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RESEARCH ARTICLE

A COMPARATIVE ANALYSIS OF LIPID PRODUCTIVITY OF SOME FRESHWATER ALGAE ON THE BASIS OF SEASONAL VARIATION

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ABSTRACT

Microalgae are a promising alternative source of lipid for biofuel production. One of the most important decisions is the choice of species to use. High lipid productivity is a key desirable characteristic of a species for biodiesel production. This paper reviews information about microalgal growth rates, lipid content and lipid productivities for 8 species of freshwater microalgae. The information provide a framework for decision-making and a starting point for further investigation of species selection. The importance of lipid productivity as a selection parameter over lipid content and growth rate individually is demonstrated. Total lipid content of freshwater algae isolated from different aquatic habitats of Ganjam district, Odisha were examined. A comparative analysis showed variation or total lipid content in different seasons. Total lipid content was high in summer season and least in monsoon. As *Microsysts* sp. *i*s a bloom causing algae, it is the best source of biofuel production due to its high temperature resistance character.

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INTRODUCTION

Sustainable production of renewable energy is being debated globally since it is increasinglyunderstood that first generation biofuels, primarily produced from food crops and mostly oil seeds,compete for arable land, freshwater or bio-diverse natural landscapes and are limited in their ability toachieve targets for biofuel production. These concerns have increased the interest in developing second and third generation biofuels such as lignocellulosics and microalgae, respectively, which potentially offer great opportunities in the longer term and do not need to compete for arable land and precious freshwater (Schenk *et al*., 2008; Chisti, 2007). Due to continuous and increasing combustion of fossil carbon, the amount ofgreenhouse gas CO_2 has increased. As a result global warming and climate change are threat eningecological stability, food security and social welfare (Chisti, 2008; Christenson and Sims, 2011). The transportation and energy sector are the two major sources, responsible for the generation of 20% and 60% of greenhouse gases (GHG) emissions, respectively, and it is expected that with the development of emerging economies the global consumption of energy will rise considerably and this will lead to more environmental damage (Stephens *et al*., 2010). Photosynthesis is the only process that can convert CO_2 into organic compounds with high energycontent, and thus can provide a source for sustainable transport fuel production. There is an urgentneed to develop technologies which are able to produce an additional five to six billion tons of organic carbon apart from the current harvest from agricultural crops (Chisti, 2008). Large-scale cultivation of microalgae may be 10–20 times more productive on a per hectare basis than other biofuel crops, are able to use a wide variety of water sources, and have a strong potential to produce biofuels without the competition for food production (Chisti, 2007). Algae can be produced either as macrophytes via marine aquaculture (Bruton *et al*., 2009) or in large-scale microalgae cultivation systems in open ponds or in photo bioreactors (Schenk *et al*., 2008). Microalgae are currently considered the most promising types of algae for biofuel production, based on their high lipid contents. Recent progress in the production of microalgae has been intensively reviewed (Brennan and Owende, 2010), and future perspectives have been presented by Stephens *et al*. (2010). Microalgae can also be cultivated as an integrated concept with wastewater treatment to optimize the energetic and financial input for the production process (McGinn *et al*., 2011). Triacylglycerides (TAGs) generally serve as energy storage in microalgae that, once extracted, can be easily converted into biodiesel through transesterification reactions (Chisti, 2008; Fukuda *et al*., 2001). These neutral lipids bear a

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common structure of triple esters where usually three long-chain fatty acids (FAs) are coupled to a glycerol molecule. Transesterification displaces glycerol with small alcohols (e.g., methanol). Recently, the rise in petroleum prices and the need to reduce greenhouse gas emission has seen a renewed interest in large-scale biodiesel production (Chen, 2011).

Lipids in Microalgae

Lipids produced by microalgae generally include neutral lipids, polar lipids, wax esters, sterols and hydrocarbons, as well as prenyl derivatives such as tocopherols, carotenoids, terpenes, quinines and pyrrole derivatives such as the chlorophylls. Lipids produced by microalgae can be grouped into two categories: storage lipids (non-polar lipids) and structural lipids (polar lipids). Storage lipids are mainly in the form of TAG made of predominately saturated FAs and some unsaturated FAs which can be transesterified to produce biodiesel. Structural lipids typically have a high content of polyunsaturated fatty acids (PUFAs), which are also essential nutrients for aquatic animals and humans. Polar lipids (phospholipids) and sterols are important structural components of cell membranes which act as a selective permeable barrier for cells and organelles. These lipids maintain specific membrane functions, providing the matrix for a wide variety of metabolic processes and participate directly in membrane fusion events. In addition to a structural function, some polar lipids may act as key intermediates (or precursors of intermediates) in cell signaling pathways (e.g., inositol lipids, sphingolipids, oxidative products) and play a role in responding to changes in the environment. Of the non-polar lipids, TAGs are abundant storage products, which can be easily catabolized to provide metabolic energy (Gurr *et al*., 2002). In general, TAGs are mostly synthesized in the light, stored in cytosolic lipid bodies, and then reutilized for polar lipid synthesis in the dark (Thompson, 1996). Micro algal TAGs are generally characterized by both, saturated and monounsaturated FAs. However, some oil-rich species have demonstrated a capacity to accumulate high levels of long-chain polyunsaturated fatty acids (PUFA) as TAG (Bigogno *et al*., 2002; Alonso *et al*., 1998). A detailed study on both accumulation of TAG in the green microalga storage into chloroplastic lipids (following recovery from nitrogen starvation) led to the conclusion that TAGs may play an additional role beyond being an energy storage product in this alga (Bigogno *et al*., 2002; Khozin-Goldberg and Cohen, 2006). Hence, PUFA-rich TAGs are metabolically active and are suggested to act as a reservoir for specific fatty acids. In response to a sudden change in the environmental condition, when the *de novo* synthesis of PUFA may be slower, PUFA-rich TAG may donate specific acyl groups to monogalactosyldiacylglycerol (MGDG) and other polar lipids to enable rapid adaptive membrane reorganization (Khozin-Goldberg and Cohen, 2006; Makewicz, 1997).

MATERIALS AND METHODS

(1) Algal isolates: The experimental species were collected from different aquatic habitats like dairy effluents, sewage drawn, old water tanks and ponds etc in different seasons (monsoon, premonsoon, post monsoon) from different parts of Ganjam district, Odisha.

(2) Identification: After collection algal samples were identified under microscope.

(3) Culture condition: The species were cultured in the laboratory and grown aseptically on dry agar (Dor *et al.*, 1987). Bluegreen algae were cultured in BG-II growth medium (Stairer *et al.*, 1971) andgreen algae were cultured in Chu 10 medium. These culture were maintained at 26 ± 4^{0}C under constant illuminator (10 h dark at 2000 Lux)

(4) Lipid extraction: Algae were ground with motor and pestle as much as possible. The ground algae were dried for 20 min at 80°C in an incubator for releasing water. Hexane and ether solution (20 and 20 mL) were mixed with dried ground algae to extract oil. Then the mixture was kept for 24 h for settling.

(5) Evaporation: The extracted oil was evaporated in vacuum to release hexane and ether solutions using rotary evaporator. The final product is lipid.

RESULTS

The total lipid content in eight species of freshwater algae is shown in Table-1. *Microcysts* sp. showed high lipid concentration followed by *Chlorella* species, whereas in *Phormidium* sp. it was lowest. Table-2 represents the total lipid content of eight species in different season. All these species were shown good lipid content in summer season than monsoon and post-monsoon seasons. Most interestingly, *Microcysts* sp. which is one of the bloom algae showed high lipid content than other species. Next to *Microcystis, Phormidoum* sp. Showed better lipid content in summer season. As in summer season temperature is too high, these algae showed the best temperature tolerance character. Table - 3 represents the total oil content of 8 algal species. *A Microcystis* sp. *alga is* a hazardous bloom causing algae but it shows high oil content which is the best source of biofuel production.

Table 1. Represents total lipid content of experimental algae

Algae	Total lipid content a, b
***Oscillatoria*sp.**	23.60 ± 0.14
***Nostoc*sp.**	18.35 ± 0.07
***Lyngbya*sp.**	10.55 ± 0.07
***Phormidium*sp.**	10.48 ± 0.10
***Microcystis*sp.**	28.15 ± 0.21
***Chlorella* sp.**	25.60 ± 0.12
***Anabaena* sp.**	8.35 ± 0.03
***Euglna*sp.**	19.21 ± 0.17

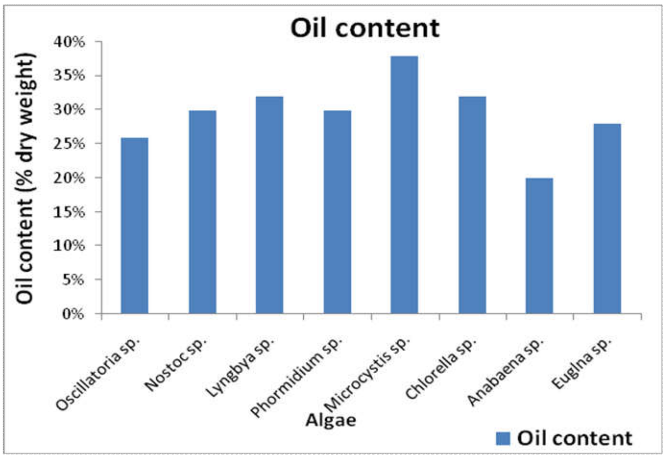
a = Mean ± standard deviation, b = Percentage of total lipid

Table 2. Total lipid content of Algae at different season

Algae	Monsoon	Post monsoon	Pre-monsoon
***Oscillatoria* sp.**	19.18 ± 0.18	23.10 ± 0.10	23.10 ± 0.10
***Nostoc* sp.**	10.27 ± 0.07	14.52 ± 0.12	20.38 ± 0.22
***Lyngbya* sp.**	11.53 ± 0.37	16.62 ± 0.12	20.33 ± 0.16
***Phormidium* sp.**	18.70 ± 0.16	24.42 ± 0.07	26.70 ± 0.14
***Microcystis* sp.**	19.48 ± 0.14	24.44 ± 0.06	30.32 ± 0.08
***Chlorella* sp.**	18.32 ± 0.12	22.42 ± 0.17	24.66 ± 0.02
***Anabaena* sp.**	8.12 ± 0.31	9.31 ± 0.33	10.71 ± 0.21
***Euglna*sp.**	19.12 ± 0.32	20.12 ± 0.72	22.53 ± 0.17

a = Mean ± standard deviation, b = Percentage of total lipid

Table 3. Total oil content of experimental algae



DISCUSSION

Harmful algae typically bloom during the warm summer season or when water temperatures are warmer than usual. As temperatures become warmer due to climate change, the growth of harmful algae may be favored over other non-harmful algae through a combination of mechanisms. Harmful algae typically bloom during the warm summer season or when water temperatures are warmer than usual. As temperatures become warmer due to climate change, the growth of harmful algae may be favored over other non-harmful algae through a combination of mechanisms: Warmer temperatures create a competitive advantage for certain types of harmful algae. As seen with the toxin-producing cyanobacteria *Microcystis*, certain harmful algae grow faster than other non-harmful algae at relatively high temperatures, some of them at temperatures above 77°F. During summer months, warming of surface waters can create a physical force strong enough to resist the wind's ability to mix the water. These layers can restrict the vertical movement of oxygen and nutrients in water. This process is known as thermal stratification. The warming of surface waters increases the frequency, strength, and duration of stratification, which favors the growth of harmful algal blooms. Warmer temperatures reduce the viscosity of water, which increases the speed at which small aquatic organisms can vertically migrate. This makes it easier for small cyanobacteria to float to the surface to form harmful algal blooms. On the other hand, a decrease in viscosity promotes the sinking of larger algae and organisms that are not capable of migrating towards the surface. This may increase the competitive advantage of cyanobacteria over other algae. From the present study it was hypothesized that the freshwater bodies are the good sources to select biofuel producing microalgae. In the present screening 8 algae were able to produce biofuel in the laboratory media. *Microcystis* sp. stood first based on biofuel producing efficacy.

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