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Development of a Simple Inclined Algal Culture System for Outdoor Cultivation

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ABSTRACT

To overcome the disadvantages of open pond and cement tube as an outdoor algal cultured system, a simple inclined algal culture system was developed. The system composed of an 18.9 L polyethylene terephthalate (PET) bottle placed on a degree adjustable stand using an air pump to generate water mixing and circulation. The system was tested by culturing 45 mL *Oscillatoria* sp. in 13 L Blue-Green-11 (BG-11) medium supplemented with 3 g L^{-1} $NaNO_3$ in plastic bottle, which was placed on different angles of inclination, viz. 45°, 60° and 90°. The system was operated outdoor under natural daylight and temperature. After 11 days, 11.5 L culture medium was poured out and 11.5 L fresh medium was refilled. Algal cell was precipitated to measure algal yield. Then, cell harvesting was done every 7 days for 6 batches. This system could culture algae continuously for 5 batches. The highest productivity was 32.23 gram wet (gw) $\cdot m^{-2} \cdot d^{-1}$, which was recorded from a second and fifth round of 45° angular system and also from third round of 60° angular system. The result showed that this simple inclined algal culture system can be done continuously for one and a half month with high productivity by 45° inclination, which was proved as a good mixing and circulation. Showing advantages over open-pond, this system was moveable and could reduce possibility of contamination.

Keywords: Algae; Culture system; Inclination; Repeated batch

1. Introduction

Autotrophic microalgae has been recommended as the most sustainable resource for food security, valuable products and environmental concerns [1]. During their growth, microalgae accumulates oil in cells,

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which can be used as bioresource for biofuel [2,3], protein and essential compounds for human nutrition and novel bioactive substances for pharmaceutical and medical applications [4,5]. In addition, the N_2-fixing cyanobacteria which is one group of microalgae can be used as biofertilizer to enhance the growth of rice, wheat and pea [6, 7, 8, 9]. Moreover, algal biomass has been employed for recovery of heavy metals from waste water and to mitigate alternative option for culture system, since it can avoid contamination, and operating system can be controlled [13]. However, the materials and construction, running, and maintenance costs of photobioreactor are highly expensive [14]. Therefore, a simple and practical algal culture system that diminishes the greenhouse gas was increasingly applied [2, 10, 11]. According to these various aspects of currently microalgae applications, studying algal production has gained more attention for researchers.

Generally, algal production is cultured in an open raceways, an open pond, or a cement tube, which is easily contaminated [12], rapid water evaporation results in chemical medium property changes, insufficient penetrated light troubling on algal growth, incomplete circulation and difficult to harvest algal cell in term of fed-batch culture. These difficulties in the control of the operating condition affect cost and time consumption. Thus, this study aimed to develop a simple semi-closed algal culture system from materials available in the market. Moreover, an effect of different inclination; 45°, 60° and 90° of culture tank on algal biomass productivity was also examined.

2. Materials and Methods

2.1 Development of algal culture system Development of algal culture system was based on equipment with easy to come by. The most important part of algal culture system was a container referring to working volume of algal culture medium. According to apparatus available in the market (e.g. basin, plastic box, plastic bottle, cement tube, aquarium and bucket), an 18.9 L screw capped plastic (polyethylene terephthalate; PET) bottle was the most suitable container. The narrow bottle neck and transparent material of the PET bottle provided advantages of decreased contamination, water evaporation, and sufficient penetrated light. Algal medium circulation was done by using an air pump with plastic tube connected to sintered sparger. In addition, angle adjustable stand was designed to use as a base (Fig. 1). Different degree of stand make algal medium asymmetry resulting in good mixing and complete circulation. Moreover, the design of adjustable stand eases algal harvesting and medium refilling.

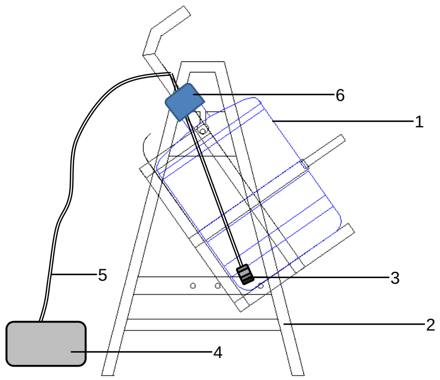

Fig. 1. Developed algal culture apparatus (1: plastic bottle, 2: angle adjustable stand, 3: sparger, 4: air pump, 5: plastic tube and 6: screw cap with pores).

2.2 Testing of algal culture system

2.2.1 Algal biomass production

The developed system was tested by culturing 45 mL of *Oscillatoria* sp. in 13 L Blue-Green-11 (BG-11) medium supplemented with 3 g·L^{-1} $NaNO_3$ in an 18.9 L culture bottle. The culture bottle was placed on different angles of inclination, viz. 45° 60° and 90°. The system was setup outdoor under natural sunlight and temperature. Air temperature varied between

23-38°C. Aeration rate was 45 $L.\ min^{-1}$. After 11 days, 11.5 L culture medium was poured out and 11.5 L fresh medium was refilled. Algae cell was harvested and reported in term of gram wet weight (gw) per liter of medium in one day ($gw{\cdot}L^{-1}{\cdot}d^{-1}$). Cell harvest was done every 7 days for 6 rounds

2.2.3 Effect of algal system inclination on gas transfer

Effect of inclination on medium circulation (gas transfer) was determined by oxygen transfer rate (OTR) and volumetric oxygen transfer coefficient (K_La). Dissolved oxygen concentration was indirectly measured by sodium sulphite oxidation method according to Rajesh et. al. 2012 [15]. Ninety and forty five angle stand algal culture systems were set up and aeration was fixed at 8 and 15 $L{\cdot}m^{-1}$. Fifteen liter of 0.05 M $Na_2S_2O_3$ was added and air pump was turned on for 15 min. Then, $CoCl_2$ was added to a final concentration of 0.003 M. Samples of 5 mL were taken every 5 min for 30 min, and titrated with 0.1 N $Na_2S_2O_3$ until light yellow solution was observed. Then, 1 mL of 2% soluble starch solution was added and continued titrating until clear. OTR and K_La was calculated as follow.

$$OTR = K_La\Delta C = K_La\ (C^{*}-C)$$

OTR = Oxygen Transfer Rate ($mg{\cdot}L^{-1}{\cdot}h^{-1}$)
K_La = volumetric oxygen transfer coefficient (s^{-1})
C = oxygen concentration in the liquid phase ($mg{\cdot}L^{-1}$)
C^{*} = saturation concentration of the oxygen in the liquid ($mg{\cdot}L^{-1}$)

3. Results and Discussion

3.1 Algal biomass production

A simple repeated batch algal culture system was composed of an 18.9 L screw capped PET bottle placed on a degree adjustable stand and using air pump to generate water circulation (Fig. 1). *Oscillatoria* sp. was cultured in developed system with three different degrees of adjustable stands. Algal biomass was harvested for 6 rounds and shown in Fig. 2. The highest algal production was recorded from 45° stand (40.0 $gw{\cdot}L^{-1}$), followed by 60° stand (39.1 $gw{\cdot}L^{-1}$). The highest total biomass per system was recorded from 45° stand (209.6 $gw{\cdot}L^{-1}$), followed by 60° stand (191.7$gw{\cdot}L^{-1}$) and 90° stand (121.9 $gw{\cdot}L^{-1}$), respectively. Algal production rate was shown in Fig. 3. The highest production rate was recorded from 45° stand (5.7 $gw{\cdot}L^{-1}{\cdot}d^{-1}$), followed by 60° stand (5.5$gw{\cdot}L^{-1}{\cdot}d^{-1}$). The highest average of productivity per system was recorded from 45° stand (4.7 $gw{\cdot}L^{-1}{\cdot}d^{-1}$), followed by 60° stand (4.3 $gw{\cdot}L^{-1}{\cdot}d^{-1}$) and 90° stand (2.8 $gw{\cdot}L^{-1}{\cdot}d^{-1}$), respectively. Therefore, system placed on stand inclination at 45° and 60° showed higher potential in algal production than at 90°. Inclination of culture tank may increase illumination surface area, which promote algal cell to utilize solar energy. However, higher solar energy may cause photoinhibitory damage of algal cell [14]. In addition, high level of dissolved O_2 concentration in culture tank may have an effect on algal productivity [16]. This has been observed in *Nannochloropsis* sp. that increasing oxygen concentration from 75% to 250% air saturation at low light intensity resulted in decreased specific growth rate and yield [17]. Increasing oxygen in the system higher competes against carbon dioxide for the Rubisco enzyme, a major agent in the process of carbon fixation to produce algal biomass [17].

3.2 Effect of algal system inclination on gas transfer

According to productivity of three different stand inclinational systems, 45° and 90° stands were selected for further study on water circulation. At identical aeration rate, inclination of stand increases K_La from $(3.75{\pm}0.08) \times 10^{-6}$ to $(4.85{\pm}0.03) \times 10^{-6}$ at 15

L·m^{-1} aeration rate and from (1.67±0.05) x 10^{-6} to (1.17±0.02) x 10^{-5} at 8 L·m^{-1} aeration rate. This result was similar to OTR, which increased from 2.63x10^{-5} to 3.40x10^{-5} at 15 L·m^{-1} aeration rate and from 1.17x10^{-5} to 8.19x10^{-5} at 8 L·m^{-1} aeration rate (Table 1).

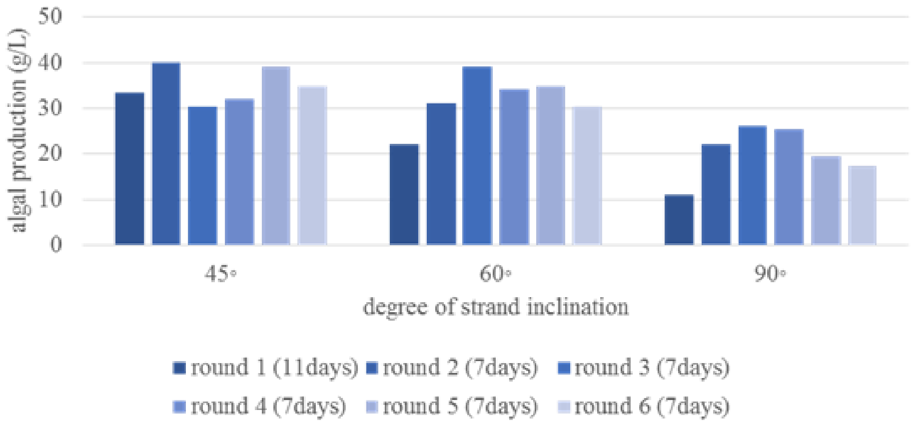

Fig. 2. *Oscillatoria* sp. production (gw·L^{-1}) from three different angle adjustment of simple repeated batch algal culture system for 46 days.

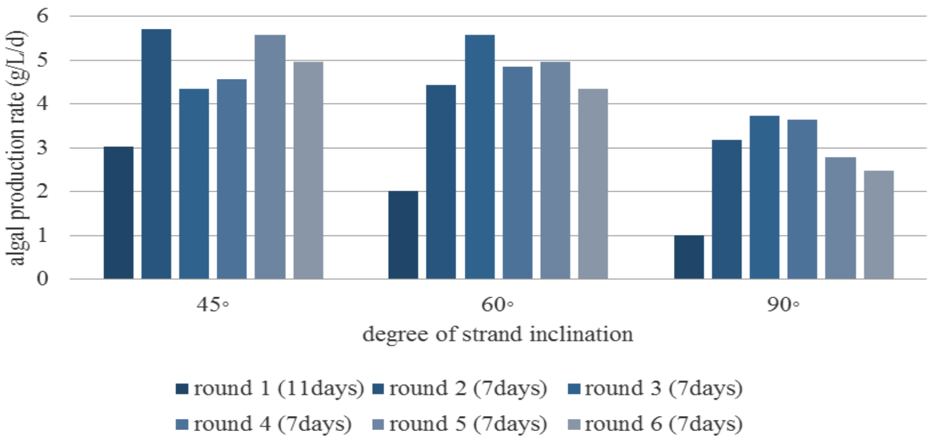

Fig. 3. Productivity (gw·L^{-1}·d^{-1}) of *Oscillatoria* sp. from three different angle adjustment of simple repeated batch algal culture system for 46 days.

The results showed that OTR in the medium of 45° stand was higher than that of 90° stand. This implied that inclination of stand improved mass transfer characteristics resulting in good medium circulation [18]. In addition, 8 L·m^{-1} aeration rate was better than 15 L·m^{-1}, because 8 L·m^{-1} created small bubbles, while 15 L·m^{-1} created big bubbles which moved faster up to the surface. The OTR at 8 L·m^{-1} aeration rate was higher than 15 L·m^{-1}. Therefore, inclination of stand produced asymmetry of medium body in container providing greater mixing. This condition enhanced algal growth than upright container. Mixing has been suggested as one of main factors to improve the mass transfer efficiency in the culture system [12]. Good mixing and nutrient circulation enhanced gas including CO_2 and O_2 transfer. Sufficient mixing improved light-dark cycling of the algal cell, which reduced the effect of photoinhibition of cells exposed to high irradiation [12, 14, 19]. Moreover, adequate mixing prevent algal cell sedimentation. Mixing in microalgal system can be improved by adding static mixers or increasing aeration rate into the culture container. This helps a lot in increasing the algal biomass. These applications enhance mass transfer and efficient distribution of light and nutrients that can be observed in tubular photobioreactor [12]. However, the algal strain is one of the factors that should be concerned, because increasing aeration rate may cause cell damage.

Table 1. Effect of stand inclination and aeration on volumetric oxygen transfer coefficient (K_La) and oxygen transfer rate (OTR)

Angle of adjustable stand	Aeration ($L \cdot m^{-1}$)	K_La (min^{-1})	OTR ($mg \cdot L^{-1} \cdot m^{-1}$)
90°	15	$(3.75\pm0.08)x10^{-6}$	$2.63x10^{-5}$
90°	8	$(1.67\pm0.05)x10^{-6}$	$1.17x10^{-5}$
45°	15	$(4.85\pm0.03)x10^{-6}$	$3.40x10^{-5}$
45°	8	$(1.17\pm0.02)x10^{-5}$	$8.19x10^{-5}$

Note: Oxygen saturation (C^*) in fresh water under steady state of this experiment was approximate 7 mg/L.

Table 2. The main differences in operation between open system, closed system and the developed system [20, 21].

	Open system	Closed system	The developed system
Type	Open race-ways	Tubular bioreactor, Polyethylene bags	a simple inclined algal culture system
Light penetration	insufficient	good	sufficient
Angular adjustment for solar collector	unable	able	able
Evaporation losses	high	low	low
Contamination	high	low	lower than open system
Circulation	depend on agitation type	depend on bioreactor type	adequate
Algal culture strain	highly selective strain	any	any
Repeated batch culture	unable (mostly contaminate)	able	able (5 rounds)
Purity of product	acceptable	very high	acceptable
Materials and construction cost	low	high	low
Running cost	low	high	low

3.3 Characteristics of development algal culture system

The simple inclined algal culture system for outdoor cultivation can be called semi-closed system which diminished the main disadvantages of open system (Table 2). Using the 18.9 L transparent plastic screw cap as the culture tank not only reduced the chance of culture contamination and evaporation losses, but also provided sufficient light penetration for solar energy. In addition, the developed system possessed advantages over the closed system in term of lower running and material costs, since it is composed of equipment that is available on the market and the operation is not complicated. Moreover, the system is moveable for preventing the opportunity loss in land uses.

4. Conclusion

The developed algal culture system is composed of an 18.9 L plastic bottle placed on a slope adjustable stand with an air pump to generate water circulation. Advantages of the system are low chance of culture contamination, low evaporation loss, high light infiltration, and low cost of construction and maintenance. The 40° inclined culture bottle can promote algal productivity and produce biomass continuously for 5 rounds. The moveable system can be used to culture mass algae outdoor using solar energy and can reduce production cost. Therefore, this developed system is suitable to culture micro algae in research unit and can be further developed as a small household biofertilizer unit.

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