Study of cultivation and growth rate kinetic for mixed cultures of local microalgae as third generation (G-3) bioethanol feedstock in thin layer photobioreactor

Wahyu Prihastuti Yuarrina¹, Yano Surya Pradana², Arief Budiman¹,²,⁵, Akmal Irfan Majid¹,⁵, Indarto¹,⁵ and Eko Agus Suyono¹,⁵

¹ Master Program of Systems Engineering, Universitas Gadjah Mada, Jalan Teknika Utara 3, Yogyakarta, Indonesia
² Chemical Engineering Department, Universitas Gadjah Mada, Jalan Grafika No. 2, Yogyakarta, Indonesia
³ Faculty of Biology, Universitas Gadjah Mada, Jalan Teknika Selatan, Yogyakarta, Indonesia
⁴ Mechanical and Industrial Engineering Department, Universitas Gadjah Mada, Jalan Grafika No. 2, Yogyakarta, Indonesia
⁵ Center for Energy Studies, Universitas Gadjah Mada, Sekip K1a, Yogyakarta, Indonesia

* Corresponding email: eko_suyono@ugm.ac.id

Abstract. The increasing use of fossil fuels causes the depletion in supply and contributes to climate change by GHG emissions into the atmosphere. Microalgae indicate as renewable and sustainable energy sources as they have a high potential for producing large amounts of biomass for third-generation biofuels (bioethanol and biodiesel) feedstock. However, there are several parameters which should be considered for microalgae cultivation, such as environmental conditions, medium composition and microalgae species. The aim of this research was to study cultivation of mixed microalgae cultures (Glagah consortium and Arthrospira maxima) in a thin layer photobioreactor. Farmpion medium, Bold’s Basal Medium (BBM) and Thoriq Eko Arief (TEA) medium were investigated as cultivation medium for bioethanol feedstock for 7 days. The results showed that the highest dry weight concentration of microalgae was in Farmpion medium (0.35 mg/ml) and the highest carbohydrate concentration of microalgae was in BBM (0.14 mg/ml). Thus, the optimum medium of microalgae cultivation for bioethanol feedstock was BBM because of the highest carbohydrate-dry weight ratio (0.88). In addition, mathematical approach by using Contois model was used to find out the growth rate of microalgae cultivation in each medium.

1. Introduction

Nowadays, renewable energy (RE) is one of hot issues in the world, which related research on this topic is always attractive to be conducted every year [1]. It is caused by the high demands of fossil-based energy.
which is not followed by the discovery of new reserves in similar capacity [2]. In addition, environmental issues related to accumulation of carbon emission in the atmosphere is also powering on development of RE [3]. In this case, Indonesia, the tropical and top-biodiversity country, has a lot of raw materials that can be utilize in developing RE. One of prominent feedstocks in developing RE is microalgae, which is potentially converted into new alternative energy [4].

Microalgae are single cell organisms contain cellulose, lipid, protein and other components which is consuming CO₂ for photosynthesis [5]. Based on these components, microalgae can be cultivated in order to produce biofuels, such as bioethanol, biodiesel, bio crude oil, called third generation (G-3) biofuels [6]. Microalgae have been proposed as G-3 biofuel feedstock because they have high biomass production per unit of light, can cultivate at any condition of land and water, and have no restriction with other sectors, such as food and pharmaceutical [7]. Moreover, microalgae can be cultivated in single culture, such as *Arthrospira* sp. and *Botryococcus* sp., or mixed cultures, such as as local strain, to reach optimum production.

Generally, microalgae can be cultivated in two methods, i.e. closed and open. In closed system, microalgae are grown in closed pond by bubbling it with air or CO₂. It will produce dry microalgae with high specification because of less contaminant during cultivation. Meanwhile, the open system will cultivate microalgae in pond which is freely contacting with air. The open system is easier to operate compared to the closed ones. In the other hand, dry microalgae product is less in quality and need to be treated for resulting in food grade or higher.

The aim of this research was to study the effect of culture medium on carbohydrate and dry weight concentration of mixed cultures microalgae which contain *Arthrospira maxima* and Glagah Consortium in thin layer photobioreactor (TL-PBR) with an open system cultivation for bioethanol feedstocks. Furthermore, the kinetics of microalgae growth rate in all medium were also investigated.

2. **Material and Methods**

2.1. Microalgae

This study used a mixed culture microalgae containing *Arthrospira maxima* and Glagah Consortium. *Arthrospira maxima* was one of the common microalgae species for mass production, which has high growth rate and biomass productivity by converting light energy into biomass efficiently. Otherwise, this species can survive both in fresh and salt water. Glagah Consortium was a natural mixed culture taken from Glagah Beach, Yogyakarta, Indonesia. This culture was then isolated in the Laboratory of Biotechnology, Faculty of Biology, Universitas Gadjah Mada. The species composing Glagah Consortium were *Cyclotella polymorpha*, *Cylindrospermopsis raciborskii*, *Corethron criophilum*, *Golenkinia radiata*, *Chlamydomonas sp.* and *Syracosphaera pirus* [8]. These cultures grew on the wide range of salinity, dry weight concentration achieving 3.42 g/L and lipid content reaching 13.58 % in cultivation medium using salt water. The aim of using mixed culture of *Arthrospira maxima* and Glagah Consortium was for mutual interaction beyond organism to complete the activity metabolism of the mixed cultures. All microorganisms in the cultures could defend as well as mutual support for growth [8].

2.2. **Culture medium**

Microalgae need culture medium, such as carbon source, vitamins, salts and nutrients, for growing [9]. Optimization of medium composition is necessary to enhance the productivity of microalgae. The main nutrient in the culture medium is nitrogen (N), which is available in inorganic matter, such as nitrite (NO₂⁻) and nitrate (NO₃⁻), or in organic matter, such as ammonium (NH₄⁺). Phosphorus (P) is also the primary nutrient for culturing microalgae. This matter is found in inorganic phosphates, such as potassium phosphate monobasic (KH₂PO₄) and potassium phosphate dibasic (K₂HPO₄), but its use cannot be assimilated with organic phosphate simultaneously [10,11]. Besides nitrogen and phosphorus, potassium is
the other primary nutrient supplied from potassium oxide (K₂O), KH₂PO₄ and K₂HPO₄. This study used three algal cultivation medium, i.e. Farmpion Green 63 Medium, Bold’s Basal Medium (BBM) and Thoriq Eko Arief (TEA) Medium, for comparing their performance.

2.2.1. Farmpion Green 63 Medium
Farmpion Green 63 Medium is an agricultural fertilizer which can be used as a medium culture microalgae. It is containing macronutrients, micronutrients and trace metals which were required for microalgae cultivation. The elements in this medium are nitrogen (N) in the form of nitrate (NO₃), phosphate (P) in the form of P₂O₅, potassium (K) in the form of K₂O, calcium (Ca), magnesium (Mg), sulfur (S) and microelements (B, Mn, Fe, Zn, Mo and Cu) [12]. This medium is affordable and easy to obtain in the fertilizer market.

2.2.2. Bold’s Basal Medium (BBM)
Bold’s Basal Medium (BBM) is the most medium used for cultivating green algae division [8], with fresh water. It uses two sources of nitrogen, i.e. sodium nitrate and ammonium molybdate. Phosphorus and potassium source is supplied from potassium phosphate monobasic (KH₂PO₄) and potassium phosphate dibasic (K₂HPO₄) [11].

2.2.3. Thoriq Eko Arief (TEA) Medium
TEA Medium is a self-generated algal culture medium by modifying of Walne’s medium and BBM as common algal culture medium. This modification was expected to increase the growth rate of microalgae and and biomass productivity [13]. As comparison, the summary of N, P, and K concentration at several medium is shown in Table 1.

| Table 1. Concentration (g/L) of Nitrogen (N), Phosphorus (P) and Potassium (K) at Several Medium |
|-------------------------------------------------|------|------|------|------------------|
| Medium                                          | N    | P    | K    | N/P ratio        |
| Farmpion Green 63 Medium                        | 21   | 21   | 21   | 1.00             |
| Bold’s Basal Medium (BBM)                       | 75   | 25   | 31   | 3.00             |
| Thoriq Eko Arief (TEA) Medium                   | 250  | 175  | -    | 1.43             |

2.3. Photobioreactor
Various cultivation methods of microalgae have been developed, one of them is photobioreactor. This study used open photobioreactor by flowing the medium forming thin layer, called thin layer photobioreactor (TL-PBR). TL-PBR was designed by referring to Doucha [14] with modification, as shown in Figure 1. The goals of thin layer photobioreactor (TL-PBR) were to avoid sedimentation of microalgae when cultivated and to increase the productivity of microalgae by optimizing light and CO₂ penetration into the medium. The installed tank capacity at this bioreactor was 600 L. To distribute the microalgae suspension properly, TL-PBR was also equipped by a pump (capacity of 75 L / min).
2.4. Analysis of Carbohydrate

Carbohydrate content was analyzed by using phenol-sulfuric acid method and spectrophotometry at wavelength of 490 nm [15].

2.5. Kinetics of Growth Rate

The relationship between substrate concentration (S) in mg/ml and growth rate of microalgae could be illustrated using Contois Equation, as shown in Equation 1. This kinetics led in facilitating future mass scale development.

\[ \mu = \frac{\mu_{\text{max}} S}{(K_s + S)} - k_d X \]  

To determine the kinetics of growth rate in Equation (1), we needed the differential equation of substrate concentration with time (t) in Equations (2) and carbohydrate concentration with time (t) in Equation (3) [6].

\[ \frac{dS}{dt} = -\frac{1}{Y_{x/S}} \frac{dx}{dt} \]  

\[ \frac{dC_{\text{carbo}}}{dt} = Y_{\text{carbo}/x} \frac{dx}{dt} \]

where, \( \mu \) was specific growth rate biomass (day\(^{-1}\)); \( \mu_{\text{max}} \) was maximum specific growth rate biomass (day\(^{-1}\)); \( K_s \) was half-saturation constant; \( k_d \) was death-rate constant (day\(^{-1}\)); \( X \) was dry weight concentration (mg/ml); \( Y_{x/S} \) was yield of biomass per mg substrate reduction; \( C_{\text{carbo}} \) was carbohydrate concentration (mg/ml); \( Y_{\text{carbo}/x} \) was yield of carbohydrate per mg biomass.

3. Results and Discussions

This research was conducted at Biotechnology Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. The cultivation process of microalgae was investigated for 7 days. Figure 2 shows the effect of culture medium on carbohydrate concentration, dry weight concentration and carbohydrate-dry weight ratio of microalgae. Figure 2(a) shows that the highest dry weight concentration was microalgae cultivated in Farmpion, followed by BBM and TEA Medium. The dry weight concentration of microalgae in Farmpion was significantly higher than BBM and TEA Medium. The composition of main components (nitrogen, phosphorus and potassium) in balanced condition, such as in Farmpion medium, would be driven dry weight concentration of microalgae faster [16].
Meanwhile, Figure 2(b) shows the continuous increasing of carbohydrate concentration in microalgae for 7 days. The results indicated that BBM was the best medium for synthesizing carbohydrate in microalgae, but it was slightly different with Farmpion. The highest carbohydrate concentration of microalgae cultivated in MBM was 0.1353 mg/ml, followed by Farmpion (0.1290 mg/ml) and TEA Medium (0.760 mg/ml). Carbohydrate, as the product of photosynthesis, required nutrients in the form of nitrogen (N), phosphorus (P) and potassium (K) [17,18]. Based on these results, the high nitrogen-phosphorus ratio (N/P ratio) in culture medium led to produce more carbohydrate and less lipid in microalgae. Nitrogen was one of important elements in driving chlorophyll formation in microalgae [12]. The formation of chlorophyll would uptake more carbon dioxide (CO$_2$) for synthesizing carbohydrate [19]. Figure 2(c) shows the effect of culture medium on carbohydrate-dry weight ratio of microalgae. This figure indicated that BBM was the best medium of microalgae cultivation for bioethanol feedstock because of the highest carbohydrate-dry weight ratio compared to other medium.
Furthermore, kinetic study was conducted to identify growth rate of mixed cultures microalge of Glagah Consortium and \textit{A. maxima} on each medium using Contois Model. This mathematical approach was used to predict growth rate of microalgae and design mass cultivation of microalgae. The relation between substrate and dry weigh concentration was expressed as in Equation 2. Meanwhile, the relation between carbohydrate and dry weigh concentration was declared as in Equation 3. The results of numerical calculation using MATLAB are shown in Figure 3, 4 and 5.

**Figure 2.** Effect of Culture Medium on: (a) Dry Weight Concentration; (b) Carbohydrate Concentration; (c) Carbohydrate-Dry Weight Ratio

**Figure 3.** Relation Between Dry Weight and Carbohydrate Concentration with Time Period Resulted from Kinetic Simulation in: (a) Farmpion Medium; (b) BBM; (c) TEA Medium

**Figure 4.** Relation between Substrate Concentration and Time Period Resulted From Kinetic Simulation in: (a) Farmpion Medium; (b) BBM; (c) TEA Medium
Figure 3 shows the relation between dry weight and carbohydrate concentration with time period resulted from kinetic simulation. As comparison of observed data and calculated data, there were deviation in all medium. In Farmpion medium, the deviation of dry weight and carbohydrate concentration was occurred in time period of 6 and 7 days. Meanwhile, the deviation of dry weight and carbohydrate concentration appeared in time period of 4 and 5 days for BBM and TEA medium. Figure 4 shows relation between substrate concentration and time period resulted from kinetic simulation. The substrate concentration was decrease everyday because it was consumed by microalgae for reproduction and/or biomass, carbohydrate, protein and other components production. However, the different composition of component in the substrate would give different composition of component in microalgae. Furthermore, Figure 5 shows relation between substrate concentration and specific growth rate resulted from kinetic simulation. From this figure, the slope of specific growth rate (dry weight concentration) was slightly differences in all medium. However, the value of specific growth rate was totally different. Specific growth rate of microalgae in Farmpion medium was the highest, followed by BBM and TEA medium respectively.

Parameters calculated from mathematical modeling and simulation of the kinetic of microalgae growth rate are shown in Table 2. From this table, Farmpion was the highest maximum specific growth rate biomass (0.082 day^-1). However, the yield of carbohydrate production per biomass production of microalgae in Farmpion medium was the lowest. Meanwhile, BBM had the highest yield of carbohydrate production per biomass production of microalgae because of the high N/P ratio.

| Parameters     | Farmpion | BBM  | TEA |
|----------------|----------|------|-----|
| µ_max          | 0.082    | 0.044| 0.039|
| S              | 63       | 131  | 425 |
| Ks             | 31.732   | 30.13| 15.495|
| Yx/s           | 0.498    | 0.5  | 0.504|
| Y_carbo/x      | 0.3310   | 0.743| 0.394|
| kd             | 0.223    | 0.187| 0.284|
| SSE            | 30.7151  | 22.4482| 16.9514|
4. Conclusions
The results showed that the highest dry weight concentration of microalgae was in Farmpion medium (0.35 mg/ml) and the highest carbohydrate concentration of microalgae was in BBM (0.14 mg/ml). Thus, the optimum medium of microalgae cultivation for bioethanol feedstock was BBM because of the highest carbohydrate-dry weight ratio (0.88). In addition, mathematical approach by using Contois model was used to find out the growth rate of microalgae cultivation in each medium.

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