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Lipid metabolism in response to individual short chain fatty acids during mixotrophic mode of microalgal cultivation: Influence on biodiesel saturation and protein profile

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HIGHLIGHTS

- Acetate fed system showed high lipid productivity (35%).
- Protein profiling confirms specific enzyme activity in VFA metabolism.
- Palmitic acid (C16:0) was dominant fatty acid in all conditions.
- VFA supplementation during stress phase increased saturation index.
- Good treatment efficiency was noticed along with lipid productivity.

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ABSTRACT

Critical influence of different short chain fatty acids as organic carbon source, during growth (GP) and nutrient stress lipogenic phase (NSLP) was investigated on biomass and lipid productivity, in mixotrophic fed-batch microalgae cultivation. Nutrient deprivation induced physiological stress stimulated highest lipid productivity with acetate (total/neutral lipids, 35/17) with saturation index of 80.53% by the end of NSLP followed by butyrate (12/7%; 78%). Biomass growth followed the order of acetate (2.23 g/l) >butyrate (0.99 g/l) >propionate (0.77 g/l). VFA removal (as COD) was maximum with acetate (87%) followed by butyrate (55.09%) and propionate (10.60%). Palmitic acid was the most dominant fatty acid found in the fatty acid composition of all variants and butyrate fed system yielded a maximum of 44% palmitic acid. Protein profiling illustrated prominence of acetyl CoA-synthetase activity in acetate system. Thus, fatty acids provide a promising alternative feedstock for biodiesel production with integrated microalgae-biorefinery.

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1. Introduction

Photosynthetic production of green fuels using microorganisms is one of the sustainable and renewable alternatives to traditional fossil fuels. Algal biomass is deemed to be one of the most promising feedstock for production of biofuels, food, feed and value added co-products. Microalgae are competent in conversion of both inorganic (CO_2) and organic carbon to value added products including lipids or triacylglycerides (TGA) (Perez-Garcia et al., 2011). With autotrophic cultivation, algae capture sunlight and fix CO_2 into useful biomolecules during adverse or environmental stress conditions (Venkata Mohan and Prathima Devi, 2014; Chokshi et al., 2015). Although microalgae can accumulate lipids under autotrophic mode, their current photosynthetic efficiencies are not viable for commercial scale production (Kruse et al., 2005). Low biomass yields, requirement of large surface area and shallow depth for better access of light, are some of the inherent limitations that persist with autotrophic algal cultivation (Venkata Mohan et al., 2015). In this context, mixotrophic mode of cultivation which can utilize both light and/or organic carbon is receiving considerable attention due to its promise of high biomass and lipid productivities (Yang et al., 2000; Venkata Mohan et al., 2014a; Bumbak et al., 2011). Acetyl-CoA pool is maintained from both the carbon sources i.e. CO_2 fixation (Calvin cycle) and extra cellular organic carbon. Mixotrophic cultures have several advantages viz. reduced photo-inhibition, improved growth rates, independence from

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photosynthesis for its carbon requirements, etc (Kong et al., 2012). The carbon from wastewater can also be considered as a viable feedstock for microalgae cultivation (Hongyang et al., 2011; Olguin, 2012; Devi et al., 2012; Chandra et al., 2014). However, supplementation of complex substrates as carbon sources mandates initial breakdown before they can be utilized for growth and subsequent lipid accumulation (Chang, 2009).

Fermentative products such as short chain fatty acids (SCFA) or volatile fatty acids (VFA) from biohydrogen production (Mohanakrishna et al., 2010; Dahiya et al., 2015) can be easily assimilated by microalgae directly for biosynthesis of long chain fatty acids through TGA formation (Venkata Mohan and Devi, 2012; Bellou and Aggelis, 2012). The bioavailability of acetate and other fatty acids is comparatively superior compared to glucose as their assimilation bypasses complex metabolic pathways (glycolysis) and does not require oxidization to simpler forms of sugar. Acetate gets metabolized by microalgae cells via acetyl-CoA in the glyoxylate cycle, and eventually gets converted to energy via Krebs cycle of mitochondria. The acetyl CoA synthetases and carboxylases (ACC) are the key enzymes responsible for conversion of acetyl CoA to lipids or fatty acids in acetate based algal metabolism (de Swaaf et al., 2003).

The main aim of this communication is to evaluate the specific function of selected VFAs (acetate, butyrate and propionate) as carbon source on both growth (GP) and starvation (NSLP) phases during mixotrophic mode of microalgae cultivation. In GP, lower concentration of SCFA (0.5 g/l) was given for optimal growth of biomass while during NSLP, the concentration was increased to 2 g/l for providing stress as well as to supplement carbon on lipid induction. Selected key enzymes involved during SCFA metabolism were also studied by protein profiling.

2. Methods

2.1. Biocatalyst

Microalgae culture collected from the outlet stream of Nacharam Cheruvu (Hyderabad) was used as parent biocatalyst (Venkata Mohan et al., 2011). The washed microalgae was restored again in rectangular plastic containers (36 cm × 24 cm × 12 cm) exposing to natural stream conditions by feeding domestic sewage (DS; COD – 420 mg/l, BOD_5 – 320 mg/l, total alkalinity –140 mg/l, chlorides – 175 mg/l, nitrates – 50 mg/l, phosphates – 50 mg/l, pH – 7.80). The cultivated algal biomass was mixed periodically by mechanical stirring (once in 3 h interval for 5 min) to avoid settling and ensure uniform exposure of all cells to sunlight. Culture with optical density (OD, 660 nm) of 2 was used to inoculate the experimental setups.

2.2. Experimental design

Three sets of experiments were operated individually with acetate, butyrate and propionate in mixotrophic fed batch mode to evaluate their effect on biomass growth and lipid productivity. Experiments were designed and operated for 14 days in dual stage mode of cultivation with 7 days of growth phase (GP) followed by 7 days of nutrient stress lipogenic phase (NSLP) in 500 ml flasks. In GP, microalgae were cultivated in 350 ml of designed synthetic wastewater (DSW; NH_4Cl – 0.5, KH_2PO_4 – 0.25, K_2HPO_4 – 0.25, $MgCl_2{\cdot}6H_2O$ – 0.3, $FeCl_3$ – 0.025, $NiCl_4$ – 0.016, $CoCl_2$ – 0.025, $ZnCl_2$ – 0.0115, $CuCl_2$ – 0.0105, $CaCl_2$ – 0.005, $MnCl_2$ – 0.015 (all units in g/l)) at pH 8.2. The VFA concentration of 0.5 g/l acetic acid (C2), butyric acid (C4) and propionic acid (C3) as carbon source were added to each flask in GP condition and during NSLP operation the concentration was increased to 2 g/l. NSLP experiments were operated with nutrient exhausted wastewater (after GP) by adding required concentrations of respective VFA for stress induction. The experimental setup without carbon or VFA (photoautotrophic mode) was operated as control and reported elsewhere (Chandra et al., 2014). All flasks were placed in orbital shaker (28 °C, 50 rpm). The light intensity of 3500 Lux was maintained using fluorescent white lamp with light:dark period of 16: 8 h. After 7 days of GP, the cultures were harvested by centrifugation and re-inoculated for the second phase (NSLP).

2.3. Lipid extraction

Solvent extraction procedure was used for extracting lipids from dried algal biomass (hot air drying at 60 °C; 24 h) after centrifugation at 3000 rpm for 5 min. After drying, the cells were grinded into fine powder using mortar and pestle and suspended in chloroform and methanol (2:1) followed by sonication using probe sonicator (Qsonica) for 5 min at ultrasonic frequencies of 20 kHz. Lipid content was confirmed by performing thin layer chromatography (TLC; aluminum-silica plates) in solvent mixture (acetone:benzene:water = 91:38:8 ml). The total lipids were extracted using chloroform–methanol (2:1) and neutral lipids by *n*-hexane and quantified gravimetrically as lipid percentage on a dry cell weight (DCW) basis.

2.3.1. Transesterification

The transesterification process was performed by refluxing (65–70 °C for 2 h) the extracted lipids in methanol in the presence of conc. H_2SO_4 (2%) as acid catalyst. After washing, diethyl ether was added to the reaction mixture to form an organic phase which was separated from aqueous phase using a separating funnel. A pinch of anhydrous sodium sulphate was added to the organic layer to remove traces of water and finally the organic phase was subjected to evaporation for solvent recovery leaving fatty acid methyl esters (FAME). The concentrated FAME samples were analyzed by GC coupled with FID (Shimadzu 17A) through capillary column using nitrogen as carrier gas (1 ml/min). The temperature of the oven was initially maintained at 140 °C (for 5 min), later increased to 240 °C at a ramping of 4 °C/min for 10 min. The injector and detector temperatures were maintained at 280 and 300 °C, respectively with a split ratio of 1:10. FAME composition was compared with the standard FAME mix (C8-C22: LB66766; SUPELCO).

2.4. Protein extraction and profiling

Protein extraction was performed according to the protocol given by Islam et al. (2004) with modification appropriate for algal cells. Algal biomass (1 g) grinded to fine powder in liquid nitrogen was transferred into 2 ml centrifuge tubes. To this 1 ml of chilled 10% TCA (prepared in acetone at 4 °C) was added and incubated for 15 min at 4 °C. The resulting sample was centrifuged and the pellet was washed with 1 ml of chilled methanol (80% with 0.1 M ammonium acetate) followed by 1 ml of chilled 80% acetone. The pellet obtained after washing was air dried at room temperature to remove residual acetone. To the dried protein pellet, phenol–SDS buffer (pH 8.0; 0.1 M Tris–HCl, 30% sucrose, 1% SDS and 5% β-mercaptoethanol and equal volume of phenol) was added by mixing and kept for 1 h at room temperature (25 °C). Subsequently, the sample was separated (10,000 rpm; 15 min; 4 °C) and the resulting upper phenol phase was transferred to a fresh centrifuge tube and incubated overnight (−20 °C) after adding 2 ml of chilled methanol with 0.1 M ammonium acetate. The resulting sample was centrifuged (10,000 rpm; 15 min; 4 °C) and the pellet was washed again with 100% methanol followed by 80% acetone. This protein pellet was used for the sodium dodecyl polyacrylamide gel electrophoresis (SDS–PAGE; 12% resolving gel). The image of stained SDS gels was captured through molecular imaging

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