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Enhancement in lipid content of *Chlorella* sp. MJ 11/11 from the spent medium of thermophilic biohydrogen production process

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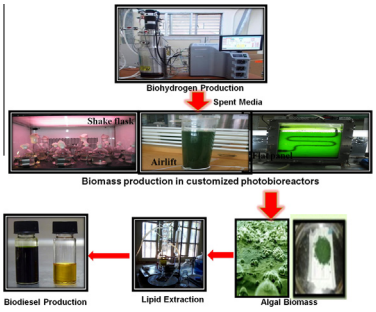
HIGHLIGHTS

- Selection of suitable physicochemical parameters for lipid accumulation.
- Effect of spent medium of dark fermentation process on lipid content of algal biomass.
- Simultaneous bioremediation and fatty acid accumulation by microalgae.
- Suitability of *Chlorella* sp. MJ 11/11 as a feedstock for biodiesel production.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study investigates the effect of spent media of acetogenic dark fermentation for mixotrophic algal cultivation for biodiesel production. Mixotrophic growth conditions were optimized in culture flask (250 mL) using *Chlorella* sp. MJ 11/11. Maximum lipid accumulation (58% w/w) was observed under light intensity, pH, nitrate and phosphate concentration of 100 μmol m^{-2} s^{-1}, 7, 2.7 mM and 1.8 mM, respectively. Air lift (1.4 L) and flat panel (1.4 L) reactors were considered for algal cultivation. Air lift showed significant improvement in biomass and lipid production as compared to flat panel reactor. The results could help in development of sustainable technology involving acetogenic hydrogen production integrated with sequential mitigation of spent media by algal cultivation for improved energy recovery.

1. Introduction

The global weather changes are gaining lots of attention in recent times. Over dependency on fossil fuels to quench the energy demands of human civilization has led to increase in the levels of greenhouse gases to alarming levels. Increase in greenhouse gases in the atmosphere has led to increase in global atmospheric temperatures under the phenomenon of global warming. The need of the hour is to find alternative fuels that are either carbon neutral or emits less greenhouse emissions. Studies on biomass based biofuels have gained importance in recent times to replace or augment the need of fossil fuels. Use of third generation biomass such as algae is gaining importance for production of biofuels as they are rich in lipids and carbohydrates (Nayak et al., 2013). Lipid rich microalgae are suitable for biodiesel production because these organisms have a very minimal nutritional requirement, higher photosynthetic efficiency and relatively faster growth rate as compared to plants. Biodiesel yield from microalgae depends on biomass concentration as well as oil content of the cells (Becker, 2004; Chisti, 2007). Media compositions have a profound effect on increasing the growth rates and productivities as well as content of other bioactive metabolites in the algal cell. Different physicochemical parameters such as nitrate concentration, phos-

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phate concentration, pH, temperature, light intensity and CO_2 concentration significantly affect the growth of microalgae in autotrophic conditions. Micronutrients required for the growth of microalgae highly influence the biomass productivities because they are the cofactors of various enzymatic reactions (Barsanti and Gualtieri, 2006). It was reported that high light irradiance stimulates accumulation of TAG (triacylglycerides) content in the algal cells. Nitrate and phosphate were also vital parameters for biomass production as these involve in the formation of structural proteins and nucleotides in algal cells (Ghosh et al., 2015). About 7–10% biomass of algae comprised of nitrogen which assimilates in the form of nitrate from media (Ghosh et al., 2015; Miyachi et al., 1964). Influence of nitrogen and phosphorous concentration on the growth and lipid content of *Nannochloropsis* sp. and *Chlorella vulgaris* was reported in literature by various authors (Reitan et al., 1994; Converti et al., 2009). Diverse organic substrates such as glucose (Wen and Chen, 2003) and salts of acetate (Garcia et al., 2011) were reported as substrates for microalgal growth and lipid accumulation apart from inorganic CO_2 (Liu et al., 2011; Prathima Devi and Venkata Mohan, 2012). Acetic acid, butyric acid and propionic acids can be easily utilised and assimilated by microalgae and can be channelized for biosynthesis of long chain fatty acids through triacylglycerides formation (Roy and Das, 2016). Recent studies investigating microalgae growth on a synthetic dark fermentation effluent medium showed very promising results. Biomass carbon yield by using *Chlorella protothecoides* as 34% and a lipid content of 48% of dry cell weight were observed when it grown heterotrophically on a mixture of acetate and butyrate (Fei et al., 2015). It was also reported that *Chlorella sorokiniana* could grow heterotrophically on acetate with a growth rate of 1.75 d^{-1} and a carbon yield of 55% w/w (Turon et al., 2015). Few studies involved on the supplementation of synthetic fatty acids by the effluents from biohydrogen reactor rich in volatile fatty acids for the lipid accumulation by heterotrophic microalgae (Prathima Devi and Venkata Mohan, 2012). The spent media generated during dark fermentation could pose a potential threat to environment. Use of volatile fatty acids (VFAs) rich spent media as substrate could be more advantageous in terms of bioremediation and the ease of energy entrapped from VFAs and converting them to liquid fuels more rapidly.

The present study dealt with the possibility of using volatile fatty acid rich spent media generated during dark fermentative hydrogen production for biodiesel production via mixotrophic growth of *Chlorella* sp. MJ11/11. Mixotrophic growth conditions were optimized to improve biomass and lipid production. Different photobioreactors (culture flask, air lift reactor and flat panel reactor) were explored for biomass production. To best of our knowledge, few reports are available where the spent media of thermophilic dark fermentation has been used for algal biodiesel production thereby improving total energy recovery. Growth characteristics in different modes of nutrition, lipid profile mapping and phosphate and nitrate removal efficiency were also evaluated during the process operation.

2. Materials and methods

2.1. Microalgae and culture medium

The culture of *Chlorella* sp. MJ 11/11 was obtained from NCCUBGA, Indian Agricultural Research Institute, New Delhi. The microalgae was grown in Tris Acetate Phosphate (TAP) medium whose composition is described below. TAP media contained 2.42 g L^{-1} Tris base, 25 ml L^{-1} TAP salt stock solution (15.0 g L^{-1} NH_4Cl, 4.0 g L^{-1} $MgSO_4{\cdot}7H_2O$, 2.0 g L^{-1} $CaCl_2{\cdot}2H_2O$, 0.375 ml L^{-1} PO_4 stock solution (28.8 g per 100 ml K_2HPO_4, 14.4 g per 100 ml KH_2PO_4), 1 ml L^{-1} Hutner trace metals (21.6 g per 100 ml H_2O EDTA: Titriplex II, 11 g per 50 ml H_2O $ZnSO_4{\cdot}7H_2O$, 5.7 g per 100 ml H_2O H_3BO_3, 2.53 g per 25 ml H_2O $MnCl_2{\cdot}4H_2O$, 0.805 g per 25 ml H_2O $CoCl_2{\cdot}6H_2O$, 0.785 g per 25 ml H_2O $CuSO_4{\cdot}5H_2O$, 0.55 g per 25 ml H_2O $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 2.495 g per 25 ml H_2O $FeSO_4{\cdot}7H_2O$), 1 ml L^{-1} Vitamins stock solution (0.5 mg L^{-1} Cyanocobalamin (B12), 100 mg L^{-1} Thiamine HCl, 0.5 mg L^{-1} Biotin), 1 ml L^{-1} glacial acetic acid.

2.2. Single parameter optimization for lipid accumulation

From literature it was found that lipid accumulation depended on the nitrate concentration, phosphate concentration, light intensity and initial pH of the media (Ghosh et al., 2015). Therefore, single parameter optimization was done by varying initial pH, light intensity, nitrate and phosphate concentrations. The experiments were performed in 250 mL conical flasks with a working volume of 100 mL and acetate (2000 mg L^{-1}) as carbon source. The experiments were performed in an illuminator shaker (INNOVA, New Brunswick, USA). The temperature and light intensity were adjusted to 25 °C and 100 μmol m^{-2} s^{-1} respectively.

2.3. Production of VFAs rich spent medium from thermophilic biohydrogen production for lipid accumulation

The spent medium was obtained from continuous biohydrogen production by thermophiles. A cylindrical glass column reactor with a working volume of 350 mL and over headspace of 150 mL was used for continuous hydrogen production. A quasi-steady state was observed at each dilution rate with respect to constant values of H_2 evolution rate, glucose and cell mass concentration in the effluent. The experiments were repeated at different flow rates to get maximum hydrogen production and sugar utilization. The reactor was purged initially with sterilized N_2 to create anaerobic conditions. The temperature of the reactor was maintained at 60 °C. HRT (hydraulic retention time) was decreased from 5 h to 1 h (Roy et al., 2014). The spent medium was utilised as a feedstock for lipid accumulation as it contained a high amount of volatile fatty acids. The pH was adjusted to 7.2 and the spent medium was then autoclaved and was used for algal cultivation.

2.4. Photobioreactors used for cultivation of algae

The microalgae were grown in customized photobioreactors to find out the most suitable configuration for cultivation. The A_d/A_r ratio of airlift reactors was 4.4 with constant surface by volume (S/V) ratio of 0.57 cm^{-1}. Diameter of the inner draft tube was 3 cm (Kumar and Das, 2012) with a working volume of 1.4 L. The flat panel reactor comprised of an iron frame sandwiched between two glass sheets (6 mm thick) with neoprene gaskets in between. Temperature was controlled using cooling coils (SS316, 6 mm I.D, 8 mm O.D., 60 cm length), which was running through the reactor having four openings, one port was for gas release, another for sampling and two ports were for process monitoring (Gilbert et al., 2011). The reactor had a working volume of 1.2 L. Reactors were developed at Indian Institute of Technology, Kharagpur, India and fabricated in Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Norway.

2.5. Analytical methods

2.5.1. Biomass analysis

Algal growth in mTAP [-acetate] medium was obtained by measuring the optical density (OD) of sample on a daily basis at 681 nm using Double Beam UV–Visible spectrophotometer (Spectroscan UV 2600, Chemito) (Ghosh et al., 2015). Dry cell weight was deter-