

## Nutrient Enrichment in Alginate Bead for Enhancement of Cell Growth and Ammonium Removal by Alginate Immobilized *Nannochloropsis* sp.

(Pengayaan Nutrien dalam Manik Alginat bagi Penambahbaikan Pertumbuhan Sel dan Penyingkiran Ammonia oleh Pegun Alginat *Nannochloropsis* sp.)

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### ABSTRACT

*Microalgae have been immobilized in alginate matrixes for the ease of harvesting of the microalgae. However, not all microalgae thrive when immobilized. Immobilized cells are sensitive to the micro-environment in alginate matrixes. Hence, this study aims to improve the growth of immobilized cells and also ammoniacal-nitrogen ( $\text{NH}_4^+\text{-N}$ ) removal by adding growth medium in alginate beads during the immobilization process. Different strength of the Guillard's f/2 medium (0%, 30%, 50%, and 100%) was enriched in alginate beads inoculated with *Nannochloropsis* sp. The immobilized cells were then cultivated in seawater with  $1000 \mu\text{M}$   $\text{NH}_4^+\text{-N}$  as the sole nitrogen source. The growth and  $\text{NH}_4^+\text{-N}$  uptake by immobilized cells enriched with different strength of f/2 medium were compared. There were no significant differences on specific growth rate and specific uptake rate of immobilized cells after nutrient enrichment in alginate beads. However, maximum cells attained and maximum  $\text{NH}_4^+\text{-N}$  removed increased when strength of f/2 medium enriched in alginate beads was increased. Significantly higher maximum cells attained and maximum  $\text{NH}_4^+\text{-N}$  removal were found in immobilized cells enriched with half strength of f/2 medium than those without nutrient enrichment. The present study demonstrated that nutrient enriched in alginate beads can be used by immobilized cells, and subsequently improves the performance of immobilized cells in marine water treatment and production of microalgal biomass simultaneously.*

*Keywords: Alginate bead; ammonia removal; immobilization; Nannochloropsis sp.; nutrient enrichment*

### ABSTRAK

*Mikroalga dipegunkan pada matriks alginat bagi memudahkan penuaian mikroalga. Walau bagaimanapun, tidak semua mikroalga dapat hidup dengan baik apabila dipegunkan. Sel yang dipegunkan adalah sensitif terhadap mikrosekitaran di dalam matriks alginat. Oleh itu, kajian ini dilakukan bertujuan untuk menambahbaik pertumbuhan sel yang dipegunkan dan juga penyingkiran nitrogen ammonia ( $\text{NH}_4^+\text{-N}$ ) dengan menambahkan medium pertumbuhan di dalam manik alginat semasa proses pemegunan. Medium Guillard f/2 dengan kepekatan yang berbeza (0%, 30%, 50% dan 100%) telah diperkayakan dalam manik alginat yang diinokulasi dengan *Nannochloropsis* sp. Sel yang telah dipegunkan kemudiannya dikulturkan ke dalam air laut yang dibekalkan dengan  $1000 \mu\text{M}$   $\text{NH}_4^+\text{-N}$  sebagai sumber nitrogen tunggal. Pertumbuhan dan pengambilan  $\text{NH}_4^+\text{-N}$  oleh sel pegun yang diperkayakan dengan kepekatan medium f/2 yang berbeza telah dibandingkan. Kadar tumbesaran khusus dan kadar pengambilan khusus bagi sel pegun tidak berbeza dengan ketara selepas pengayaan nutrien dalam manik alginat. Namun begitu, jumlah sel maksimum yang diperolehi dan  $\text{NH}_4^+\text{-N}$  maksimum yang disingkirkan telah meningkat apabila kepekatan medium f/2 yang diperkayakan di dalam manik alginat meningkat. Jumlah sel maksimum yang diperolehi dan  $\text{NH}_4^+\text{-N}$  maksimum yang disingkirkan adalah nyata lebih tinggi di dalam sel pegun yang diperkayakan dengan kepekatan separuh medium f/2 berbanding dengan sel pegun tanpa pengayaan nutrien. Kajian ini menunjukkan bahawa pengayaan nutrien dalam manik alginat dapat digunakan oleh sel pegun dan seterusnya meningkatkan prestasi sel pegun dalam rawatan air marin dan penghasilan biojisim mikroalga secara serentak.*

*Kata kunci: Manik alginat; Nannochloropsis sp.; pemegunan; pengayaan nutrien; penyingkiran ammonia*

## INTRODUCTION

The difficulty and high cost in harvesting microalgae are one of the main constraints for the cost-effective production of microalgal biomass (Khan et al. 2018; Tan et al. 2020) and sustainable phycoremediation (Emparan et al. 2019). For this, microalgae have been widely immobilized in sodium alginate gel hardened with calcium for the ease of harvesting process (De-Bashan & Bashan 2010; Lv et al. 2017; Pérez-Martínez et al. 2010; Soo et al. 2017). The alginate has been regarded as an excellent polysaccharide for immobilization process due to its mild procedure during the process. Immobilized microalgae do not suffer extreme changes of physical-chemical conditions which allow higher cell viability (Pane et al. 1998). Even so, not all microalgae thrive when immobilized (Moreno-Garrido et al. 2005). Several factors could affect the survival and growth of immobilized microalgae, including temperature, water pH, light intensity, among others; particularly, nutrients could directly influence the microalgae growth (Zhuang et al. 2018). The restriction of nutrients to immobilized cells even in an abbreviated time frame could influence the survival and growth of immobilized cells.

Nutrient enrichment in alginate solution is a novel approach to improve the performance of the immobilized cell. To date, CO<sub>2</sub> supplement has been used to improve nutrient uptake of alginate immobilized *Scenedesmus bicellularis* in water treatment (Kaya et al. 1996). The authors found that intermittent CO<sub>2</sub> enrichment during nutrient deprivation of immobilized microalgae cells would stimulate photosynthesis and subsequently accelerate tertiary wastewater treatment. The idea of nutrient enrichment in alginate beads in the present study is contrary to starvation of immobilized cells prior to the water treatment. A starvation period has been used to improve nutrients uptake of *Chlorella* in water treatment (Hernandez et al. 2006; Zhang et al. 2012). However, negative effects such as declined population of microalgae

and damage to cells could happen if starvation period is not controlled well.

Satisfying the nutrient requirement of immobilized cells during acclimatization period could be an optimization technique in microalgae immobilization. Nutrient added in beads serves as a supplement for immobilized microalgae. As immobilized cells are very sensitive to their micro-environment, nutrient enrichment in bead creates a better circumstance for immobilized cells to survive. To the best of our knowledge, this is the first study where nutrient was enriched in alginate beads during microalgae immobilization. This study aimed to investigate the feasibility of nutrient enrichment in Ca-alginate beads to improve the growth of immobilized *Nannochloropsis* sp. and subsequently, to enhance the ammoniacal-nitrogen removal process in marine water treatment.

## MATERIALS AND METHODS

## MICROALGAE SPECIES AND CULTURE

*Nannochloropsis* sp. was obtained from the marine microalgae stock culture in the Universiti Malaysia Terengganu (UMT), Malaysia. The microalgae were cultivated under standard culture conditions as described in Hii et al. (2011). In brief, the Guillard's *f/2* medium (Smith et al. 1993) was used for the microalgae stock culture *via* batch culture technique with a constant illumination of 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  from a cool-white fluorescent light at 28 °C, salinity of 30 ppt, and pH value of 8. Table 1 lists the composition of essential nutrients in the *f/2* medium with slight modification where silicate, Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O, was not added in the medium because *Nannochloropsis* sp. does not contain silicate cell wall. The growth phase and cell density of microalgae stock culture were monitored *via* optical density method at 600 nm.

TABLE 1. Composition of Guillard's *f/2* medium (Smith et al. 1993) with some modifications

Nutrients	Chemicals	Final concentration (mg L <sup>-1</sup> )
Nitrate solution	NaNO <sub>3</sub>	75
Phosphate solution	NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	5
Trace Metal/EDTA Solution	Na <sub>2</sub> C <sub>10</sub> H <sub>14</sub> O <sub>8</sub> N <sub>2</sub> ·H <sub>2</sub> O (Na <sub>2</sub> EDTA)	4.36
	CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.01
	CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01
	FeCl <sub>3</sub> ·6H <sub>2</sub> O	3.15
	MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.18
	Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.006
	ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.022
Vitamin Solution	Thiamin HCl (Vitamin B <sub>1</sub> )	0.1
	Biotin (Vitamin H)	0.0005
	Cyanobalamin (Vitamin B <sub>12</sub> )	0.0005

#### IMMOBILIZATION OF MICROALGAE CELL

Immobilization of microalgae was conducted as described in Soo et al. (2017). Alginate solution was prepared by dissolving 5% (w/v) sodium alginate (Fisher Scientific, USA) in warm distilled water and autoclaved at 121 °C and 15 psi pressure for 20 min. An aliquot of exponentially growing *Nannochloropsis* sp. culture was harvested by centrifugation at  $1542 \times g$  for 15 min. The cells were washed twice with pasteurized seawater and resuspended in the pasteurized seawater before the cell densities were determined spectrophotometrically at 600 nm. The concentrated *Nannochloropsis* sp. cell suspension (< 1 mL/100 mL of alginate solution) was mixed with the above-mentioned alginate solution to obtain an alginate-cell suspension. Alginate-cell suspension was added drop wise into a 2% (w/v)  $\text{CaCl}_2$  solution by using a peristaltic pump (MASTERFLEX), from a height of 2.5 cm and at a rate of one drop per second. The 4 mm beads were kept stirring in  $\text{CaCl}_2$  solution for 30 min to allow complete hardening of the alginate. The beads were washed several times with pasteurized seawater to eliminate the remaining  $\text{CaCl}_2$ . The initial cell density in the bead was approximately  $0.4 \times 10^6$  cell bead<sup>-1</sup>.

#### EXPERIMENTAL DESIGN

Four types of beads enriched with different strengths of the Guillard's f/2 medium were formed in the present study. Three different strengths of Guillard's f/2 medium (30%, 50%, and 100%) were added into each alginate solution prior to bead making process. Alginate solution without Guillard's f/2 medium enrichment was used as control. In triplicate, each type of beads (100 mL) was cultured in seawater (300 mL) that enriched with 1000  $\mu\text{M}$   $\text{NH}_4^+\text{-N}$  as a sole nitrogen source. The nutrient content of seawater was determined prior to the experiment. As the nutrient concentration in seawater was low ( $\text{NH}_4^+\text{-N}$  < detection limit,  $\text{NO}_3^-\text{-N}$  < 65  $\mu\text{M}$ , and  $\text{PO}_4^{3-}\text{-P}$  < 1.0  $\mu\text{M}$ ), the 1000  $\mu\text{M}$   $\text{NH}_4^+\text{-N}$  added in the seawater was regarded as the sole source of nitrogen in the experiments. Approximately 3000 beads were formed from a 100 mL of alginate solution. All the flasks were positioned randomly on an orbital shaker (250 rpm) under standard culture conditions as described above. The water pH value was recorded with a digital pH meter (WTW SERIES) and maintained at pH 8 to pH 9 with 0.01 M autoclaved HCl throughout the experiment. The immobilized cell was cultured for a total of 216 h.

Eight to ten samplings were conducted throughout the experiment. At each sampling times, 5 mL of beads and 15 mL of seawater sample (ratio 1:3) were taken from each treatment. The alginate beads and seawater samples were taken proportionally to the initial experiment

setup. The ration of the amount of culture volume and number of beads left was always constant. The number of cells in beads was counted by an improved Neubauer haemocytometer after dissolving 10 alginate beads in a 0.5 M tri-sodium citrate solution. The  $\text{NH}_4^+\text{-N}$  concentration in seawater sample was determined by Phenate method (Parsons et al. 1984). To determine cell leakage from the bead, 10 mL of water sample was concentrated into 1 mL sample by centrifugation at the end of the experiment. The cell leakage was assessed by counting the algal cells in the concentrated water sample by an improved Neubauer haemocytometer. The diameter of beads was measured by using a digital caliper (GERE) at the beginning and the end of the experiment.

#### DATA AND STATISTICAL ANALYSIS

The specific growth rate (SGR) and specific uptake rate (SUR) of immobilized cells were obtained by a linear regression of the logistic model following Jimenez-Perez et al. (2004) as follows:

$$\ln((K/Nt) - 1) = a - bt$$

where K is the maximum cell attained (cell bead<sup>-1</sup>) or maximum  $\text{NH}_4^+\text{-N}$  uptake ( $\mu\text{M}$ ) in the culture; Nt is the cell density (cell bead<sup>-1</sup>) or  $\text{NH}_4^+\text{-N}$  concentration ( $\mu\text{M}$ ) at time t; a is the intercept; and b is the specific growth rate (h<sup>-1</sup>) or specific uptake rate (h<sup>-1</sup>).

A one-way ANOVA followed by a Tukey post-hoc test was used to compare the maximum cell attained, SGR, maximum  $\text{NH}_4^+\text{-N}$  removed, and SUR of immobilized cells enriched with 0%, 30%, 50%, and 100% of f/2 medium. The difference was significant if p value  $\leq 0.05$ . All the statistical analyses were carried out by using the Statistical Software for Social Sciences (SPSS Version 22, SPSS Inc. 1995).

#### RESULTS AND DISCUSSION

Figure 1 illustrates that *Nannochloropsis* sp. survives and grows after immobilized in alginate bead, albeit less growth was observed for those without nutrient enrichment. The maximum cell attained by immobilized cell increased from  $8.2 \times 10^5 \pm 4.3 \times 10^4$  cell bead<sup>-1</sup> to  $9.5 \times 10^5 \pm 2.4 \times 10^4$  cell bead<sup>-1</sup> when the strength of f/2 medium enriched in alginate bead increased from 0% to 100%. No significant difference (p value > 0.05) on maximum cell attained between immobilized cells that enriched with 0% (control) and 30% of f/2 medium. The maximum cell attained by immobilized cell was significantly higher (p-value  $\leq 0.05$ ) than that in control when immobilized cell was enriched with half and full

strength of f/2 medium. At the end of the experiment, immobilized *Nannochloropsis* sp. with nutrient enrichment removed higher concentration of  $\text{NH}_4^+\text{-N}$  as compared to the control. The maximum concentration of  $\text{NH}_4^+\text{-N}$  removed by immobilized *Nannochloropsis* sp. was steadily increased with increasing nutrient enrichment (Table 2). Immobilized cells enriched with half and full strength of f/2 medium removed significantly higher  $\text{NH}_4^+\text{-N}$

concentrations than that in control ( $p\text{-value} \leq 0.05$ ). Nevertheless, there were no significant differences ( $p\text{-value} > 0.05$ ) on maximum cell attained and maximum  $\text{NH}_4^+\text{-N}$  removed by immobilized cells that enriched with half and full strength of f/2 medium. The results demonstrated that substantial improvement in maximum cells attained and maximum  $\text{NH}_4^+\text{-N}$  removal by the immobilized cells can be accomplished by adding half strength of f/2 medium in alginate beads during immobilization.

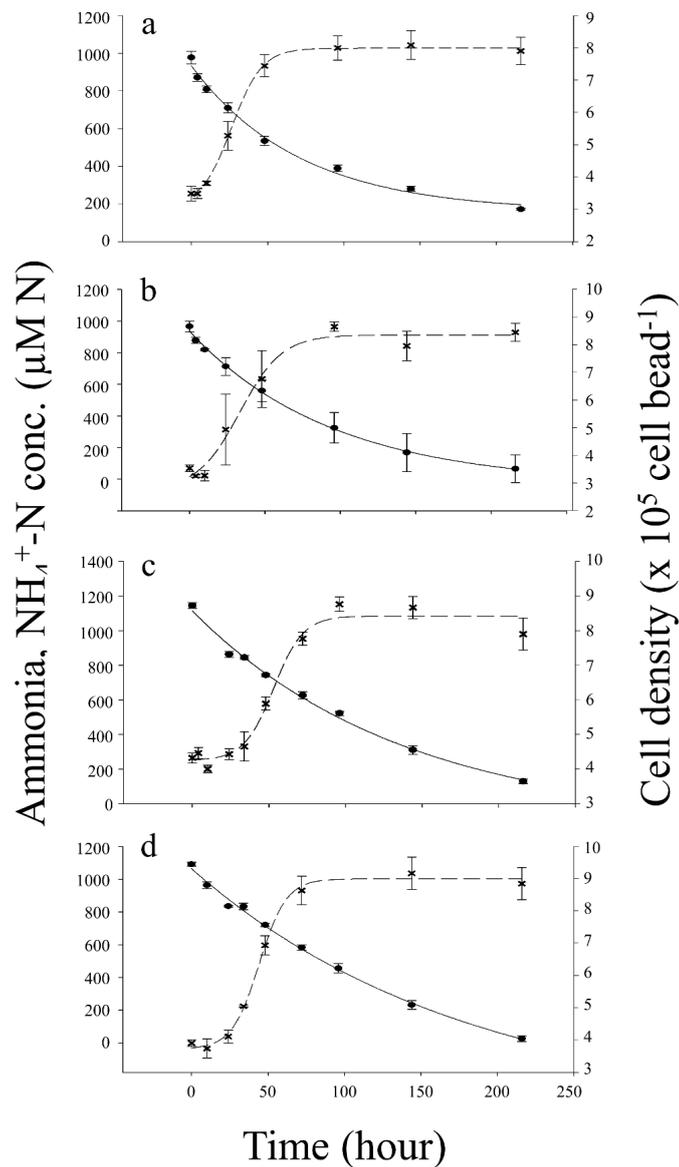


FIGURE 1. Cell growth (dash line) and  $\text{NH}_4^+\text{-N}$  uptake (solid line) by immobilized *Nannochloropsis* sp. enriched with (a) 0%, (b) 30%, (c) 50% and (d) 100% of f/2 medium. All treatment was supplied with  $1000 \mu\text{M}$   $\text{NH}_4^+\text{-N}$  as sole nitrogen source.  $N = 3$  incubations

TABLE 2. Summary of the results and one-way ANOVA of immobilized *Nannochloropsis* sp. enriched with different strength of f/2 medium. The data are means  $\pm$  standard deviation of N = 3 incubations

Strength of f/2 medium enriched in alginate bead (%)				One-way ANOVA				
0 (Control)	30	50	100	Source of variation	df	MS	F	p
Maximum cell attained, cell bead <sup>-1</sup>								
8.2 <sup>a</sup> $\times 10^5 \pm 4.3 \times 10^4$	8.7 <sup>ab</sup> $\times 10^5 \pm 1.7 \times 10^4$	8.9 <sup>bc</sup> $\times 10^5 \pm 1.5 \times 10^4$	9.5 <sup>c</sup> $\times 10^5 \pm 2.4 \times 10^4$	Effect	3	8.5 $\times 10^9$	11.551	<b>0.003</b>
				Error	8	7.4 $\times 10^8$		
SGR, h <sup>-1</sup>								
0.063 <sup>a</sup> $\pm 0.022$	0.039 <sup>a</sup> $\pm 0.016$	0.029 <sup>a</sup> $\pm 0.002$	0.042 <sup>a</sup> $\pm 0.014$	Effect	3	0.001	2.758	0.112
				Error	8	0		
Maximum ammonium concentration removed, $\mu\text{M}$								
798.7 <sup>a</sup> $\pm 28.6$	907.9 <sup>ab</sup> $\pm 80.4$	1014.7 <sup>bc</sup> $\pm 26.4$	1063.8 <sup>c</sup> $\pm 5.9$	Effect	3	41747.2	20.821	<b>0.001</b>
				Error	8	2005.1		
SUR, h <sup>-1</sup>								
0.025 <sup>a</sup> $\pm 0.002$	0.029 <sup>a</sup> $\pm 0.005$	0.021 <sup>a</sup> $\pm 0.002$	0.025 <sup>a</sup> $\pm 0.003$	Effect	3	0	3.565	0.067
				Error	8	0		

<sup>a</sup>Means followed by the same letter within the same row is not significantly different according to a one-way ANOVA test followed by Tukey's test. Significant value of p-value  $\leq 0.05$  is indicated in bold

The present study showed that nutrient enrichment in alginate beads during cell immobilization had no effect on the SGR and SUR of immobilized *Nannochloropsis* sp. Table 2 shows that there was no significant difference (p-value  $> 0.05$ ) on SGR of immobilized cells after enrichment of f/2 medium in alginate beads, with a mean of 0.043 h<sup>-1</sup>. Similarly, SUR of NH<sub>4</sub><sup>+</sup>-N was not significantly different (p-value  $> 0.05$ ) in immobilized cells after nutrient enrichment in alginate beads, ranging from 0.021  $\pm$  0.002 h<sup>-1</sup> to 0.029  $\pm$  0.005 h<sup>-1</sup>. Studies had shown that growth rate and nutrient uptake rate of microalgae are depends on other factors such as nutrient sources and concentrations (Hii et al. 2011; Kwon et al. 2020; Xin et al. 2010). Banerjee et al. (2019) showed that alginate concentration used in microalgae immobilization could also influence their specific growth rate and nutrient uptake rate.

The present study demonstrated that immobilized cell can utilize nutrients enriched in beads. Subsequently, maximum cell attained and NH<sub>4</sub><sup>+</sup>-N removed by immobilized cell increased substantially. It is hypothesized that nutrients enriched in beads help to overcome nutrient limitation of cells when they are being immobilized in alginate beads and incubated in seawater. Although studies have been reported that no restrictions on nutrient diffusion through alginate gel beads was found (Jiménez-Pérez et al. 2004; Ruiz-Marin et al. 2010), other studies

have also demonstrated the existence of restriction to nitrate diffusion through alginate gel beads (Garboya et al. 2002). Despite everything, the availability of nutrients in beads can serves as a temporary nutrient source and utilized by immobilized cells at least at the initial incubation time.

The Guillard's f/2 medium contains nitrogen, phosphorus, trace metals, and vitamins essential for microalgae growth (Table 1). The present study demonstrated that immobilized cell in alginate bead could utilize those essential nutrients enriched in alginate beads as supported by the better growth of immobilized cell with nutrient enrichment. In this study, immobilized cell is incubated in seawater with ammonium being the sole nitrogen source for cell growth. The enrichment of essential nutrients including trace metals and vitamins in the alginate bead is hypothesized to serve as essential supplements for immobilized cell. This is particularly important when immobilized cell was used to treat wastewater that usually contains high nitrogen and phosphorus concentration but might lack of essential trace metals and vitamins for the growth of immobilized cell. Hence, the essential nutrient enrichment in alginate beads is vital for better growth of immobilized cells.

All beads were stable in diameter throughout the experiment; differences of beads in mean diameter were less than 5% (Table 3). All beads enriched with nutrient showed a slight decrease in diameter, ranging from 0.24

to 3.41% at the end of the experiment. Calcium alginate beads without f/2 medium enrichment increased by 0.26% in bead diameter at the end of the experiment. The cell leaching ranged from 0.09 to 0.42% in all cultures. Bead stability is one of the major barriers for the application of alginate beads in marine water (Soo et al. 2017). The instability of beads often gives rise to disintegration of beads and subsequent cell leaching in water treatment. The rupture of beads impedes the water treatment process, reducing the efficiency of nutrient removal. The cells leaching from beads will also reduce the harvesting of microalgal biomass. The present study demonstrated that f/2 medium enrichment in alginate solution prior to bead making has no negative impact on bead stability. The alginate bead was stable throughout the nine days experiment with minimum cell leaching is observed. It can

sustain the growth of the immobilized cell to stationary growth phase which is ready for harvesting.

Previous studies have demonstrated that free living *Nannochloropsis* sp. can be cultivated in aquaculture wastewater (Dourou et al. 2018; Velichkova et al. 2016). The present study demonstrated a promising application of the present immobilized *Nannochloropsis* sp. in alginate beads for marine water treatment and production of microalgal biomass simultaneously. The alginate bead can be easily harvested from water treatment system when immobilized *Nannochloropsis* sp. is entering stationary growth phase with relatively constant cell density. Hence, it is recommended that a replacement of a new batch of immobilized beads every four to five days to ensure the optimum performance of immobilized microalgae for nutrient removal and biomass production simultaneously.

TABLE 3. The stability of alginate bead after immobilized with *Nannochloropsis* sp. and percentage of cell leakage

Strength of f/2 medium enriched in alginate bead (%)	Initial bead size (mm)		Final bead size (mm)		Bead changes (%)	Maximum cell attained (100 mL)		Total cell leached (300 mL)		Cell leakage (%)
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
	0 (Control)	4.04	0.060	4.05		0.040	0.26%	$2.3 \times 10^9$	$9.1 \times 10^7$	
30	4.07	0.015	3.93	0.154	-3.41%	$2.4 \times 10^9$	$1.9 \times 10^8$	$1.0 \times 10^7$	$1.4 \times 10^6$	0.42%
50	4.08	0.006	4.07	0.020	-0.24%	$2.5 \times 10^9$	$9.3 \times 10^7$	$3.1 \times 10^6$	$4.1 \times 10^5$	0.13%
100	3.94	0.032	3.91	0.024	-0.76%	$2.7 \times 10^9$	$2.2 \times 10^8$	$2.5 \times 10^6$	$6.0 \times 10^5$	0.09%

#### CONCLUSION

The present study demonstrated that nutrient enrichment in alginate beads could be a promising method to sustain and enhance the performance of immobilized cells. Essential nutrients enriched in beads play a significant role in cell growth at least at the initial of the incubation of immobilized cells. There were no significant changes on SGR of immobilized cell after enrichment of f/2 medium; yet the maximum cell attained by immobilized cell with nutrient enrichment gradually increased with increasing strength of f/2 medium. As more cells were produced with nutrient enriched in alginate beads, more ammonium was removed by immobilized cell enriched with f/2 medium; though there was no significant difference on SUR of ammonium by immobilized cell. The present study demonstrated that half strength of f/2 medium was enough to significantly enhance the performance of immobilized cells.

#### ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Science, Technology and Innovation (MOSTI) for funding this study through e-science Project; 05-01-12-SF0001.

#### REFERENCES

- 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: 107241.
- De-Bashan, L.E. & Bashan, Y. 2010. Immobilized microalgae for removing pollutants: Review of practical aspects. *Bioresource Technology* 101(6): 1611-1627.
- Dourou, M., Tsolcha, O.N., Tekerekopoulou, A.G., Bokas, D. & Aggelis, G. 2018. Fish farm effluents are suitable growth media for *Nannochloropsis gaditana*, a polyunsaturated fatty acid producing microalga. *Engineering in Life Sciences* 18(11): 851-860.

- Empanan, Q., Harun, R. & Danquah, M.K. 2019. Role of phycoremediation for nutrient removal from wastewaters: A review. *Applied Ecology and Environmental Research* 17(1): 889-915.
- Garboya, I., León, R. & Vilchez, C. 2002. Diffusion characteristics of nitrate and glycerol in alginate. *Colloids and Surfaces B: Biointerfaces* 25(1): 1-9.
- Hernandez, J.P., de-Bashan, L.E. & Bashan, Y. 2006. Starvation enhances phosphorus removal from wastewater by the microalga *Chlorella* spp. co-immobilized with *Azospirillum brasilense*. *Enzyme and Microbial Technology* 38(1-2): 190-198.
- Hii, Y.S., Soo, C.L., Chuah, T.S., Mohd-Azmi, A. & Abol-Munafi, A.B. 2011. Interactive effect of ammonia and nitrate on the nitrogen uptake by *Nannochloropsis* sp. *Journal of Sustainability Science and Management* 6(1): 60-68.
- Jiménez-Pérez, M.V., Sánchez-Castillo, P., Romera, O., Fernández-Moreno, D. & Pérez-Martínez, C. 2004. Growth and nutrient removal in free and immobilized planktonic green algae isolated from pig manure. *Enzyme and Microbial Technology* 34(5): 392-398.
- Kaya, V.M., Goulet, J., de la Noüe, J. & Picard, G. 1996. Effect of intermittent CO<sub>2</sub> enrichment during nutrient starvation on tertiary treatment of wastewater by alginate-immobilized *Scenedesmus bicellularis*. *Enzyme and Microbial Technology* 18(8): 550-554.
- Khan, M.I., Shin, J.H. & Kim, J.D. 2018. The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories* 17(36): 1-21.
- Kwon, G., Nam, J.H., Kim, D.M., Song, C. & Jahng, D. 2020. Growth and nutrient removal of *Chlorella vulgaris* in ammonia-reduced raw and anaerobically-digested piggery wastewaters. *Environmental Engineering Research* 25(2): 135-146.
- Lv, J., Feng, J., Liu, Q. & Xie, S. 2017. Microalgal cultivation in secondary effluent: Recent developments and future work. *International Journal of Molecular Sciences* 18(1): 79.
- Moreno-Garrido, I., Campana, O., Lubián, L.M. & Blasco, J. 2005. Calcium alginate immobilized marine microalgae: Experiments on growth and short-term heavy metal accumulation. *Marine Pollution Bulletin* 51(8-12): 823-829.
- Pane, L., Feletti, M., Bertino, C. & Carli, A. 1998. Viability of the marine microalga *Tetraselmis suecica* grown free and immobilized in alginate beads. *Aquaculture International* 6(6): 411-420.
- Parsons, T.R., Maita, Y. & Lalli, C.M. 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Oxford: Pergamon Press. pp. 3-8.
- Pérez-Martínez, C., Sánchez-Castillo, P. & Jiménez-Pérez, M.V. 2010. Utilization of immobilized benthic algal species for N and P removal. *Journal of Applied Phycology* 22(3): 277-282.
- 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.
- Smith, L.L., Fox, J.M. & Granvil, D.R. 1993. Intensive algae culture techniques. In *CRC Handbook of Mariculture. Volume 1. Crustacean Aquaculture*. 2nd ed. edited by McVey, J.P. Boca Raton, Florida: CRC Press, Inc. pp. 3-14.
- 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: 1-7.
- Tan, J.S., Lee, S.Y., Chew, K.W., Lam, M.K., Lim, J.W., Ho, S.H. & Show, P.L. 2020. A review on microalgae cultivation and harvesting, and their biomass extraction processing using ionic liquids. *Bioengineered* 11(1): 116-129.
- Velichkova, K.N., Sirakov, I.N., Beev, G.G., Denev, S.A. & Pavlov, D.H. 2016. Treatment of wastewater originating from aquaculture and biomass production in laboratory algae bioreactor using different carbon sources. *Sains Malaysiana* 45(4): 601-608.
- Xin, L., Hong-Ying, H., Ke, G. & Jia, Y. 2010. Growth and nutrient removal properties of a freshwater microalga *Scenedesmus* sp. LX1 under different kinds of nitrogen sources. *Ecological Engineering* 36(4): 379-381.
- Zhang, E., Wang, B., Ning, S., Sun, H., Yang, B., Jin, M. & Hou, L. 2012. Ammonia-nitrogen and orthophosphate removal by immobilized *Chlorella* sp. isolated from municipal wastewater for potential use in tertiary treatment. *African Journal of Biotechnology* 11(24): 6529-6534.
- Zhuang, L.L., Yu, D., Zhang, J., Liu, F.F., Wu, Y.H., Zhang, T.Y., Dao, G.H. & Hu, H.Y. 2018. The characteristics and influencing factors of the attached microalgae cultivation: A review. *Renewable and Sustainable Energy Reviews* 94: 1110-1119.

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Received: 21 February 2020

Accepted: 18 August 2020