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Biomass yield, oil productivity and fatty acid profile of *Chlorella lobophora* cultivated in diverse eutrophic wastewaters

K. Santhosh Kumar, S. Prasanthkumar, J.G. Ray*

Laboratory of Ecology & Ecotechnology, School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India

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ABSTRACT

Experimental trial of biomass yield, oil productivity and fatty acid profile of *Chlorella lobophora* in natural eutrophic wastewaters from three different sources was carried out using Bold's Basal Medium as the control. Eutrophication of water is a serious environment issue in agricultural as well as in many industrial zones in the world. But algal biomass production remains the most cost-effective and sustainable means to deal with eutrophic wastewater. Algal culture is the potential method of combining wastewater treatment, nutrient removal, resource recovery and simultaneous yield of biomass and bio-oils. Algal biomass has nutraceutical and biofuel applications. The major objective of the present experimentation was to test the suitability of different eutrophic wastewaters towards understanding the optimum requirements of microalgae for best biomass and oil yield. Influence of variations in nutrient composition of the wastewaters on chemical profile of the oil are also analyzed. Different water quality parameters were found significantly correlated to biomass characteristics of the alga. Oil productivity as well as chemical profile of the oil also exhibited interrelationships to water quality parameters. The oil of algal biomass taken from all kinds of wastewater sources is found suitable for biodiesel through transesterification. Overall, the method stands as model for preliminary screening of algae for economic utilization of wastewaters towards attaining high biomass yield with desired oil quality, especially for biodiesel production.

1. Introduction

Eutrophication is a common effect of excess nutrients in water (Lapointe et al., 2015; Wallace et al., 2015; Smith, 2016). Eutrophic waste waters are notorious for high amount of algal biomass with characteristic water blooming. Green algae are one of the common water blooming species, which are highly specialized microalgae adapted to various ecological habitats (Ramanan et al., 2016). Among the green algae, Chlorophytes is one of the largest phyla with many species and wide geographical distribution (Study et al., 2015; Tesson et al., 2016). Certain kinds of green algae are always common in many different kinds of eutrophic waters. Few species of green algae exhibit high photosynthetic efficiency and its lipid production magnitude is greater than that of terrestrial crops (Hu et al., 2008; Brownbridge et al., 2014). Many of them have the ability to produce substantial amounts of triacylglycerol as storage lipids (Selvarajan et al., 2015). These lipids have various applications in industry as renewable source of energy and fuel. The high yield and high density of algal biomass is widely exploited in the production of biofuels (Slade and Bauen, 2013; Kenny and Flynn, 2014). Algal biomass production can meet current energy demands (Ullah et al., 2014, 2015), and at the same time, algal cultivation is considered a fast process of carbon sequestration (Aresta et al., 2005; Wang et al., 2010; Barry et al., 2015), quite important in controlling the climate change.

Algae as aquatic species, don't need arable land for cultivation and grow abundantly in polluted freshwater systems (Pittman et al., 2011) so that space for agricultural crops will not be affected, if they are cultivated excessively. Cultivation of algae in polluted water is the best of tertiary treatment to clean the same, and the biomass and biofuel yield will be an additional economic benefit (Park et al., 2011; Makareviciene et al., 2014). Tertiary treatment of water aims at removal of non oxidizable dissolved materials in waste waters to the desired quality level. Variety of methods such as sedimentation, other physical and chemical removal, pH regulations as well as biological means of removal of nutrients are utilized in the process. Tertiary treatment is essential in the reuse of water for drinking and certain agriculture, industrial, recreational uses and water recharge purposes. Chlorophytic microalgae especially Chlorella species are already in use for tertiary treatments of wastewaters and are reported very efficient in the removal of nutrients (Makareviciene et al., 2011; Mahapatra et al.,

* Corresponding author.
E-mail address: jgray@mgu.ac.in (J.G. Ray).

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2014; Calicioglu and Demirer, 2016). However, the potential of eutrophic wastewater to meet the specific growth requirements of algae for optimum productivity of biomass and oil or specific oil characteristics is not yet properly examined.

Among the known useful microalgae, Chlorella species are proved to be highly industrially valuable for the production of lipids and biomass (Pavel et al., 2012; Sri-uam et al., 2015; Kim et al., 2016). They are tolerant to high concentrations of CO_2, adaptable to various environmental conditions, useful in the treatment of industrial effluents and purification of wastewater systems (Dalrymple et al., 2013; Wu et al., 2014). Potential strains of Chlorella have been studied enormously and are shown to be effective in recovering nitrogen and phosphorus from wastewaters (Cai et al., 2013; Praveen and Loh, 2016). Chlorella species cultivated in industrial, municipal and agricultural wastewaters yield a remarkable difference in their lipid and biomass productivity (Chiu et al., 2015). Environmental conditions and media compositions are of great influences in the production of pigments, oils and fatty acid compositions in algal cells (Sudhakar and Premalatha, 2012; Olofsson et al., 2012; Gaurav et al., 2013).

Major objectives of the present investigation included examination of the potentials of eutrophic wastewaters as natural media, assessment of specific environment factors on biomass yield, oil productivity, chemical characteristics of the oil and the feasibility of conversion of the oil to biodiesel. The experiment also had a general goal to standardize the species for optimum oil yield of desirable quality in eutrophic waters. In the present experiment, *Chlorella lobophora* VM Andreyeva, one of the well known, easily cultivable and productive green algae, common in eutrophic waters of Kerala (Santhoshkumar et al., 2015) is used as the test organism. The experiment was carried out in three different eutrophic wastewater samples collected from urban, rural and agriculture areas. Bold's Basal Medium (BBM) was used as the control. Algal cultivation in diverse eutrophic waters, which differ in specific nutrients, is thus integrated with a test for examining the influence of specific nutrient levels on biomass yield and total oil productivity as well as chemical characteristics of the oil in *Chlorella lobophora*. The oil extracted was not only characterized, but the feasibility of biodiesel production from the extracted oil was also carried out.

2. Materials and methods

2.1. Sample collection, isolation and Identification of algal strain

A freshwater sample containing local strains of *Chlorella lobophora* was collected in a sterilized one litre bottle from the bloomed fresh water pond (12.584245°N 74.981770°E) of Kasaragod district of Kerala, India. The alga was identified by using the online algae database of Guiry and Guiry (2014); 50 mL of the water sample were centrifuged at 4032 g for 5 min at room temperature to concentrate the algal cells. A high magnification digital compound microscope (Motic BA 310) was used for observation of the algal species. Serial dilution of the water sample followed by micro capillary isolation method (Stein, 1973) and streak plate method were used for the isolation of the alga in pure form (Santhosh Kumar et al., 2016). The isolated strain was cultured in Bold's Basal Medium (BBM) (Andersen, 2005) at pH of 7.30. Pure culture of the strain in BBM is maintained in the algal culture facility centre in the Ecotechnology Laboratory, School of Biosciences, Mahatma Gandhi University.

2.2. Analysis of eutrophic waters

Bloomed water samples (eutrophicated) in triplicates were collected in the summer season from an urban pond (9.311176°N, 76.431253°E -Karuvatta, Alappuzha), rural pond (9.647147°N, 76.513966°E-Kaipuzha, Kottayam) and agriculture (Rice) fields (9.635046°N, 76.499100°E- Villoonni-Kottayam) of Kerala. Biological Oxygen Demand (BOD) and dissolved free carbon dioxide were analyzed as per standard methods (Trivedy and Goel, 1986). The pH was measured by using a Eutech Multi-Parameter PCSTest™ 35 portable instrument. Total nitrogen was measured using Micro Kjeldahl method as per APHA (1999); Na and K were measured using a flame photometer (Systronics Flame Photometer-128). Phosphorus, calcium, magnesium, copper, iron, manganese, cobalt, boron and zinc in waters were measured using Atomic Absorption Spectroscopy (Thermo Scientific™ iCE™ 3000 series AAS). Each eutrophic water sample, after filtration through Whatman No1. filter paper and usual sterilization by autoclaving at 120 °C at 15 psi for 20 min was used for experimental culture of the alga.

2.3. Biomass yield of Chlorella lobophora

Pure culture of the alga in triplicates (in the control and in experimental treatments) was carried out in one litre flasks. Sterilized BBM was used as the control. Sterilized eutrophic water samples from rural pond, urban pond and agriculture (Rice) fields were used as the experimental treatments. All the cultures were incubated under controlled conditions of light (8000 lx) and temperature (24 ± 2 °C). Biomass yield was measured on completion of 30 days of growth. On the 31st day, the biomass from all the controls and experimental treatments were collected by centrifugation at 7168 g for 5 min at room temperature. The solid biomass from both the control and experiments were further air dried. Percentage increase in biomass per day per litre was calculated as the biomass yield of the alga and it was compared with that of the control.

$$\%\ \textbf{of Biomass yield of algae/L/day} = \frac{\textbf{Final dry weight}}{\textbf{Initial dry}\ \text{weight} \times \text{total}\ \textbf{number of culturing}\ \text{days} \times \text{Vol.}} \times 100$$

2.4. Algal oil extraction

The biomass was collected by centrifugation at 7168 g for 5 min at room temperature from the culture and it was then oven dried (approximately at 40 °C for 24 h). The extraction of crude oil from dried algal biomass was carried out as per Bligh and Dyer (1959). Ten grams of oven dried biomass was taken into a round bottom flask and added 100 mL of chloroform: methanol (2:1 v/v) mixture into the biomass. The biomass was then kept soaked in the organic solvents at room temperature under continuous shaking at 150 rpm for 4 h in a rotary shaker (Roteck RRS-06); afterwards the mixture was centrifuged at 4023 g at room temperature for 5 min. Residual biomass was separated from the extract and then the oil along with the solvent was transferred into a separating funnel. About 40 mL of distilled water was added to this mixture to separate the oil from the solvent. The oil got separated as an organic phase in the bottom layers, which was then collected into a bottle. The separated biomass and the oil were made free of the solvent by using a rotary evaporator.

The air dried residual biomass free of the solvent was further subjected to the hot method of extraction for the collection of the remaining lipids. The biomass was placed in a Soxhlet extractor and extracted with 75 mL of hexane, refluxed under 70 °C for 2 h. The extracted oil components were collected and the oil was made free of the solvent by using a rotary evaporator. Finally, the two extracted oil samples were mixed together to get the total oil extract. Percentage of oil in algae (%) was calculated by using the following formula (Abubakar et al., 2012):

$$\frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100$$