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Bioremediation and lipid synthesis through mixotrophic algal consortia in municipal wastewater

Durga Madhab Mahapatra [a,b], H.N. Chanakya [b,c], T.V. Ramachandra [a,b,c,*]

[a] Energy and Wetlands Research Group, Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India
[b] Centre for Sustainable Technologies (astra), Indian Institute of Science, Bangalore 560012, India
[c] Centre for infrastructure, Sustainable Transportation and Urban Planning [CiSTUP], Indian Institute of Science, Bangalore 560012, India

HIGHLIGHTS

- Bioremediation by mixotrophic algal consortia grown in sewage fed bioreactor.
- Significantly removed TOC (86%), TN (90%), Amm.-N (89%), TP (70%) and OP (76%).
- Observed higher biomass productivity and lipid with FAME suitable for biofuel.
- Ca, Fe, P and Cl might have helped in cell clumping in the culture.
- Algae flocculated at the end of the culture.

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ABSTRACT

Algae grown in outdoor reactors (volume: 10 L and depth: 20 cm) were fed directly with filtered and sterilised municipal wastewater. The nutrient removal efficiencies were 86%, 90%, 89%, 70% and 76% for TOC, TN, NH_4-N, TP and OP, respectively, and lipid content varied from 18% to 28.5% of dry algal biomass. Biomass productivity of ~122 mg/l/d (surface productivity 24.4 g/m^2/d) and lipid productivity of ~32 mg/l/d were recorded. Gas chromatography and mass spectrometry (GC–MS) analyses of the fatty acid methyl esters (FAME) showed a higher content of desirable fatty acids (bearing biofuel properties) with major contributions from saturates such as palmitic acid [C16:0; ~40%] and stearic acid [C18:0; ~34%], followed by unsaturates such as oleic acid [C18:1(9); ~10%] and linoleic acid [C18:2(9,12); ~5%]. The decomposition of algal biomass and reactor residues with an exothermic heat content of 123.4 J/g provides the scope for further energy derivation.

1. Introduction

Treatment and disposal of wastewater generated in habitations are one of the key environmental challenges faced in urban localities due to a burgeoning population of the recent decade. Nutrient laden wastewater generated in municipalities has been either untreated or partially treated and directly fed into the nearby water bodies regularly, resulting in nutrient enrichment and algal blooms. Conventional wastewater treatment options are energy and capital intensive as well as inefficient in their capacity of removing nutrients completely. In this context, algal processes are beneficial in terms of removing nutrients through carbon sequestration and a resultant biomass production (Woertz et al., 2009). Algae grows rapidly and assimilate nutrients (C, N and P) available in wastewater (Mahapatra et al., 2013a,b) for which they are useful in nutrient remediation. This algal biomass provides aeration in the water body in addition to having a good valorisation potential with biofuel prospects (Mahapatra et al., 2013a,b).

Higher growth rates and the ability to accumulate lipids have made algae a viable substrate for biofuel when compared to other biofuel feed-stocks (Damiani et al., 2010; Chanakya et al., 2012; Ramachandra et al., 2013). Microalgae rich in lipids and hydrocarbons are being exploited now to mitigate the impending fuel oil

Abbreviations: TOC, Total Organic Carbon; TN, Total Nitrogen; NH_4-N, Ammonium Nitrogen; TP, Total Phosphate; OP, Ortho Phosphate.

* Corresponding author at: Energy & Wetlands Research Group, CES TE15, Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560019, India. Tel.: +91 080 22933099/22933503x107.
E-mail address: cestvr@ces.iisc.ernet.in (T.V. Ramachandra).
URL: http://ces.iisc.ernet.in/energy (T.V. Ramachandra).

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crisis (Ramachandra et al., 2009) as they are renewable, carbon neutral and viable substitutes for fossil fuels. Algae based biofuel generation coupled with wastewater treatment would also counter the diminution of environmental externalities and additional energy expenses (Chen et al., 2011).

The major aspects in establishing an integrated wastewater treatment and biofuel production system are the selection of appropriate species, requirement of modulated illuminating conditions, rapid nutrient removal ability, enhanced biomass productivity, and an efficient algal biomass harvesting capability. Native mixed algal species growing abundantly in wastewater are versatile to changing oxic-anoxic conditions, predominately following a mixotrophic mode of nutrition intake with high biomass productivity throughout the year. Mixotrophic species grow profusely in the carbon (organic C and CO_2) dominant environment. Mixotrophic algae unlike dense phototrophic cultures grew even in a light limiting environment (Wang and Lan, 2011), removing nutrients (C, N and P), thereby, possessing high cell densities and productivities (Woertz et al., 2009; Prathima Devi et al., 2012). These helped in the synthesis of higher lipids and removal of organics in wastewater (Bhatnagar et al., 2010). Earlier studies on nutrient removal and lipid production from wastewater were on uni-algal species like, *Chlorella* sp. (Wang et al., 2010), *Neochloris* sp. (Wang and Lan, 2011) etc. which grew under closed conditions in laboratories, wherein mixed cultivation systems of algal-bacterial consortium (Su et al., 2011) and algal-fungal (Zhou et al., 2012a) consortium existed as against mixed algal species/algal consortia (Perez-Garcia et al., 2010; Venkata Mohan et al., 2011). However, investigations on more efficient methods for algal biomass concentration and harvesting require further research.

Nutrient uptake by algae from wastewater has been assessed through cellular biochemical composition and elemental analysis. This helps in the understanding of nutrient transformations such as carbon allocation, lipid synthesis and accumulation. The spectroscopic techniques (like FTIR, etc. – Stehfest et al., 2005; Dean et al., 2010) are non-destructive, accurate, efficient and fast for compositional analysis. Algal cells, even after extracting lipid, would have fuel values, which is evident from thermal analysis of species, viz., *Chlorella* sp. (Sostaric et al., 2012) and derivatives from *Botyococcus braunii* (Salmon et al., 2009). This highlights that there is a scope for integrated energy analysis of mixed algal consortium of wastewater systems. The focus of the current work has been the assessment of bioremediation and biofuel potential of algal consortia, optimal harvest mechanism and derivation of thermal properties of algal biomass.

2. Methods

2.1. Wastewater sampling and analysis

Wastewater collected from the inflow channels (of Bellandur lake, Koramangla region, South of Bangalore, India) was allowed to settle for 2 days. The supernatant was sterilised, filtered and used as the culture media (as per Zhou et al., 2012b). Wastewater used for culturing were analysed through standard protocols (APHA, 2005) and the parameters were pH 6.3 ± 0.1, redox potential (ORP) -163 mV, total volatile solids (TVS) 432 ± 66 mg/l, total suspended solids (TSS) 540 ± 84 mg/l. Similarly, the analysis of wastewater broth through HACH protocol yielded Total Organic Carbon (TOC) of 219 ± 14 mg/l, Chemical Oxygen Demand (COD) of 676 ± 17.2 mg/l, Total Nitrogen (TN) of 64.6 ± 4.2 mg/l, Nitrate-Nitrogen (NO_3-N) of 1.07 ± 0.15 mg/l, Nitrite-Nitrogen (NO_2-N) of 0.32 ± 0.04 mg/l, Ammonium-Nitrogen (NH_4-N) of 51.5 ± 5.5 mg/l, Total Phosphorus (TP) of 28 ± 4.3 mg/l and Ortho-phosphates (OP) of 22.2 ± 2.4 mg/l.

2.2. Culturing algal consortia and growth conditions and reactor design

Algal samples were collected from wastewater fed urban lakes. From these samples, algal species were identified by morphological keys using the light and electron microscopes. The water sample collected from the lake was centrifuged at 1816*g* and the algal pellet containing the algal consortia was washed and centrifuged repeatedly with deionised water. Subsequently, the algal cells were inoculated in Bolds Basal (BB) media. The algal consortia grown in BB media comprised of bacillariophyceae (*Cyclotella meneghiniana*, *Nitzschia palea*), chlorophyceae (*Chlorococcum* sp., *Scenedesmus quadricauda*, *Scenedesmus obliques*, *Arthrodesmus curvatus*, *Golenkinia radiata*, *Kirchneriella lunaris*, *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Chlorococcum humicola*, *Chroococcus* sp., *Monoraphidium* sp., *Ankistrodesmus falcatus*), cyanophyceae (*Oocystis* sp., *Phormidium tenue*, *Spirulina maximus*) and euglenophyceae (*Trachelomonas* spp., *Euglena* spp., *Phacus longicauda*, *Phacus caudatus*). Inoculum comprising of these algal consortia were then added to the reactor ($\sim 10^6$ cells/ml; inoculum volume 40 ml; reactor working volume 10 L).

The algal reactor was made up of translucent polypropylene with a storage capacity >12 l (measuring 32 cm × 16 cm × 24 cm depth; working volume 10 L), covered with a glass sheet (the spacing between the glass sheet and the reactor was 20 cm). The reactor was kept on rooftop with a direct access to sunlight (light: dark period of 12 h: 12 h), and the culture was mixed uniformly thrice a day at regular intervals. The diurnal illuminance was recorded at the horizontal surface of the reactor using a lux meter. Illuminance ranges from 600 lux (during sunrise and sunset) to ~1,00,000 lux (during the mid-day). Algal cultures were maintained at ambient temperatures (22–30 °C) and the growth was monitored daily for two weeks. 100 ml of broth was collected daily and centrifuged. The reactor volume was maintained at 10 L with deionised water (to compensate evaporation losses and also 100 ml of broth). The filtered supernatant from centrifuged broth was used for nutrient analysis. The algal pellet was subjected to repeat washing following which it was dried and weighed (dry weight). Subsequently, the total lipid content, spectroscopic and elemental analyses were conducted. Algal biomass concentration (cell dry weight in the culturing medium in terms of g/l) was estimated by gravimetry.

2.3. Growth, productivity and lipid measurements

The probable model for biomass growth in 12 days is given by Eq. (1).

$$B_2 = B_1 e^{SGR(T_2 - T_1)} \quad (1)$$

where T_2–T_1 (day) represents the time difference between the two measurements,

B_2 and B_1 (g/L) represent the concentration of biomass at T_2 (12th day) and T_1 (1st day), respectively. SGR – specific growth rate helps in estimating likely biomass on the *n*th day.

The biomass productivity (B_{prod}) during culturing is given by Eq. (2)

$$B_{prod} = (B_2 - B_1)/T_2 - T_1 \quad (2)$$

where, B_{prod} is the biomass productivity (g/l/d), T_2–T_1 (day) represents the time difference between the two measurements, B_2 and B_1 (g/l) represent the concentration of biomass at T_2 (12th day) and T_1 (1st day), respectively.

Cell suspensions were pelletised through centrifugations at 7267*g* for 10 min, and stored at −20 °C for further use. Lipids from these pellets were extracted through Bligh Dyer's method which