zhang, Q. Nie and M. Xian, 2009. Biodiesel production from oleaginous microorganisms. Ren. Ene., 34(1) 1-5.

Zhila, N.O., G.S. Kalacheva and T.G. Volova, 2005. Effect of nitrogen limitation on the growth and lipid composition of the green alga *Botryococcus brauni* Kutz IPPAS H-252. Rus. J. Pla. Phys., 52(3): 311-319.

Li, M., R. Gong, X. Rao, Z. Liu and X. Wang, 2006. Effects of nitrate concentration on growth and fatty acid composition of the marine microalga *Pavlova viridis* (Prymnesiophyceae). Ann. Microbiol., 55(1): 51-55.

Xu, H., X. Miao and Q. Wu, 2006. High quality biodiesel production from a microalgae *Chlorella protothecoides* by heterotrophic growth in fermenters. J. Biotech., 126(4): 499-507.

Miao, X and Q. Wu, 2006. Biodiesel production from heterotrophic microalgal oil. Biores. Technol., 97(6): 841-846.

Li, X., H. Xu and Q. Wu, 2007. Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. Biotech. Bioeng. 98(4):764 -771.

Mohamed, M.S., L.Z. Wei and A.B. Ariff, 2011. Heterotrophic cultivation of microalgae for production of biodiesel. Rec. Pat. Biotech., 5(2): 95-107.

Chisti, Y., 2008. Biodiesel from microalgae beats bioethanol. Tren. Biotech., 26(3): 126-131.

Wen Z.Y and F. Chen, 2003. Heterotrophic production of eicosapentaenic acid by microalgae. Biotech. Adv., 21(4): 273-294.

Muller-Feuga, A., 2004. Microalgae for aquaculture: the current global situation and future trends. In Handbook of Microalgal culture. Richmond, A., Eds.; Blackwell Science: p.p. 352-364.

Chen, F and G.Q. Chen, 2006. Growing phototrophic cells without light. Biotech. Lett., 28: 607-616.

John, D.M., B.A. Whitton and A.J. Brook, 2002. The Freshwater Algal Flora of the British Isles. An Identification Guide to Freshwater and Terrestrial Algae", No 288, London: University Press; p.p. 329-330.

Borowitzka, M.A., 1999. Commercial production of microalgae: ponds, tanks, tubes and fermenters. J. Biotech., 70: 313-134.

Greenspan P, E.P. Mayer and S.D. Fowler, 1985. Nile Red a selective fluorescent stain for intracellular lipid droplets. J. Cell Biol. 100: 965-973.

Fajardo, A.R., L.E. Cerdán, A.R. Medina, F.G.A. Fernández, P.A.G. Moreno and E.M. Grima, 2007. Lipid extraction from the microalga *Phaeodactylum tricornutum*. Eur. J. Lipid Sci. Tech. 109: 120–126.

Weis, V.M., E.A. Verde and W.S. Reynold, 2002. Characterization of a short form perdinin-chlorophyll-protein (PCP) cDNA and protein from the symbiotic dinoflagellate *Symbiodinium muscatinei* (Dinophyceae) from the sea anemone *Anthopleura elegantissima*". J. Phycol., 38: 57-163.

Lowry, O.M., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with Folin Phenol reagent, J. Bio. Chem., 193: 265-275.

Dubios, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances., Anal. Chem., 28: 350-356.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Methods in enzymology. Packer, L.; Douce, R., Eds.; Academic press, London; .p. 350-382.

Chen, F and M.R. Johns, 1991. Effect of C/N ration and aeration on the fatty acid composition of heterotrophic *Chlorella sorokiniana*. J. App. Phy., 3: 203-209.

Ugwu, C.U., H. Aoyagi and H. Uchiyama, 2008. Photobioreactors for mass cultivation of algae. Biores. Technol. 99: 4021-4028.

TABLE 1: Biomass and main chemical components of *Chlorella* sp. grown under photoautotrophic and heterotrophic conditions

Parameters	**Autotrophic culture conditions**	**Heterotrophic culture conditions**
Biomass (gL^{-1})	2.45 ± 0.26	8.90 ± 0.83
Cell components	(% of dry weight)	
Lipid	13.59 ± 0.51	59.80 ± 0.37
Protein	48.71 ± 0.46	12.65 ± 1.35
Carbohydrate	13.56 ± 1.64	14.91 ± 1.15
Chlorophyll	06.28 ± 0.38	1.68 ± 0.54

Data represent mean ± SD of three replicates. Data recorded on 18 days of culture.

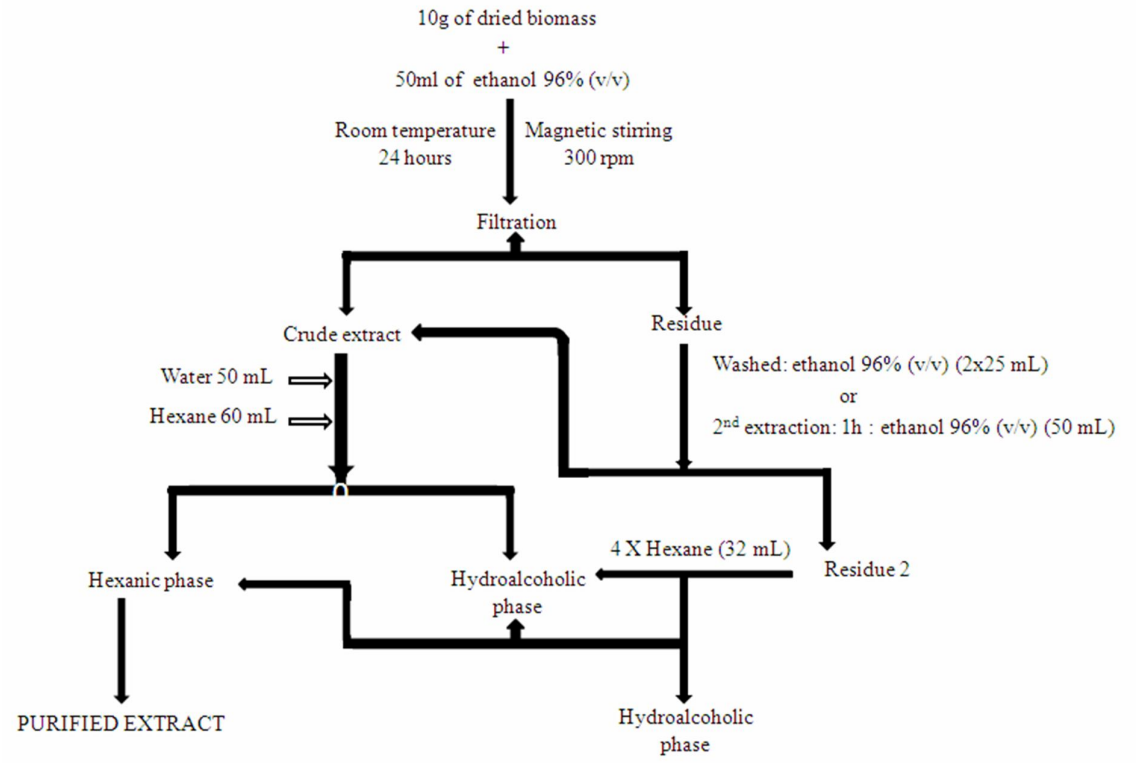
British Journal of Engineering and Technology
URL: http://www.bjet.baar.org.uk /current-issue.html
ISSN: XXXX-XXXX
Vol. 1, No. 1, pp 251 – 264, **October 2012**

TABLE 2: Properties of biodiesel after the transesterification of lipids from *Chlorella* sp. grown under photoautotrophic and heterotrophic conditions

Properties	**Autotrophic culture conditions**	**Heterotrophic culture conditions**
Density	0.89 g/cm³	0.86 g/cm³
Viscosity	11.4 cSt at 38 °C	9.8 cSt at 38 °C
Solidifying point (^{0}C)	-14	-14
Acid value (mg KOH/g)	0.410	0.395
Heating value (MJ/kg)	41	41

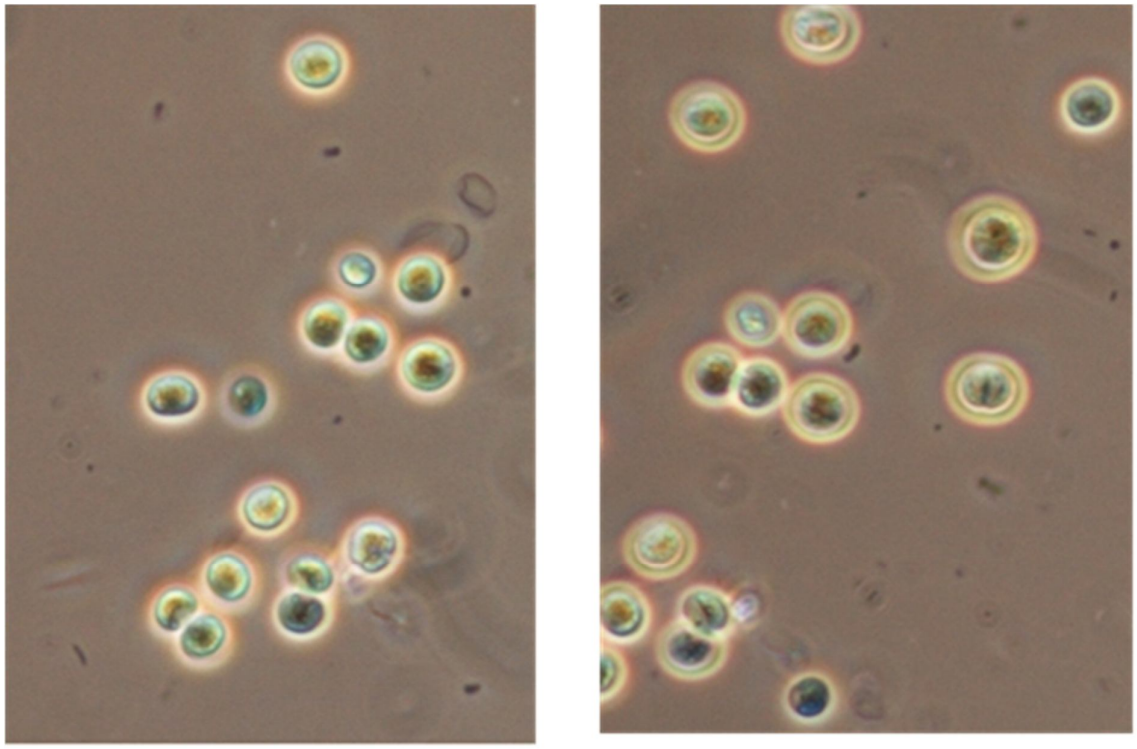
British Journal of Engineering and Technology
URL: http://www.bjet.baar.org.uk /current-issue.html
ISSN: XXXX-XXXX
Vol. 1, No. 1, pp 251 – 264, **October 2012**

FIGURE 1: Protocol for the extraction and quantification of lipids from microalgae.



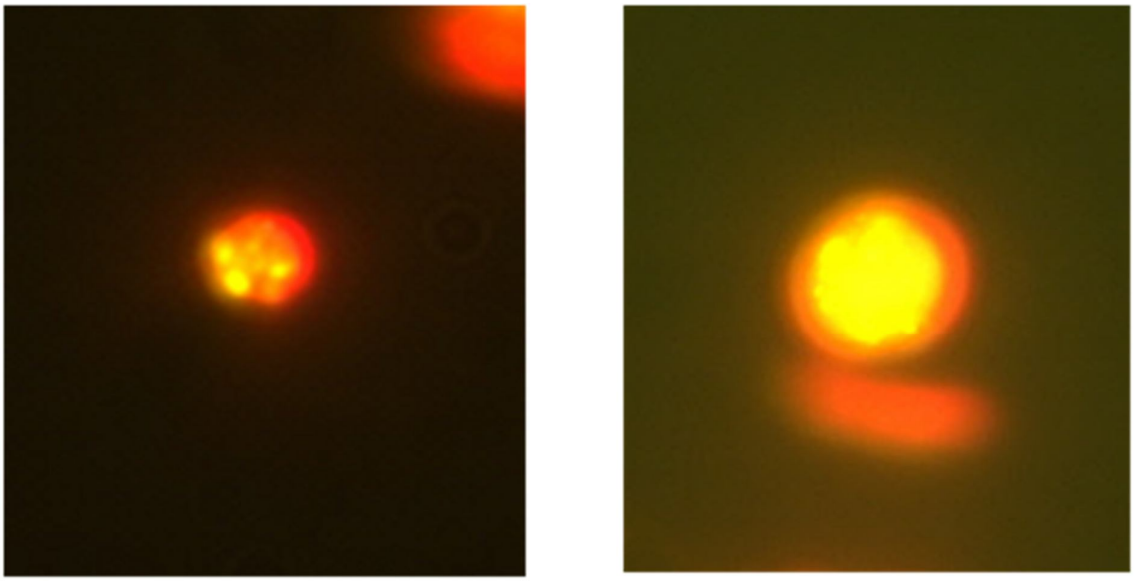
British Journal of Engineering and Technology
URL: http://www.bjet.baar.org.uk /current-issue.html
ISSN: XXXX-XXXX
Vol. 1, No. 1, pp 251 – 264, **October 2012**

FIGURE 2: Density of the cell of *Chlorella* sp. (A) grown in autotrophic conditions (B) grown in heterotrophic conditions



British Journal of Engineering and Technology
URL: http://www.bjet.baar.org.uk /current-issue.html
ISSN: XXXX-XXXX
Vol. 1, No. 1, pp 251 – 264, **October 2012**

FIGURE 3: Nile red staining of *Chlorella* cell (A) cells grown in autotrophic conditions (B) cells grown in heterotrophic conditions.



A B