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Research paper

Biomass yield and biochemical profile of fourteen species of fast-growing green algae from eutrophic bloomed freshwaters of Kerala, South India

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ABSTRACT

Eutrophic, bloomed waters are known for fast-growing microalgae of high biomass yield. The biochemical composition of algae may vary from species to species. Identification of fast-growing local algal species, their experimental culture for assessing biomass yield and biochemical screening of the same for desirable metabolites is crucial to the prospects of algal technology. The freshwater algal diversity of Kerala - one of the biodiversity hotspots of the world, remains poorly explored. In this context, we assessed the yield and biochemical profile of hitherto uninvestigated 14 fast-growing microalgae of eutrophic bloomed freshwaters of Kerala. The biomass yield, carbohydrate, protein, pigment and lipid content of these species were significantly different. The alga *Pseudococcomyxa simplex* showed the highest biomass yield of 196.5 ± 3.04% increase L^{-1} day^{-1}. The alga *Kirchnerialla lunaris* with 58.95% protein was found superior to the other algae in this regard. The species *Scenedesmus obliquus* was significantly higher in total lipids (32.05% of dry biomass) than the other algae. The alga *Monoraphidium griffithii* with 42.92% of omega groups of fatty acids in its lipid appeared a highly valuable species. The algae, *Radiococcus nimbatus* (12.77 ± 2.31 mg g^{-1} of chlorophyll *a*), *Myrmecia bisecta* (5.87 ± 0.01 mg g^{-1} of chlorophyll *b*) and *Monoraphidium griffithi* (7.50 ± 0.02 mg g^{-1} carotenoid) appeared superior to the others in pigment content. Fourier-Transform-Infrared Spectroscopy of the biodiesel prepared from the lipids of all the algae confirmed the biodiesel feasibility of the same. The bioresource potentials of the 14 algal species revealed are new to science.

1. Introduction

Many species of algae are well-known bioresources, especially for nutraceutically valuable compounds, oil and minerals [1]. Since thousands of years, several algae have been used as a direct source of human food or for the preparation of various kinds of nutrient-rich functional foods in different parts of the world [2]. In general, the nutritional value of many species of microalgae is quite high, as they contain a high proportion of proteins, carbohydrates, lipids and vitamins [3]. Today, many algae are commercially cultivated for food [4], nutraceutical [5] and biofuel purposes [6]. Algae have now emerged as a stable economic crop [7].

Isolation of species and production of specific biomolecules from algal biomass for food and feed [8] is not new. Many species of poly-unsaturated fatty acid yielding green algae are currently used for the feedstock preparations in the aquaculture industry [9]. Algae, rich in essential fatty acids are beneficial in the treatment of some illness and metabolic disorders in vertebrates [10–12]. Nutraceutical and toxicological evaluations of specific algal biomass have demonstrated microalgae as a valuable feed supplement, which can easily replace the conventional protein supplements in animal feeds [13]. Algal proteins and other energy supplements from algal biomass form a prebiotic for enhancing production and maintaining the health of livestock [14]. In general, algae can provide a high yield of nutrient-rich biomass, which is readily convertible to animal feed at a minimum cost of production [15,16]. In addition to these aspects, certain green algal species are rich sources of pigments, antioxidants, vitamins, immune-stimulants as well as specific plant hormones such as the Chlorella-Growth-Factor compounds [17] for various human purposes and medicinal applications. Many species of algae with high photosynthetic efficiency can produce oil in the form of tri-acyl-glycerol as storage lipids [18], which are valuable in the biofuel industry as a renewable source of energy and fuel. The annual productivity and the lipid content of certain species of algae are found to be far higher than that of seed crops [19].

Currently, the biomass and biofuel extraction from algae is well known as an industrially reliable process. Although reports on the biochemical potentials of few limited numbers of species exist in the literature [20], that of a large number of algae remain entirely

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unexplored [21]. Therefore, exploration of the specific nutraceutical and oil characteristics of local species continues a significant research topic for global utilities. The commercial exploitation for diverse products of particular interest from the unexplored species depends on a thorough knowledge of both qualitative and quantitative aspects of the biochemical profile of such species regarding carbohydrate, lipid, protein and pigment about biomass yield and productivity of the alga.

Kerala as one of the biodiversity hotspots of the world is rich in green algal resources [22] and the exploration of biochemical aspects of certain of these algal biodiversity is already known [23]. Such studies have revealed the significance of exploring the food, feed, nutraceutical and biofuel potentials of as many new species as possible for global commercial utilities of this local algal wealth. The major objectives included assessment of the biomass yield and biochemical characterisation of the 14 fast-growing species isolated from the eutrophic bloomed freshwaters of Kerala. Another important objective was to analyse the yield of valuable biochemicals and the biodiesel feasibility of the lipid content of these different native species of green algae.

2. Materials and methods

The collection of all the algae was from different bloomed freshwater bodies of Kerala, India (Table 1). One litre of bloomed water was collected from each site for analysis. About 50 mL of water sample was centrifuged at 4100 × g for 5 min at room temperature (27°C- 30 °C) to concentrate the algal cells. High magnification digital compound microscope (Motic BA 310) was used for observation of the algal species. The algae were identified by using algal keys [24,25] and online algae database [26]. The identified algae were isolated from fresh samples by using serial dilution and micro-capillary isolation method [27] followed by streak plate method [23]. Once the isolation was confirmed, the isolated species were cultured in Bold's Basal Medium (BBM) [28], its composition consist of chemicals: Stock (1)- $NaNO_3$-2.5 g 100 mL^{-1}, $MgSO_4$.7 H_2O-750 mg 100 mL^{-1}, NaCl-250 mg 100 mL^{-1}, K_2HPO_4 -0.75 g 100 mL^{-1}, KH_2PO_4-1.75 g 100 mL^{-1}, $CaCl_2.2H_2O$-25 mg 100 mL^{-1}, Stock (2)- Alkaline EDTA solution consisting of EDTA-5 g 100 mL^{-1}, KOH− 3.1 mg 100 mL^{-1}, Stock (3)- Boron solution H_3BO_3-1.14 g 100 mL^{-1} and Stock (4)- Acidified iron solution consisting of $FeSO_4.7H_2O$ solution- 498 mg 100 mL^{-1} of distilled water and 2 drops of conc. H_2SO_4 and Stock (5)- trace element mixture prepared by dissolving 882 mg of $ZnSO_4.7H_2O$, 144 mg of $MnCl2.4H_2O$, 71 mg of MoO_3, 157 mg of $CuSO_4.5H_2O$ and 49 mg of $Co(NO_3)_2.6H_2O$ in 100 mL of distilled water. For the preparation of 1 L of BBM medium, 10 mL each of macronutrients from stock one and one ml each from Stock 2–5 were taken (64 mL) in a standard flask and made up to 1 L by addition of 936 mL distilled water. Pure cultures of the identified strains of all the species are maintained in the green algal repository at the Ecotechnology Laboratory, School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India.

Table 1
Geographical and taxonomic references of the 14 microalgal species from bloomed water bodies of Kerala, which are investigated in terms of biomass yield and biochemical composition.

Sl. No	Algal species	Type of water body	Latitude and Longitude	Taxonomic reference
1	*Scenedesmus obliquus* (Turpin) Kutzing	Reservoir	8.574361 N, 77.021146 E	[57]
2	*Scenedesmus acuminatus* (Lagerheim) Chodat	Temple pond	9.674254 N, 76.560600 E	[57]
3	*Scenedesmus bijuga* (Turpin) Lagerheim	Temple pond	9.869219 N, 76.668974 E	[58]
4	*Scenedesmus armatus* (Chodat) Chodat	Lake	8.412563 N, 76.994024 E	[59]
5	*Myrmecia bisecta* Reisigl	Urban pond	8.477329 N, 76.940996 E	[60]
6	*Tetrastrum komarekii* Hindák	Temple pond	9.858871 N, 76.400177 E	[61]
7	*Radiococcus nimbatus* (De Wildeman) Schmidle	Reservoir	8.536630 N, 77.145705 E	[25]
8	*Monoraphidium litorale* Hindak	Urban pond	10.036764 N, 76.411802 E	[61]
9	*Monoraphidium contortum* (Thurte) Komarkova-Legnerova	River	10.112482 N, 76.348158 E	[62]
10	*Monoraphidium griffithii* (Berkeley) Komarkova-Legnerova	Rural pond	8.729094 N, 76.711096 E	[62]
11	*Kirchneriella lunaris* (Kirchner) Mobius	Temple pond	10.151597 N, 76.223197 E	[63]
12	*Oocystis lacustris* Chodat	Rural pond	12.31012 N, 75.13349 E	[64]
13	*Pseudococcomyxa simplex* (Mainx) Fott	Temple pond	9.674254 N, 76.560600 E	[65]
14	*Pseudotetradesmus quaternaries* Hirose et Akiyama	Rural pond	10.014110 N, 76.339629 E	[66]

2.1. The biomass yield and biomass productivity

The cultures were carried out in 1-L flasks using BBM in triplicate. All the culture vessels were incubated under controlled conditions of light (8000 Lx), temperature (24 ± 2 °C) and pH (7.30). The biomass yield was measured on completion of 30 days of growth. After the incubation, the biomass was collected by centrifugation at 4100 × g for 10 min at room temperature (27°C- 30 °C) (REMI, R-24) and the concentrated algal pellets were washed with distilled water, the solid biomass was further air-dried at room temperature (27°C- 30 °C) for 2–3 days until constant weight was attained and stored at −20 °C in a deep freezer (Samsung-ModelNo.SRS600NLS) for further analysis. The percentage increase in biomass per day per litre was calculated as the yield of algae as per the Equation Eq. (1)

$$\%\ \text{increase of Biomass yield of algae } L^{-1}\,day^{-1} = \frac{Final\ dry\ weight\,(mg)}{Initial\ dry\ weight\,(mg)\ x\ \ Volume\ of\ culture\ (L)\ x\ total\ number\ of\ culturing\ days} \text{x } 100 \quad (1)$$

The volumetric biomass productivity $P_{Biomass}$ of alga was calculated as per the equation (Eq). (2) [29]:

$$Biomass\ productivity\,(mg\ L^{-1}day^{-1}) = (X_2 - X_1).\,(t_2 - t_1)^{-1} \quad (2)$$

Where, X_1 and X_2 were the biomass dry weight concentrations (mg L^{-1}) on days t_1 (starting point of cultivation) and t_2 (endpoint of cultivation), respectively.

2.2. The extraction of total carbohydrate and its estimation

The carbohydrate extraction and estimation were done by using the methods of DuBois et al. (1956) [30]. Exactly 50 mg air-dried sample was taken and treated with five mL HCl (2.5 N), and the mixture was incubated at 100 °C in a boiling water bath for 3 h, then cooled to room temperature. It was then made up to 100 mL using distilled water in a standard flask and centrifuged at 2931 × g for 5 min at room temperature. The supernatant was collected, and the total carbohydrate content of the sample was estimated from the standard graph prepared with different concentrations of glucose.

2.3. The extraction of protein and its estimation

The crude protein content in the samples was estimated as per Micro-Kjeldahl method [31]. Exactly 50 mg of the air-dried samples were placed in the Kjeldahl flask and added five mL of 96% H_2SO_4 along with the mixtures of copper sulfate and potassium sulfate used as