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Case Study

Estimating lipids for bioprospecting: A case study with Deepor beel algae

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Abstract: Microalgae have been considered as ideal candidates for biodiesel production and other bio-products. They have received extensive research for bioprospecting during the past few decades. Chlorophycean algae are one of the few microalgae employed for many biotechnological applications. Therefore, the present work was aimed to analyze the growth and lipid contents of four Chlorophycean algae isolated from Deepor beel Ramsar site, Assam (India) to assess their potentiality for industrial means. Of the four species, *Carteria cerasiformis* showed the highest lipid content (24.4 ± 1.45 %).

Keywords: Bioprospecting, Chlorophyceae, Deepor beel, Growth, Lipid contents, Lipid productivity

Introduction

Recently, algae have been given wide interest for bioprospecting due to their several inherent advantages. They are relatively easy to cultivate and do not need to compete with food production (Ho *et al*., 2014). They can adapt to any change in environmental conditions by producing a great variety of secondary metabolites (Ahmed *et al*., 2014).

Algae are one of the major natural sources for a vast array of valuable compounds such as proteins, carbohydrates, lipids, chlorophylls, carotenoids and phycobilins (Del Campo *et al*., 2007). For which algae has been widely used in fish and animal feedstock, human nutrition, medical treatments, cosmetic industry, biodiesel production (Spolaore *et al*., 2006). Few are used in agriculture industry (Milledge, 2011). *Chlorella*, *Dunaliella*, *Haematococcus*, *Isochrysis*, *Nannochloropsis*, *Porphyridium*, *Scenedesmus* sp. are known to contain significant amounts of antioxidative polysaccharides and polyunsaturated fatty acids and have already been exploited for commercial applications (Stranska-Zachariasova *et al*., 2016).

Lipids are biological molecules which are soluble in organic solvents. Microalgae are known to accumulate high lipid content ranging from 25-75% of its dry weight (Malcata, 2011), due to which microalgal biomass is nowadays considered to be a good source of biofuel. Recent study revealed that algae have the potential to produce 100 times more oil than any other plants (Bartley *et al*., 2013; Mubarak *et al*., 2015). But lipid yield, composition and fatty acid profiles can substantially vary depending upon the type of microalgae used as well as cultivation conditions of the particular strain such as light intensity, temperature, ratio of light/dark cycle, aeration rate and growth media (Halim *et al*., 2012; Onay *et al*., 2016). Besides its application as an alternative biodiesel feedstock, microalgae are also considered as an alternative source of essential fatty acids, particularly omega-3 fatty acid in order to replace fish oil (Solana *et al*., 2014), which is important for human's health. The omega-3 and omega-6 fatty acids present in microalgae may reduce the risk of many human associated diseases including cancer, cardiovascular disease, inflammatory and autoimmune diseases (Simopoulos, 2002).

Bioprospecting of microalgae encompasses searching and collection of unique microalgal strains from different aquatic environments for exploiting their potential applications in production of value-added products (Mutanda *et al*., 2011). The present work was therefore attempted to gain insights into the lipid contents of four Chlorophycean microalgae of Assam isolated from Deepor beel Ramsar site.

Materials and Methods

Cultivation and growth condition of the organisms

Microalgal samples were collected from Deepor beel Ramsar site, Assam (India). Though Deepor beel is endowed with rich algal biodiversity, only four microalgae were selected for this preliminary work. They were *Carteria cerasiformis*, *Chlamydomonas* sp., *Chlorococcum* sp. and *Lobochlamys segnis*. The microalgal samples were allowed to grow separately in a 250 ml Erlenmeyer flask containing 100 ml modified Bold's Basal medium (BBM). Modified BBM contained (g L^{-1}): $NaNO_3$, 0.297; $MgSO_4$, 0.052; K_2HPO_4, 0.105; KH_2PO_4, 1.8; $CaCl_2$, 0.022; NaCl, 0.022; EDTA, 0.049; KOH, 0.031; and trace

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elements solution contained (g L^{-1}): H_3BO_3, 0.011; $FeSO_4$, 0.005; $ZnSO_4$, 0.009; $Co(NO_3)_2$, 0.001; $MnCl_2$, 0.001 and $CuSO_4$, 0.001; and one drop of H_2SO_4 L^{-1}. The medium was sterilized in an autoclave at 121°C for 90 minutes before inoculation.

Frequent streaking and subculture was done in order to obtain pure form of the culture. For each species, 10 ml of the mother culture was diluted in a 250 ml Erlenmeyer flask containing 100 ml of the fresh sterile media as mentioned above. This culture was maintained at a constant temperature of 27±1 °C with continuous photoperiod at an irradiance of 42 µmol photons $m^{-2}s^{-1}$ provided by cool white fluorescent lamps. Each sample was grown in triplicates for each experimental test. Microalgal cells were harvested by centrifugation at 6000 rpm for 10 minutes at room temperature after 14 days for lipid analysis. All experimental tests were carried out under aseptic laboratory conditions. Identification of the species was done following the monographs of Perumal and Anand (2009).

Estimation of dry weight and specific growth rate

Dry cell weight (DCW) was estimated following the standard protocol as stated by Patnaik and Mallick (2015). A known volume of the culture was centrifuged at 5000 rpm for 10 minutes. The pellet was resuspended in distilled water and filtered through a Whatman GF/C filter paper (1.2 µm pore size). The algal cells were harvested and dried at 60°C in an oven until constant weight was obtained and were cooled down to room temperature in a desiccator before weighing and was calculated as g L^{-1} DW. The biomass productivity (mg L^{-1} d^{-1}) was calculated as:

$$P_{biomass} = \frac{\Delta x}{\Delta t}$$

Where $P_{biomass}$ is the biomass productivity (mg L^{-1} d^{-1}), Δx is the variation in biomass concentration (g L^{-1}) within a cultivation time of Δt (d).

The specific growth rate (µ) was calculated according to the equation:

$$\mu = \frac{\ln (N_2/N_1)}{t_2-t_1}$$

Where N_1 is the initial biomass concentration at t_1, and N_2 is the biomass concentration at t_2 of the selected time intervals respectively.

Estimation of lipid content

The lipid concentration of the microalgal biomass was analyzed by a procedure adapted from Folch *et al.,* (1949). This method involves using of chloroform and methanol as solvent mixture in 2:1(v/v) ratio. The algal cells was washed with deionized water, lyophilized and weighed. A known amount of the dry samples were homogenised with 5 ml of chloroform: methanol mixture. The extract was then filtered through Whatman GF/C filter paper and the filtrate was transferred into a separating funnel. 3 ml of saline water (1% NaCl) was added to the filtrate and allowed to stand undisturbed in room temperature for few minutes. The lower organic phase ($CHCl_3$) layer containing the lipid components was collected in a clean pre-weighed (W_1) small beaker. The solvent was left overnight in a dessicator to evaporate the solvent near to dryness, leaving lipid at the bottom of the beaker. The weight of this beaker with the lipid extract was reweighed (W_2). The difference between the initial weight and the final weight (W_2-W_1) gives the total lipid content of the sample. The lipid productivity (mg L^{-1} d^{-1}) was calculated as:

$$\mathbf{P_{lipids} = P_{biomass} \times C_f}$$

Where P_{lipids} = lipid productivity

C_f = final lipid concentration

Statistical analysis

The entire test was conducted in triplicate independent cultures to confirm their reproducibility and the results of the entire analysis were expressed as mean ± standard deviation of three experiments.

Results and Discussion

Because of their high value for biofuel, nutraceutical and pharmaceutical formulations; lipids is one the most important algal components for biotechnology (Durvasula *et al.*, 2015). That is why this study was undertaken with the four Chlorophycean species as the test organisms, each revealing different results. Chlorophyceae are amongst the most frequently used microalgae for bioprospecting. No optimization cultivation parameters were made to enhance the lipid content of the species in this study. The data obtained are the results of the species grown in modified BBM under a low light intensity of 42 µmol photons $m^{-2}s^{-1}$ for 14 days.

Growth of the organisms

The specific growth rate (SGR), biomass concentration and biomass productivity of the four Chlorophycean microalgal species are shown in Table 1. No studies have yet been reported regarding the biochemical analysis of the species *C. cerasiformis* and *L. segnis*. This is by further the first report of this type related to these two species. Exponentially growing cells were used as inocula. The time interval of the biomass concentration taken for the determination of SGR and biomass productivity was 7th and 14th day of the culture. The biomass concentration of the respective species taken on 7th day were 0.29 ± 0.03 g L^{-1} DW for *C. cerasiformis*, 0.48 ± 0.02 g L^{-1} DW for *Chlamydomonas* sp., 0.37 ± 0.03 g L^{-1} DW for *Chlorococcum* sp. and 0.21 ± 0.02 g L^{-1} DW for *L. segnis*. The biomass concentration of the species ranged from 0.75 ± 0.03 to 0.52 ± 0.04 g L^{-1} after 14 days of cultivation which is in agreement with previous findings by

Ngangkham *et al.,* (2012), Kim and Hur (2013), Salama *et al.,* (2013), and Fakhry and El Maghraby (2015) in different species. Growth rate of any microalgae may vary depending on the composition of their growth medium and time of incubation (Ho *et al.*, 2014).

The biomass productivity obtained in this study ranged from 152 ± 7.0 to 109 ± 5.2 mg $L^{-1}d^{-1}$. Similar result was observed by Rodolfi *et al.,* (2008) in which he screened 30 microalgae strains and obtained biomass productivity between 0.04 and 0.37 g $L^{-1}d^{-1}$. The SGR ranged from 0.63 ± 0.04 to 0.39 ± 0.06 d^{-1} among the selected species during the study period. This finds support from the reports published by Vello *et al.,* (2014) and Patnaik and Mallick (2015). It was found that out of the four Chlorophycean algae, the growth rate of *Chlamydomonas* sp. was comparatively higher, which reached upto 0.75 ± 0.03 g L^{-1} of biomass concentration, 152 ± 7.0 mg $L^{-1}d^{-1}$ of biomass productivity and 0.63 ± 0.04 d^{-1} of SGR on the 14th day in the present study.

Lipid contents

Lipids are essential for all living organisms as components of membranes, energy storage compounds and as cell signaling molecules (Eyster, 2007). They have been recognized as essential components in human and animal nutrition and are used as feed additives in aquaculture. Lipid content is highly species specific and their composition are influenced by culture media and environmental factors such as nutrient availability and light intensity and is also known to change with age of a culture (Griffiths *et al.*, 2011; Goiris *et al.*, 2012). To investigate the potentiality of the tested organisms for various biotechnological applications, total lipid content was analyzed on dry weight basis after 14 days of culture and was expressed as % out of DCW.

Table 1. Growth, lipids and lipid productivity of the test organisms

Species Name	Biomass concentration (g L^{-1})	Biomass productivity (mg $L^{-1}d^{-1}$)	Specific growth rate (d^{-1})	Lipid concentration (%)	Lipid productivity (mg $L^{-1}d^{-1}$)
Carteria cerasiformis	0.54 ± 0.03	123 ± 8.1	0.55 ± 0.05	24.4 ± 1.45	95.76 ± 8
Chlamydomonas sp.	0.75 ± 0.03	152 ± 7.0	0.63 ± 0.04	10.8 ± 1.26	44.18 ± 3
Chlorococcum sp.	0.68 ± 0.01	128 ± 7.6	0.48 ± 0.04	10.23 ± 1.25	43.78 ± 5
Lobochlamys segnis	0.52 ± 0.04	109 ± 5.2	0.39 ± 0.06	22.5 ± 1.34	92.61 ± 6

Table 1 showed the lipid content and lipid productivity of the tested organisms. In the present work, *C. cerasiformis* showed a relatively high lipid content than the other three species with a value of 24.4 ± 1.45 %. The results obtained correspond to a number of previous studies for a number of Chlorophytic species. This finding is in good agreement with the results as reported by Alkhamis and Qin (2016) in which the lipid contents of *Tisochrysis lutea* was found to be 22.0 ± 1.06 % grown in phototrophic conditions. It is also in concomity with the findings of Orr and Rehmann (2015) where the lipid content of *Chlorella vulgaris* ranged from 15 ± 0 to 24 ± 3 % grown in six different media. In this work, the lipid content of *Chlorococcum* sp. was found to be 10.23 ± 1.25 % which agrees well with the results reported by Hawati *et al.,* (2012) where the lipid content of *Chlorococcum* sp. in control condition was found to be 10.3 %, and also with that of *Chlorella sorokiniana* H-84 strain with lipid content of 10.0 % as reported by Matsukawa *et al.*, (2000).

Lipid productivity should be considered as the most appropriate factor to facilitate species selection for biodiesel (Huerlimann *et al.*, 2010). It is correlated well with biomass productivity, which substantiates its usefulness as a suitable indicator for biodiesel production (Vello *et al.*, 2014). The lipid productivity of the four selected Chlorophycean microalgae was reported to range from 95.76 ± 8 to 43.78 ± 5 mg $L^{-1}d^{-1}$ which were at par with that of Ho *et al.,* (2014), Talebi *et al.,* (2013), Zhao *et al.,* (2012) and Rodolfi *et al.,* (2008). The highest calculated value of 95.76 ± 8 mg $L^{-1}d^{-1}$ as obtained in this study was far above the average lipid productivity (50 mg $L^{-1}d^{-1}$) as shown in the available literature (Chtourou *et al.*, 2015). *C. cerasiformis* and *L. segnis* yielded less growth rate but achieved higher lipid content (24.4 ± 1.45 %) and (22.5 ± 1.34 %) respectively than the other two microalgae during the study period. Existing result revealed that lipid content can be high even when the growth rate and lipid productivity is low (Rodolfi *et al.*, 2008).

Not all lipids of microalgae can be used for the production of biodiesel. They can be used for other purposes such as, be used in the cosmetic industry, aquaculture industry, food industry and the pharmaceuticals industry (Varfolomeev and Wasserman, 2011). In comparison to the other species as mentioned above, the growth and lipid contents of the tested organisms of the present study seems pretty good and may therefore be recommended for further analysis.

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