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MICROALGAE GROWTH AND NUTRIENT RECOVERY OF *CHLAMYDOMONAS REINHARDTII* 11/32C CULTIVATED UNDER LABORATORY-CONTROLLED CONDITION

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ABSTRACT

Microalgae have been identified as one of the most promising sources for future wastewater treatment and generate many valuable products. Photosynthetic microalgae utilize energy from the sun and assimilate nutrients like carbon (C), N and P from wastewater. Microalgae can grow rapidly in wastewater and produce biomass that can be used to produce biofuels, fine chemical products, and bio-fertilizers. This study was conducted to investigate the nutrient recovery from wastewater through microalgae biological uptake. The green microalgae of C. *reinhardtii* 11/32C used in this study and cultivated in Bold's Basal Media (BBM). The algae culture was placed in a 2 liter of photobioreactor for approximately in 14 days. The green C.*reinhardtii* 11/32C were cultivated in the following conditions: photoperiod 16 hr light, 8 hr dark (16:8); light intensity 250±5 μEm-2s-1; and temperature 20±3°C which run in triplicates. The liquid samples were collected every two days over a period of 14 days for Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), Nitrate (NO_3^-), Phosphate (PO_4^{3-}), Chlorophyll-a, Total Phosphorus (TP) and Total Nitrogen (TKN). The results revealed that green microalgae can uptake nutrient at the rate of 2.13 mg N/l/d and 0.5 mg P/l/d. These results could recover up to 5.9 % and 1.3% of N and P in their cell, respectively.

KEY WORDS : Bold-basal media, *Chlamydomonas reinhardtii*, Microalgae growth, Nutrient recovery.

INTRODUCTION

In a climate change era, sustainability is an essential principle in natural resources management which involved controlling the environmental impact and the socio-economics (Brennan and Owende, 2010). In general, environment quality is declined due to human activities. A considerable amount of water used for agricultural, municipal, and industrial purposes will generate wastewater that contains pollutants, including nitrogen (N) and phosphorus (P) (Cai *et al*., 2013). In addition to the wastewater generation, the global human sewage contributes approximately 87% and 88% in forms of N and P, respectively (Morée *et al.,* 2013). These portions must be managed and treated correctly, which otherwise will cause eutrophication in the water bodies. Therefore, it is worth to explore the possibility to harvest any quantity of N and P from wastewater through nutrient recovery in microalgae cell to achieve long-term sustainability and to improve surface water quality (Yulistyorini, 2016).

Concerning global nutrient requirement, as well as renewable energy and water reuse form wastewater, microalgae are the robust microorganisms to recover nutrient and carbon from various wastewater type to produce biomass feedstock for biofertiliser or even for biofuel (Brennan and Owende, 2010; Cai *et al.,* 2013; Batstone *et al.,* 2015; Mo and Zhang, 2013). Nutrient

recovery from wastewater through microalgae assimilation is a promising approach to integrate the algae for the bioremediation of wastewater and the production of biofuels and added value bio-products (Yulistyorini, 2016; Unc *et al.,* 2017).

This study aims to investigate the ability of the green freshwater microalgae to recover nutrient in their cell to produce algal biomass feedstock. The research was conducted under the laboratory-controlled condition, which focused on nutrient recovery in the green microalgae cultivated in photobioreactors.

MATERIALS AND METHOD

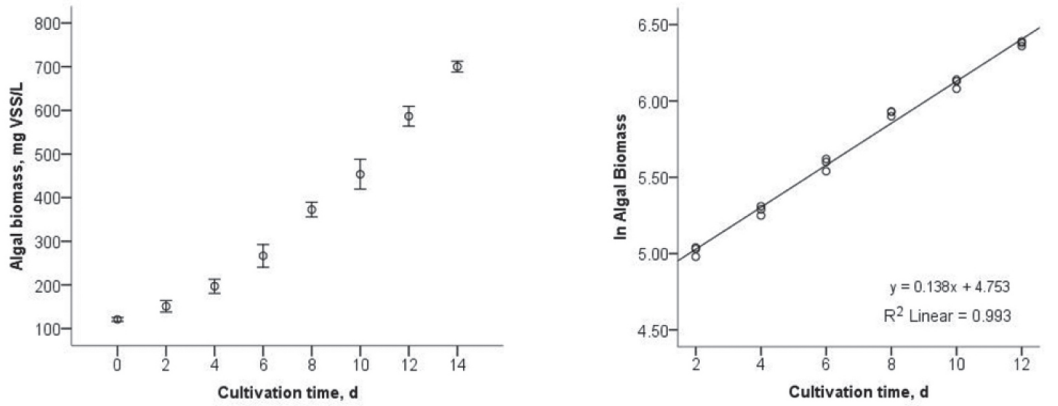
The green microalgae of *Chlamydomonas reinhardtii* 11/32C used as photosynthetic microorganism in this study. This alga was selected because of its ability to store the excess of P in their cell (Komine *et al.,* 2000; Ruiz *et al.,* 2001). *C.reinhardtii* 11/32C was cultivated under controlled laboratory conditions using standard Bold's Basal Media (BBM) in a 2-litre photobioreactor (PBRs). The standard BBM contained a 7.5 mL of 45 g l^{-1} $NaHCO_3$ with the nutrient medium of 42 mg N l^{-1} and 53 mg P l^{-1}. The green *C.reinhardtii* 13/32C was cultivated in the following conditions: photoperiod 16 hr light, 8 hr dark (16:8); light intensity 250±5 $\mu Em^{-2}s^{-1}$; and temperature 20±3 °C which run in triplicates.

The liquid samples were collected every two days for 14 days for Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), Nitrate (NO_3^-), Phosphate (PO_4^{3-}), Chlorophyll-a, Total Phosphorus (TP) and Total Nitrogen (TKN) using the methods outlined in (APHA, 2012).

RESULTS AND DISCUSSION

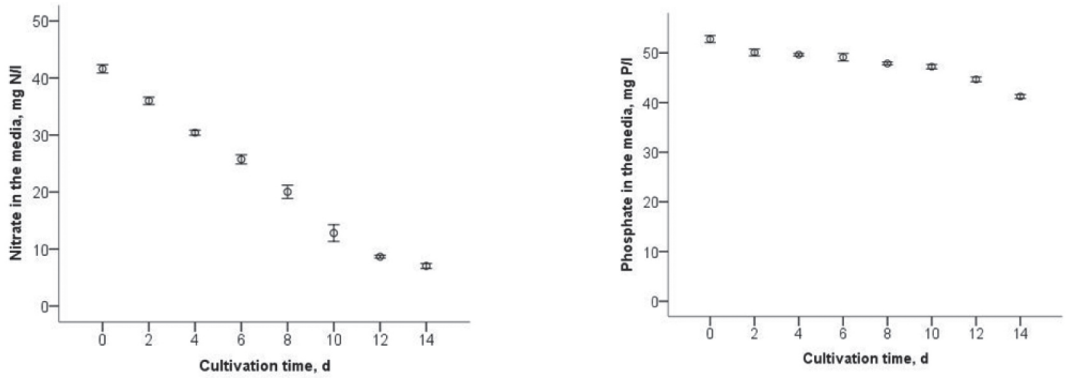
A well-defined growth pattern of *C. reinhardtii* 11/32C cultivated in artificial wastewater (standard BBM) is shown in Fig. 1a. There was a lag phase of about two days required by the algae for the new environmental adaptation. The algal biomass concentration increased exponentially from day two until day 12 that followed a linear regression of natural logarithm organic biomass (ln VSS) was plotted against cultivation time (d) (see Fig. 1b; regression coefficient, $R^2 = 0.99$). In the exponential growth phase, the rate of specific algal growth (μ) was 0.14 $d^{-1}$with biomass productivity was 44 mg VSS L^{-1} d^{-1}. As a consequence, these growths were followed by an increment in N and P uptake in algal cells and decreased of their concentrations in the media (Fig. 2).

The graphs reveal that 83% of the NO_3^- contained in the media (mean initial concentration of 42 mg N L^{-1}) was taken up and incorporated in the algal biomass after 14 days (Fig. 2a). Simultaneously, PO_4^{3-} concentration slightly decreased from 53 to 41 mg P L^{-1} from which only 22% of PO_4^{3-} was taken up by *C.reinhardtii*11/32C (Fig. 2b). Under tested conditions, *C.reinhardtii*11/32C was able to uptake NO_3^- at 2.13 mg N L^{-1} d^{-1} and PO_4^{3-} at 0.5 mg P L^{-1} d^{-1}, which indicates that the microalgae were able to accumulate 5.8% N and 1.6% P (dry weight). The content of N and P in the algal cells was validated by Energy-dispersive X-ray spectroscopy (EDX) analysis. Figure 3 below shows that N and P contents in algal cells cultivated after 14 days were 5.9% and 1.3% respectively, confirming experimental results.



A b

Fig. 1. (a) algal biomass growth; (b) specific growth rate of*C.reinhardtii*11/32C cultivated in standard BBM



A B

Fig. 2. (a)NO_3^- (NO_3^--N L^{-1}); (b) PO_4^{3-} (PO_4^{3-}- P L^{-1}) assimilated by *C.reinhardtii*11/32C in standard BBM

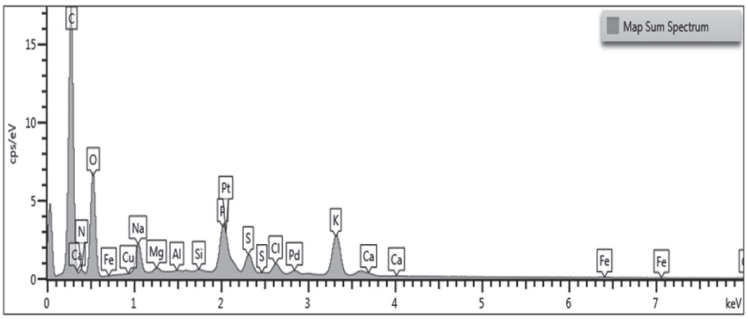


Fig. 3. EDX analysis for N and P content in *C.reinhardtii*11/32C

C. reinhardtii 11/32C used in this study due to its molecular development and genetic tools that useful in various studies. They have flagella structure and function, chloroplast, photosynthetic mechanism and can grow under stress conditions (Harris, 2001; Harris, 2008). This study revealed its ability to recover N and P from synthetic growth media. Under the favourable environment, 83% of N-NO_3^- and 22% of P-PO_4^{3-} were removed from the media, *C.reinhardtii* 11/32C stored 5.9% and 1.3% for N and P respectively. This evidence conceded that phycoremediation offers advantages in removing pollutant from wastewater and convert them as N and P content in microalgae cells (Shahid *et al.*, 2020). The biomass may contain highly valuable substance,including polyunsaturated fatty acids, pigments, carbohydrates, amino acids, and high sugar content (Shahid *et al.*, 2020; Levasseur *et al.*, 2020). *C.reinhardtii* 11/32C cultivated in photobioreactors also shown better in removing nutrients than *C. reinhardtii* grew in an Erlenmeyer flask using synthetic wastewater, which was reported to remove 42-55% N and 12-13% P (Kong *et al.*, 2010).

The use of NO_3^- as a sole nitrogen source in preliminary tests shown that *C.reinhardtii* 11/32C can assimilate nitrate as inorganic nitrogen and not only ammonium. This reaction presented that *C.reinhardtii* 11/32C can use as consortium microorganism with nitrifying bacteria. Vargas, Donoso-Bravo (2016),reported that nitrifying bacteria oxidized NH_4^+ to NO_3^- in raw wastewater up to 60%, while microalgae assimilated 40% of nitrate in a steady-state condition.Moreover, the mechanisms for nitrate metabolisms include the transportation of NO_3^- into the cell where nitrate reductase (NR) catalyzed reduction to NO_2^- which eventually is transported into the chloroplast. Then nitrite reductase (NiR) helped to catalyze further reduction to NH_4^+ to form carbon skeleton (Cai *et al.*, 2013). It means that *C.reinhardtii* 11/32C have enough energy to perform the reduction process in their cells.

Furthermore, these findings also proved that *C.reinhardtii* 11/32C could uptake nutrients from its surrounding environment and to stored reserves at over 1% of P dry weight in their cells. This reserve of P is stored inside the cells as polyphosphate granules (Eixler *et al.*, 2006); in this study, luxurious P uptake was confirmed to take place under non-stress environmental conditions (i.e., no limiting growth conditions).

CONCLUSION

In the present study, synthetic media was used as a source of nutrients for microalgae cultivation to mimic wastewater as a source of nutrient for microalgae growth. *C.reinhardtii* 11/32C can effectively assimilate the nutrient for their growth and store the organic nitrogen and phosphorus in their cell. The nutrient uptake rate reached at 2.13 mg N L^{-1} d^{-1} and at 0.5 mg P L^{-1} d^{-1} for N and P respectively in which generated N and P content of 5.9% and 1.3 %. *C.reinhardtii* 11/32C revealed to be able to accomplish luxurious P uptake under the non-stress condition and proved their possibility to use the microalgae system to retrofit in the existing wastewater treatment.

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