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Response of growth and fatty acid compositions of *Chlorella pyrenoidosa* under mixotrophic cultivation with acetate and glycerol for bioenergy application

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ABSTRACT

Chlorella pyrenoidosa was grown in mixotrophic condition in presence of sodium acetate and glycerol. Nearly, six fold enhancement in biomass productivity and a remarkable 32 fold increment in lipid productivity were recorded in cultures grown with sodium acetate (10 g m^{-3}) in comparison to autotrophic culture. With glycerol (0.5% by volume fraction), the biomass productivity and lipid productivity were three times and twenty times higher as compared to control. Glycerol proved to be more beneficial in lipid accumulation produces lipid content of 17.3%, which is about seven times higher to that of the control. While, lipid content with sodium acetate was 13.5%, more than five times of control. Oil samples collected from *C. pyrenoidosa* were converted into fatty acid methyl ester (FAME) through acid based transesterification and characterised by GC–MS. The fatty acid profiles of mixotrophic cultures showed its suitability for biofuel production than autotrophic cultures. The content of palmitic acid (C-16) and oleic acid (C-18) (indicator of biodiesel quality) is much higher in mixotrophic cultures than autotrophic cultures. *C. pyrenoidosa*, has shown significant improvement in growth and quantity as well as quality of lipid with acetate and glycerol. This provides a better way to produce biofuel at reduced overall cost since these substrates can be obtained as waste byproducts of some processes like biohydrogen production and biodiesel production.

1. Introduction

Microalgae are an attractive option for biodiesel production and have gained immense attention during the past decade [1]. Majority of the strains are photoautotrophic in nature and grow only in presence of light. However, there are certain strains which are mixotrophic in nature and can utilise photosynthesis and catabolism of organic substrate simultaneously to fuel cell growth [2]. Both the modes of growth (autotrophic and heterotrophic) can co-exist in cells under mixotrophic condition [3]. Lower biomass production in autotrophic cultures has directed the researchers towards mixotrophy with different carbon sources which results in better biomass production [4].

The preferred choice, glucose, is highly beneficial in enhancing the biomass as well as lipid productivities of microalgae [5,6]. However, the drawback with using glucose lies in the fact that cost of glucose adds up to 80% of the total medium cost and makes the cultures prone to contamination [7]. Other cheap substrates like sorghum juice [8] rice straw

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hydrolysate [9] have been successfully attempted to reduce the cost of algae-based biodiesel production. But, the conflict of food and fuel still exists with such agricultural wastes like in case of first- generation biofuels.

Glycerol, a by-product from current biodiesel production, has been found to be a potential carbon source for raising the growth and lipid content in algae [10,11]. Another industrial by-product, acetate that comes from fermentative hydrogen production has also been demonstrated to be an effective carbon source to boost biomass and lipid productivities of mixotrophic microalgal cultures [5].

Chlorella pyrenoidosa is a green microalga and can grow photoautotrophically under nitrogen containing medium. Previous study has revealed that the species is capable to grow in mixotrophic condition using glucose and rice straw hydrolysate [9]. High lipid productivity under mixotrophy shows its potential for bioenergy production. To our knowledge, there is no data available for mixotrophic growth and lipid production of *C. pyrenoidosa* using acetate and glycerol. Our study proposes the use of organic substrates for growth and lipid enhancement in algae. *C. pyrenoidosa* cells were grown in mixotrophy with sodium acetate (NaAc) and glycerol. The oil obtained under different conditions was converted into fatty acid methyl esters (FAME) which is requisite biodiesel by simple chemical process known as "transesterification". The triacylglycerols (TAGs) present in the oil react with alcohol in the presence of a catalyst to produce methyl esters and glycerol is released as byproduct [12]. The effect of uptake of carbon sources in presence of light on biomass, lipid productivity and fatty acid profiles of this alga was studied and compared. This is the first study on growth and lipid quality of *C. pyrenoidosa* under acetate and glycerol supported medium.

2. Materials and methods

2.1. Species and medium

C. pyrenoidosa NCIM 2738, obtained from National Collection of Industrial Microorganism, National Chemical laboratory, Pune, India and was grown photoautotrophically in Fogg's medium [13]. Cultivation was conducted in 5 L Erlenmeyer flasks with 3 L autoclaved media. The cultures were performed in a temperature controlled incubator at 25 °C providing 24 h continuous illumination (40 W, white tube light) of 40.5 μmol m^{-2} s^{-1} and were shaken intermittently. No extra carbon di-oxide was provided to the cultures except naturally existing in the atmosphere. Inoculation was done using exponentially growing cells such that the initial optical density of the cultures is 0.1. This was referred to as autotrophic control culture.

2.2. Cultivation under mixotrophic conditions

Sodium acetate trihydrate (MERCK, India) and glycerol (MERCK, India) were added as carbon source to the media to study the growth, lipid accumulation and fatty acid composition of *C. pyrenoidosa* under mixotrophic condition. 5 g m^{-3} and 10 g m^{-3} of NaAc and 0.5% and 1.0% volume fraction of glycerol were taken in the medium by keeping other media components as in Fogg's media and other culture conditions same as in autotrophic growth.

2.3. Growth analysis

Growth of *C. pyrenoidosa* was measured at an optical density of 660 nm spectrophotometrically (UV/Vis Shimadzu) after every 24 h for both autotrophic and mixotrophic cultures. After 7 days of inoculation, mixotrophic cultures with NaAc and glycerol were harvested by centrifugation (Eppendorf 5810R) at 5000 rpm for 5 min. The pellet was then washed twice with distilled water to remove any debris and salts and dried in oven at 105 °C to obtain dry cell mass. Biomass concentration and biomass productivity were calculated according to the formula:

$$\text{Biomass Concentration } (\text{g m}^{-3}) = \text{mass of the culture/volume}$$

$$\text{Biomass productivity } (\text{g m}^{-3}\ \text{d}^{-1}) = \text{mass of the culture/volume} \times \text{days}$$

2.4. Lipid analysis

Lipids were extracted from biomass using Bligh and Dyer method [14]. 50 cm^{-3} Methanol and 25 cm^{-3} chloroform were added to the dried and crushed algal powder and kept on shaker at 2.5 Hz for 16 h. The mixture was then centrifuged at 2420× *g* for 5 min to recover solvent phase. The pellet was again subjected to methanol and chloroform for second extraction. The supernatants were then pooled and 50 cm^{-3} chloroform was added and then shaken properly. 50 cm^{-3} Distilled water was then added for phase separation and lower organic phase was collected and dried in rotary evaporator. The dried lipid extract was weighed and lipid content and lipid productivity were calculated as:-

$$\text{Lipid content}(\%) = \text{mass of lipid (g)} \times 100/\text{mass of algae culture (g)}$$

$$\text{Lipid production}(\text{g m}^{-3}) = \text{mass of lipid (g)/volume } (\text{m}^{-3})$$

$$\text{Lipid productivity } (\text{g m}^{-3}\ \text{d}^{-1}) = \text{cumulative biomass production (g)} \times \text{lipid content (\%)/working volume } (\text{m}^{-3}) \times \text{cultivation time (d)}$$