Nitrogen Removal from Shrimp Culturing by Attached *Spirulina* Mat and the Factors Effect on Attachment

C. WANTAWIN*, K. NARDPIRIYARAD*, M. RUENGJITCHATCHAWALYA**, S. SIRIRAKSOPHON*** and A. MIMURA****

**Department of Environmental Engineering, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand (Email: chalermraj.wan@kmutt.ac.th, khunkee@hotmail.com)*
***Department of Biotechnology, School of Bioresources and Technology, KMUTT, Bangkok 10140, Thailand (Email: marasri.rue@kmutt.ac.th)*
****Biochemical Engineering and Pilot Plant Research and Development Unit, National Center for Genetic Engineering and Biotechnology, KMUTT, Bangkok 10150, Thailand. (Email: sunun@pdti.kmutt.ac.th)*
*****Department of Applied Chemistry and Biotechnology, University of Yamanashi, Japan (Email: mimura56@ab11.yamanashi.ac.jp)*

The attachment of *Spirulina platensis* BP, a cyanobacteria that having high protein, on a Recycled Polyester Mat (RPM) has been studied and applied for ammonia removal in shrimp culturing water. Factors affecting attachment including light intensity, salinity, and water velocity were tested in circulated batch systems. The results showed that light intensity affected attachment and the attachment occurred obviously in stationary phase or in low nutrient condition. The dry weight of *S. platensis* per area of RPM at light intensity of 2,300 and 4,000 lux were 74 and 25 g/m^2, respectively. The attached *S. platensis* on RPM was tested for ammonia removal using synthetic shrimp culturing water with ammonia loading of 0.9 gN/m2-d in circulated batch system and water velocity of 0.02 m/s by varying salinity of 0, 10, 15, 20 and 30 ppt. The ammonia removal rates obtained at salinity conditions of 0, 10, 20 and 30 ppt were 0.7, 0.7, 0.5 and 0.4 gN/m^2/d, respectively. The results illustrated no different rates at condition of 0 and 10 ppt and found less removal rates at 20 and 30 ppt. The concentration of suspended solid that daily washed out was found stable in all experiment reactors. The detachment rates examined in conditions of 0.02 m/s water velocity at salinity 0, 10, 15, 20 and 30 ppt were 2, 17, 24, 29 and 48 g-SS/m2-d, respectively. There was no change in detachment rate when varying water velocity from 0.02 to 0.14 m/s in the condition of 15 ppt salinity and 1.8 gN/m^2-d ammonia loading. The final dry weight of *S. platensis* was analyzed and found that the weight increased when salinity increased (1,263 and 2,071 g/m^2 at salinity 15 and 30 ppt, respectively). The fraction of nitrogen to dry weight, however, was less in the condition of higher salinity level (28 and 19 mgN/g-cell at salinity 15 and 30 ppt,

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respectively). The results implied other produced substances involving in attachment of *S. platensis*. Determination on different feeding system, circulated batch system and circulated semi feed system (2 times per day), demonstrated that the latter system could served twice ammonia loading that from 1.8 to 3.6 gN/m^2-d for effluent ammonia approximately of 0.1 mgN/l. The ammonia removal rate of circulated batch system and circulated semi feed system were 1.5 and 3 gN/m^2-d, respectively.

Keywords: ammonia removal; attachment; salanity; *Spirulina platensis*

Introduction

High production of cultured shrimp induce the culturing with high intensity and following with the requirement of higher water quality in order to reduce the stress on the pond ecosystem. Open system with high water exchange is usually applied however closed or recirculating seawater with zero or nearly zero water discharge is coming up due to the issue of environmental friendly. For each metric tonne of shrimp produced, at the commonly achieved food conversion ratio (FCR) of 2:1, approximately 1,250 kg of organic material, 87-118 kg of nitrogen and 24-28 kg of phosphorus are generated as waste (Anon, 1991; Lin *et al*., 1991). This means that about 63-78 percent of nitrogen and 76-86 percent of phosphorus fed to shrimp under intensive cultivation contaminate the environment. Shrimp tolerates high amount of nitrate (>1,5000 μM) (Cavalli *et al*., 1996) but high ammonia concentration are lethal or may serious inhibit their food intake and growth (Ostrensky and Wasielesky, 1995; Cavalli *et al*., 1996). Unionized ammonia in the concentration of more than 0.1 mg/l become toxic to shrimp and require removal from culturing system (Boyd, 1992). Moreover, shrimp expose to high ammonia concentrations seems to reduce their resistance to disease (Brock and Main, 1994). On the other hand, remained nitrogen, both ammonia and nitrate, in the shrimp farm effluent can cause the damage on water resource by inducing eutrophication. To keep dissolved nitrogen at low levels, large amount of water must be exchanged daily, increasing the costs of shrimp production.

An alternative way to maintain high water quality in shrimp pond is biological treatment. Usually ammonia removal is an important function of microalgae in aquaculturing system since microalgae use ammonia and phosphorus for growth. However, certain limitation must be taken into account; microalgae separation is difficult and drained water from culturing pond increase organic loading to water reservoirs and cause water pollution. To overcome this constrain, immobilization technology for nutrient treatment by microalgae has been developed. Several materials were applied for biocarriers. Some used porous structure such as alginate, carageenan for microalgae entrapment. (Chevalier and de la Noüe, 1985a, b; Kaya *et al*., 1995; Tam *et al*., 1994; Lau *et al*., 1998; Tam and Wong, 2000) and others used surface texture such as cellulose, polyurethane, polyethylene for external attachment (Còrdoba *et al*., 1995; Craggs *et al*., 1997; Sawayama *et al*., 1998). This microalgae attached media will be applied to treat ammonia and phosphorus in sea water shrimp ponds and Spirulina is chosen in this study due to its high salinity toleration

(Chuntapa *et al.*, 2003) and high nutritional values for nourishment in shrimp culturing. The purpose of this research is to develop in-situ shrimp culturing water treatment using synthetic polymer biomat predominant with Spirulina for removal of nitrogen and phosphorus with regard to reduce the water exchange and enrich the supplement for shrimp growth.

Methods

Spirulina platensis *culture*

Stock culture of *S. platensis* was obtained from algae laboratory of Department of Biotechnology, King Mongkut's University of Technology Thonburi, Thailand. *S. platensis* maintained in Zarrouk's medium (Zarrouk, 1966) that replaced $NaNO_3$ by NH_4Cl. *S. platensis* incubated in incubator shaker under 120 rpm, 35°C and 72 μE/m^2/s illumination and accustomed with desired
salinity before used.

Synthetic shrimp culturing water

Zarrouk's medium (without NH_4Cl and K_2HPO_4, respectively) was diluted with tap water to10% of total volume and later mixed NH_4Cl and K_2HPO_4 to concentration of 1 mgN/l and 0.3 mg-P/l, respectively.

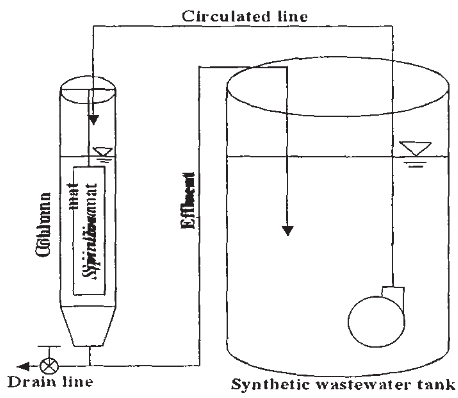

Fig. 1. Circulation reactor.

Circulation reactor

Cylindrical column made of acrylic (diameter 5 cm and height 60 cm) was jointed with 100-l plastic tank. There was submerged pump in this tank for circulated water to the column as shown in Fig. 1. Continuous illumination under 72–90 μE/m^2/s. The *S. platensis* mat (3×33 cm) was stretched with plastic line and hung in the middle of column.

Water quality analysis

Ammonia Nitrogen and Phosphorus in water were measured everyday. Suspended solid in water was measured in the last day of treatment cycle. Salinity and pH were also monitored. Analysis methods follow Standard Methods for the examination of water and wastewater, 18th ed. (APHA, 1992).

Preliminary test

Attachment of *S. platensis* on different media (Commercial Polyester on Urethane Mat, CPUM; Virgin Polyester Mat, VPM; Recycled Polyester Mat, RPM) was observed. Stock culture of *S. platensis* was prepared using Zarrouk's medium. Two initial cell concentrations of *S. platensis* (261 and 478 mg/l) were added into 250-ml flask 100 ml. Each flask added 2 pieces of the same media size 2×3 cm and without media for control. Batch experiment was carried out under light intensity 2300 and 4000 lux (41 and 72 $\mu E/m^2/s$) and shaken at 100 rpm. Temperature was maintained at 35°C. The samples were collected for determination of turbidity of cell suspension (OD560). Dry weights, at 105°C for 4 hours, of *S. platensis* were measured at the end of experiment.

Trial I: Effect of salinity on attachment and nutrient removal rate of* S. platensis *mat

Detachment and ammonia removal rate of *S. platensis* attached on RPM under varying salinity 0, 10, 20 and 30 ppt were studied. Water velocity was 0.02 m/s by recirculate water from 70-l synthetic wastewater with initial ammonia concentration of 1 mgN/l. The operation was Circulated Batch System (CBS) with 4 days cycle time and ammonia loading of 0.9 gN/m^2-d.

Trial II: Effect of feeding strategies on nutrient removal rate of* S. platensis *mat

Nutrient removal rate of *S. platensis* attached on RPM with different two feeding strategies, Circulated Semi Feed System (CSFS) and Circulated Batch System (CBS), was compared. This trial conducted under salinity 15 ppt and water velocity 0.02 m/s.

Circulated Semi Feed System (CSFS). Ammonia nitrogen and phosphorus solution was pumped directly to column 2 times per day. Total working volume of synthetic wastewater was 70 litre. Nitrogen and phosphorus loading were 3.6 mgN/m^2-d and 1 mgP/m^2-d, respectively.

Circulated Batch System (CBS). Ammonia nitrogen and phosphorus solution was mixed with synthetic wastewater and then pumped to column. Total working volume of synthetic wastewater was 35 litre. Nitrogen and phosphorus loading were 1.8 mgN/m^2-d and 0.5 mgP/m^2-d, respectively.

Trial III: Effect of water velocity on attachment and nutrient removal rate of* S. platensis *mat

Detachment and nutrient removal rate of *S. platensis* attached on RPM under varying water velocity 0.02 and 0.14 m/s were studied. Salinity 15 ppt and 35-l synthetic wastewater was maintained.

Trial IV: Effect of salinity on attachment and nutrient removal rate of* S. platensis *mat under water velocity 0.14 m/s

Detachment and nutrient removal rate of *S. platensis* attached on RPM under varying salinity 15 and 30 ppt were studied. Water velocity 0.14 m/s and 35-l synthetic wastewater was maintained.

Results and Discussion

Preliminary test

The dry weight per area of *S. platensis* attached on CPUM, RPM and VPM under light intensity 2300 and 4000 lux were shown in Fig. 2 and Fig. 3, respectively. The results indicated that light intensity affected to attachment than initial cell concentration. The lower light intensity resulted in higher attachment. Interesting observation in this experiment is mass attachment occurred obviously in stationary phase. RPM media gave the highest attachment of *S. platensis* at light intensity 2300 that total was 74 g/m^2 and 64 g/m^2 during stationary phase.

Trial I: Effect of salinity on attachment and nutrient removal rate of* S. platensis *mat

Nitrogen removal rates and detachment rates were shown in Table 1. High salinity results to enlarge the cycle time to 4 days in order to achieve ammonia effluent less than 0.1 mg/l. With long treatment cycle of 4 days the loading of ammonia in this trial in each run was only 0.9 gN/m^2-d. Increase of salinity resulted in more detachment of *S. platensis* from RPM and less nitrogen removal rate. At steady state, *S. platensis* could adapt to

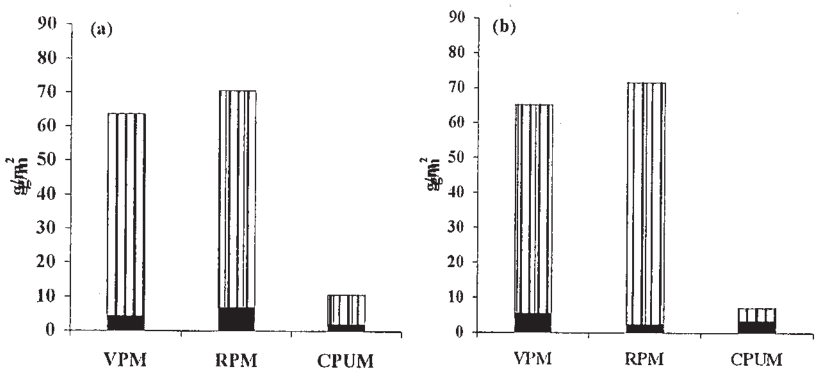

Fig. 2. Mass attachment of *S. platensis* per area of CPUM, RPM and VPM media under light intensity 2300 lux and initial cell concentration (a) 261 mg/l and (b) 478 mg/l, respectively.
■ during log phase (9 days), ll during stationary phase (4 days).

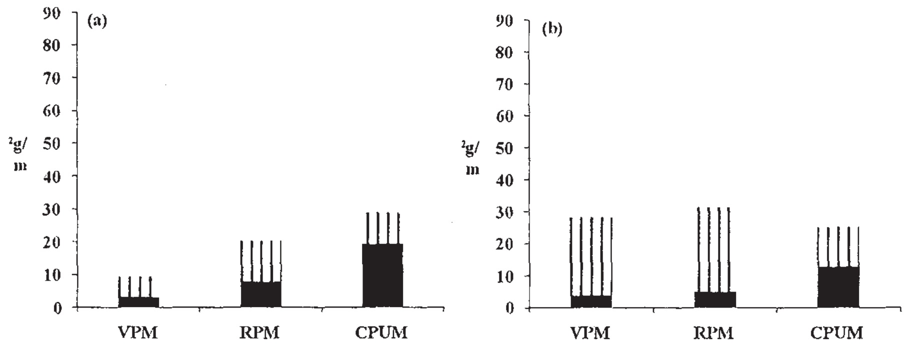

Fig. 3. Mass attachment of *S. platensis* per area of CPUM, RPM and VPM media under light intensity 4000 lux and initial cell concentration (a) 261 mg/l and (b) 478 mg/l, respectively.
■ during log phase (7 days), ll during stationary phase (3 days).

Table 1. Nutrient removal rate and detachment rate of *Spirulina* attached on RPM mat.

Trial	Feeding strategy	NH_3 loading (gN/m^2-d)	P loading ($gP/m^2/d$)	Water velocity (m/s)	Salinity (ppt)	NH_3 removal rate (gN/m^2-d)	P removal rate (gP/m^2-d)	Detachment rate ($gSS/m^2/d$)
I	CBS	0.9	0.25	0.02	0	0.7	-	2
					10	0.7	-	17
					20	0.5	-	29
					30	0.4	-	48
II	CBS	1.8	0.5	0.02	15	1.5	0.11	25
	CSFS	3.6	1			3.1	0.25	62
III	CBS	1.8	0.5	0.02	15	1.5	0.11	25
				0.14		1.6	0.09	26
IV	CBS	1.8	0.5	0.14	30	1.7	0.10	48

photosynthesis under high salinity but at lower resulted in lower nitrogen removal rate. Karnchanarak (1994) reported that photosynthesis rate of *Spirulina* exposured to NaCl salt at salinity 44 ppt was 85.21% of initial rate. Zeng and Vonshak (1998) found that *S. platensis* exposured to high salinity upto 44 ppt under light intensity 100-200 $\mu E/m^2$-s, photosynthetic and respiratory system was inhibited at initial and then recovered with new lower steady state.

Trial II: Effect of feeding strategies on nutrient removal rate of* S. platensis *mat

Nutrient removal rate and detachment rate was shown in Table 1. Under both feeding strategies *S. platensis* could treated ammonia nitrogen from 1 to 0.1 mgN/l within 24 hours and slightly phosphorus removal (Fig. 4).

The CSFS mode had more advantages because it could serve double of nutrient loading compare to CBS mode. CSFS mode results to high load or high concentration of nutrient at the beginning of cycle and induce to increase of utilization rate of *S. platensis*.

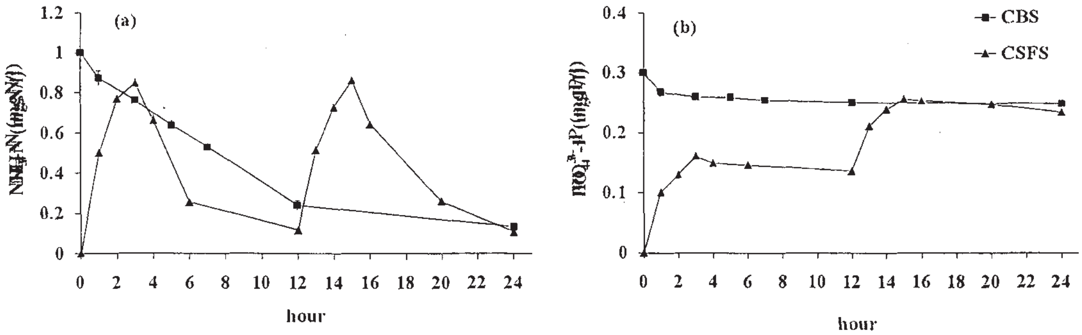

Fig. 4. Profile under feeding strategies CBS and CSFS of (a) nitrogen and (b) phosphorus, respectively.

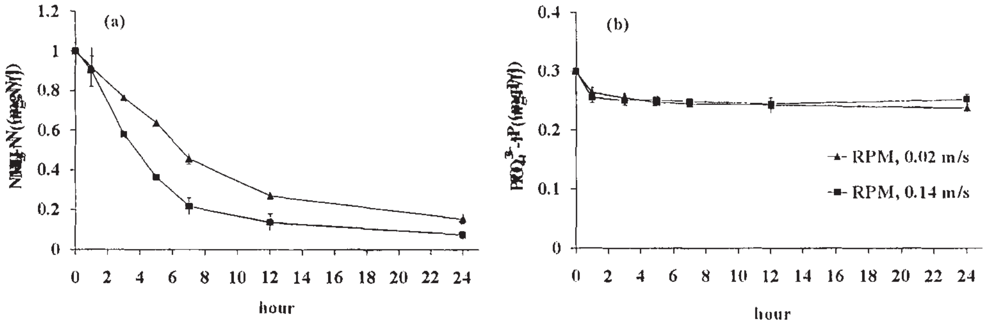

Fig. 5. Profile under water velocity 0.02 m/s and 0.14 m/s of (a) nitrogen and (b) phosphorus, respectively.

Trial III: Effect of water velocity on attachment and nutrient removal rate of* S. platensis *mat

Nutrient removal rate and detachment rate was shown in Table 1 Ammonia nitrogen can be can be reduce from 1 to 0.1 mgN/l within 24 hours under both water velocities. But it decreased more rapidly in first 7 hours at water velocity of 0.14 m/s (Fig. 5). This may caused of lower mass transfer resistant or thinner layer between nutrient bulk and surface of biofilm due to high shear force. Although higher shear force, it did not affect to detachment rate inversely higher accumulation of *S. platensis* on mat occurred.

Trial IV: Effect of salinity on attachment and nutrient removal rate of* S. platensis *mat under water velocity 0.14 m/s

Nutrient removal rate and detachment rate was shown in Table 1. *S. platensis* mat

Table 2. Dry weight of *S. platensis* attached on RPM media and its nitrogen content under salinity 15 and 30 ppt and water velocity 0.02 and 0.14 m/s.

Salinity (ppt)	Water velocity (m/s)	g-*Spirulina*/m^2	gN/g-*Spirulina*	gN/m^2
15	0.02	556	32	17,792
	0.14	1,263	28	35,364
30	0.14	2,071	19	39,349

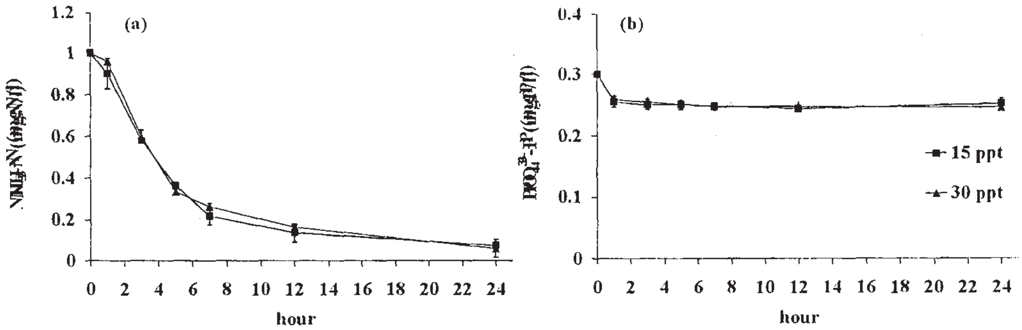

Fig. 6. Profile under salinity 15 ppt and 30 ppt maintained water velocity 0.14 m/s of (a) nitrogen and (b) phosphorus, respectively.

could treat ammonia nitrogen from 1 to 0.1 mgN/l within 24 hours (Fig. 6) although in high salinity (30 ppt). The same as others trials, slightly phosphorus was removed. But interesting results in this trial, at high salinity, was the increase of accumulation of *S. platensis* mass on RPM mat with lower nitrogen content per mass (Table 2).

This indicated that *S. platensis* produced another substance such as carbohydrate within cell for reserved energy to pump out exceed Na^+ (Stal and Reed, 1987; Zeng and Vonshak, 1998.) Other works (De Phillippis and Vincenzini, 1998; Nicolaus, *et al.*, 1999) also reported the production of exopolysaccharides (EPS) for protect cell during stress condition or more attached firmly under high shear force.

Conclusion

Recycled polyester mat was the media that giving the highest attachment mass per area under low light intensity (2300 lux) and *S. platensis* attached rapidly in stationary phase. Salinity affected to the detachment of *S. platensis* from RPM media. Detached mass increased as salinity increased. Attached *S. platensis* on RPM media was not only endurable under high water velocity condition but also more accumulative. Nitrogen removal rate decreased as salinity increased and used several days (4 days) to achieve to less than 0.1 mgN/l. High ammonia concentration at the beginning of cycle from CSFS mode results to 90% nitrogen removal within 24 hours with the double loading compare to CBS mode. In all trials, slightly phosphorus can be removed. It was found that at high salinity (30 ppt) nitrogen content per mass of *S. platensis* decreased while its dry weight on RPM mat increased. This indicated that *S. platensis* might produce another substance for surviving under high salinity and water velocity such as polysaccharide etc.

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