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INVESTIGATION OF THE INFLUENCE OF MICROALGAL CULTURE MEDIUM ON BIOMASS PRODUCTION

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SUMMARY

Microalgae have been shown to produce a variety of products, including biofuels, proteins, and beta-carotene. However, increasing their biomass production still requires a lot of effort from researchers. Biomass from different culture media has presented challenges and possible solutions to improve microalgae production efficiency. In addition to factors such as light and temperature, nutrients also affect microalgal culture media. This study has developed basic experiments revolving around the fluctuations of essential nutrients such as carbon (C), nitrogen (N), phosphorus (P) during the cultivation of *Chlorella vulgaris* on Bold's Basal Medium (BBM). As a result, reducing or increasing the N and P concentrations by half, and replacing C in $NaHCO_3$ with glucose significantly changed the growth curve of *C. vulgaris.* There were significant fluctuations in the microalgal characteristics of the lag phase and the stable phase, with the former being longer and the latter being shorter compared to BBM. Additionally, the maximum biomass obtained was 1.95 g/L dry weight when microalgae were cultivated in medium containing glucose (M1), where the C concentration was replaced by glucose, and 1.56 g/L when the P content was increased 1.5 times compared to 1.35 g/L of dry weight in BBM. However, reducing or increasing nitrogen content by half inhibited cell growth, leading to low biomass efficiency of only 1.07 g/L and 1.21 g/L, respectively. On the other hand, changes in the essential nutritional composition of the culture medium also affected the efficiency of settling of *C. vulgaris*, this value was higher than 80% in all media compared to BBM.

Keywords: Biomass, microalgal cultivation, microalgal harvesting

INTRODUCTION

The biomass culture of microalgae in wastewater has contributed significantly to managing freshwater ecosystems by providing a more environmentally friendly approach to reducing the eutrophication potential of human being waste streams-compared to current treatment measures. On the other hand, it is possible to collect biomass for applications in human life, such as producing bio-oil, fertilizer, and high-value chemical compounds in functional foods. The production process of microalgae biomass includes two main processes: upstream and downstream. Among these,

biomass production is an important part of upstream steps. Previous studies have shown that the cost of the biomass harvesting process is 20–30% of the total production cost. The main factors influencing biomass production are light, CO_2 and nutrients. Many choices in culture system approaches have provided convincing evidence that algal culture systems influence biomass recovery efficiency. As an alternative to open ponds, microalgal strains cultured in closed photobioreactors (PBRs) achieved a higher productivity of 10 times, approximately 0.8 to 1.5 g L^{-1} day^{-1}. Many previous studies have demonstrated that laboratory-scale PBRs have better control over the growth medium in which biomass is mixed by air, gas lift, or pumps. However, they have high construction costs and are difficult to scale up, require high energy input for gas exchange (Cuaresma *et al.*, 2011; Münkel *et al.*, 2013; Rodolfi *et al.*, 2009). Along with light, CO_2 and nutrients, maximizing growth required to maximize growth. Particularly, CO_2 can be supplied through direct foaming, but its distribution in the culture medium is an additional cost factor. Another challenge is removing excess O_2 in air, which inhibits photosynthesis (Wang *et al.*, 2012).

Other arguments suggest that current photosynthetic properties may represent optimal growth for plants. The balance between plant growth and stress tolerance in highly variable and potentially stressful environments can also prevent the maximization of photosynthesis and, thus, potential growth. Tolerance to environmental stress in microalgae cultures may be due to changes in nutrients. Fluctuations in the carbon or nitrogen composition of nutrients can change the photosynthesis of microalgae, thus leading to a decrease or increase in the performance of biomass production (Yang *et al.*, 2018; Zarrinmehr *et al.*, 2020). The aim of this study was to investigate the influence of carbon, nitrogen, and phosphorous compositions to shed light up the fluctuations of biomass performance.

MATERIALS AND METHODS

Strain and growth conditions

For the starter culture, *Chlorella vulgaris* SAG 211-19 was cultured in 250 mL of BBM medium with ingredients as follows: 1151.98 mg/L $NaNO_3$ (corresponding to 189,7 mg/L of N), 140.00 mg/L $MgSO_4$. $7H_2O$, 100.00 mg/L KH_2PO_4 (corresponding to 22.8 mg/L of P), 840.00 mg/L $NaHCO_3$ (corresponding to 120 mg/L of C), 25.00 mg/L $CaCl_2.2H_2O$, and 0.5 mL Hutner solution; white light of LEDs at a flux of 150 μmoL/m^2/ s for 12 hours, the rest of the day. It is easy to access further manipulations with a correlation between the absorbance at 680 nm of microalgal growth with its chlorophyll-a (Nguyen *et al.*, 2014).

The microalgal culture medium was prepared by replacing the carbon in $NaHCO_3$ with glucose 450 mg/L (M1), reducing and increasing by half the nitrogen content in the $NaNO_3$ (M2 and M3), as well as the phosphorous in KH_2PO4 (M4 and M5).

Experimental setup

An initial content of 5% v/v microalgae with a cell density (OD_{680}nm) of 0.8 in the starter culture was adjusted for a biomass culture in an Erlenmeyer of 250 mL with standard media of BBM as a sample control, and medium M1 to M5. The microalgal growth was recorded daily the absorbance to assess the final biomass before harvesting. In particular, the growth data of each sample

was extracted to evaluate the life cycle of microalgae in different media. The biomass was collected visually once the microalgal cells reached the phase of death, the biomass was collected.

Methods

Settling efficiency

The settling efficiency of microalgal cells, which evaluates the performance of cell flocculation or cell removing out of media, is estimated according to the Beer-Lamber law as follows:

$$E, \% = (1 - \frac{A_f}{A_o}) \times 100$$

Where A_f, A_o represent the absorbance of the microalgal cell suspension at 680nm at the final and initial harvesting suspension, respectively.

Analysis

The biomass dry matter (DM) was obtained by filtering the microalgal suspension through GF/F filters, drying, and weighing. The pigments were measured by extracting cells using methanol for 4 h. The total chlorophyll a content was determined according to the method (Ritchie, 2006). Measurements were recorded as the average of triplicate experiments, and the error bars were calculated.

RESULTS AND DISCUSSION

Cell growth evaluation

The correlation between microalgal absorbance at 680 nm and chlorophyll a, as well as the number of cells, was described by the following equation: y = 0.1056 x with y is the absorbance at 680 nm, x is chlorophylla (mg/L), and R^2 = 0.9971. The growth of microalgae in different media for 14 days was shown in Figure 1. Results in Figure 1 illustrated the cell proliferation in 9 days of cultivation. After 9-day culture, the microalgae entered the death phase, it was a suitable time to harvest cells. However, it is evident that changes in the nutritional composition of the culture medium have a clear effect on the cell state in the lag phase and exponential phase. Compared to the control sample, the acceptance of the new environment by *C. vulgaris* cells requires a longer time, causing a longer lag phase time. Except for *C. vulgaris,* which grew strongly in M5 medium, the cells showed poor growth compared to the control sample. Specifically, the substitution of glucose for $NaHCO_3$ molecules accelerated bacterial infection, causing the medium M1 to become pale milky white after 6 days of cultivating microalgae. At this time, the microalgae began to grow more slowly due to competition from other microorganisms. Meanwhile, reducing or increasing half the composition of $NaNO_3$ (compared to the control sample also resulted in poorer growth). In contrast to increasing the nitrogen concentration in $NaNO_3$ by 1.5 times (medium M3), the microalgae exhibited weaker growth compared to the control sample. *C. vulgaris* exhibited the strongest growth when the phosphorus concentration of KH_2PO_4 in medium M5 by 1.5 times. According to Satpati *et al*. 2016, the maximum biomass yield of *Chlorella* sp. was increased to 1.56 g/L compared to the control sample of 1.31 g/L when the concentration of P in the culture media was 1500 mg/L. Meanwhile, the findings of Hamedi et al., 2016 showed that changing the medium, compared to the standard BG11 helped increase the number of *C. vulgaris* cells by 52% and dry weight by 54% (Hamedi *et al.,* 2016; Li e*t al*., 2019).

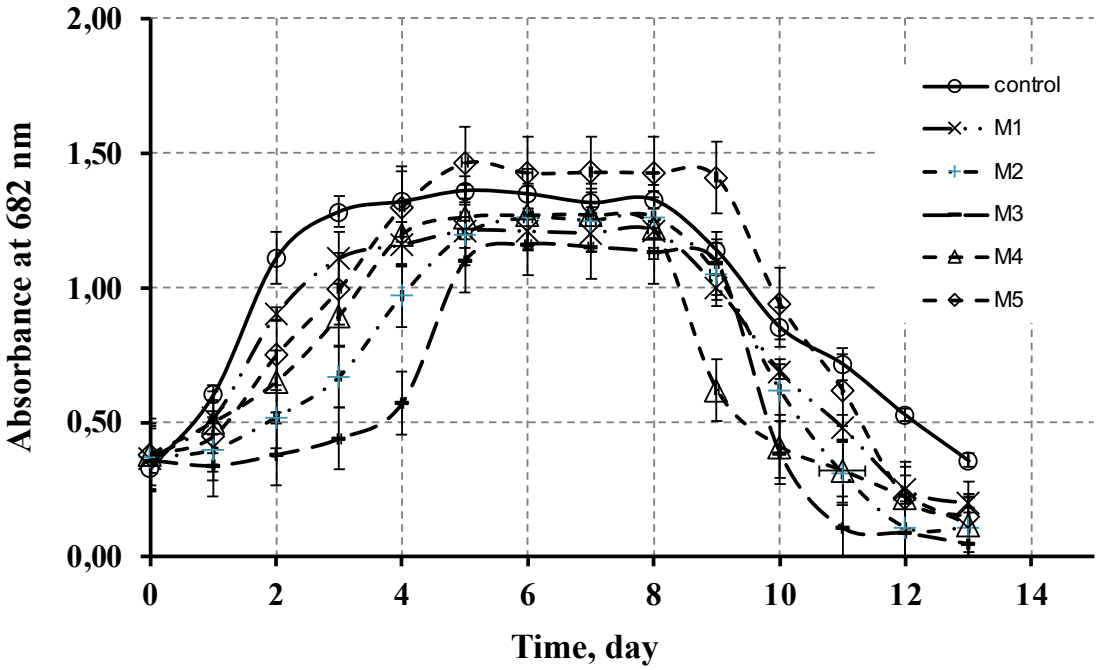

Figure 1. Growth schema of microalgal cultivation in different media.

Determination of biomass and cell harvesting

The importance of nutrient supply in controlling the growth kinetics of phytoplankton has prompted many studies. In this study, the biomasses of *C. vulgaris* cultured in five media were recorded to evaluate the effect of essential nutrients on microalgal growth and biomass acquisition. The results obtained in Figure 1 indicate the difficulty in the growth process of *C. vulgaris* cells in a C, N and P starvation environment. Microalgae entered the death phase more easily than when cultured in BBM standard medium. With data collected from 2 sampling points shown in Figure 2, (biomass measurement was on the 4th and another one was the last day when all culture processes were stopped), it was observed that the biomass of microalgae in M1 was the highest at 1.94 g/L in all experimental samples, even though its biomass at the time of the exponential phase was to a lesser at 0.62 g/L. On the other hand, when the P content in sample M5 was increased 1.5 times, the biomass obtained was slightly higher than the control sample at 1.59 g/L and 1.37 g/L, respectively. The increased biomass of microalgae in M5 compared to other samples might be due to the symbiosis of bacterial infection when the culture environment contains glucose. Many studies have proven that during photosynthesis it is necessary to provide nutrients for cell division. Bacteria can act as enzymes to accelerate this process. As reported by Li et *al.*, 2019, microalgae growth was significantly inhibited by increasing the ammonium concentration in the autotrophic culture medium (Li *et al.*, 2019). The final biomass dry weight (1679 mg/L) with 50 mg/L ammonium (corresponding to 38.88 mg/L of N) was

significantly lower than the control group, but the microalgae showed a gradual increase in biomass. The maximum specific growth rate (μ_m, 0.61/day) and average specific growth rate (μ_m, 0.20/day) with 50mg/L ammonium were close to the specific growth rate in the control samples. In addition, some results have shown that N uptake in microalgae in the form of NH_4^+ is easier than in the form of NO_3^-. However, whether NO_3^- can serve as the primary nitrogen resource for cell growth or has an adaptive function to nitrogen uptake conditions depends not only on the available N source but also on other nutrients and environmental conditions (Li *et al.*, 2019; Procházková *et al*., 2014). Meanwhile, phosphorus is also an essential nutrient for microalgae growth and cell division, and its requirements vary widely among different microalgae species. According to Roopnarain *et al*., 2014, the optimal phosphorus concentration for microalgae ranges from 0.001 g/L to 0.179 g/L. Phosphorus limitation is an effective stress of envỉonmental condition to induce lipid accumulation. In addition, microalgae are highly efficient in absorbing inorganic phosphate to participate in cellular structures, such as the production of cellular components like phospholipids, DNA, RNA and ATP (Mulbry *et al*., 2008). It was also reported that microalgae growth and phosphate uptake were linearly proportional to biomass yield (Chu *et al*., 2013).

In figure 3, the results indicated that the settling efficiency of microalgae also depends on changes in nutrient levels. The substitution of carbon in glucose for that in $NaHCO_3$ creates favorable conditions for bacteria to invade, leadingto microalgae settling very easily when *C. vulgaris* is reaching into the death phase.

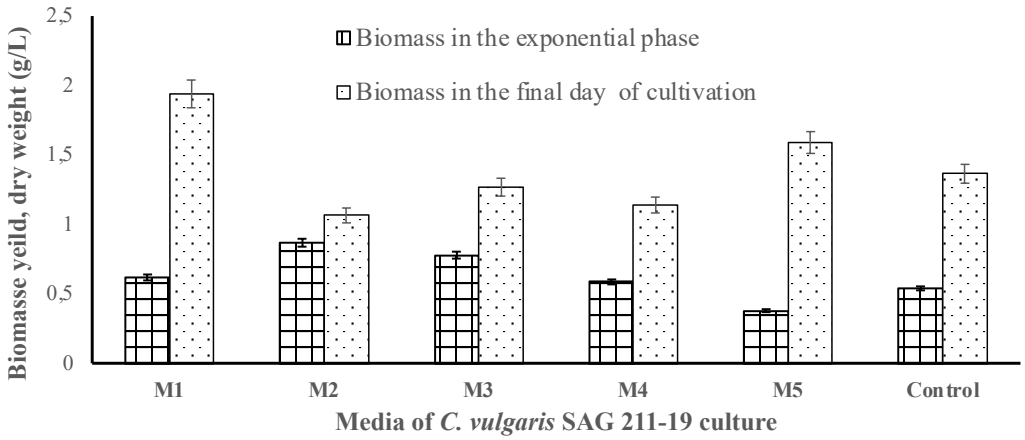

Figure 2. Determination of microalgal biomass after fluctuations in essential nutrients.

Moreover, fluctuations in essential components in the culture medium, such as N and P, also accelerate microalgal cells into the death phase. In previous studies, biomass recovery efficiency also affects the total cost of the microalgal production process. This study also investigated the sedimentation ability of microalgae to examine the influence of the culture environment on the self-sedimentation ability of microalgae. In Figure 3, it can be observed that the settling ability of *C. vulgaris* in most cultures at the

end of microalgal production gave an efficiency of settling about 80%, although at the end of the exponential phase, this efficiency is much different. Furthermore, the efficiency of settling of M1 medium has a value higher than 90%. This has also been explained in many studies that bacteria in the environment enhance the ability to aggregate cells together to form biofloc by forming biofilm, leading to microalgal settling to the bottom of the culture vessel easily (Nguyen *et al.*, 2018; 2019; 2020).

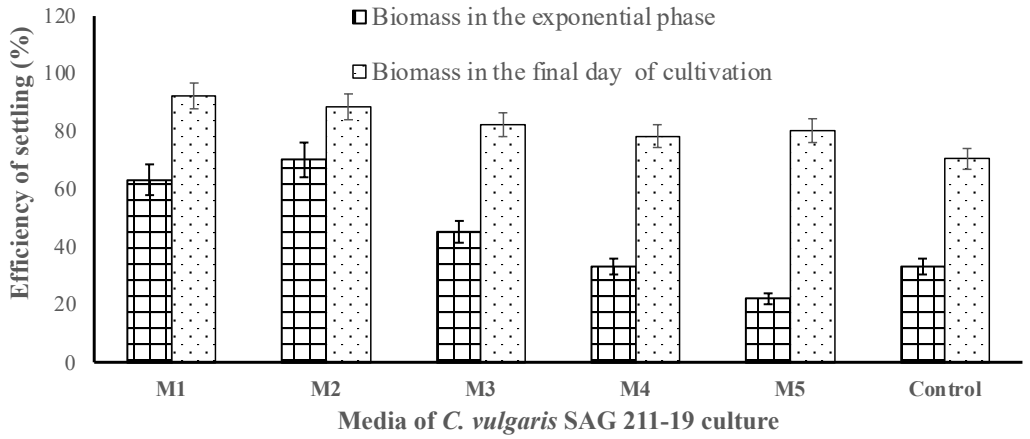

Figure 3. Settling ability of *C. vulgaris* after fluctuations in essential nutrients.

In this study, *C. vulgaris* SAG 211-19 was investigated for a new approach to establish biomass production. Harvesting microalgal biomass normally depends on physical factors of culture conditions, such as temperature, light, and nutrients. Similar to other studies, the variation of essential components in nutritional factors significantly changes the growth process of *C. vulgaris*, resulting in the obtained biomass compared to the standard BBM medium.

CONCLUSION

In conclusion, compared to the biomass yield of these media, this work has provided results the maximum biomass yield of 1.95 g/L dry weight when cultivating microalgae with glucose instead of $NaHCO_3$. Definitely, the efficiency of cell settling was recorded up to 90% when regulating C, N and P concentration in essential nutrients in the culture medium.

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