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Nutritional mode influences lipid accumulation in microalgae with the function of carbon sequestration and nutrient supplementation

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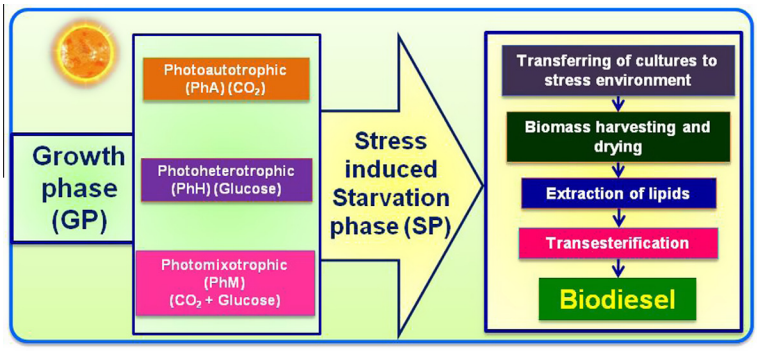
HIGHLIGHTS

- Microalgae nutritional mode effects biomass growth and lipid productivity.
- Mixotrophic nutritional mode showed higher biomass growth.
- Nutrient limitation enhanced lipid productivity from growth phase to starvation phase.
- Microalgae diversity and fatty acid composition varied with the nutritional modes.
- Good carbon/nutrient removal was observed during growth phase.

GRAPHICAL ABSTRACT



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ABSTRACT

Effect of nutritional mode viz., photoautotrophic, photoheterotrophic and photomixotrophic on the biomass growth and lipid productivity of microalgae was studied. Experiments were designed and operated in biphasic mode i.e., growth phase (GP) followed by stress induced starvation phase (SP). Nutritional mode documented marked influence on biomass growth and subsequent lipid productivity. Mixotrophic mode of operation showed higher biomass growth (4.45 mg/ml) during growth phase while higher lipid productivity was observed with nitrogen deprived autotrophic mode (28.2%) followed by heterotrophic (26.1%) and mixotrophic (19.6%) operations. Relative increments in lipid productivities were noticed in SP operation from GP in mixotrophic operation (2.45) followed by autotrophic (2.2) and heterotrophic (2.14) mode of operations. Higher concentrations of chlorophyll *b* and presence of lipid accumulating species supported the lipid biosynthesis. Algal fatty acid composition varied with function of nutritional modes and depicted eighteen types of saturated (SFA) and unsaturated fatty acids (USFA) with wide fuel and food characteristics.

1. Introduction

Microalgae synthesize their food material employing three modes of nutrition viz., autotrophic, heterotrophic and mixotrophic classified based on the utilisation of organic carbon source and luminosity (Farooq et al., 2013; Devi et al., 2012; Kong et al., 2012). Microalgae also have the flexibility to switch their nutritional mode in accordance to the surviving environment (Venkata Mohan et al., 2013). Autotrophs have the capability to convert physical (sunlight/photons) and chemical (CO_2 and H_2O) energy sources into carbohydrates (Lee et al., 2013). The synthesized carbohydrates form a base for the construction of all other carbon containing bio-molecules to compensate the cellular requirements. These autotrophs are relatively self-sufficient and self-sustainable due to their ability to obtain energy from sunlight (Yoo et al., 2010). On contrary, heterotrophic organisms utilize organic carbon

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substrates as primary energy sources for their growth and survival. These organisms also have the ability to utilize organic carbon produced by autotrophs as energy source for their metabolic functions (Qiao and Wang, 2009). Heterotrophic cultivation of microalgae offers several advantages over the other including elimination of light requirement, good control of the cultivation process, higher growth and low-cost for biomass harvesting (Brennan and Owende, 2010; Devi et al., 2012; Venkata Mohan et al., 2011). Also, the major advantage of heterotrophic mode is the facilitation of wastewater treatment along with lipid synthesis which gives an edge of its application in the present state of escalating pollution levels (Garcia et al., 2011).

Microalgae can also function under mixotrophic mode by combining both the autotrophic and heterotrophic mechanisms. Mixotrophic growth can be defined as the simultaneous assimilation of both CO_2 and organic carbon leading to the concurrent operation of respiratory and photosynthetic metabolisms. Mixotrophic cultures show reduced photo inhibition and improved growth rates over autotrophic and heterotrophic cultures (Garcia et al., 2011; Venkata Mohan et al., 2011). The advantage of the mixotrophic nutrition is its flexibility towards photosynthesis and carbohydrate utilization. Mixotrophic cultivation is beneficial due to the feasibility of using wastewater as substrate. This mechanism also offers efficient substrate degradation owing to its ease of cultivation in wastewater and ability to utilize the carbon content existing in it (Kong et al., 2012; Venkata Mohan et al., 2011). Application of either autotrophic or heterotrophic or mixotrophic nutritional modes using specific strains with defined media (Kong et al., 2012; Farooq et al., 2013) and mixed consortia with wastewater (Devi et al., 2012 ; Venkata Mohan and Prathima Devi, 2012) was reported. Enhancement of lipid productivity by applying different concentrations of nitrogen and urea using *Chlorella* sp. as biocatalyst was reported (Hsieh and Wu, 2009; Nigam et al., 2011). Nutritional modes play a major role in enhancing the biomass growth and lipid productivity of microalgae. The flexibility of microalgae to shift its nutritional mode makes its survival easy even when operated in wastewater.

In this context, the present study was designed to evaluate the functional role of various nutritional modes on the biomass growth and subsequent lipid productivity of microalgae by employing two phase operation viz. growth phase (GP) and stress induced starvation phase (SP). Autotrophic, heterotrophic and mixotrophic nutritional modes were evaluated separately based on varying carbon [organic, inorganic] and macronutrients [nitrogen (N), phosphorus (P), potassium (K)] supplementation during GP. Carbon and nitrogen are essential building blocks for the cellular activities while phosphorus facilitates the energetics of cellular reaction. Potassium maintains the cell osmoticum and involves in the metabolic functions (Zeng et al., 2011). Starvation phase (SP) was operated by depriving the nutrients and carbon to induce lipid accumulation. Biomass growth was monitored during GP while lipids were extracted and analyzed during the end of GP and SP of operations. The change in the fatty acid profile with the function of experimental conditions was also evaluated.

2. Methods

2.1. Biocatalyst

Mixed microalgae culture from a lentic water body (Nacharam Cheruvu, Hyderabad) collected in pre-monsoon season was used as biocatalyst in all the experiments. The culture selection was based on the previous study (Venkata Mohan et al., 2011). Prior to experimentation, the culture was washed and pelletized by centrifugation (3000 rpm; 10 min at 30 °C) to remove the associated debris.

2.2. Experimental methodology

Experiments were designed and operated in autotrophic, heterotrophic and mixotrophic modes to evaluate their effect on biomass growth and lipid productivity of microalgae (Supplementary Fig. 1). The nutritional modes were distinguished based on the supplementation of organic, inorganic carbon sources and macronutrients. Autotrophic condition (PhA) was maintained by supplementing CO_2 and macro nutrients, heterotrophic condition (PhH) was operated with organic carbon source (glucose) while mixotrophic condition (PhM) was evaluated by supplementing carbon (glucose, CO_2) and nutrients (NPK). Experimental conditions and concentrations studied are as depicted in Table 1. CO_2 supplementation was carried out at a flow rate of 1 ml/min for every 4 h as optimized in the previous study (Devi and Venkata Mohan, 2012). Experiments were operated in biphasic mode comprising of sequential growth phase (GP) and stress induced starvation phase (SP) each accounting for eight days of retention time. The experimental conditions were supplemented with carbon source and macro nutrients during GP while no nutrients/carbon source was provided in SP, to facilitate stress conditions. Batch experiments were performed using a series of conical flasks with a total/working volume of 0.25/0.18 l. Prior to start-up, each flask was inoculated with 20 ml of microalgae culture (2.05 mg/ml) along with 160 ml of domestic sewage (pH, 7.5; COD, 400 mg/l; TDS, 750 mg/l, nitrates, 115 mg/l and phosphates, 48 mg/l) to heterotrophic and mixotrophic conditions as feed while it was replaced with tap water in autotrophic condition. Prior to loading, the pH of the feed was adjusted to 8.2 using 3 N NaOH. After loading all the constituents, carbon sources and macronutrients were supplemented to the feed (tap water/domestic sewage) according to the experimental variations designed and closed with sterile cotton plugs. Operations were carried out at a light:dark period of 12:12 h. In the light phase, flasks were mounted on a temperature controlled shaking incubator (120 rpm) in the presence of a fluorescent light (4 klux) at 28 °C. In dark phase, the light source was turned off to facilitate dark conditions while the rest of the operating conditions remained same. After GP of 8 days, the resulting algal biomass was separated by centrifugation (5000 rpm; 5 min at 28 °C) and reinoculated into the flasks containing tap water (160 ml) to induce stress conditions for another 8 days (SP). After SP, the resulting biomass was separated by

Table 1
Experimental variations operated during the study.

S.no	Experimental variation	Carbon source	Nutrient source	Concentration (mg/l)	Mode of nutrition
1.	N	Inorganic	Sodium nitrate	500	Autotrophic (PhA)
2.	P	Inorganic	Inorganic phosphate	500	Autotrophic (PhA)
3.	NP	Inorganic	Sodium nitrate, inorganic phosphate	250 + 250	Autotrophic (PhA)
4.	NPK	Inorganic	Inorganic form	500	Autotrophic (PhA)
5.	C	Organic	Glucose	500	Heterotrophic (PhH)
6.	CN	Organic & inorganic	Glucose, sodium nitrate	250 + 250	Mixotrophic (PhM)
7.	CP	Organic & inorganic	Glucose, phosphate	250 + 250	Mixotrophic (PhM)
8.	CNP	Organic & inorganic	Glucose, Sodium nitrate, inorganic phosphate	166 + 166 + 166	Mixotrophic (PhM)