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Immobilized Microalgae using Alginate for Wastewater Treatment

Amanatuzzakiah Abdul Halim* and Wan Nor Atikah Wan Haron

Department of Biotechnology Engineering, Kulliyyah of Engineering, International Islamic University Malaysia (IIUM), Jalan Gombak, 53100 Kuala Lumpur, Malaysia

ABSTRACT

Organic and inorganic substances are released into the environment because of domestic, agricultural, and industrial activities which contribute to the pollution of water bodies. Removal of these substances from wastewater using conventional treatment involves high energy cost for mechanical aeration to provide oxygen for aerobic digestion system. During this process, the aerobic bacteria rapidly consume the organic matter and convert it into single cell proteins, water, and carbon dioxide. Alternatively, this biological treatment step can be accomplished by growing microalgae in the wastewater. *Chlorella vulgaris* immobilized in calcium alginate was used to study the removal efficiency of main nutrients in wastewater such as ammonium and phosphate that act as an important factor in microalgae growth. The immobilized cells demonstrated higher percentage of ammonium and phosphate removal of 83% and 79% respectively, compared to free-suspended cells (76% and 56%). COD removal recorded was 89% and 83% for immobilized cells and free-suspended cells, respectively. The kinetics parameters of nutrients removal for immobilized *C. vulgaris* in synthetic wastewater were also determined. The specific ammonium removal rates (R_A) and phosphate removal rates (R_P) for *Chlorella vulgaris* in synthetic wastewater were 8.3 $mg.L^{-1}day^{-1}$ and 7.9 $mg.L^{-1}day^{-1}$, respectively. On the other hand, the kinetic coefficient for each nutrient removal determined were $k_A = 0.0462\ L.mg^{-1}\ day^{-1}\ NH_4$ and $k_P = 0.0352\ L.mg^{-1}\ day^{-1}\ PO_4^{3-}$. This study proves the application of immobilized microalgae cells is advantageous to the wastewater treatment efficiency. Furthermore, optimization on the immobilization process can be conducted to further improve the nutrients removal rates which potentially can be applied in the large-scale wastewater treatment process.

Keywords: Alginate, ammonium removal, cell immobilization, COD, microalgae, nutrients removal, phosphate removal

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E-mail addresses:
amana@iium.edu.my (Amanatuzzakiah Abdul Halim)
atikahwanharon@gmail.com (Wan Atikah Wan Haron)
* Corresponding author

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INTRODUCTION

Microalgae are unicellular photosynthetic organisms consist of both prokaryotic and eukaryotic and commonly grow in various aquatic environments. Photosynthetic algae are important to the global nutrients cycle such as carbon and oxygen and about 40% of photosynthesis is performed by microalgae (Benedetti et al., 2018). They can easily grow with adequate amounts of carbon (C), nitrogen (N), and phosporus (P) with other essential trace elements (Stockenreiter et al., 2016). In recent years, microalgae have been widely applied in various biotechnology areas such as aquaculture, cosmetic, food and pharmaceutical industries (Rizwan et al., 2018). In addition to that, microalgae have shown a great potential to treat the industrial wastes such as petroleum, heavy metals, dyes, and toxic gases (Kaur et al., 2019; Qiu et al., 2019). These versatile microalgae can also be used in the treatment of agro-industrial (Halim et al., 2019) and aquaculture effluent (Tejido-Nunez et al., 2019).

Water pollution that mainly consists of nitrogen and phosphorus has become a serious issue to environment which contributes to eutrophication. Eutrophication is known as the nutrient enrichment that may change the structure and function of aquatic ecosystems (Pacheco et al., 2015). It can cause damage to biodiversity, ecosystem sustainability and affects human health. Alternatively, these pollutants can be utilized for microalgae growth. Both water medium and necessary nutrients are freely available from the wastewater that are ideal for microalgae cultivation. With various mode of metabolism, microalgae can remove a common pollutant from the water course. Therefore, integrating wastewater treatment with algae cultivation may have the economic potential and simultaneously promoting green technology to treat wastewater as well as contributing to the algae-based chemicals production.

Utilization of free suspended microalgae cells in wastewater treatment has a great potential in removing the nutrients in the wastewater. Despite of the low cost and shorter hydraulic retention time (HRT) compared to the conventional wastewater treatment, this system has low cell loadings and biomass removal issue. The nature of the cells that are negatively charged and small in size (5-50 μm) prevent cells aggregation (Wu et al., 2012). Therefore, the removal of the algal biomass from the treated water requires high energy intensive and costly operation. Various methods have been used to address the biomass removals such as centrifugation, filtration, flotation, and flocculation. On the other hand, immobilization technique can be used to solve the biomass harvesting issue and simultaneously retaining the high-value biomass for further processing.

Various methods of microalgae immobilization have been reported (Kaparaku, 2017). Among them are adsorption on a surface, encapsulation, entrapment within a matrix and containment within a polymer. Majority of the works used entrapment method by employing natural and synthetic polymers. The utilization of alginate bead has been considered as one of the most suitable methods for cell immobilization. This is due to their characteristics

of mild gelation properties, inert aqueous matrix with high porosity that help preserve the physiological properties and functionality of the encapsulated cells (Gao et al., 2016). Moreover, the entrapped microalgae are protected from mechanical stress while nutrients and metabolites can diffuse through the semi-permeable bead (Acarregui et al., 2012). Studies have shown that immobilized cells are more efficient in removing N and P with higher cell loading as compared to free suspended cells (De-Bashan & Bashan, 2010). In this study, microalgae were immobilized in alginate beads and the removal efficiency of the main nutrients and COD was compared with free suspended microalgae cells. The kinetics parameters of nutrients removal for immobilized *C. vulgaris* in synthetic wastewater were also determined.

METHOD

Microalgae Strains

Chlorella vulgaris (Algae Research Laboratory, Institute of Ocean and Earth Sciences, University Malaya) was grown in Tris–acetate–phosphorus (TAP) media and 150 rpm in a rotary shaker. The microalgae cells were grown at room temperature (25°C) in 12 hours light and 12 hours dark cycles.

Immobilization of Microalgae

150 mL of microalgae containing $5x10^6$ cells/mL was centrifuged at 3500 rpm for 10 minutes. The cells were suspended and mixed with 80 mL of 2% alginate solution, and slowly mixed with a stirrer for 15 minutes. The alginate beads were obtained by dropping the alginate–algae mixture from a syringe into 2% $CaCl_2$ solution. The beads formed were left for 1 hour in the solution for hardening process. These beads were washed off several times with sterile distilled water until their pH reached pH 7.

Synthetic Wastewater Preparation

Synthetic wastewater for the experiment was prepared using the following components (mg/L): Glucose (256.41), NH_4Cl (35.33) and KH_2PO_4 (43.8) to obtain 300 mg/L of Chemical Oxygen Demand (COD), 10 mg/L of ammonium and 10 mg/L of phosphorus. The pH was adjusted to 7 with KOH (Hernandez et al., 2006; Mujtaba et al., 2015).

Determination of Microalgae Growth

Cell number was determined once in every two days, using a hemacytometer under a microscope. 10 µl of cell suspension (or 1 drop from a transfer pipette) was added to the hemacytometer and cells were observed under the microscope. For immobilized cells in alginate beads, ten beads were taken from flask and solubilised by immersion in 1 mL of

4% $NaHCO_3$ solution for 30 minutes before the cells were observed under microscope. In determining the optical density, 1 mL of the sample was taken every two days and measured by spectrophotometer at 680 nm of wavelength.

Analytical Tests

The performance of wastewater treatment by microalgae system was indicated by the percentage of COD and nutrients (i.e., ammonium and phosphorus) removals. The percentage of removal was calculated as shown in Equation 1.

$$\%\ of\ removal = \frac{Initial\ concentration\ (m\ g/L) - Final\ concentration\ (m\ g/L)}{Initial\ concentration\ (m\ g/L)} \times 100 \quad [1]$$

Standard methods for the examination of wastewater were used to analyse the COD, ammonium, and phosphorus removals (APHA, 1999). COD, ammonium, and phosphorus were respectively analysed by closed reflux of colorimetric method, phenate method and ascorbic acid method.

RESULTS AND DISCUSSION

Growth of *C. Vulgaris* in TAP Media

The growth profile of free suspended and immobilized *Chlorella vulgaris* in TAP media is shown in Figure 1. Two main growth phases were observed during 10 days of experiment (log phase and stationary phase). No lag phase was observed for both microalgae cultures. The cells number increased exponentially and reached the maximum cells concentration of 53×10^6 cells/mL and entered the stationary phase on day eight of the experiment for free suspended cells. Similar trend was observed for immobilized *C. vulgaris* which showed rapid growth with maximum cells concentration of 64×10^6 cells/mL. Immobilized cells showed better growth than free suspended cells as reported by other studies (Aguilar-May & Sanchez-Saavedra, 2009; Singh, 2003). Ruiz-Marin et al. (2010) reported similar observation on the cell growth where the cells number increased rapidly once the beads were added into the medium. However, immobilized cells may also undergo lag phase because of cell adaptation to the new environment (Shen et al., 2017). The specific growth rate of the immobilized cells is comparable to the free suspended cells during the exponential phase which are 0.13 day^{-1} and 0.10 day^{-1}, respectively. This result shows that the growth of the encapsulated algal cells was not reduced compared to the free cells. The medium nutrients can diffuse through the semi-permeable beads allowing the cell to undergo the cell division process. On the contrary, some entrapment matrices made from synthetic material may produce small pore bead size that restricts nutrients diffusion which affect the

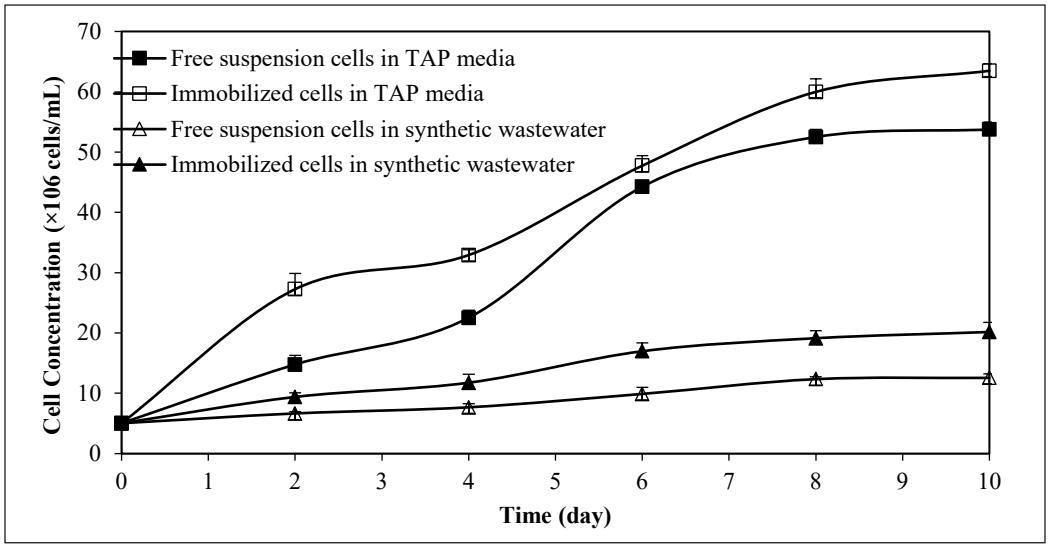

Figure 1. Growth profile of *Chlorella vulgaris* in TAP media and synthetic wastewater. Error bar shows one standard deviation of mean (n=3)

cell morphology and viability (Shen et al., 2017). Moreover, high cell loading may impede the cell growth as there is limited space for the cell to grow or possibly due to restriction on nutrient movement (Leenen et al., 1996). Nevertheless, the results prove that alginate can be used efficiently for cell immobilization.

Growth of *Chlorella vulgaris* in Synthetic Wastewater

The growth profile of both free suspended cells and immobilized cells cultivated in synthetic wastewater is illustrated in Figure 1. Similar trend of growth was observed for both cultures as compared to the growth in TAP media. Maximum concentration for free suspended cells and immobilized cells were 13×10^6 cells/mL and 20×10^6 cells/mL respectively. The increase in cell numbers indicated that the algal cells were able to undergo cell division when cultivated in a limited nutrient medium. While the specific growth rate of free suspension cells and immobilized cells were 0.119 day^{-1} and 0.122 day^{-1} respectively. The maximum number of cells grown in synthetic wastewater were slightly lower than the one cultivated in TAP media which was expected as TAP media contains optimum nutrients needed for microalgae growth while the synthetic medium only consist a few nutrients. However, these data show that synthetic wastewater were able to support the growth of the cells with the minimum nutrients of ammonium, phosphate, and carbon source (glucose).

Nutrients Removal

The removal of ammonium by the free suspended and immobilized cells is shown in Figure 2. Nitrogen in the form of ammonium is essential to many functional components

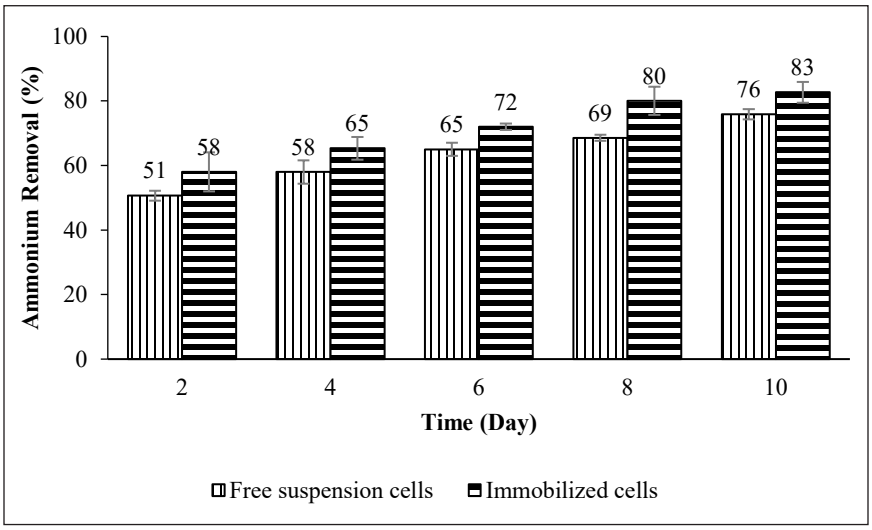

Figure 2. The percentage removal of ammonium by free suspension cells and immobilized cells. Error bar shows one standard deviation (n=3)

of algae and reported to be the main source of nitrogen for algae. The nutrient uptake occurs through direct assimilation between the cells and ammonium based on the form and concentration available (Sanz-Luque et al., 2015). Both cells type achieved rapid removal of 51-58% on day 2 and gradually increased until day 10. The rate of ammonium removal is at the highest during the first two days when the nutrient availability was high. The high ammonia concentration triggered the rapid uptake where the cells passed from a least concentrated to a more concentrated medium. As the day progressed, the nutrient uptake slows down and achieved a total of 76% and 83% ammonium removal on day 10 for free and immobilized cells, respectively. The results show the immobilized *C. vulgaris* has slightly higher percentage removal than the free suspended cells. These values were well correlated with the cell concentration profile in Figure 1. Similar trend was reported by several studies on higher ammonium removal for alginate-immobilized microalgae as compared to the free suspended cells (Banerjee et al., 2019; Soo et al., 2017).

The phosphate uptake by the cells started slowing around 21% and 26 % on day 2 and achieved final removal of 56% and 79% on day 10 for free cells and immobilized cells respectively (Figure 3). The percentage nutrient removal by microalgae cells is lower for phosphate compared to the ammonium especially for free suspended cells. In general, ammonium is the most preferable nitrogen source as it requires less energy for its uptake compared to the other form of nutrients (Delgadillo-Mirquez et al., 2016). Similar trend was observed by other studies which reported higher removal of ammonium compared to phosphate (Samsudin et al., 2018; Shen et al., 2017). Rapid removal of COD was observed on day 2 by freely suspended (61%) and immobilized (71%) *C. vulgaris* (Figure 4). The percentage removal gradually increased until day 10 with maximum removal of 89% by

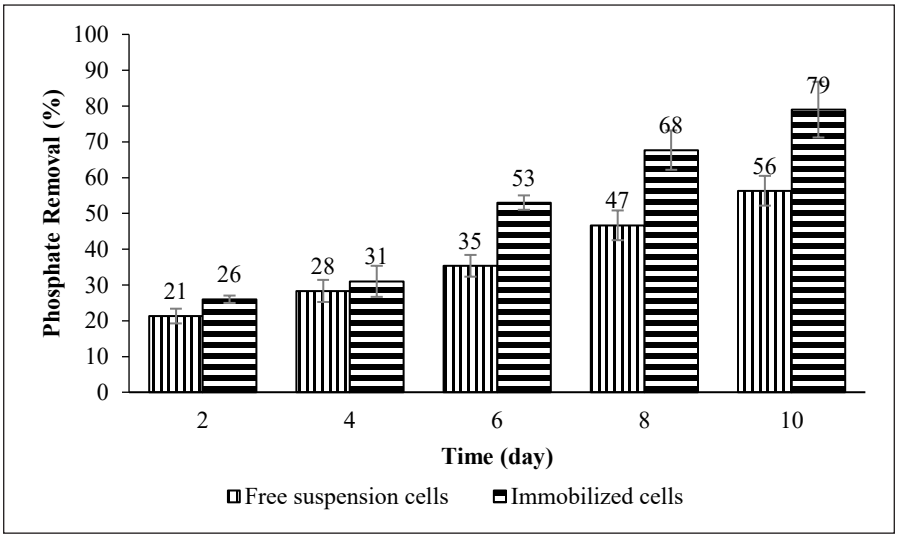

Figure 3. The percentage removal of phosphate by free suspension cells and immobilized cells. Error bar shows one standard deviation (n=3)

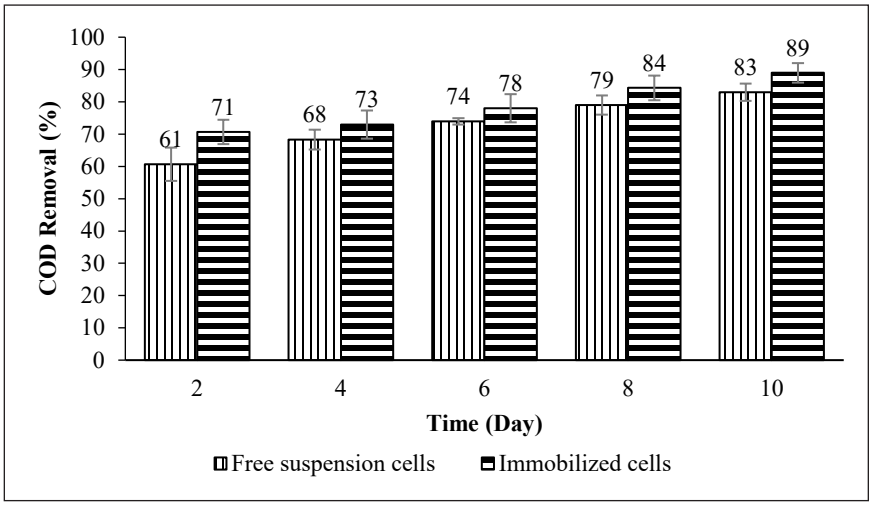

Figure 4. The percentage removal of COD by free suspension cells and immobilized cells. Error bar shows one standard deviation (n=3)

the immobilized cells and 83% for free cells. On the first 2 days of the cell cultivation, the cells were actively doubling their number in which reflected the higher uptake of glucose that provide high amount of energy needed for the cell's activities, hence the consumption of glucose as the carbon source was very high within the short period. This indicates high efficiency removal of COD by microalgae cells.

Based on the results discussed above, the performance of nutrient removal of immobilized microalgae is relatively higher compared to the free cells. Immobilized

Chlorella vulgaris exhibits uptake rates of 23%, 7% and 6% higher than suspended free algae for phosphate, ammonium, and COD, respectively. This result is consistent with the previous findings in other study (Covarrubias et al., 2012). The enhanced removal performance can be described based on the chemical interactions between alginate matrix and the nutrients. The presence of carboxyl group (COO^-) attracts cations and the free binding site on the surface of the alginate matrix provides the medium for physical adsorption for both NH^{4+} and PO_4^{3-} ions before assimilation process occur by the encapsulated algae (Banerjee et al., 2019). Higher adsorption rate is expected with increment in alginate concentration. The optimum adsorption rate was achieved at 3% alginate concentration with high removal of nutrients conducted using *Chlorella vulgaris* (Banerjee et al., 2019). The removal of nutrients by the alginate bead alone was reported to be minimal compared to the alginate-immobilized microalgae cells (Banerjee et al., 2019). Even though the contribution of alginate in removing nutrients are evident, the primary mechanism of nutrient removal through microalgae uptake is more significant compared to the chemical and physical processes. Moreover, the utilization of immobilized cells using alginate matrix provides protective environment against the mechanical shear stress and external microorganisms contamination which enhanced the overall nutrients removal performance.

Kinetics of Nutrient Removal by Immobilized *Chlorella vulgaris*

In this study, the ammonium and phosphate removal rates were determined using Equation 2.

$$R = \frac{S_o - S_i}{t_i - t_o} \quad [2]$$

Where R represents the removal rate of nutrients (ammonium and phosphate), S_o represents the initial concentration of nutrients, S_i is the nutrient concentration at time t_i and t_i is the time when there was no significant change taking place for nutrient concentration. The specific ammonium removal rates (R_A) and phosphate removal rates (R_P) for *Chlorella vulgaris* in synthetic wastewater were 8.3 $mg.L^{-1}day^{-1}$ and 7.9 $mg.L^{-1}day^{-1}$, respectively.

In this study, ammonium and phosphate removal fitted well with the second order reaction of equal initial concentration of nutrients (ammonium and phosphate). The defining second-order differential Equation 3 and 4 as below:

$$-r_A = -\frac{dC_A}{dt} = kC_A^2 = kC_{A0}^2(1 - X_A)^2 \quad [3]$$

Which on integration yields

$$\frac{1}{C_A} - \frac{1}{C_{A0}} = \frac{1}{C_{A0}} - \frac{X_A}{1 - X_A} = kt \quad [4]$$

Therefore, the reaction of ammonium and phosphate can be expressed by Equation 5.

$$C_A = C_{A0}\, e^{-kt} \qquad [5]$$

where C_A represents nutrient concentration at time t_i, C_{AO} represents the initial concentration of nutrient, and k is the second order reaction constant.

Experimental data given in Figure 2 were plotted in the form of $1/C_A$ versus time as shown in Figure 5. From the slope and intercept of best fit line of this plot, kinetic coefficient of ammonium removal by *C. vulgaris* were determined with k_A = 0.0462 L.mg^{-1} day^{-1} NH_4 (R^2 = 0.9806).

Similarly, from the experimental data in Figure 3, the coefficient for phosphate removal was found with k_P = 0.0352 L.mg^{-1} day^{-1} PO_4^{3-} from the intercept and slope of best fit line of $1/C_A$ versus time, (R^2 = 0.8711) as illustrated in Figure 6.

The yield coefficient for ammonium and phosphate removal was calculated using Equation 6.

$$Y = \frac{C - C_o}{S_0 - S} \qquad [6]$$

where Y is the yield of biomass linked with the consumption of nutrients, S_O is initial nutrient concentrations and S is final nutrient concentration. C represents the concentration of cells with respect to the nutrient S and C_O is the initial concentration of the cells. Therefore, the kinetics parameters of nutrients removal for *C. vulgaris* in synthetic wastewater are summarized in Table 1.

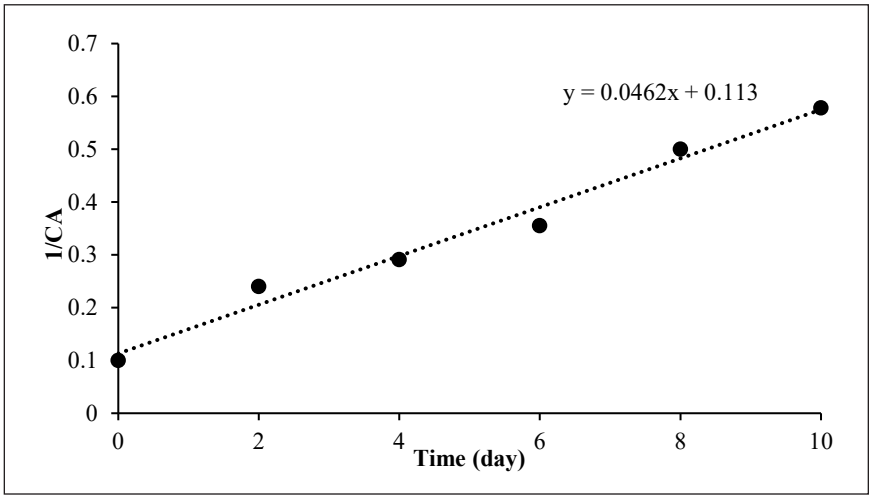

Figure 5. Determination of kinetic coefficient for ammonium removal by *C. vulgaris*

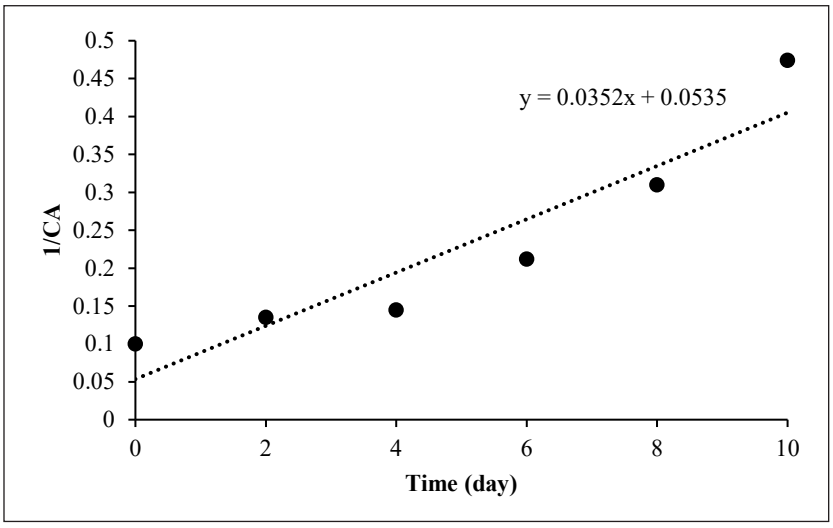

Figure 6. Determination of kinetic coefficient for phosphate removal by *C. vulgaris*

The specific ammonium removal rates (R_A) and phosphate removal rates (R_P) for *C. vulgaris* obtained were 8.3 mg.L^{-1}day^{-1} and 7.9 mg.L^{-1}day^{-1}, respectively. Other studies reported various results depending on the condition of the experiments such as the initial nutrient concentration, reaction time, and bead's condition. Aslan and Kapdan (2006) reported 10.5 mg NH_4 L^{-1}day^{-1} and 2.0 mg PO_4^{3-} L^{-1}day^{-1} while Lau et al. (1998) obtained 5.44 mg NH_4 L^{-1}day^{-1} and 1.3 mg PO_4^{3-} L^{-1}day^{-1}, both were using *C. vulgaris* cells. On the other hand, the biomass yield based on ammonium and phosphate consumption obtained were 0.0407 mg.mg^{-1}NH_4 and 0.0318 mg.mg^{-1} PO_4^{3-} respectively. The ratio of $\frac{Y_A}{Y_P}$ (0.781) indicates the amount of the ratio of P/A required (mg P consumed/mg A consumed) to produce the same unit amount of biomass. The kinetic coefficient for ammonium and phosphate removal were determined based on second order reaction model with k_A = 0.0462 L.mg^{-1} day^{-1} NH_4 and k_P = 0.0352 L.mg^{-1} day^{-1} PO_4^{3-}. In other study the kinetic coefficients reported were k_A = 0.00249 L.mg^{-1} day^{-1} NH_4 and k_P = 0.192 L.mg^{-1} day^{-1} PO_4^{3-} for ammonium and phosphate removal by *C. vulgaris* respectively (Banerjee et al., 2019). The difference value between these kinetic coefficients is due to the different conditions of the experiments as well as the model used.

Table 1
Kinetics parameters of nutrients removal for immobilized C. vulgaris in synthetic wastewater

Kinetics Parameters	Data
Initial N/P (mass ratio)	1
R_A (mg.L^{-1}day^{-1})	8.3
R_P (mg.L^{-1}day^{-1})	7.9
Y_A (mg mg^{-1} A)	0.0407
Y_P (mg mg^{-1} P)	0.0318
Y_P/ Y_A	0.781
k_A (L.mg^{-1} day^{-1} NH_4)	0.0462
k_P (L.mg^{-1} day^{-1} PO_4^{3-})	0.0352

CONCLUSION

This present study demonstrated that alginate immobilized *C. vulgaris* showed higher nutrients (ammonium and phosphate) and COD removal efficiency from the synthetic wastewater than the free cells. The percentage removal of ammonium and phosphate for immobilized cells were about 83% and 79%, respectively compared to free suspension cells which were 75% for ammonium removal and 56% for phosphate removal. Similarly, with the COD, over 80% of glucose were removed from the synthetic wastewater by both the alginate immobilized microalgae cells and free cells within 10 days. The main reasons for high removal performance could be correlated to the high metabolic activities of the immobilized cells and the interaction between the supporting materials and the nutrients ions in wastewater. The kinetics data determined in this study include, the specific ammonium removal rates (R_A) and phosphate removal rates (R_P) which were 8.3 $mg.L^{-1}day^{-1}$ and 7.9 $mg.L^{-1}day^{-1}$, respectively. Moreover, the kinetic coefficient for each nutrient removal obtained were $k_A = 0.0462$ $L.mg^{-1}\,day^{-1}$ NH_4 and $k_P = 0.0352$ $L.mg^{-1}\,day^{-1}$ PO_4^{3-}. The findings from this study provide some insights on the utilization of immobilized cells in wastewater treatment. Nevertheless, this method can be further integrated with several components such as CO_2 biofixation and biofuel production to efficiently manage the environmental issues.

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REFERENCES

Acarregui, A., Murua, A., Pedraz, J. L., Orive, G., & Hernandez, R. M. (2012). A perspective on bioactive cell mircoencapsulation. *BioDrugs, 26*, 283-301. https://doi.org/10.1007/bf03261887

Aguilar-May, B., & Sánchez-Saavedra, M. P. (2009). Growth and removal of nitrogen and phosphorus by free living and chitosan-immobilized cells of the marine cyanobacterium *Synechococcus elongates*. *Journal of Applied Phycology, 21*, 353-360. https://doi.org/10.1007/s10811-008-9376-7

APHA. (1999). *Standard methods for the examination of water and wastewater part 4000 inorganic nonmetallic constituents*. American Public Health Association. https://doi.org/10.2105/SMWW.2882.067

Aslan, S., & Kapdan, I. K. (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological Engineering, 28*, 64-70. https://doi.org/10.1016/j.ecoleng.2006.04.003.

Banerjee, S., Tiwade, P. B., Sambhav, K., Banerjee, C., & Bhaumik, S. K. (2019). Effect of alginate concentration in wastewater nutrient removal using alginate-immobilized microalgae beads: Uptake kinetics and adsorption studies. *Biochemical Engineering Journal, 149*, Article 107241. https://doi.org/10.1016/j.bej.2019.107241

Benedetti, M., Vecchi, V., Barera, S., & Dall'Osto, L. (2018). Biomass from microalgae: The potential of domestication towards sustainable biofactories. *Microbial Cell Factories, 17*(1), 1-18. https://doi.org/10.1186/s12934-018-1019-3

Covarrubias, S. A., de-Bashan, L. E., Moreno, M., & Bashan, Y. (2012). Alginate beads provide a beneficial physical barrier against native microorganisms in wastewater treated with immobilized bacteria and microalgae. *Applied Microbiology and Biotechnology, 93*, 2669-2680. https://doi.org/10.1007/s00253-011-3585-8

De-Bashan, L. E., & Bashan, Y. (2010). Immobilized microalgae for removing pollutants: Review of practical aspects. *Bioresource Technology, 101*(6), 1611-1627. https://doi.org/10.1016/j.biortech.2009.09.043

Delgadillo-Mirquez, L., Lopes, F., Taidi, B., & Pareau, D. (2016). Nitrogen and phosphate removal from wastewater with a mixed microalgae and bacteria culture. *Biotechnology Reports, 11*, 18-26. https://doi.org/10.1016/j.btre.2016.04.003

Gao, H., Khera, E., Lee, J. K., & Wen, F. (2016). Immobilization of multi-biocatalysts in alginate beads for cofactor regeneration and improved reusability. *Journal of Visualized Experiments*, (110), Article 53944. https://doi.org/10.3791/53944

Halim, A. A., Samsudin, A., Azmi, A. S., & Nawi, M. M. N. (2019). Nutrient and chemical oxygen demand (COD) removals by mircoalgae-bacteria co-culture system in palm mill oil effluent (POME). *IIUM Engineering Journal, 20*(2), 22-31. https://doi.org/10.31436/iiumej.v20i2.1109

Hernandez, J., Luz, E., & Bashan, Y. (2006). Starvation enhances phosphorus removal from wastewater by the with *Azospirillum brasilense. Enzyme and Microbial Technology, 38*, 190-198. https://doi.org/10.1016/j.enzmictec.2005.06.005

Kaparapu, J. (2017). Micro algal immobilization techniques. *Journal of Algal Biomass Utilization*, *8*(1), 64-70.

Kaur, H., Rajor, A., & Kaleka, A. S. (2019). Role of phycoremediation to remove heavy metals from sewage water: Review article. *Journal of Environmental Science and Technology, 12*, 1-9. https://doi.org/10.3923/jest.2019.1.9

Lau, P. S., Tam, N. F. Y., & Wong, Y. S. (1998). Effect of carrageenan immobilization on the physiological activities of *Chlorella vulgaris*. *Bioresource Technology, 63*(2), 115-121. https://doi.org/10.1016/S0960-8524(97)00111-9

Leenen, E. J. T. M., Dos Santos, V. A. P., Grolle, K. C. F., Tramper, J., & Wijffels, R. H. (1996). Characteristics of and selection criteria for support materials for cell immobilization in wastewater treatment. *Water Research*, 30(12), 2985-2996. https://doi.org/10.1016/s0043-1354(96)00209-6

Mujtaba, G., Rizwan, M., & Lee, K. (2015). Simultaneous removal of inorganic nutrients and organic carbon by symbiotic co-culture of *Chlorella vulgaris* and *Pseudomonas putida*. *Biotechnology and Bioprocess Engineering, 20*(6), 1114-1122. https://doi.org/10.1007/s12257-015-0421-5

Pacheco, M. M., Hoeltz, M., Moraes, M. S. A., & Schneider, R. C. S. (2015). Microalgae: Cultivation techniques and wastewater phycoremediation. *Journal of Environmental Science and Health. Part A, Toxic/hazardous Substances & Environmental Engineering, 50*(6), 585-601. https://doi.org/10.1080/10934529.2015.994951

Qiu, S., Wang, L., Champagne, P., Cao, G., Chen, Z., Wang, S., & Ge, S. (2019). Effects of crystalline nanocellulose on wastewater-cultivated microalgal separation and biomass composition. *Applied Energy, 239*, 207-217. https://doi.org/10.1016/j.apenergy.2019.01.212

Rizwan, M., Mujtaba, G., Memon, S. A., Lee, K., & Rashid, N. (2018). Exploring the potential of microalgae for new biotechnology applications and beyond: A review. *Renewable and Sustainable Energy Reviews, 92*, 394-404. https://doi.org/10.1016/j.rser.2018.04.034

Ruiz-Marin, A., Mendoza-Espinosa, L. G., & Stephenson, T. (2010). Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresource Technology, 101*(1), 58-64. https://doi.org/10.1016/j.biortech.2009.02.076

Samsudin, A., Azmi, A. S., Nawi, M. N., & Halim, A. A. (2018). Wastewater treatment by microalgae-bacteria co-culture system. *Malaysian Journal of Microbiology, 14*(2), 131-136. https://doi.org/10.21161/mjm.97818

Sanz-Luque, E., Chamizo-Ampudia, A., Llamas, A., Galvan, A., & Fernandez, E. (2015). Understanding nitrate assimilation and its regulation in microalgae. *Frontiers in Plant Science*, 6(10), 1-17. https://doi.org/10.3389/fpls.2015.00899

Shen, Y., Gao, J., & Li, L. (2017). Municipal wastewater treatment via co-immobilized microalgal-bacterial symbiosis: Microorganism growth and nutrients removal. *Bioresource Technology, 243*, 905-913. https://doi.org/10.1016/j.biortech.2017.07.041

Singh, Y. (2003). Photosynthetic activity, and lipid and hydrocarbon production by alginate-immobilized cells of Botryococcus in relation to growth phase. *Journal of Microbiology and Biotechnology, 13*(5), 687-691.

Soo, C. L., Chen, C. A., Bojo, O., & Hii, Y. S. (2017). Feasibility of marine microalgae immobilization in alginate bead for marine water treatment: Bead stability, cell growth, and ammonia removal. *International Journal of Polymer Science, 2017*, Article 6951212. https://doi.org/10.1155/2017/6951212

Stockenreiter, M., Haupt, F., Seppälä, J., Tamminen, T., & Spilling, K. (2016). Nutrient uptake and lipid yield in diverse microalgal communities grown in wastewater. *Algal Research, 15*, 77-82. https://doi.org/10.1016/j.algal.2016.02.013

Tejido-Nuñez, Y., Aymerich, E., Sancho, L., & Refardt, D. (2019). Treatment of aquaculture effluent with *Chlorella vulgaris* and *Tetradesmus obliquus*: The effect of pretreatment on microalgae growth and nutrient removal efficiency. *Ecological Engineering, 136*, 1-9. https://doi.org/10.1016/j.ecoleng.2019.05.021

Wu, Z., Zhu, Y., Huang, W., Zhang, C., Li, T., Zhang, Y., & Li, A. (2012). Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. *Bioresource Technology, 110*, 496-502. https://doi.org/10.1016/j.biortech.2012.01.101.