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Diverse acidogenic effluents as feedstock for microalgae cultivation: Dual phase metabolic transition on biomass growth and lipid synthesis

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HIGHLIGHTS

- Mixotrophic cultivation of mixed microalgae showed high biomass productivity with fermented distillery waste in growth phase.
- Mixotrophic and heterotrophic cultivation with fermented dairy waste showed significant influence on lipid productivity.
- Heterotrophic cultivation with fermented dairy waste during stress phase showed higher neutral lipids productivity.

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ABSTRACT

In this study, a biorefinery process integrating dark fermentation with microalgae cultivation (dual phase metabolic transition) was demonstrated with real-field wastewater. Acid rich fermented effluents (distillery waste (FDW1); dairy waste (FDW2)) were used as feedstock for microalgae cultivation. Experiments were performed with FDW1 during growth phase (GP) in mixotrophic mode and FDW2 during stress phase (SP) in both mixotrophic and heterotrophic modes. Mixotrophic cultivation with FDW1 documented significantly higher biomass productivity (5.3 g/l). Total lipid (TL) percentage was high in mixotrophic (34%) mode and neutral lipid (NL) was high in heterotrophic (13%) mode of cultivation during SP with FDW2. Overall, the microalgae growth is favoured with effluents containing high acetate, and low butyrate concentrations. Mixotrophic cultivation enhanced both biomass growth and lipid production along with simultaneous treatment.

1. Introduction

Sustainable platform for bio-based products (food, feed, and chemicals) and bioenergy (fuels, heat and electricity) may constitute a potential solution for addressing energy, climate and environmental issues. Integrating both acidogenic fermentation and photosynthetic processes treating waste and wastewater is a good example of the environmental biorefinery concept where energy and bio-molecules are produced concomitantly with waste remediation (Ghimire et al., 2015; Venkata Mohan et al., 2016; Sambusiti et al., 2015). Volatile fatty acids (VFA)/Carboxylic acids like acetate, butyrate and propionate can be used as substrates to cultivate microalgae (mixotrophic and heterotrophic) (Venkata Mohan and Prathima Devi, 2012) for the production of biodiesel and suggested as a very promising sustainable approach for producing gaseous and liquid biofuels (Park et al., 2014). Carbon supplementation in the form of VFA and involves in the biosynthesis of long-chain fatty acids through triacylglycerides (TAG) formation (Thauer et al., 1977; Chandra et al., 2014). Acidogenic fermentation effluents also contain substantial amounts of N and P that are required to sustain the microalgae cultivation (mixotrophic and heterotrophic). Mixotrophic cultivation utilizes both organic and inorganic carbon sources for cellular growth in the presence of light as the energy source providing high cell densities and higher lipid contents as compared to other cultivation conditions (autotrophic and heterotrophic) (Cheirsilp and Torpee, 2012; Chiranjeevi and Venkata Mohan, 2015). Heterotrophic mode of cultivation results in high lipid productivity and cell densities when cultivated in closed bioreactors under controlled conditions with organic carbon substrates such as glucose, acetate, glycerol, etc (Perez-garcia et al., 2010; Venkata Mohan et al., 2015; Chiranjeevi and Venkata Mohan, 2016) as energy sources, thereby avoiding the light requirements. Two stage cultivation strategies are being developed in which first stage targets at high density biomass cultivation by utilizing maximum carbon and nutrients present in the media/feed followed by enhancement of total/neutral

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lipid content by exposing the cells to higher carbon condition or by addition of lipid elicitors molecules (Prathima Devi et al., 2012).

In the present study, the feasibility of mixed microalgae to utilize acidogenic effluent generated from biohydrogen production was evaluated. Fermented distillery effluents contain major fraction of carbon (in the form of short chain fatty acids), along with nutrients (Nitrogen and Phosphorus) and can be considered as potential feedstock for mixotrophic microalgal cultivation during the growth phase for maximum biomass productivity. Comparatively less concentration of carbon and nutrients containing fermented dairy effluent was used as a substrate during the nutrient stress phase of microalgae cultivation in both the heterotrophic and mixotrophic mode of operations for high lipid productivity. Microalgal utilization of dark fermentation effluents from acidogenic biohydrogen reactor is a promising biorefinery approach for a sustainable biofuel production.

2. Experimental methodology

2.1. Feedstock/wastewater

Two types of fermented feedstocks viz., fermentative distillery wastewater (FDW1) and fermented dairy wastewater (FDW2) were collected from acidogenic H_2 producing bioreactor and used for evaluating the biomass and lipid production potential of microalgae during growth and starvation phase. Acid rich effluents were collected from a bench-scale reactor operated with distillery and dairy effluents at pH 6 with organic load viz., 15 and 3.3 COD g/l. The reactor was operated in suspended growth mode with a total/working volume of 34/29 l and a gas holding capacity of 5 l. Operations were carried out in sequencing/periodic discontinuous batch mode with a total cycle period of 48 h [hydraulic retention time (HRT)].

Comparatively distillery effluents documented higher VFA accumulation than dairy effluents [FDW1-pH, 7.4: ORP, −11: COD, 21.38 g/l: VFA, 1.77 g/l; FDW2-pH, 4.5: ORP, 146: COD, 1.5 g/l, VFA-2.36 g/l)] were collected after 48 h of reactor operation and used as a substrate for microalgal biomass growth and lipid accumulation. The required concentration of VFA (7.5 g/l) was adjusted by diluting with tap water to operate the growth phase (GP) in the mixotrophic mode of nutrition by adjusting the pH to 7. After the end of the GP, the reactor was fed with the FDW2 for stress phase of operation with VFA concentration of 2.0 g/l at pH 7.0. Prior to experimentation, VFA composition were analyzed by High Performance Liquid Chromatography (HPLC) (FDW1-acetic acid: 60%, butyric acid: 8%, propionic acid: 10%, formic acid: 22%; FDW2-acetic acid: 49%, butyric acid: 41%, propionic acid: 5%, formic acid: 5%).

2.2. Experimental details

Experiments were designed and performed to evaluate the role of FDW1 on microalgae biomass growth and lipid accumulation during growth phase (GP) and FDW2 during stress phase (SP) each with eight days of cultivation period. GP was operated with mixotrophic mode while stress phase (SP) refers to an operation in both heterotrophic and mixotrophic cultivation modes separately. Batch experiments for microalgal cultivation were conducted in sterilized 250 ml of borosilicate flask. Flasks were autoclaved (20 min; 121 °C; 1.05 kg/cm steam pressure) prior to the inoculation to avoid the bacterial contamination. Microalgal culture, majorly belonging to the class chlorophytes collected from Nacharam Lake (Pedda Cheruvu), Hyderabad, was used as parent inoculum as explained elsewhere (Venkata Mohan et al., 2011). Sterilized flasks after feeding wastewater were inoculated with microalgae inoculums (10% v/v; OD, 0.1). To avoid bacterial contamination antibiotic (Ampicillin: 0.2 g/l) was added to the each experimental setup once in every alternate day. All the experimental were operated at ambient temperature (32 ± 1 °C) with feeding pH of 7 and were kept in a temperature controlled shaking incubator (100 rpm). Light (4000 lux) was provided with external LED for both GP and SP of mixotrophic cultivation. During GP operation, biomass, cell density, pigment analysis (chlorophyll *a* and *b*) along with total cellular carbohydrate were estimated once in every alternate day. Lipid analysis was done at the end of GP and SP. All the experiments were carried out in triplicates and the results presented here represent an average of three independent and identical operations.

2.3. Analysis

At the end of GP (mixotrophic) and SP (both mixotrophic and heterotrophic), the biomass was separated (5000 rpm; 5 min; 28 °C), and the resulting biomass after solar drying was powdered by blending. The blended powder was further sonicated (40 kHz; 2 min), and lipids were extracted using chloroform and methanol (2:1) as solvents using modified Bligh and Dyer method (Venkata Mohan and Prathima Devi, 2012). Hexane was used for neutral lipid extraction. Thereafter the samples were centrifugated (8000 rpm/5 min) and separate lipid layer transferred to round bottom flask (pre-weighed). The total and neutral lipids were determined gravimetrically in percentage by dry weight of flask and lipid productivity (%) was calculated based on the ratio of total lipid extracted from dry cell weight (% DCW) of algal biomass. Algal biomass was quantified with dry cell weight apart from the regular monitoring by measuring OD at 600 nm and in correlation with the biomass quantification pigment analysis (chlorophyll *a:* 647 nm; chlorophyll *b:* 664 nm) were performed at regular interval. Internal carbohydrates were estimated by Anthrone method by disrupting algal cells (sonication, 40 kHz for 2 min) followed by centrifugation (5000 rpm for 5 min at 28 °C), and the supernatant solution was estimated to know the dissolved concentrations of carbohydrates. COD, nitrates, phosphates, and pH were analysed as per standard procedures (APHA, 1998).

3. Results and discussion

3.1. Biomass

3.1.1. Mixotrophic growth phase (GP)

Acidogenic effluent (VFA as major fraction; FDW1) from biohydrogen production process showed marked influence on the mixotrophic biomass production during GP. Microalgae showed rapid growth from initial (0.5 g/l) to the seventh day (5.3 g/l) of cultivation, after then, the growth started to decrease (8th day; 4.9 g/l) might be due to exhaustion of carbon (87.6%), nitrogen (35%) and phosphorus (80.3%) (Fig. 1). FDW1 constitutes major fraction of acetic acid (60%) as key metabolite which gets assimilated to produce acetyl-CoA. Acetate fed microalgae cultivation showed maximum biomass productivity compared with other synthetic volatile fatty acids used (Venkata Mohan and Prathima Devi, 2012). Due to its low molecular weight, structural simplicity and easy degradability enables its entry into multiple pathways contributing to biomass productivity during the mixotrophic cultivation (Buchanan et al., 2000). Acetate get uptaked by the microalgae directly into the cellular glyoxysome by monocarboxylic proton transporter (mct1) system and transforms into acetyl-CoA, by the acetyl-CoA synthetase (Garcia et al., 2005; Boyle and Morgan, 2009; Yang et al., 2000; Richmond, 1986). The dual carbon assimilation (inorganic atmospheric CO_2 and organic carbon uptake from