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Full Length Research Paper

Effect of algal density in bead, bead size and bead concentrations on wastewater nutrient removal

M. S. Abdel Hameed

Botany Department, Faculty of Science, Beni Suef University, Beni suef, Egypt. E-mail: drmohamedsa@yahoo.com.

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Laboratory experiments were performed to study nitrogen and phosphorus uptake by the unicellular green microalga *Chlorella vulgaris* immobilized in calcium alginate beads. Different cell stockings in beads, different bead sizes and different algal bead concentrations in wastewaters were tested. Significant higher nutrients reductions were found in bioreactors containing algal beads than the blank alginate beads (without algae). The bioreactor containing algal beads (4 mm diameter) with 1.5 x 10^6 cells $bead^{-1}$ (cell stocking) at concentration of 10.66 beads ml^{-1} wastewater (1:3 bead: wastewater, v/v) achieved complete removal of NH_4^+-N and about 95% reduction in phosphate removal within the experimental period (48 h). Algal uptake and adsorption on alginate gels were the major processes involved in the removal of N and phosphate from wastewater. Increasing cell stocking in beads did not cause any improvement in the efficiency of treatment, but caused some leakage problems. Also, increasing the beads concentrations in wastewater caused reductions in light penetration and enhanced self-shading effects and the beads settled at the bottom of the reactor.

Key words: Algae, cell stocking, bead concentration, ammonia, phosphate, removal, and wastewater.

INTRODUCTION

Due to increasing human population, upcoming industries and agriculture practices there is an increase in eutrophication of aquatic ecosystem by the release of chemicals, fertilizers in the aquatic ecosystem (Thakur and Kumar, 1999). Removal of ions is highly expensive and energy consuming effort. Use of biological systems serves as a cheap and efficient way of nutrient removal from wastewater.

Microalgae have received more attention in recent years, especially in the tropical and subtropical regions, as an alternative biosystem for wastewater treatment (Fallowfield and Garrett, 1985; de la Noue et al., 1992; Abdel Hameed and Hammouda, 2007). Algal systems have traditionally been employed as a tertiary treatment process (Lavoie and de la Noue, 1985; Oswald, 1988) and have recently been proposed as a potential seconddary treatment system (Tam and Wong, 1989). One of the major problems in using microalgae for wastewater treatment is their recovery from the treated effluent (de la Noue et al., 1992; Laliberte et al., 1994). Immobilization technology, which entraps the microalgal cells into a matrix, solves the harvest problems (Chevalier and de la Noue, 1985). This technology offers a greater degree of operational flexibility and easy separation (Mallick and Rai, 1993). Many authors (de la Noue and Proulx, 1988; Abdel Hameed, 2002) reported higher nutrient removal efficiency in the immobilized algal biomass than the freely suspended cells of the same algal species.

Nutrient removal efficiency could be increased depending upon many factors, including algal species, immobilization matrix, cell and bead concentration, aeration, and retention time. Many authors reported that *Chlorella* is a common and effective species for the immobilization and nutrient removal purposes (Robinson et al., 1988; Tam et al., 1994; Lau et al., 1997, 1998; Abdel Hameed, 2002). Alginate is the most frequent polymer used for algal immobilization. Studies have adequately verified cell viability in the alginate matrix (Mallick and Rai, 1993; Romo and Perez-Martinez, 1997; Vilchez et al., 2001). In a freely suspended algal treatment system, the removal efficiency is often directly related to the cell mass. Increasing the algal biomass would improve the removal efficiency and shorten the retention time (Talbot and de la Noue, 1993; Lau et al., 1995). On the contrary, the superconcentrated cell stocking in the beads, posed a serious leakage problem (Robinson et al., 1986; Lau et al., 1997) and affects the treatment efficiency by the number of beads in wastewater (algal bead concentration). Therefore, the aim of this study is to examine the effect of cell stockings in immobilized beads, size of the beads and different concentrations of alginate immobilized *Chlorella* beads on the removal of ammonia, nitrate and phosphate from wastewater.

MATERIALS AND METHODS

Stock suspension of *Chlorella vulgaris* was cultivated in commercial Bristol medium (pH 6.5 - 7.2) under aseptic conditions, aerated by filtered air at a rate of 40 ml min^{-1} and maintained at temperature of 25 ± 2ºC, with light intensity of 180 E s^{-1} m^{-2} and a 16/8 h light/dark cycle. After two weeks cultivation, algal cells were harvested by centrifugation at 3500 rpm for 15 min. The cell residues were washed with deionized water and resuspended in deionized water prior to use. The cell density in the suspension was 9 x 10^7 cells ml^{-1}. Different quantities of the algal cells were resuspended in 50 ml deionized water to give different cell densities. Each diluted algal suspension was mixed with an equal volume of 4% sodium alginate to yield a mixture of 2% algal alginate suspension. Each mixture was dropped into a solution of 0.1 M $CaCl_2$ using a peristaltic pump to form uniform algal beads (4 mm diameter). Three different types of beads with different cell stockings were formed. Low stocking beads contained 3 x 10^5 cells $bead^{-1}$. The medium stocking beads were prepared with cells 5 times that of the low stocking giving beads with a cell density of 1.5 x 10^6 $bead^{-1}$. The high stocking beads contained 3 x 10^6 cells $bead^{-1}$ (10 times that of low stocking). The $CaCl_2$ solution was gently stirred for 2 h in order to stabilize the beads. The beads were then collected by filtration and kept in a solution containing 20 mM NaCl and 10 mM $CaCl_2$ until use (Adlercreutz and Mattiasson, 1982). Blank beads were made without any algal cells as control. Four sets (3 for the different beads with different cell stockings and the fourth for the control beads) of conical flasks (2 l) each containing 1.6 l wastewater (primary treated) was used as reactors. Each set was in triplicate. The bioreactors were kept in the same above conditions. Samples were taken every 4 h intervals for nutrients analysis and for algal count (using hemacytometer) in wastewater as indication for cell leakage.

To study the effect of algal beads concentration in wastewater on the treatment efficiency, beads (4 mm) with cell stockings of 1.5 x 10^6 cells $bead^{-1}$ were formed as above. 32 beads were made out of each 1 ml of alginate algal suspension indicating that each algal bead occupied 0.03125 ml volume. According to the bead volume, four different beads: wastewater ratios were calculated. The first reactor (A) contains 10.66 beads ml^{-1} wastewater (1:3 ratio), the second reactor (B) contains 16 beads ml^{-1} wastewater (1:2 ratio), the third reactor (C) contains 32 beads ml^{-1} wastewater (1:1 ratio), the fourth reactor (D) contains 64 beads ml^{-1} wastewater (2:1 ratio) and the fifth reactor contains 32 free beads as the control (1:1). All the reactors were in triplicates and kept at the same above conditions. Samples were taken every 4 h intervals for nutrients analysis and for algal count (using hemacytometer) in wastewater as indication for cell leakage.

In order to study the effect of bead size on nutrients removal, three different beads were formed as above using different needles with different internal diameters. The formed beads were 2.8, 4 and 6 mm diameter and each has stocking cell density of 1.5 x 10^6 cells $bead^{-1}$. Each bead size was used in a reactor in the ratio of 1:3 (bead: wastewater) and the control reactor contains beads (4 mm) free of algae in the same ratio. All the reactors were in triplicates and kept at the same above conditions.

pH value was recorded with an Orion 210 digital pH meter in all the different reactors at different time intervals. All the analyses of ammonia, phosphate and nitrate were performed according to standard methods for the examination of waters and wastewaters (APHA, 1992).

Statistical analysis

The mean and standard deviation values of the triplicates for each treatment were calculated. The parametric one-way analysis of variance (ANOVA) was used to evaluate the effects of the different bioreactors at particular treatment time. Tukey's 'Honest Significant Difference' (Tukey HSD) test was used when the ANOVA result showed a significant difference among treatments at $p < 0.05$. The statistical computer program R was used to study the simple statistics, analysis of variance (ANOVA), and Tukey HSD of the different treatments.

RESULTS

Effect of different cell stockings in immobilized beads on its efficiency in wastewater removal

The reductions in wastewater ammonia in the immobilized algal bioreactors were significantly (Tukey, HSD $p < 0.05$) better than control beads (Figure 1a). The percentages of ammonia removal after 24 h were 92.4, 72.3, 65.5 and 40% for the medium stocking beads, high stocking beads, low stocking beads and control, respectively. The medium stocking beads achieved 100% removal after 48 h. The differences were significant between the medium stocking beads and all other types of beads (Tukey, HSD $p < 0.05$). The behavior of the low and high stocking beads was nearly similar, although the high stocking recorded higher ammonia removal but the difference was insignificant.

The phosphate concentration in wastewater declined rapidly in the recator containing medium stocking beads after 16 h of treatment (Figure 1b). The decline continued to the end of experiment (48 h) with the highest percentage removal (94.7%). Again, the differences between the medium stocking beads and other bead types were significant (Tukey, HSD $p < 0.05$). The high and low stocking beads removed phosphate efficiently and achieved 76 and 67% respectively after 48 h (Table 1). The control beads recorded only 35% phosphate removal at the end of the experiment.

Different pattern was seen in case of nitrate removal. Nitrate concentration increased in the wastewater after 4 hrs in the control and low stocking beads reactors, remained constant in the high stocking and reduced by 9% only in the medium stocking beads reactor (Figure 1c). The nitrate concentrations fluctuated up and down in both the control and low stocking beads reactors to reach 3.1 and 2.8 mgl^{-1} respectively at the end of the experiment. Both medium and high stocking beads reactors achieved 96.4 and 55.5% nitrate removal, respectively after 48 h. Significant differences were recorded

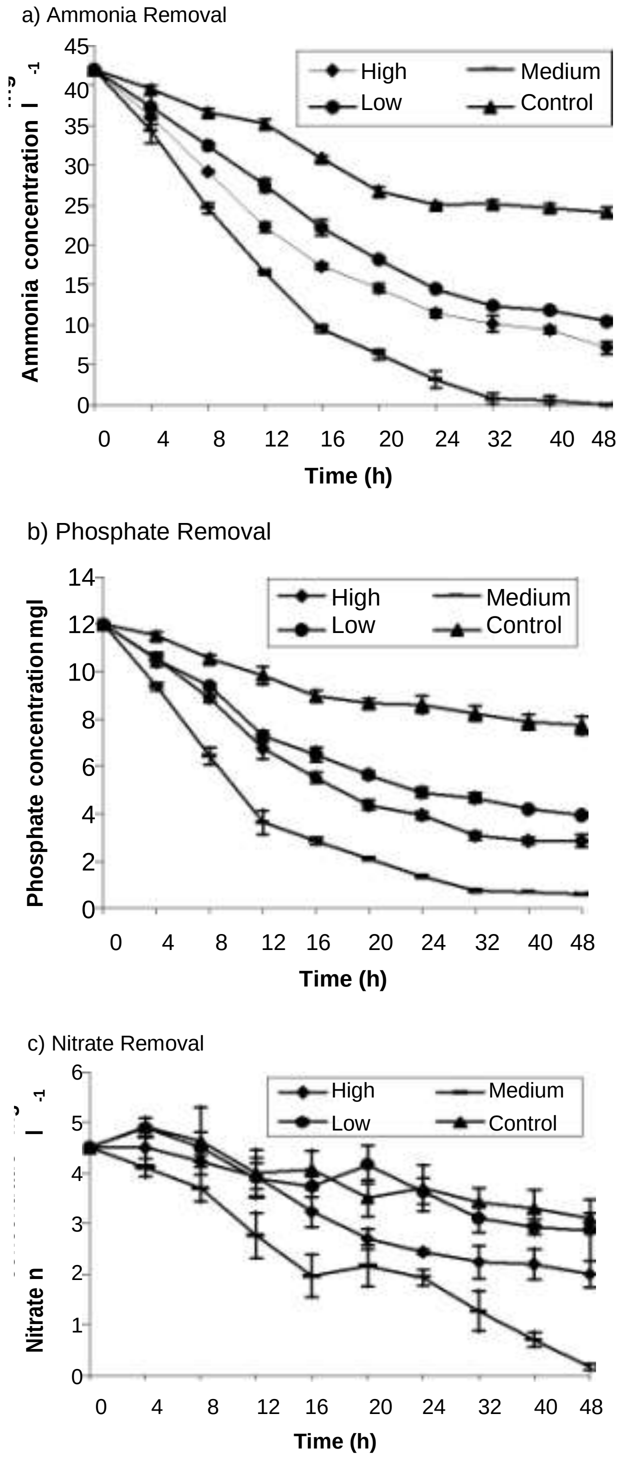


Figure 1. Residual concentrations of: a) ammonia, b) phosphate and c) nitrate in wastewater during the experimental period in different bioreactors. (High = 3 x 106 cells bead-1; Medium = 1.5 x 106 cells bead-1; Low = 3 x 105 cells bead-1 and Control = blank beads).

between nitrate removal in the medium stocking beads reactors and the other types of reactors (Tukey, HSD $p < 0.05$).

Effect of algal beads concentrations in reactors on wastewater treatment efficiency

Reactors A and B were similar in ammonia removal from wastewater. They both achieved 100% ammonia removal after 32 and 48 h treatments, respectively (Figure 2a). Although the B reactor took longer time to achieve 100% ammonia removal than reactor A, the difference was statistically insignificant. Ammonia concentrations declined gradually to reach 4.8 and 12.3 mgl^{-1} in C and D reactors respectively. The percentages of removal in C and D reactors were 88.6 and 70.6%, respectively. The control reactor was successful in removing only 40% of ammonia. The differences in the efficiency of ammonia removal between the reactors A, B and C, D, E were significant (Tukey, HSD $p < 0.05$).

All the reactors with algal beads (A, B, C and D) were efficient in phosphate removal from wastewater. The situation was complicated between these four reactors (Figure 2b). They all achieved about 95% phosphate removal after 48 h (Table 1). The differences among the four reactors were insignificant. The control reactor was able to reduce the phosphate concentration to 7.6 mgl^{-1} at the end of the experiment. The differences between the control reactor and all the other reactors were highly significant (Tukey, HSD $p < 0.01$).

The nitrate concentration declined rapidly by 56% in the first reactor (A) after 16 h. The decline was slower in the B reactor (15.5%) after the same period. Despite this high difference between the two reactors (A and B), they successfully reached 96 and 90% removal respectively at the end of the experiment (Figure 2c). The overall difference between A and B reactors were statistically insignificant. The nitrate concentration in reactor C fluctuated and recorded its maximum removal (57%) after 24 h of treatment then fluctuated again till the end of the experiment with a final removal of 52.5%. Similar pattern was seen in D reactor as nitrate concentrations fluctuated over the experimental time to reach its lowest after 48 h (1.6 mgl^{-1}).

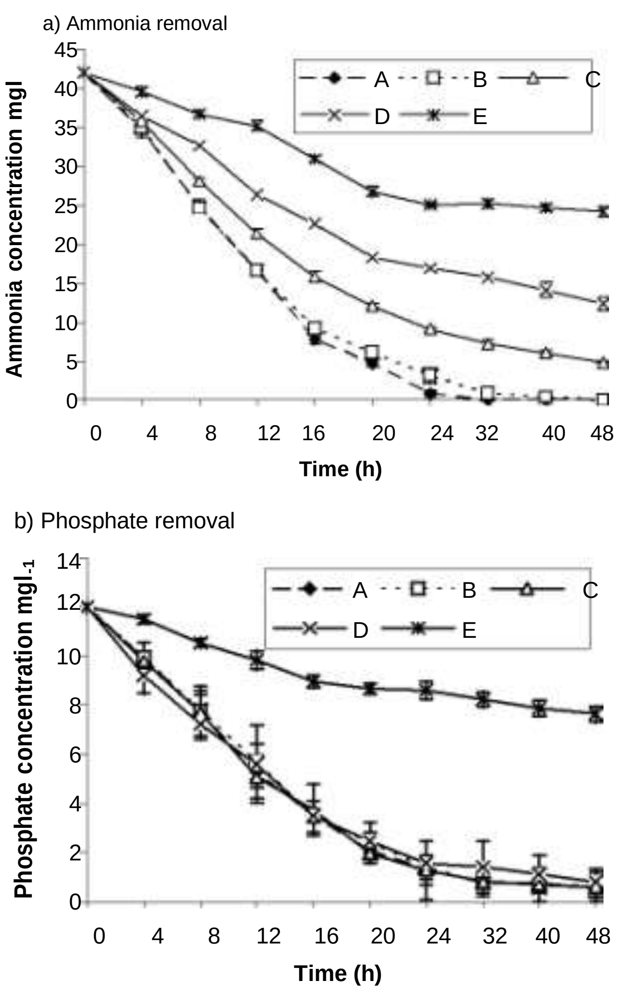
Effect of bead size on the efficiency of nutrients removal from wastewater

Both medium and big size algal beads were very efficient in ammonia removal from wastewater (Figure 3a). Their pattern of removal was nearly similar. They both reached 99% ammonia removal after 48 h treatments. The difference between their rate of ammonia removal and the removal rate in the small and control beads was significant (Tukey, HSD $p < 0.05$). The reactor-containing small size algal beads achieved more than 50% ammonia removal within 16 h. The removal efficiency increased to reach its maximum (79.5%) after 48 h. The control beads achieved about 40% ammonia removal at the end of the experiment.

More that 57% of phosphate concentrations were remov-

Table (1). Summary of the treatment efficiencies (percentage removal) of the different types of reactors for the three experiments after 48 h treatment.

Treatment	Time (h)		
	Ammonia	Phosphate	Nitrate
<u>Experiment 1</u>			
Low stocking beads	75	67	36
Medium stocking beads	100	94	96
High stocking beads	82	76	55
Control beads	42	35	30
<u>Experiment 2</u>			
A (1:3 algal beads: wastewater)	100	95	96
B (1:2 algal beads: wastewater)	100	95	90
C (1:1 algal beads: wastewater)	88	95	52
D (2:1 algal beads: wastewater)	70	93	64
E control beads	40	36	31
<u>Experiment 3</u>			
Small size beads	79	77	41
Medium size beads	99	95	96
Big size beads	99	94	96
Control beads	40	35	31



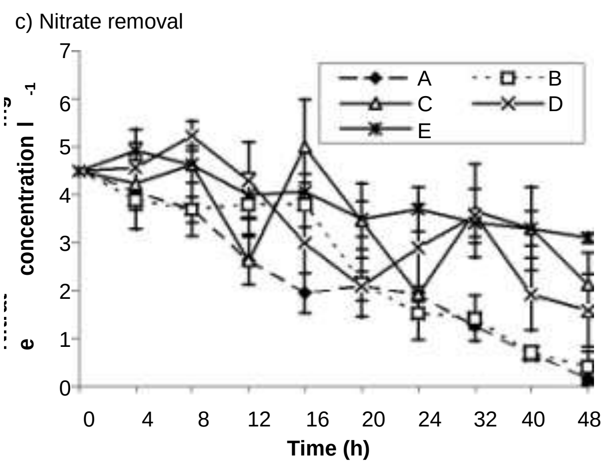


Figure 2. Residual concentrations of: a) ammonia, b) phosphate and c) nitrate in wastewater during the experimental period in different bioreactors with different algal bead concentrations. (A = 1:3 bead : wastewater, v/v; B = 1:2; C =1:1; D = 2:1 and E = 1:1 blank algal beads : wastewater as control).ed from the reactors containing medium and big size algal beads within 12 h of treatment (Figure 3b). This 57% removal was achieved later after 16 h treatment in the small size algal beads reactor. The performance of this reactor increased with time to record its highest percentage phosphate removal (78%) after 40 h of treatment. The medium and big size algal beads reactors were more efficient as the percentage of removal at the end of the experiment was 95% for each reactor (Table 1). The control reactor differed significantly from the three

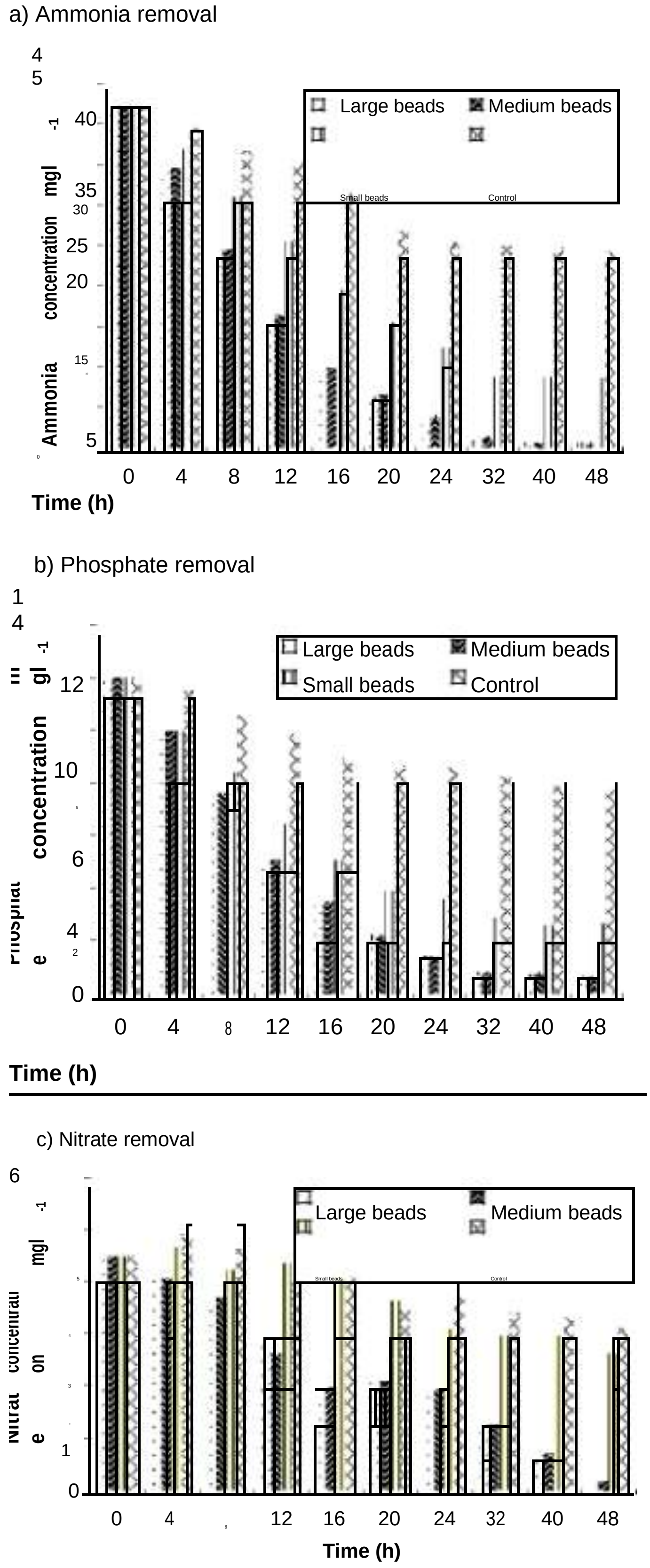


Figure 3. Residual concentrations of: a) ammonia, b) phosphate and c) nitrate in wastewater during the experimental period in different bioreactors with different algal bead sizes. (Large beads = 6 mm; Medium beads = 4 mm; Small beads = 2.8 mm and the control 4 mm blank beads).

types of algal beads in phosphate removal (Tukey, HSD $p < 0.05$). The efficiency of treatment in the control reac-tor was only 35% after 48 h of treatment.

The behavior of both reactors containing medium and big size algal beads was similar with slight differences. They both achieved 96% nitrate removal at the end of the experiment (Figure 3c). According to Tukey, HSD ($p < 0.05$), their removal efficiencies were significantly differ-ent from the reactors containing small size algal bead size and control beads. The nitrate concentrations in wastewater fluctuated up and down in the reactors conta-ining small and control beads to reach its final concentrations of 2.6 and 3.1 mgl^{-1}, respectively.

pH changes in wastewater during the treatment periods

The pH in all the reactors was alkaline (Table 2). The pH values ranged between 7.9 usually recorded in the control reactors in the different experiments and 9.3 recorded in different reactors with different types of beads.

DISCUSSION

Immobilized *C. vulgaris* beads were efficient in removing NH_4^+-N from wastewater comparing to those without algae, indicating that uptake of ammonium and assimi-lation into algal cells are essential processes. McCarthy et al. (1977) reported that NH_4^+-N is generally preferred as nitrogen source for algae. Medium cell stocking beads (1.5 x 10^6 bead^{-1}) with diameter 4 mm in the ratio of either 1:3 or 1:2 (beads: wastewater) were very efficient in ammonia removal (about 100%). Increasing the cell stocking in beads reduced their efficiency in nutrients removal and caused cell leakage (monitored in the reactor containing high stocking beads after 32 h treatment). Increasing algal cells entrapped within the beads didn't cause any significant improvement in nutrient removal (Chevalier and de la Noue, 1985; Lau et al., 1997). On the contrary, superconcentrated cell stockings in the beads posed serious leakage problem (Robinson et al., 1986; Lau et al., 1997). Lukavsky et al. (1986) reported that nutrients such as ammonia and phosphate could first be adsorbed on the surface of the bead, then penetrate slowly into alginate and be contin-ually sorbed into cells. Superconcentrated cell stockings may restrict to some extent the nutrient diffusion through the alginate pores (Jimenez-Perez et al., 2004). De-Bashan et al. (2005) concluded that the size of the microalgal population controls the uptake of nitrogen in *C. vulgaris* cells - the higher the population (regardless the experimental parameters), the less nitrogen each cell takes up. As the reactors were constantly aerated and the pH in all reactors was alkaline, removal of ammonia by volatilization was possible. Previous studies reported

Table 2. Changes in pH values (mean and standard deviation, n = 3) during the treatment periods in the different reactors for the three experiments.

Treatment \ Time (h)	8	16	24	32	40	48
Experiment 1						
Low stocking	8.7±0.2	8.8±0.1	8.85±0.16	8.6±0.25	8.5±0.12	8.6±0.2
Medium stocking	9 ±0.1	8.9±0.11	9.2±0.05	9.3±0.06	9.1±0.06	9±0.14
High stocking	9 ±0.15	8.7±0.05	8.8±0.1	9.1±0.08	9±0.09	8.9±0.11
Control	8.1± 0.2	8.2±0.13	7.9±0.2	8±0.14	8.1±0.06	8±0.07
Experiment 2						
A (1:3)	9±0.2	9.3±0.03	9.1±0.05	8.9±0.1	9±0.13	9.2±0.2
B (1:2)	9.1±0.12	8.9±0.25	9.2±0.1	8.8±0.23	9±0.2	9.1±0.16
C (1:1)	8.9±0.19	9.1±0.08	9.2±0.08	9±0.2	8.9±0.16	9±0.24
D (2:1)	9.3±0.1	9.3±0.05	8.9±0.18	8.7±0.25	9±0.28	8.9±0.3
E control	8±0.2	7.9±0.15	7.9±0.3	8.1±0.05	8±0.08	7.9±0.25
Experiment 3						
Small beads	8.6±0.25	8.8±0.17	9±0.02	9.1±0.11	8.8±0.18	9±0.02
Medium beads	8.9±0.18	9±0.1	9.2±0.14	8.9±0.23	9.1±0.3	8.9±0.06
Big beads	9±0.1	8.9±0.25	9.3±0.08	8.9±0.18	9.1±0.16	8.8±0.25
Control	8.2±0.02	8±0.04	7.9±0.2	8±0.2	8.1±0.05	8.1±0.25

that air-striping of ammonia is a possible mechanism for N removal in an intensely aerated microalgal system with alkaline pH (McCarthy et al., 1977; Lau et al., 1995, 1997, 1998). Increasing bead concentrations caused sig-nificant reductions in the treatment efficiency. Dense beads would reduce the amount of light penetrating through the reactor, and enhance the self-shading effects, which limit the metabolic activities of algal cells. In the reactors with high beads concentrations (C, D) large numbers of beads settled at the bottom of the reactors due to the heavy weight of the large numbers of beads and the supplied air was not sufficient to comple-tely suspend all the beads in these reactors. Similar observations were recorded by Tam and Wong (2000).

The rate of nitrate removal was slow. Nitrate even accumulated in some reactors at time intervals. Again the reactors with medium cell stockings, medium size (4 mm) with beads concentrations in the ratio of either 1:3 or 1:2 (beads: wastewater) were capable of reducing nitrate concentrations in wastewater efficiently. Their perform-ance even improved after the exhaust of most of the ammonia. It has been found that algae preferentially utilize ammonium and other reduced forms of nitrogen and leave nitrite and nitrate in wastewater (Matusiak et al., 1976). Other studies had also indicated that NO_3^--N is utilized by algal cells and so removed from wastewater only after the concentration of ammonia nitrogen falls below a certain threshold or is depleted from the media (Przytocka-Jusiak et al., 1984; Maestrini et al., 1986). The possibility of loosing nitrate as N gas by denitrifi-cation process would be unlikely in the present system due to constant aeration and as the reactors were kept under aerobic conditions by the photosynthetic algal cells. Tam and Wong (2000) reported similar results.

The reduction of phosphate was similar to the pattern of reduction of wastewater ammonia. Rapid decline was monitored in the reactors containing medium beads with medium stocking beads. Figure 3b indicated that the beads concentrations in wastewater did not affect the efficiency of phosphate removal, as all the reactors containing algae significantly remove phosphate from wastewater with no significant differences. Tam and Wong (2000) reported that the uptake of phosphorus by algae is related to algal biomass. Also phosphate might be precipitated as calcium phosphate due to the pre-sence of calcium ions in the alginate matrix (Jimenez-Perez et al., 2004) and wastewater together with elevated pH values. Lau et al., (1997) reported that the wastewater calcium concentrations increased dramatically in bioreac-tors containing alginate beads. At pH values around 8 and higher, phosphate precipitation is an important phenomenon (Megharaj et al., 1992; Moutin et al., 1992), which was the case in the present study.

CONCLUSION

The present study demonstrates that *C. vulgaris* immobi-lized in alginate beads were more effective in removing N and P from wastewater than blank alginate beads. The optimum cell stocking is 1.5 x 10^6 cells $bead^{-1}$ and the optimum bead size is 4 mm. Bead concentrations in the ratios of 1:3 (algal beads: wastewater, v/v) is ideal, efficient and economic to use in bioreactors for wastewater treatment. In addition to algal cells, the calcium alginate (immobilization matrix), the alkaline pH that prevailed in the experiment and the constant aeration of wastewater also contributed to the removal of nutrients from wastewater.

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