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Influence of citrate on *Chlorella vulgaris* for biodiesel production

Thangapandi Marudhupandi [a,*], Velusamy Gunasundari [a], Thipramalai Thankappan Ajith Kumar [a,c], Kapila R.A. Tissera [b]

[a] Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences Annamalai University, Parangipettai 608502, Tamil Nadu, India
[b] E-tropical Fish International (Pvt.) Ltd., Sri Lanka
[c] National Bureau of Fish Genetic Resources, Indian Council of Agriculture Research, Lucknow, Uttar Pradesh 226002, India

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ABSTRACT

The present study was carried out on microalgae *Chlorella vulgaris* to investigate the effect of citrate metabolite as a nutrient for biodiesel production. The oil obtained from *C. vulgaris* is a promising fuel substitute and can be used as biodiesel. *C. vulgaris* was cultured in conway medium at different concentrations of citrate, namely 0.5, 1.0 and 2 g/L were added to the culture medium in different experimental groups. Subsequently, the growth, proximate composition and biomass content of the cultured cells were analyzed. The maximum biomass (1.68 ± 0.044 g/L) in the cultured cells was obtained at a citrate concentration of 2.0 g/L in the culture medium. However, the highest oil yield (28.62%) was obtained at the 1owest citrate concentration. Physico-chemical parameters such as pH, viscosity, density, acid and iodine values of the extracted oil were analyzed. In addition, a freeze-dried sample of the algae was analyzed to ascertain its biochemical composition. The maximum lipid content ($37.03 \pm \pm 0.88$%) was obtained at the minimum experimental concentration of citrate (0.5 g/L) in the culture media. An increase in citrate concentration in the media resulted in a decrease in total lipid content of the cells, but increased the carbohydrate content up to 29.43 ± 0.72%. Fatty acid profiles of the control and experimental groups were analyzed. The present study was suggested that the presence of lowest concentration of citrate in the culture media can improve the accumulation of lipids in *C. vulgaris*.

1. Introduction

The diminishing reserves of crude oil, liquid fuels derived from the plant material -biofuels are an attractive source of energy (Scott et al., 2010). Biodiesel, which consisting of fatty acid methyl ester, is renewable, biodegradable, non-toxic as it produces less sulfur dioxide and unburned gases than fossil fuel (Fu et al., 2013). According to a World Bank Report (2008), about 6.5 billion liters of biodiesel were produced globally in 2006. The European Union had contributed 75% and the United State of America (USA), 13%. However, the present contribution of biodiesel to the global transportation fuel consumption was only 0.14% and its favorable policies of the major countries in the world are expected to increase this contribution by 5 times in 2020 (Courchesne et al., 2009).

In comparison with conventional terrestrial plants, oil-rich microalgae have shown to be a promising alternative source for lipids for used as biodiesel. They are also widespread and have the capacity to yield comparatively higher oil contents (Rodolfi et al., 2010). In comparison with terrestrial biomass, the advantage of microalgae is much higher due to its photosynthetic competence, higher growth rates and improved CO_2 alleviation (Brennan and Owende, 2009).

Fatty acids are the building blocks for TAG. All other cellular lipids synthesized in the chloroplast use a separate set of enzymes, of which acetyl CoA carboxylase (ACCase) is the key in regulating the synthesis of fatty acids (Hu et al., 2008). The lower lipid content and biomass of microalgae is considered as major obstacles in biodiesel production from microalgae, thus increasing the production cost (Sheehan et al., 1998). Many studies have been conducted with a view of increasing the lipid content in algal cells such as nitrogen deprivation and phosphate limitation (Rodolfi et al., 2010), silicon deficiency (Griffiths and Harrison, 2009), iron supplementation (Liu et al., 2008) and application of varying carbon dioxide (CO_2) concentrations (Tsuzuki, 1990) have been tested to achieve a higher yield of fatty acids in algal cells. In addition, Feng et al. (2005) reported that the glucose was added initially to the culture medium to achieve an enhanced fatty acid production in *Chlorella vulgaris*.

Citrate plays a vital role in the intermediary metabolism activity by catalyzing the efflux of citrate from the mitochondrial matrix

* Corresponding author: Mobile: +91 8870366413.
E-mail address: tmarudhu@gmail.com (T. Marudhupandi).

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to the cytosol in exchange for tricarboxylates, dicarboxylates or phosphoenolpyruvate (Kramer and Palmieri, 1992). In the cytosol, citrate yields acetyl-CoA, which represses adenosin triphosphate dependent (ATP) citrate lyase activity. This in turn modulates the glycolytic flux by inhibiting phosphofructokinase. This is a positive allosteric affecter of acetyl-CoA carboxylase, and it activates fatty acid synthesis. With this background, the present study was conducted to evaluate the effect of citrate on biomass and lipid content in *C. vulgaris*.

2. Materials and methods

2.1. Microalgae and culture conditions

An inoculant of marine microalgae *C. vulgaris* was obtained from the marine ornamental fish hatchery of the Centre for Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, India. The algae was cultured in Conway medium (Walne, 1974) with 1 ml citrate solutions at different concentrations, (0.5, 1 and 2 g/L) in different groups. The control group did not contain citrate. All cultures were maintained at 28 ± 1 °C in 250 ml flasks containing 100 ml culture under illuminated at 2500 l × with 12 h light, 12 h dark and shaken at 120 rpm on an orbital shaker. Cultures were harvested on day 4 (96 h).

2.2. Cell growth and dry cell weight

The growth status of the culture was measured by using a UV–visible spectrophotometer (Thermoscientific, Evolution 201, USA) at an absorbance of 660 nm. The dry cell weight of microalgal biomass was analyzed by the method proposed by Chiu et al. (2009) with minor modification. Microalgal cells were harvested and centrifuged at 2000 rpm for 10 min. Centrifuged samples were washed twice with distilled water and freeze-dried. The specific growth rate was calculated by using the following formula proposed by Tang et al. (2011).

Specific growth rate μ (d^{-1}) was calculated from the following equation:

$$\text{Specific growth rate } (\mu) = (\ln X_1 - \ln X_0)/t_1 - t_0$$

where, X_1 and X_0 were the biomass concentration (g L^{-1}) on day's t_1 and t_0, respectively.

2.3. Determination of proximate composition

Triplicate samples of freeze-dried cells (10 mg) from each of the experimental cultures were analyzed for total lipid, protein and carbohydrate contents. Total lipid contents were estimated by the method of Folch et al. (1957), protein was estimated by the method of Lowry et al. (1951) and carbohydrates were analyzed by Anthrone method (Seifter et al., 1950).

2.4. Oil extraction

Oil extraction from microalgal biomass (2 g) was carried out by the method of Bligh and Dyer (1959). The percentage of oil yield was calculated as

$$\text{Yield of oil } (\%) = \text{Weight of the oil extracted } (g) / \text{Weight of the dried biomass } (g)$$

2.5. Physico-chemical parameters of algal oil

The physico-chemical parameters of algae oil such as pH, viscosity, density, acid and iodine values were analyzed by standard methods of analysis (AOCS, 1998).

2.6. Analysis of fatty acid profile by GC–MS

The fatty acid components were analyzed with the help of GC–MS. Fatty acid methyl esters (FAME) were obtained by esterification of the lipids (Ichihara and Fukubayashi, 2010). One micro litter of the sample was injected into capillary columns of 25 mm × 2 mm × 0.33 μ film thickness. The equipment used was the Gas Chromatograph model 6890 N of Agilent Technologies, USA. Injection temperature was 220 °C. The initial column temperature was maintained at 60 °C for 4 min and the temperature was subsequently increased to 250 °C with a gradient of 20 °C min^{-1}. Hydrogen was used as the carrier gas at a flow rate of 30 ml/min. Fatty acid profiles of the samples were identified by comparing the commercial Eucary data base with MIS software package (MIS Ver. No. 3.8, Microbial ID. Inc., Newark, Delaware).

3. Results and discussion

The proximate composition of the control and experimental groups of *C. vulgaris* given in Table 1. The protein content of the control and experimental groups were ranged from 44.14 ± 0.92 to 46.11 ± 1.24%. No considerable deviations were present in the protein content among the different groups. However, the carbohydrate and lipid contents showed perceptible deviations depending upon the concentration of citrate in the culture media. In comparison with the control, the maximum cell-carbohydrate content (29.43 ± 0.72%) was observed with the highest tested concentration of citrate (2 g/L) in the media. In contrast, the maximum lipid content of cells (37.03 ± 0.88%) was seen to be associated with the minimum tested concentration of citrate (0.5 g/L). The similar tendancy that the reduced lipid content ranged from 22.5% to 15.9% was observed in *C. vulgaris* while increasing the concentration of KNO_3 ranged from 0.2 to 5.0 mM (Ming et al., 2010). The present study suggested that the variation in lipid and carbohydrate contents in the various tested groups could be the influence of different levels of citrate in the culture media and the high carbohydrate content seen at 2 g/L might be due to high ATP and nicotinamide adinine dinucleotide (NADH) in *C. vulgaris* that could have repressed glycolysis.

The effect of citrate concentrations on the growth of *C. vulgaris* was estimated by measuring turbidity of the culture solution at 660 nm with a spectrophotometer (Fig. 1). Control showed the minimum growth and the experimental groups growth rate was increased with increase in concentration. The maximum biomass concentrations and high specific growth rates of control and experimental groups were shown in Table 2. Maximum biomass concentration (1.68 ± 0.004 g/L) and highest specific growth rate (1.107 ± 0.016 d^{-1}) was obtained in the group containing 2 g/L of citrate in the culture media. It was higher than the maximum reported biomass concentration in *C. vulgaris* which, is 1.2 g/L (Ming et al., 2010). In the present study, the biomass concentration and specific growth rate had increased at the higher concentration (2 g/L) of citrate but the lipid content in that group was lower. At the lowest concentration (0.5 g/L) of citrate that enhanced

Table 1
Biochemical composition (% dry weight biomass) of *C. vulgaris* at different concentration of citrate and control. Value indicates mean ± SD.

Samples (g/L)	Carbohydrates (%)	Protein (%)	Lipid (%)
Control	9.76 ± 0.3	44.14 ± 0.92	23.13 ± 0.24
0.5	12.06 ± 0.66	43.78 ± 0.82	37.03 ± 0.88
1.0	21.71 ± 0.52	45.25 ± 1.06	24.3 ± 0.70
2.0	29.43 ± 0.72	46.11 ± 1.24	16.04 ± 0.38

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