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Optimum Storage Condition for *Spirulina* Mat before Applying to Ammonia-Nitrogen Removal from Simulated Shrimp Culturing Water

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ABSTRACT

The polyester mats fully attached by *Spirulina* were stored under different conditions before using for ammonia removal from simulated shrimp culturing water. Three factors on storage were studied; light intensity of 1000, 2000 and 3000 lux; substrate concentration of 1X, 0.5X, 0.3X and 0.1X Zarrouk's medium; storage time of 2, 4, and 6 weeks. During storage, detachment of excess cells due to growth resulted in accumulation of suspended solids at 6 weeks in the range of 155 to 202 mg/l. More suitable condition for growth during storage yielded more suspended solids in solution. Circulated batch reactor with flow velocity of 0.14 m/s and initial ammonia nitrogen of 1 mg/l was applied for investigating the ammonia removal by stored *Spirulina* mats. Steady state effluent ammonia concentration of 0.127 mg-N/1 from fresh *Spirulina* mat reactor (control experiment) was achieved within 20 days. The control relative ammonia removal efficiencies from *Spirulina* mats, kept six weeks before applying in 1x Zarrouk's medium under 3000, 2000 and 1000 lux reactor at the end of experiment (25 days), were 93.8%, 92.3% and 91.9%, respectively. While the efficiencies by *Spirulina* mats, under 1000 lux light intensity, after storing in 0.5x, 0.3x and 0.lx Zarrouk's medium were 92.0%, 91.8% and 87.9%, respectively. Concentration of daily washed out suspended solid was found stable in all reactors. Optimal condition for storage of *Spirulina* for long term period (six weeks) was 1000 lux light intensity and 0.1x Zarrouk's medium.

Key words: *Spirulina* mat, storage condition, ammonia removal, shrimp culturing water

INTRODUCTION

Thailand has become the leading country in shrimp production since 1991. The cultured species is mainly *Penaeus monodon* or tiger shrimp. Shrimp culturing farms have a number of interaction with natural environment especially nutrients residue in effluent culturing water. Many applications of suspended microalgae on wastewater nutrient removal have been successfully carried out (Wong *et al.*, 1995; Lincoln *et al.*, 1996). However, algal harvesting is difficult and a final effluent with algae suspension could affect the water reservoirs.

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Consequently, several researchers introduced the immobilization of microalgae to bypass the harvesting or recovery of algae in algal wastewater treatment system (Travieso *et al.,* 1995; Chen, 2001). *Spirulina* was employed for this study due to the capability in removing nutrients, non-toxics to their surroundings as well as high salinity tolerance (Katephokasiri *et al*., 2004). Nutrient removal in shrimp ponds by *Spirulina* has been studied by several researches either on suspended or immobilized growth (Chuntapa *et al*., 2003; Wantawin *et al*., 2004).

Storing and transporting time of *Spirulina* mats before applying to the pond as well as the time during shrimp pond preparation before next crop could reduce the mat capability on ammonia removal. Therefore, the optimal conditions, including storage time, light intensity and medium concentration, were investigated in order to ensure the stability of *Spirulina* cell on mats when employ to the field after storage.

MATERIALS AND METHODS

S. platensis culture

Stock culture of *S. platensis* strain BP was obtained from Algal Biotechnology laboratory, King Mongkut's University of Technology Thonburi at Bangkhuntein, Bangkok, and maintained in Zarrouk's medium (Vonshak, 1986) under 3000 lux light intensity at RTM with 9 ppt salinity. The algal cells was gradually acclimatized to higher salinity up to 15 ppt.

Circulated batch reactors

Several acrylic tube columns with 5 cm diameter and 60 cm length were employed as the reactors. Without and with circulation were operated in the experiments of storage and treatment respectively. The 50 liters water tank filled with 35 liter of simulated shrimp culturing water was employed during nutrient removal efficiencies tests. The 36 watt fluorescence lamps were set up on both sides of reactors for the light source. Simulated shrimp culturing water was pumped from water tank into the top of acrylic column, an effluent from the column was discharged back to the water tank. The schematics of circulated batch reactor for the experiments during nutrient treatment tests is shown in Figure 1.

Immobilization of *S. platensis* on mat

S. platensis strain BP was cultured in Zarrouk's medium until reach to 0.2 OD_{560}. After that transfer to reactor in which 3×30 cm^2 fibrous polyester mat was vertically hanged. The light intensity of 3000 lux was used and 15 ppt Zarrouk's medium was changed every 9 days. It took 27 days for immobilization after that *Spirulina* mat was ready prepared for storage experiments.

Simulated shrimp culturing water

Simulated shrimp culturing water was prepared from modified Zarrouk's medium by diluting to 0.1x with tap water. NH_4Cl with concentration of 1.0 mg N/l was in placed of $NaNO_3$ in 0.1x modified Zarrouk' s medium. The salinity was adjusted to 15 ppt by NaCl and

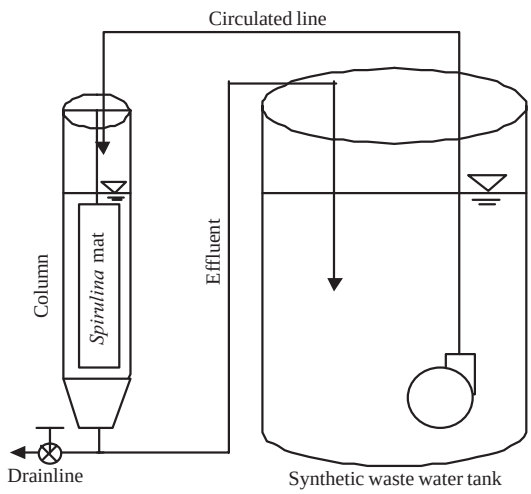

Figure 1 Circulated batch reactor.

measured by salinity-conductivity-temperature meter YSI model 33.

Light intensity and storage time

First of three sets of *Spirulina* mats, each was kept for 6 weeks in stagnant 1x Zarrouk's medium with light intensity of 3000, 2000 and 1000 lux or 54, 36 and 18 $\mu E/m^2/s$ (1 klux = 18 $\mu E/m^2/s$) respectively. Each mat reactor was covered by plastic cloth in order to prevent effect of another light source. Two and four weeks after beginning of the first set, the second and third sets were kept under the same conditions as the first, but storage periods were changed to 4 and 2 weeks, respectively. Therefore, all three sets (9 *Spirulina* mats) of different storage time periods had been ended at the same day and were further investigated within 25 days for their capabilities on ammonia removal comparing to the fresh immobilized *Spirulina* mat.

Ammonia nitrogen and suspended solids were monitored every day until the steady state of fresh *Spirulina* mat was reached.

Substrate concentration and storage time

First of three sets of *Spirulina* mats, each was kept for 6 weeks under light intensity of 1000 lux with 0.5x, 0.3x and 0.1x Zarrouk's medium concentration. Two and four weeks after beginning of the first set, the second and third sets were prepared under the same conditions as the first, only storage periods were changed to 4 and 2 weeks, respectively. All had been ended after six weeks. Efficiency of nutrient removal by *Spirulina* mats was evaluated within 25 days. Ammonia nitrogen and suspend solids were monitored every day until the steady state of fresh *Spirulina* mat was reached.

Ammonia-nitrogen and total suspended solid analyses

Ammonia and total suspended solid were analyzed by the Nesslerization method and by oven-dried at 103-105°C (APHA *et. al*., 1992), respectively.

Statistic analysis

The one-way analysis of variance (ANOVA) at $P \geq 0.05$ using SPSS ver. 10 software was employed for analyzing the results.

RESULTS AND DISCUSSIONS

Total suspended solid during storage

At the same substrate concentration, suspended solid in reactor under light intensity of 3000 lux was a little more than under light intensity of 2000 and 1000 lux as shown in Figure 2a. The higher suspended solids resulted from detachment of excess growth cells due to more photosynthesis induced by higher light intensity (Danesi *et al*., 2004).

Under the same light intensity, different substrate concentration resulted in different level of total suspended solids. At initial of storage period, there were no significant difference ($P>0.05$) of suspended solid concentrations among the reactors as shown in Figure 2b. However, at sixth week, the suspended solids of 201 mg/l in reactor with *Spirulina* mats in 0.1x Zarrouk's medium was significant higher ($P\leq0.05$) than that of other three reactors with *Spirulina* mats in 1.0x, 0.5x and 0.3x Zarrouk's medium, of which suspended solids were 155, 161 and 172 mg/l, respectively. The lower initial substrate concentration, the higher cumulative suspended solids in solution indicating that substrate was not enough for cell maintenance resulted in resuspension of some died cells in the medium.

Ammonia-nitrogen removal by stored *Spirulina* mat

The efficiency of ammonia removal in circulated batch reactor by the *Spirulina* mats previously stored under different light intensities was investigated and compared to the fresh one

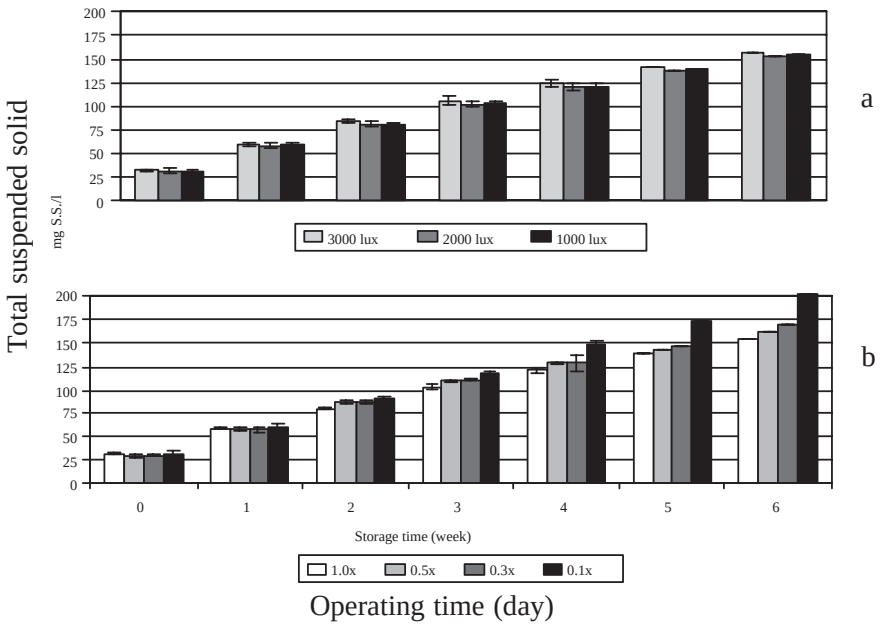

Figure 2 Total suspended solid of *Spirulina* mats during 6 week storage at 1x Zarrouk's medium concentration under 3000, 2000 and 1000 lux light intensity (a) and storage under 1000 lux light intensity, in 1.0x, 0.5x, 0.3x and 0.1x Zarrouk's medium concentration (b).

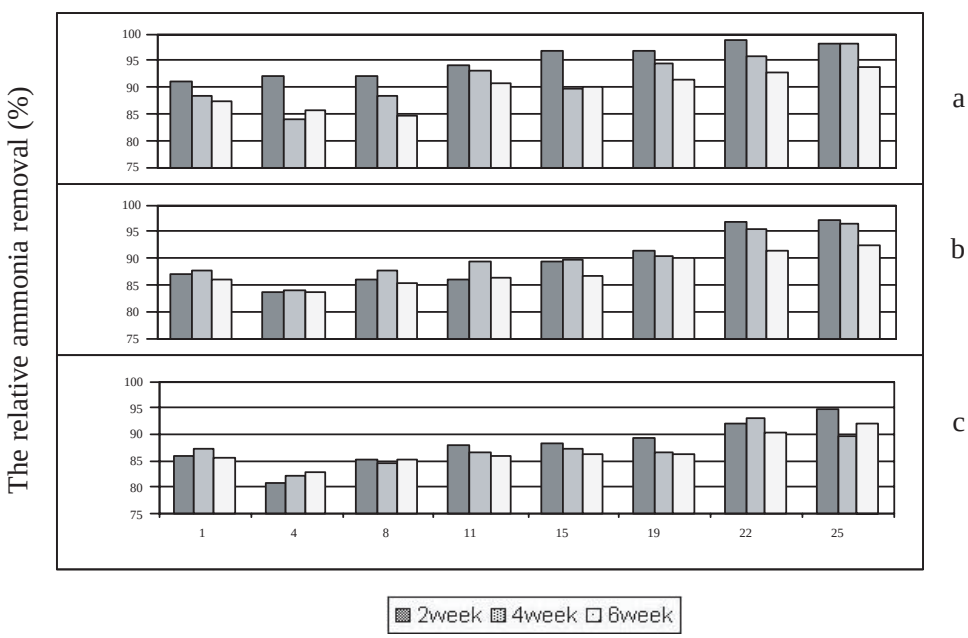

Figure 3 The relative ammonia removal comparing with the control by stored *Spirulina* mat under storage condition of 1x Zarrouk's medium, 3000 lux (a), 2000 lux (b) and 1000 lux (c) light intensity.

employed as the control. The relative ammonia-nitrogen removals by *Spirulina* mats previously stored in 1xZarrouk's medium under 1000, 2000 and 3000 lux light intensity and storage periods of 2, 4 and 6 weeks were shown in Figure 3a, 3b and 3c, respectively. Within the first eight days, the ammonia-nitrogen remained in effluent varied according to the transient period for *Spirulina* cells acclimatizing to the new environment. On twenty fifth day, relative ammonia removal by *Spirulina* mats previously stored under 3000, 2000 and 1000 lux light intensity were in the range of 93.8-98.9, 92.3-97.3 and 91.9-94.8%, respectively. The result corresponded to a report shown that *Spirulina* cells kept under higher light intensity was more active than that of under lower light intensity (Vonshak *et al*., 1996). Moreover, in accordance with ANOVA statistic results, there was difference between the relative ammonia removal on twenty fifth day by *Spirulina* mats stored under 3000 and 1000 lux light intensity especially at two weeks storage time ($P \leq 0.05$).

Focusing on the factor of storage time, different transient times for acclimatizing were required. Comparing *Spirulina* mats among different storage time under the same light intensity of 3000 lux, the relative ammonia removal by two week stored *Spirulina* mat was 98.9% which was higher than 98.1% and 93.8% by four and six weeks stored *Spirulina* mats, respectively. On the contrary, there was no difference of ammonia removal capacities among *Spirulina* mats previously kept for two, four and six weeks neither storage under light intensities of 2000 lux nor 1000 lux (97.3%, 96.5%, 92.3% and 94.3%, 93.0%, 91.9%, respectively).

Average suspended solids during nutrient removal test were 19, 21, 22 mg/l and 20, 20, 22 mg/l for mats previously two, four and six week stored under light intensity of 2000 lux and 1000 lux, respectively. The suspended solids were quite similar to those from the control experiments (18 mg/l). For 3000 lux stored mats, average suspended solids detached from mats previously stored for two and four weeks during nutrient removal tests, were similar to those of 2000 and 1000 lux stored mats. According to more attached cell generated during storage for six weeks under light intensity of 3000 lux, more average suspended solids of 28 mg/l was obtained during nutrient removal test.

The efficiency of ammonia removal by *Spirulina* mats previously stored under different substrate concentrations was also investigated comparing to the fresh *Spirulina* mat. Figure 4 shows the relative ammonia removal of *Spirulina* mats previously stored under 1000 lux light intensity at 0.1x, 0.3x, 0.5x and 1.0x Zarrouk's medium concentrations.

Rapid uptake of nutrient by microalgae after the period of starvation incubation was reported in *Kappaphycus alvarezii* (Dy and Yap, 2001). The surge ammonium uptake of the cultured seaweed without additional enrichment was found higher variability in uptake during the first hour of incubation than that of the cultured alga with additionally exposed to enriched medium before the experiment. In this work, however, ammonia was in placed of nitrate in medium during nutrient tests v.s. storage times. The longer storage (starvation) time, all stored mats showed lower ammonia removal efficiency than the fresh mats (relative efficiencies < 100%) as shown in Figure 4. On the first day of nutrient removal tests, uptake ammonia nitrogen was high, especially the two week stored mats, and gradually decrease in subsequent sampling day. During acclimatization state the effluent ammonia concentration was not stable. After that, the percentage of removal trended to increase. The relative ammonia removal of *Spirulina* mats previously stored for 2 weeks in 0.3x and 0.1x Zarrouk's medium concentration were 94.6, 92.8, 92.8 and 92.4%, respectively ($P > 0.05$). For short time storage, the substrate was enough to maintain the cell activity hence all two weeks storage *Spirulina* mats were still active as

the fresh one.

For longer storage time, the relative ammonia removal by *Spirulina* mats previously stored for four weeks in 1.0x, 0.5x and 0.3x Zarrouk's medium concentration were also insignificant different (93.9%, 91.3% and 91.2%, respectively). However, the relative ammonia removal of *Spirulina* mats in the lowest Zarrouk' s medium (0.1x) was reduced to 87.8% which was significantly lower than the others ($P \leq 0.05$).

Effluent nitrate concentrations of fresh *Spirulina* mat (control) reactor and stored *Spirulina* mat were 0.042 ± 0.004 mg-N/l and in the range of 0.038 - 0.051 mg-N/l, respectively. Low nitrate implied that the ammonia removal according to the above results come from *Spirulina* mat activities, not from nitrifying bacteria.

Total suspended solids during efficiency test by *Spirulina* mats previously stored under 1000 lux light intensity were measured. The trends of suspended solids of 0.5x and 0.3x Zarrouk's medium stored mats were similar to 1.0x ones. Average suspended solids during nutrient removal tests for those mats previously stored for two, four and six weeks in 0.5x and 0.3x Zarrouk's medium were 19, 22, 23 mg/l and 23, 23, 25 mg/l, respectively. On the other hand, the average suspended solids during nutrient removal tests of the mat previously stored in 0.1x Zarrouk's medium were higher, especially for long storage time of two, four and six weeks, as 22, 24 and 30 mg/l, respectively.

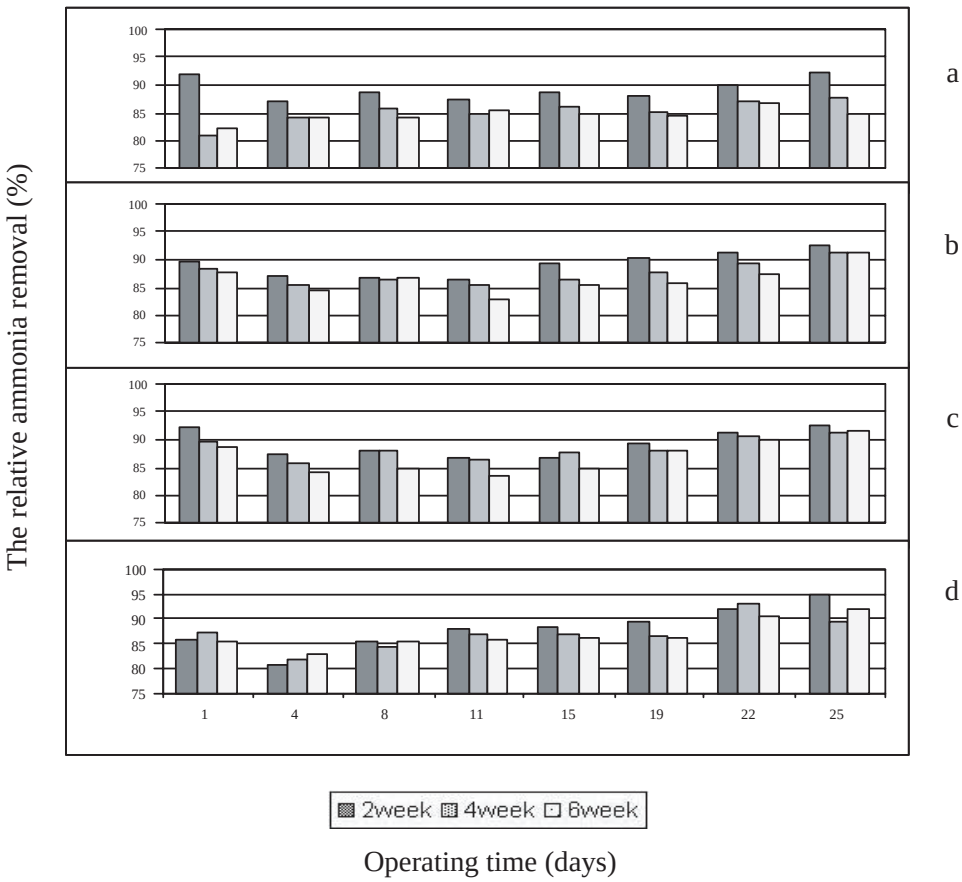

Figure 4 The relative ammonia removal by storage *Spirulina* mats under storage condition of 1000 lux light intensity, in 0.1x (a), 0.3x (b), 0.5x (c) and 1.0x (d) Zarrouk's medium concentration.

CONCLUSION

Ammonia removal efficiency of fresh *Spirulina* mat was better (88%) than stored mats. Storage condition which resulted in the highest removal efficiency was 3000 lux light intensity and 1x Zarrouk's medium at 2 weeks storage (98.9% of control). The lowest ammonia removal efficiency was occurred when mat was previously stored under 1000 lux light intensity, in 0.1x Zarrouk's medium for 6 weeks (85% of control).

The relative ammonia removal by stored *Spirulina* mats should be increased to be 100% (the same as control), if those processes were operated for a longer time. *Spirulina* mats previously stored under lower light intensity and for longer storage times required more transient time.

From this study, *Spirulina* mats can be stored under 1000 lux light intensity, in 0.1x Zarrouk's medium concentration and for six weeks storage time according to ammonia removal efficiency of 85% and effluent ammonia concentration of 0.15 mg/l which still be accepted (compared with fresh *Spirulina* mat). On the other hand, that condition employed the lowest energy consumption and the lowest substrate concentration during storage.

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