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Semi-continuous cultivation of *Chlorella vulgaris* using chicken compost as nutrients source: Growth optimization study and fatty acid composition analysis

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ABSTRACT

In the present study, cultivation of *Chlorella vulgaris* was extensively explored under semi-continuous approach to enhance its growth. The microalgae were cultivated in a photobioreactor supplemented with compost derived from chicken waste as an alternative nutrients source in both batch and semi-continuous cultivations. It was found that *Chlorella vulgaris* grew well up to 17 cycles of semi-continuous cultivation based on the following conditions for each cycle (3 days per cycle): 30% (v/v) removal of cultivation medium and 0.04 L/L of chicken compost in cultivation medium of pH 3. The average biomass productivity attained through these conditions was 0.0736 g/L/day, which was higher than batch cultivation (0.0568 g/L/day). Besides, the average total lipid content from each cycle under semi-continuous cultivation was maintained in the range of 25–35 wt%. The success of lipid extraction from the microalgae biomass was evidenced by the result of Fourier Transform Infrared Red (FT-IR) analysis that revealed lower peak intensity of carbon, especially in the range 2809–3012 cm^{-1} after lipid extraction. It was also worthwhile to mention that the fatty acid methyl ester (FAME) compositions of *Chlorella vulgaris* consisted mainly of C16:0 and C18:3 that were not significantly altered during the semi-continuous cultivation. Both saturated and unsaturated fatty acids in the harvested biomass accounted for approximately 26.3% and 73.3%, respectively, within 5 cycles of the cultivation.

1. Introduction

Microalgae are recognized as one of the promising feedstock for the production of biofuel, valuable pigments, nutraceutical and therapeutic products [1–3]. Microalgae are able to synthesize different types of compound within their cells, such as protein, amino acids, fatty acids, vitamins and various bioactive constituents [4,5], that are highly demanded in the field of pharmaceuticals, cosmetics and biofuel industries. In recent years, studies with regard to microalgae have spurred interest among researchers due to their high biomass productivity, ease of cultivation [4,6], high lipid content and ability to bio-fix carbon dioxide [7]. Besides, it is also proven that the microalgae lipid can be converted to biodiesel; a type of renewable biofuel that is non-toxic to the environment [8]. A few microalgae species had been reported to contain high lipid content and suitable for biodiesel production are *Botryococcus braunii*, *Chlorella vulgaris*, *Nannochloropsis* sp. and *Scenedesmus obliquus* [1–3]. Among all, *Chlorella vulgaris* is widely studied owing to its small cell size (easy to circulate in cultivation medium), ease to grow and ability to withstand extreme environment. For example, Kalhor et al. reported that the growth of *Chlorella vulgaris* could be enhanced by using wastewater polluted with crude oil as nutrients source. As a result, a maximum of 0.41 g dry biomass and additional of 20% lipid was recorded as compared with the control sample [9]. Also, Marudhupandi et al. reported that high lipid content (37%) could be attained when *Chlorella vulgaris* was cultivated in Conway chemical medium supplemented with 0.2 g/L of citrate [10]. These remarkable results indicated that *Chlorella vulgaris* serves as a potential microalgae species for large scale cultivation and biodiesel production [2].

Nevertheless, one of the limitations to cultivate microalgae for large scale biodiesel production is the cost of chemical nutrients. The cost of

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nutrients could contribute up to 10–20% of the entire cultivation cost [2,11], making it a major factor to be considered in many microalgae cultivation processes. In view of that, wastewater and compost had been suggested as alternative nutrients sources in order to reduce the overall biodiesel production cost. Different from wastewater, compost derived from biomass waste contains consistent nutrients composition which serves as an excellent nutrients source to promote the growth of microalgae in each cultivation cycle [12]. Zhu et al. reported that livestock waste compost was able to provide sufficient nutrients for the growth of *Chlorella* sp. that leaded to high biomass and lipid productivity of 0.29 g/L/day and 0.1 g/L/day, respectively [13].

On the other hand, cultivation method is another important factor affecting the cell growth and biochemical compositions of microalgae. Batch cultivation mode is commonly used in microalgae cultivation, yet it requires long cultivation time and produces large amounts of water that need to be processed after each harvesting cycle [14]. Therefore, semi-continuous cultivation is recommended by researchers as opposed to batch process as it is a more feasible method especially for large scale cultivation. Apart from higher biomass yield, separation of microalgae cells from the culture medium is also less hassle due to smaller amount of harvested medium. Under semi-continuous cultivation, a portion of the cultivation medium will be regularly discharged whereas the remaining culture is further utilized as the seed in continuing the cultivation [15]. With a proper control of the culture medium feeding rates, the achievement of high microalgae biomass productivity via semi-continuous cultivation mode is possible [16,17]. Hence, this is anticipated to improve the techno-economic of microalgae cultivation for biodiesel production in large scale [18].

The potential of semi-continuous cultivation of microalgae had been reported in recent years, especially in wastewater treatment [19], CO_2 bio-fixation [20], carbohydrate and lutein production [21,22]. It was reported that semi-continuous cultivation of *Chlorella vulgaris* could simultaneously remove total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD) from undigested dairy manure [19]. Besides, semi-continuous cultivation of *Chlorella minutissima* with specific control on the light intensity and CO_2 concentration could increase 19% of lutein productivity of the microalgae [22]. Although semi-continuous cultivation of microalgae for lipid production had been reported recently, however, the findings were still limited to growth optimization using waste as the nutrients source, number of semi-continuous cycles and fatty acid composition analysis [23,24]. In fact, the published reports were focusing more on the nutrients uptake by microalgae and downstream processing, such as harvesting and transesterification optimization study for biodiesel production.

The aim of the present study is to cultivate *Chlorella vulgaris* by using chicken compost as nutrients source through semi-continuous cultivation. A few important parameters, namely amount of nutrients added into the culture medium, volume of culture medium removed and the culture medium removal duration were optimized to attain higher microalgae biomass yield. Subsequently, the possibility of reusing the culture medium was also investigated. The effect of semi-continuous cultivation method on the lipid composition of microalgae was also determined to reveal the possibility of this method in large scale biodiesel production.

2. Material and methods

2.1. Microalgae seed cultivation

Chlorella vulgaris were obtained from Prof. Lee Keat Teong (School of Chemical Engineering, Universiti Sains Malaysia). The microalgae was preserved and grown in Bold's Basal Medium (BBM) consisting of: (1) 10 mL per litre of culture medium with the following chemicals: $NaNO_3$ (25 g/L), $CaCL_2{\cdot}2H_2O$ (2.5 g/L), $MgSO_4{\cdot}7H_2O$ (7.5 g/L), K_2HPO_4 (7.5 g/L), KH_2PO_4 (17.5 g/L), NaCl (2.5 g/L) and (2) 1 mL per litre of culture medium with the following chemicals: EDTA anhydrous (50 g/L), KOH (31 g/L), $FeSO_4{\cdot}7H_2O$ (4.98 g/L), H_2SO_4 (1 mL), H_3BO_3 (11.4 g/L), $ZnSO4_4{\cdot}7H_2O$ (8.82 g/L), $MnCl_2{\cdot}4H_2O$ (1.44 g/L), O_3 (0.71 g/L), $CuSO_4{\cdot}5H_2O$ (1.57 g/L), $Co(NO_3)_2{\cdot}6H_2O$ (0.49 g/L). The initial pH of the medium was adjusted to the range of 3.0–3.5. The seed culture was grown in a 1 L Duran bottle containing 500 mL of culture medium, aerated with compressed air and illuminated with cool white fluorescent light (light intensity of 60–70 μmol $m^{-2}s^{-1}$) with surrounding temperature ranging from 25 to 28 °C [25].

The correlation equation of microalgae biomass concentration and absorbance measured by spectrophotometer (Shimadzu UV-2600) at the wavelength of 688 nm (OD_{688}) is shown in Eq. (1):

$$\text{Biomass concentration, g/L} = 0.3983 \times OD_{688},\ R^2 = 0.9777 \quad (1)$$

2.2. Compost nutrients preparation

Chicken compost was purchased from the local market. A 10 g of the chicken compost was immersed in 600 mL of tap water and stirred for 24 h using a magnetic stirrer. Non-soluble particulates were filtered using filter paper (Double Rings 101). The resulting filtrate would be the compost medium which was subsequently used in the batch and semi-continuous cultivation studies. The characteristics of the compost medium are shown in Table 1.

2.3. Batch and semi-continuous cultivation

Batch cultivation was performed with the pre-determined optimum cultivation conditions as follows: pH 3 and 0.04 L/L of chicken compost in 1 L photobioreactor. After 12 days of batch cultivation, the microalgae were partially harvested and a similar volume of fresh water and pre-determined amount of compost nutrients were replenished into the photobioreactor for continuous growth. The pH of the cultivation medium was monitored from time to time and adjusted by using 1 M sodium hydroxide and 1 M sulphuric acid for the entire experiments. The semi-continuous cultivation experiments were optimized for different parameters, such as amount of nutrients added into the cultivation medium, amount of the culture medium being removed, duration of the semi-continuous cycle and the effect of utilizing the recycle medium. Fig. 1 summarizes the process flow of the semi-continuous cultivation in the present work. All the experiments were conducted in triplicate, including sample analysis, to ensure validity of the data.

The specific growth rate of the microalgae, μ, was calculated by using Eq. (2) [25]:

$$\mu = \frac{In\frac{N_2}{N_1}}{t_2 - t_1} \quad (2)$$

where N_1 and N_2 represented the microalgae biomass dry weight density (g/L) at time t_1 and t_2, respectively.

The biomass productivity (P) of the microalgae was calculated by using Eq. (3) [26]:

Table 1
Characteristics of chicken compost.

Test Description	Unit	Chicken waste compost	Method Used
Nitrogen (as N)	%w/w	3.3	MS4 17: 1994
Phosphorus (as P_2O_5)	mg/kg	20.5	MS4 17: 1994
Potassium (as K_2O)	mg/kg	701.6	MS4 17: 1994
Calcium (as CaO)	mg/kg	1053.7	MS4 17: 1994
Magnesium (as MgO)	mg/kg	77.0	MS4 17: 1994
Sulphur (as S)	%w/w	Nil	AOAC 980.02, 17th Ed
Boron (as B_2O_3)	mg/kg	46.5	AOAC 982.01, 17th Ed
Zinc (as Zn)	mg/kg	9.2	AOAC 965.09, 17th Ed
Manganese (as Mn)	ppm	Nil	AOAC 965.09, 17th Ed
Copper (as Cu)	mg/kg	1.0	AOAC 965.09, 17th Ed