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Cultivation of microalga, *Chlorella vulgaris* under different auto–hetero–mixo trophic growths as a raw material during biodiesel production and cost evaluation

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ABSTRACT

The biomass and lipid productivities of green microalga, *Chlorella vulgaris* TISTR 8580 cultivated in the Bold's basal medium under three different culture conditions namely AC (autotrophic), HC (heterotrophic) and MC (mixotrophic) cultivations in a 20 L rectangular bioreactor were investigated. Maximum cell growths were obtained on day 10, 3 and 5 under AC, HC and MC, respectively. The *C. vulgaris* cells in heterotrophic condition contained the highest amount of lipids (32.9%) and the lowest amount of proteins (14.5%). The composition of *C. vulgaris* cells under MC condition was found to be between those found at AC and HC conditions. The most efficient energy production system in terms of lipid production to energy consumption was MC. The biodiesel yield was about 93.3% and fatty acid in methyl esters were found as 35.33% palmitic acid, 19.01% oleic acid, 19.21% linoleic acid, 11.68% linolenic acid and 14.77% others.

1. Introduction

The ever increasing demand and production of biodiesel, an environmentally sustainable biofuel, has triggered concerns over the availability of feedstocks such as soybean, coconut, jatropha rapeseed (canola), palm, sunflower, animal fats and even waste cooking oils to meet anticipated future growth in demand. The combined supply of these fats and oils is sufficient to replace only a few percentage of the petrodiesel market [1]. In recent years the food versus fuel issue has gained significant interests, i.e., that a potential source of food (edible vegetable oil) should not be used for fuel purposes due to effects on food prices and land-use change. Considerations such as these have stimulated interest in the development of other lipid sources as feedstocks for biodiesel production, especially jatropha and algae [2–7].

The production of biodiesel from microalgae as a non-edible biodiesel feedstock has generated significant interest. This is because microalgae i) have higher oil yields (up to 10–20 times) than land plants; ii) are able to fix waste CO_2; iii) can grow in variable climates and non-arable land using brackish, salt or wastewater instead of freshwater; and iv) would not compromise production of food, fodder and other products derived from crops [2,8].

According to, *Chlorella vulgaris*, a readily available and abundant green microalga, is one of many microalgae species capable of producing oils and lipids necessary for biodiesel processing. It is one of the fastest growing microalgae and is easy to cultivate [9]. This strain can grow under AC (autotrophic), HC (heterotrophic), as well as under mixotrophic conditions (HC) giving them the capability to metabolically shift in response to changes in environment. Lipids content in *C. vulgaris* under various growth conditions is up to ~57% by weight of dry biomass [10]. As with most microalgae, heterotrophic growth of *C. vulgaris* compared to the autotrophic growth resulted in higher biomass production and induced higher accumulation of lipid [11,12].

The technical problems facing biodiesel include poor cold flow and oxidative stability and these properties are largely dependent on the FA (fatty acid) composition of biodiesel feedstocks. Considerable research has focused on solving or alleviating these problems and one of the approaches is to modify the fatty acid profile by physical means, genetic modification of the feedstock or use of alternative feedstocks with different fatty acid profiles [13]. The FA

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profile of biodiesel from *C. vulgaris* has been studied recently [14] and shows a FA profile (46% oleic acid, 18% palmitic acid, 13% linolenic acid, and 9% linoleic acid) that is more likely to yield biodiesel with improved cold-flow properties and oxidative stability. The high proportion of oleic acid is a direct result of the FA biosynthesis in heterotrophic *C. vulgaris* which suggests that combined AC and HC can be used to produce algal biomass with a suitable FA profile for biodiesel [14,15].

In this work is an attempt to investigate and characterize the fatty acids profile of *C. vulgaris* cultivated under 3 different AC, HC and MC. Subsequently, the biomass and lipid productivities are determined in the 3 different growth conditions and finally a preliminary economic assessment among different cultivation modes is evaluated.

2. Materials and methods

2.1. Microalgal strain

The pure culture microalgae of *C. vulgaris* TISTR 8580 in suspension form was purchased from the TISTR (Thailand Institute of Scientific and Technological Research), Bangkok, Thailand. The algal suspension then was enriched in a 250 mL shake flask (140 rpm) containing 100 mL Bold's basal liquid medium at pH 6.8 and controlled temperature at 25 °C with continuous fluorescence light intensity of 3000 lux. Bold basal media was prepared following Miao and Wu, 2006 [11] and comprised of (g L^{-1}): 0.25 $NaNO_3$, 0.074 K_2HPO_4, 0.0175 KH_2PO_4, 0.024 $CaCl_2 \cdot 2H_2O$, 0.073 $MgSO_4 \cdot 7H_20$, 0.025 NaCl, 0.005 $FeSO_4$ and 0.045 EDTA. The growth cell was monitored until obtained 10^6 cells mL^{-1} biomass and meant that the algal cell was ready for use as seed starter.

2.2. Microalgae cultivation

The initial 10^6 cells mL^{-1} algal biomass obtained from the logarithmic phase of growth was inoculated in a 300-mL liquid medium. Three different culture conditions; AC, HC and MC were undertaken. In the AC, carbon and nitrogen sources of CO_2 and 25 g L^{-1} sodium nitrate ($NaNO_3$) were added to the medium with a supply of 5000 lux light intensity using interval periods of 16 h and 8 h in the dark. In case of HC, the algal biomass was cultivated in the dark all times and supplemented with carbon and nitrogen sources of 10 g L^{-1} sucrose and 0.10 g L^{-1} $NaNO_3$. Meanwhile, for MC, the algal biomass was exposed to light (5000 lux) at interval periods of 16 h light and 8 h dark. Sucrose (10 g L^{-1}), unless stated otherwise, and $NaNO_3$ (0.1 g L^{-1}) were added to the medium.

2.3. Analytical techniques

Cell growth was monitored by measurement of daily changes at mean of the absorbance (540 nm) of the suspension with spectrophotometer (PG Instrument Limited, T60, UK) and cell numbers were counted under a microscope with hemacytometer. The components of algal biomass cell such as carbohydrate, protein and moisture contents were analyzed using standard procedures of Phenol sulfuric method, Lowry's method and Weender analysis respectively. The total intracellular lipids in algal biomass cells were extracted using n-hexane Soxhlet extraction. The yield of the microalgae lipid (%) was calculated using the following equation, $Y = W_L/W_{DAB}$ where W_L andW_{DAB} are the weight of the extracted lipids (g) and of the dry algal biomass (g) respectively.

2.4. Ex-situ transesterification of microalgal lipids

Biodiesel was conducted after oil extraction from algal biomass followed by *ex-situ* transesterification. It should be noted that for the *In-situ* transesterification that the wet algal biomass was directly used without extraction step, was also coupled carried out however, the data not shown here. The basic reaction was carried out on hot plate stirrer with a gently mixed via magnetic bar using 3:1 (v/v) ratio of methanol to oil, 0.5% (w/v) NaOH catalyst (based on oil weight) at 60 °C and 60 min for reaction time. The chemical composition of biodiesel, FAMEs (fatty acid methyl esters), was analyzed using gas chromatography (GC-17A, Shimadzu, Japan).

3. Results and discussion

3.1. Autotrophic, heterotrophic and mixotrophic growths of C. vulgaris

C. vulgaris can grow autotrophically or heterotrophically utilizing sugars as carbon and energy sources [16]. The growth parameters of the algal cells grown autotrophically, heterotrophically or mixotrophically in batch cultures were investigated. The alga was grown in the same basal medium that supported well the growth of the alga in the three growth modes (see Fig. 1a). The highest biomass was reached on days 10, 5 and 3 for AC, MC and HC respectively. As shown in Table 1, autotrophic cells grew in the fastest indicated by the high specific growth rate (0.31 d^{-1}) and cell biomass (0.8 g L^{-1}). In contrast, the alga grew slowly under heterotrophic conditions supplemented with 10 g L^{-1} of sucrose, with a specific growth rate of 0.18 d^{-1} and a cell biomass concentration of 0.4 g L^{-1}. The alga under the MC had a similar specific growth rate to that achieved under autotrophic mode with an intermediate biomass concentration of 0.6 g L^{-1}.

As shown in Fig. 1b, heterotrophic growth of *C. vulgaris* resulted in not only the disappearance of the chlorophyll in cells (inferred from the green color) but also accumulation of high lipid content in cells. The mixotrophic cells, on the other hand, were lighter green in color. Lipid content in heterotrophic cells reached as high as 32.9%, which was about two times that in autotrophic cells (15.4%) (see Table 1). The lipid-soluble compounds from the autotrophic cells appeared in dark green with chlorophyll and carotenoid as the major components, whereas the lipid-soluble compounds from the heterotrophic cells appeared in a state of light yellow grease, which were mainly lipid compounds (referred as microalgal oil). This observation is in agreement with other previous researchers [10,11] who studied cultivation of *Chlorella protothecoides* under heterotrophic growth and effect of iron on lipid accumulation in *C. vulgaris*, respectively.

The highest protein content was found in AC (42.84%) while only 14.47% obtained in HC and 35.2% in MC. In contrast, the lipid content in HC cells reached as high as 32.85%, which was about two times compared to AC cells (15.37%). It was confirmed that under HC growth, *C. vulgaris* was induced to accumulate lipid instead of protein storage which was naturally found in AC growth of microalgae. It had been reported [17] that the autotrophic microalgae had low lipid content in comparison with those under heterotrophic and mixotrophic conditions.

The lipid productivity, a product of biomass productivity and lipid content, under different growth conditions were compared (see Table 1). The highest lipid productivity (45.6 mg L^{-1} d^{-1}) was achieved when cells were grown under the MC and generally in the absence of light, lower lipid productivities were achieved. This suggests that light stimulated its growth as indicated by higher biomass and lipid productivities and corroborates with the findings reported elsewhere [12,18] The lipid productivity obtained in this study lines in the same range as those observed using *C. vulgaris* [10,12,19] but lower than the productivity of up to 147 mg L^{-1} d^{-1} achieved by Feng et al., 2011 [20] who cultivated *C. vulgaris* in artificial wastewater medium. This is due to the

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