THE EXPERIMENTS ON BRINE SHRIMP (*ARTEMIA* SP.) CULTURE TO CONTROL THE QUALITY OF WASTE WATER FROM INTENSIVE SHRIMP FARMING

การทดลองเลี้ยงไรน้ำเค็ม (*Artemia* sp.) เพื่อควบคุมคุณภาพน้ำทิ้งจากการเลี้ยงกุ้งกุลาดำ

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ABSTRACT

*Brine shrimp (*Artemia *sp.) culture experiment in waste water from shrimp farming was operated for 37 days without water circulation and aeration supply. Six circular concrete tanks of 350 l were used. The prior period of the experiment was designed without water exchange to study the difference in water quality between two treatments. The one was control, designed without* Artemia, *and the other one was stocked at a density of 100 individuals/l (pre-adult stage). Three replications were conducted for each treatment. The results showed the significant ($p<0.05$) difference in pH, nitrite, nitrate, ammonia, orthophosphate, total phosphate, dissolved oxygen, biological oxygen demand (BOD), chlorophyll a, chlorophyll b and chlorophyll c content.* Artemia *was able to grow from the initial length of 2.36 mm to the maximum length of 6.06 mm on the 6th day. The brood egg appeared on the 7th day and maximum biomass was on the 5th day. BOD was decreased from the initial level of 8.34 - 3.40 mg/l within the detention time of 6 days.*

Waste water exchange was designed in the experiment to study pollutant removal rate and Artemia *production.* Artemia *was cultured for 23 days and was able to grow from 1.88 - 5.34 mm in length. Brood egg and maximum biomass appeared on the 20th day. The filamentous bacterium infested* Artemia *on the 23rd day. Average production of* Artemia *on the 23rd day was 19.72 g/tank. The pollutant removal rates were the highest*

during the 15th – 23rd day of 0.11mg/ind/day, 4.83 μg/ind/day, 0.53 μg/ind/day and 1.55 μg/ind/day for the removal rate of BOD, chlorophyll a, chlorophyll b and chlorophyll c, respectively.

บทคัดย่อ

ทดลองเลี้ยงไรน้ำเค็มด้วยน้ำเสียจากนากุ้งโดยไม่ใช้ระบบน้ำหมุนเวียนและการเพิ่มอากาศ ในถังทดลองคอนกรีตกลม บรรจุน้ำประมาณ 350 ล. ใช้เวลา 14 วัน เป็นการทดลองเพื่อทดสอบความสามารถของไรน้ำเค็มในการลดมลสารในน้ำเสียจากนากุ้ง เปรียบเทียบระหว่างชุดทดลองที่เลี้ยงไรน้ำเค็มอัตรา 100 ตัว/ล. และชุดทดลองที่ไม่เลี้ยงไรน้ำเค็ม ด้วยจำนวนซ้ำ 3 ซ้ำ พบว่าคุณภาพน้ำจะแตกต่างกันอย่างมีนัยสำคัญ ($p<0.05$) คือ pH ไนไตรท์ ไนเตรท แอมโมเนีย ออร์โธฟอสเฟต ฟอสเฟตรวม ออกซิเจนละลายน้ำ BOD ปริมาณสารแขวนลอย คลอโรฟิลล์เอ คลอโรฟิลล์บี และ คลอโรฟิลล์ซี ไรน้ำเค็มสามารถเติบโตได้โดยไม่ต้องเปลี่ยนน้ำ ให้มวลชีวภาพสูงสุดในวันที่ 5 และไรมีไข่ในวันที่ 7 โดยมีการเจริญเติบโตจากความยาวเฉลี่ย 2.36 มม. เพิ่มขึ้นสูงสุดเป็น 6.06 มม. ในวันที่ 6 ค่า BOD ลดลงจาก 8.34 มก./ล. เหลือ 3.40 มก./ล. ในเวลา 6 วัน จึงเลือกระยะเวลานี้เป็นตัวกำหนดในการเปลี่ยนถ่ายน้ำเพื่อเพิ่มอาหารแก่ไรน้ำเค็มในการทดลองต่อไป ซึ่งพบว่าไรน้ำเค็มเติบโตจาก 1.88 มม. จนมีความยาว 5.34 มม. ให้มวลชีวภาพสูงสุดและไรมีไข่ในวันที่ 20 ของการเลี้ยงเช่นกัน ไรจะเกิดโรคจาก filamentous bacterium ในวันที่ 23 และเก็บผลผลิตเฉลี่ยได้ 19.72 ก./ถัง อัตราการบำบัดน้ำเสียสูงในช่วงระยะ 15–23 วัน ของการเลี้ยงโดยมีค่าสูงสุดคือ 0.11 มก./ตัว/วัน, 4.83 ไมโครกรัม/ตัว/วัน, 0.53 ไมโครกรัม/ตัว/วัน, 1.55 ไมโครกรัม/ตัว/วัน สำหรับค่า BOD คลอโรฟิลล์เอ คลอโรฟิลล์บีและคลอโรฟิลล์ซี ตามลำดับ

INTRODUCTION

The pond culture pattern of shrimp farming in Thailand can be broadly classified into 3 categories based on the level of production : extensive, semi-intensive and intensive. Features of intensive shrimp culture include seed supply, from hatchery, high stocking density, formulated diets, application of aeration and water exchange. Intensive shrimp culture is designed for maximize production in the limited land by using these technologies. However, increasing the number of shrimp ponds, usually increases the seawater supply and also affects the quality of seawater. A major limiting factor in intensive shrimp culture is water quality because of in-adequate water quality control mechanisms for pollutant removal and detoxification.

Intensive management requires applications of fertilizers and feeding with pellet

into the pond culture; both practices cause eutrophication of shrimp ponds and increasing of BOD[24]. The pellet from feeding remains, excretion from the shrimps which is called "organic matter" and phytoplankton accumulates causing a BOD increase.

Water quality problems in aquaculture may be divided into problem area: the water supply, media (water in culture pond) and pond outlet which are interrelated. The important water quality parameters in intensive shrimp ponds are salinity, dissolved oxygen, pH, ammonia, nitrite, suspended solids, BOD, phytoplankton abundance etc.

The treatment plant for the waste water from shrimp farm is not practical, because of great deal of the discharge of the pond outlet and low BOD level.

A brine shrimp is a non selective filter feeding crustacea with high tolerance, a short period of culture and a high benefit. The brine shrimp can filter and digest the organic particles; thus, pollutant removal by *Artemia* sp. feeding is the main purposed and *Artemia* sp. harvest is a by-product.

The brine shrimp is live food and suitable for mariculture which is used in shrimp and marine fish hatchery[25]. In Thailand, the cyst of brine shrimp is commonly used in the hatcheries and costs about 1,200-1,800 baht/kg and the cost of live brine shrimp is about 50-100 baht/kg.

This experiment was carried to conduct the comparative study of water quality with and without brine shrimp culture and to study the pollutant removal rate of brine shrimp using waste water from shrimp farms as a food source.

MATERIALS AND METHODS

Materials

The waste water from shrimp farm which had been cultured for 90-120 days taken from a private farm at Ranot District, Songkhla Province.

- *Artemia* sp. cyst from San Francisco Bay tribe

Experimental procedure

First step experiment (Preliminary experiment)

The experiment was held for 14 days. Those conditions were without feeding by supplementary food, aeration and water exchange. All experimental tanks were left outdoors in the same conditions as shrimp farm.

A. Experimental unit

Each circular concrete tank was used to install shrimp farm waste water for 315 l. There were 2 treatments and 3 replications in the experiment which were 3 tanks of the control, without *Artemia* and 3 tanks of the treatment with *Artemia* (pre-adult stage) at a density of 100 ind/l.

B. Determination of water quality, plankton analysis and *Artemia* growth

Water quality: the waste water in every tank was collected daily during 7.00-9.00 a.m. and analysed for pH, salinity, nitrite, ammonia, orthophosphate and total phosphate, but dissolved oxygen and temperature were measured directly at dawn (5.30 - 6.00 a.m.)

Plankton: the waste water in every tank was collected during 7.00-9.00 a.m. and analysed daily.

Artemia growth: *Artemia* was sampled from every tank in the treatment. The *Artemia* density was determined daily during 1.00-3.00 p.m. The *Artemia* growth of body length and weight were done as the same time of plankton sampling and were also collected for daily analysis.

Secondary step experiment (Feasibility experiment)

This experiment was operated for 23 days. The waste water examination was only one treatment with *Artemia* density of 100 ind/l. Each 2.0 tons of waste water from the shrimp farm for 5 time-transportations was taken to exchange the media after the *Artemia* consumed the particulate suspendance until the BOD was degraded at detention time (4 mg/l)[15]. This detention time is determined from the result of the first step experiment.

A. Experimental unit

Those same experimental tanks as the first step experiment were used and also left outdoors. Six replications were designed for this experiment. The waste water was determined to drain out at 15 cm-level, and exchange with the new one by filling at the 40-45 cm-level. The level of water was needed to record for calculation of water volume.

B. Determination of water quality, plankton analysis and *Artemia* growth

Water quality: the waste water in every tank was collected for immediate analysis while the effluent was drained out and the new waste water was already filled up. The parameters were analysed such as pH, salinity, nitrite, nitrate, ammonia, orthophosphate, total phosphate, suspended solids, BOD, total chlorophyll contents and water temperature.

Plankton analysis: the waste water in every tank was collected for immediate analysis while the effluent was drained out and the new waste water was already filled up. The *Artemia* growth of determination by body length and weight was done at the same time of plankton sampling.

Method of sampling and sample preparation

Water sampling: one liter of water sample was siphoned through a 200 μ plankton net, in the middle level of the tank by using a pipe. Then the samples were brought to the laboratory to be immediately analysed.

Water samples were prepared by filtration through filter paper Whatman GF/C to determine of nitrite, nitrate, ammonia, orthophosphate and total phosphate.

Plankton and *Artemia* sampling: phytoplankton, zooplankton and *Artemia* were sampled by one liter volume, then filtered through 25 μ plankton net. The samples were preserved in 5% formalin solution.

Method of water quality analysis

Nitrate, nitrite, ammonia and orthophosphate were determined by method of Stickland and Parsons.[26]

Total phosphate and BOD were determined by method of APHA, AWWA and WPCF.[2]

Chlorophyll a, b and c contents and phytoplankton quantity were measured by method of APHA, AWWA and WPCF.[2]

Phytoplankton and zooplankton were classified by the method of Cupp[10], Desikachary[11], Fergoson[12], Kudo[16] and Shirota[22]

Suspended solid was measured by the method of Chuan and Sugahara.[8]

Method of growth and mortality determination

The 30 individuals of *Artemia* were examined in each tank of the treatment. The body length was measured by profile microscope and determined the length from the carapace to the fucal.

The individual weight was examined after the length measurement. The *Artemia* samples were prepared by isolation during measurement of the body length and transferred to the small bottle which contained distilled water. The samples were filtered through Whatman Filter Paper No. 1 about 30 ind/tank and dried in the oven at 60°C for 2h, then they were left in desiccator for 1h. The weight was measured by a high sensitive balance, modified from Benijts et al.[3]

The *Artemia* mortality was determined at the first rearing through out the last day. The mortality at the other day was analysed as follows:

$$\text{Mortality (\%)} = D_i - D_n \times 100/D_i$$

D_i = the density of *Artemia* on the first rearing day

D_n = the density of *Artemia* on the other rearing days

Data analysis

At the first step of the experiment, every variable of water quality between the control and the treatment were compared and analysed day by day, using a t-test.

At the secondary step of experiment, the water quality was comparatively reported both before and after *Artemia* culture.

The statistical analysis of pollutant removal rates were conducted by completely randomized designed (F-test) and various pollutant removal rates which were different were tested by Duncan's New Multiple Range Test.[9]

RESULTS

First step experiment

Water quality such as : temperature, salinity, pH, nitrite, nitrate, ammonia, orthophosphate, total phosphate, dissolved oxygen, BOD, suspended solid, chlorophyll a, chlorophyll b and chlorophyll c in the control and the treatment were shown in Table 1. The plankton abundance in the control and the treatment were shown in Tables 2 & 3. The first step experiment found that detention time of 6 days was the most suitable period for the highest decrease in BOD in the treatment and this detention time was selected for exchanging water media in the second step experiment.

Secondary step experiment

Water quality

The pH and salinity during this experiment, varied from 7.21 - 7.81 and 33.5 - 36.8 ppt, respectively (Table 4).

The water temperature was 28.0 - 31.7°C, as the water was being exchanged. The difference of temperature between before and after drainage was 0.7, 0.0, 1.5 and 3.83°C at the 1st, 2nd, 3rd and 4th order batch of water exchange, respectively (Table 4).

Nitrite in the 2nd, 3rd and 4th order batch of water exchange were increased 300.00%, 100.00% and 400.00%, respectively. However, in the 1st and the 5th order batch of water exchange, there was a decrease of 50.00% and 80.00%. Both waste water and effluent had the same range of 0.001-0.005 mg NO_2/l (Table 4).

Nitrate in the 2nd, 3rd and 4th order batch of water exchange increased 350.00%, 33.33% and 63.22%, respectively. However, nitrate in the 1st and the 5th order batch of water exchange decreased 47.06% and 100.00%. The waste water and effluent were 0.004 - 0.029 mg/l and 0.000 - 0.038 mg/l (Table 4).

Ammonia in comparison of waste water and effluent, increased in the 1st, 2nd, 3rd and 4th order batch of water exchange of 563.83%, 476.15%, 382.89% and 741.08%, respectively. Ammonia in waste water and effluent were 0.14 - 1.07 mg/l and 0.61 - 1.56 mg/l (Table 4).

Orthophosphate in comparison of waste water and effluent increased in the 1st, 2nd, 3rd and 4th order batch of water exchange of 550.00%, 600.00%, 700.00% and 750.00%, respectively. The orthophosphate in waste water and effluent were 0.002 - 0.016 mg/l and 0.000 - 0.038 mg/l (Table 4).

Total phosphate in comparison of waste water and effluent increased in the 1st, 2nd, 3rd and 4th order batch of water exchange of 6.12%, 123.81%, 148.15% and 1187.50%, respectively. In the 5th order batch of water exchange, it decreased to 50.00%.

Total phosphate in waste water and effluent were 0.008 - 0.021 mg/l and 0.047 - 0.103 mg/l.

Biochemical oxygen demand in comparison of waste water and effluent decreased in the 1st, 2nd, 3rd, 4th and 5th order batch of water exchange of 88.45%, 95.00%, 84.92% and 1.35%, respectively. BOD in waste water and effluent were 4.00 - 8.88 mg/l and 0.04 - 8.74 mg/l (Table 4).

The suspended solids in waste water and effluent were 193.5 - 135.3 mg/l and 124.2 - 171.2 mg/l.

Chlorophyll a content in comparison of waste water and effluent decreased in the 1st, 2nd, 3rd, 4th and 5th order batch of water exchange and accounted for 977.42%, 1129.06%, 721.88%, 1135.94% and 229.81%, respectively. Chlorophyll a in waste water and effluent were 66.8 - 165.2 μg/l and 6.2 - 50.09 μg/l (Table 4).

Chlorophyll b content in comparison of waste water and effluent decreased in the 1st, 2nd, 3rd, 4th and 5th order batch of water exchange and accounted for 1733.33%, 73.33%, 220.00%, 275.86% and 103.99%, respectively. Chlorophyll b in waste water and effluent were 8.2 - 23.5 μg/l and 2.9 - 11.52 μg/l (Table 4).

Chlorophyll c content in comparison of waste water and effluent decreased in the 1st, 2nd, 3rd, 4th and 5th order batch of water exchange and accounted for 320.78%, 476.19%, 428.43%, 223.68% and 125.96% respectively. Chlorophyll c in waste water and effluent were 24.6 - 64.4 μg/l and 7.6 - 28.5 μg/l (Table 4).

Plankton abundance

The plankton composition consisted of 22 genera and the main groups were *Chlorococcus, Trichodesmium, Chlorella, Cosinodiscus, Nitzschia* etc. The zooplankton comprised 11 groups and main groups were occupied by copepod and rotifer. The total of zooplankton in waste water was 4986, 1123, 494, 6 and 1420 ind/l for the 1st, 2nd, 3rd, 4th and 5th batch, respectively. The zooplankton in the effluent totally was 3875, 2491, 160, 621 and 32 ind/l for the 1st, 2nd, 3rd, 4th and 5th batch, respectively (Table 5).

The genera of phytoplankton still remaining in the effluent were *Climacosphenia, Gyrosigma* and *Oscillatoria* which accounted for 63%, 37%, and 5%, respectively. Zooplankton, the groups which remained in the effluent were copepod and ostracod which accounted for 42% and 12%. The increasing genera of those plankton in effluent were *Volticella, Zoothamnium, Actinophaenium* and rotifer. Rotifer in the effluent was approximately 10 times of the amount in waste water (Table 6).

Pollutant removal rate

BOD removal rates averaged 0.0348, 0.0410, 0.0651, 0.1100 and 0.0244 mg BOD/ind/day during the period of the 1st, 2nd, 3rd, 4th and 5th batch, respectively.

The analysis of data indicated that the various averages of BOD removal rate were significantly ($p<0.05$) different (Table 7).

The chlorophyll a removal rate averaged 0.3414, 1.6894, 1.2076, 1.3080 and 4.8355 μg/ind/day for the 1st, 2nd, 3rd, 4th and 5th batch, respectively (Table 7).

The analysis of data indicated that the various averages of chlorophyll a removal rates were significantly ($p<0.01$) different. Chlorophyll a removal rate of the 5th batch was significantly ($p<0.05$) higher than the others.

The chlorophyll b removal rates averaged 0.0303, 0.0993, 0.1001, 0.0693 and 0.5344 μg/ind/day for the 1st, 2nd, 3rd, 4th and 5th batch, respectively. The analysis of data indicated that the various chlorophyll b removal rates were significantly ($p<0.01$) different. The statistical analysis showed the chlorophyll b removal rate of the 5th batch was the highest (Table 7).

The chlorophyll c removal rates averaged 0.1403, 0.7586, 0.3868, 0.3224 and 1.5506 μg/ind/day, for the 1st, 2nd, 3rd, 4th and 5th batch, respectively. The analysis data showed that the various chlorophyll c removal rates were significantly ($p<0.01$) different (Table 7).

***Artemia* growth**

Artemia weight increased from the 1st - the 4th batch, but it decreased in the 5th batch. The records of dry weight were 0.00007, 0.000071, 0.000106, 0.000206, 0.000384 and 0.000305 g/ind for the initial, 1st, 2nd, 3rd, 4th and 5th batch, respectively.

The length of *Artemia* from the initial batch to the 5th batch increased and accounted for 1.88, 3.79, 4.26, 5.19, 5.34 and 5.81 mm, respectively.

The levels of *Artemia* mortality decreased from the initial batch to the 5th batch, which were 0%, 11.6%, 27.38%, 27.67%, 56.56% and 62.57%, respectively.

The 1.86 mm total length of the post metanauplius stage were fed. The *Artemia* reached adult stage (4.26 mm) and precoupulation on the 12th day. On the 20th day, few females carried the egg brood, and after that became unhealthy and were sluggish. The body colour faded from reddish brown after the 4th order batch of water exchange (the 20th day of rearing). On the 23th day, the *Artemia* was infested with a filamentous bacterium.

The *Artemia* in the various tanks was harvested on the 23th day. The averages of live weight and number were 19.74 g/tank and 3,505.67 ind/tank.

DISCUSSION

The comparative ammonia values showed that the control could be able to be self purified and degraded. The ammonia which dissolved in the water is diffused and

changes to gas formation.[6] The phytoplankton of the control was more than the treatment and can absorb ammonia in photosynthesis. The ammonia was accumulated due to the *Artemia* mortality. The high protein content (47-60%) and decaying, *Artemia* and its excretion, produced the excess ammonia concentration. The nitrite and nitrate in the control were more than the treatment. The high ammonia concentration might be an inhibitor of the nitrifers, because nitrite and nitrate in the treatment were lower than the control.[23] The low BOD in the treatment was influence to the nitrification and reflected the low nitrite and nitrate concentration of the treatment.[20]

The important reason for the increasing nitrate in the control was due to the excess of blue green algae in the control. Blue green algae was able to fix the nitrogen gas to nitrate. In Table 3, the amount of *Trichodesmium* on the 5th - the 14th day was more than the treatment.

Both orthophosphate and total phosphate in the treatment were more than the control, because dead *Artemia* released the phosphorus compounds.[7] The utilization of domestic waste water for freshwater flea culture, also produced the increased orthophosphate from the control unit.[18]

Generally, the BOD is influenced from carbonaceous and nitrogenous organic matter.[17] The BOD of waste water from shrimp farm was mainly influenced from carbonaceous organic matter from phytoplankton, according to cell abundance. The degradation of phytoplankton cells might affect BOD decreasing. BOD removal depended on the decrease of phytoplankton. The detention time of this biological treatment was 6 days, according to the decrease of BOD from 11.90 - 3.14 mg/l by *Artemia* filterability. The results showed that the degradation of BOD between the 1st and the 2nd experiment was not fixable on the 6th day.

The DO in the treatment was less than the control due to the *Artemia* respiration.[18] The interrelationship of low DO and high ammonia concentration was reported that DO of 2.3 mg/l and ammonia of 33.5 mg/l caused 90% of tiger shrimp prawn to die within 96 h. Increasing DO to 5.7 mg/l and ammonia of 33.9 mg/l, caused only 3.33% mortality.[1]

The pH in the control was higher than the treatment, it might be described in two basic causes :

- Influence of ammonia concentration

The nitrification caused the decrease of pH in the treatment modified by Higgin and Burns.[13]

- Influence of carbon dioxide concentration

The carbon dioxide caused by excess *Artemia* respiration, was hydrolysed to carbonic acid.

The suspended solids in the treatment was less than the control. The observation of water colour in the treatment was clear but the colour of control was still greenish

brown and it was similar to the suspended solids value throughout the secondary step experiment. To be reasonable that *Artemia* was not able to remove any groups on plankton such as *Euglena, Gymnodinium, Nitzschia Chlorella, Aphanocapsa* and rotifer etc.

The chlorophyll a content is the main component of various phytoplankton.[4,19] In the treatment the chlorophyll a decreased after the 4th day, according to the degradation of suspended solids and phytoplankton. Thus, decreasing of chlorophyll a content during the 4th day - the 7th day reasonably supported that *Artemia* was able to filter plankton more than the prior day.

The chlorophyll b content is the component of green algae.[4,19] The green algae in the 1st step experiment comprised *Aphanocapsa Scenedesmus, Cosmarium, Spirogyra* and *Chlorella.* The chlorophyll b content decreased during the 4th - the 14th day, indicating that the *Cosmarium* and *Chlorella* had gone. This result supported that *Artemia* was able to feed on *Cosmarium* and *Chlorella.*[14] During the 2nd step experiment, *Spirogyra, Scenedesmus* and *Chlorella* were removed.

The chlorophyll c content is the component of diatom, Euglenaceae and dinoflagellate.[4,19] In the 2nd step experiment, *Melosira, Cosinodiscus, Skelletonema, Cheatoceros, Amphipora, Navicula* and *Streptotheca* were the decreasing diatoms. *Gymnodinium* was not decreased due to effect on its well locomotion and *Artemia* was not able to feed, especially *G. lohmanni* was too large for filterability. In the 1st step experiment, *G. lohmanni* was dominated over on the 11th day - the 14th day. Thus, it may be indicated that the remain species might be a trouble to the *Artemia.*

In the 1st step experiment, *Artemia* weight and length increased and reached adult stage within 5 days. Few *Artemia* formed brood egg after the 8th day with approximately 20-30 egg/ind. The comparative fecundity of the same strains as this study, which was fed by rice bran suspension showed that the average fecundity was 73.6 egg/ind. The growth and biomass were decreased after the 12th day, according to the increasing of ammonia and decreasing of suitable food sources. The determination of harvesting might be on the 5th - the 6th day.

In the 2nd step experiment, maximum *Artemia* weight was on the 20th day of 0.3841 mg/ind. The initial size, BOD and plankton species affected *Artemia* growth. According to different initial sizes (2.36 mm and 1.86 mm for the 1st step and the 2nd step experiment, respectively), the culture period was approximately 3 - 4 times longer to reach the same adult size.

Artemia mortality was influenced by the following factors :

- Temperature effect : The wide range of diurnal temperature and long periods of culture affected mortality.[27] The wide range temperature (3.7°C) occurred during water exchange in the 5th batch of the 2nd step experiment. This condition might be

affected *Artemia* metabolism and cause infestion by external parasitic protozoa and bacteria.

- External parasites: The observation by microscope found that Vorticellidae and Scyphididae were parasites of *Artemia*. *Zoothamnium* is the common genus in shrimp pond and always infests the crustracea.[5]

- Competition community: The zooplankton in the waste water was phytoplankton feeders, with rotifer and copepod as the main zooplankton groups. The zooplankton was larger size and more active movement than *Artemia*. The same particular food sources were consumed by the zooplankton and caused *Artemia* to starve. In the 1st step experiment, dinoflagellate was dominant over because *Artemia* was not able to feed.

- Bacterial infestion: The shortage of immunity for *Artemia* caused bacterial infestion. This might be due to the unsuitable condition, such as temperature change, protozoa infestion and starvation, which occurred during the 2nd step experiment.

The removal rate depended on filterability of *Artemia*. The increasing length and weight influenced on the removal rate. The advanced developmental stage of *Artemia* is also differentiated and the locomotive and filtering organs are more advanced.[21]

The maximum and minimum of BOD removal rates were belonged to the 4th and the 5th batch, respectively. Filterability was gradually increased from the 1st, 2nd, 3rd and 4th batch, respectively. The accumulation of nitrogeneous organic matter from dead *Artemia* caused BOD to increase. The low BOD removal rate was revealed in the last batch.

The maximum and minimum of chlorophyll a, chlorophyll b and chlorophyll c removal rates belonged to the 5th and the 1st batch, respectively. Those chlorophyll contents on the 5th batch were well removed because of *Artemia* filterability.

The *Artemia* stocking density of 100 ind/l reared in the waste water from intensive shrimp farm produced a maximum biomass of 36.63 mg/l (in the 1st step experiment) and 20.02 mg/l (in the 2nd step experiment). Both of two experiments could not produce the offspring (nauplii). It might be that the limited condition, nutrition and infestion of protozoa were not suitable for recruitment. However, the *Artemia* was able to grow and produced biomass.

RECOMMENDATIONS

Artemia filterability only degraded a few pollutants, such as BOD, chlorophyll contents, nitrate and nitrite. On the other hand, ammonia nitrogen and phosphate were increased. The protection of water pollution should include seaweed for the biological treatment component to absorb the excess ammonia and phosphate. The effluent from

those two biological treatment components might provide suitable seawater for a close system of water supply.

Plankton composition in shrimp farm should be examined before culture.

Artemia is able to feed only in small particles. The waste water from shrimp farm may sometimes be dominated by unsuitable plankton. In addition to some toxic phytoplankton might be harmful for *Artemia*. Therefore, the pre-examination of plankton species is essential for *Artemia* culture.

Generally, the water movement system for intensive *Artemia* culture is applied for using in mass production. The sediment removal, remove of ammonia, and aeration are the practicles to improve the production.

In biological treatment unit, production may be improved by these criteria. The outdoor culture condition as biological treatment may cause diurnal thermal stratification. The influence of high temperature damages *Artemia* production. Thus, using water movement can decrease high water temperature and raise feeding filterability of *Artemia*.

The suitable density of *Artemia* culture in biological treatment needs to be studied to gain the maximum production and highest efficiency of pollutant removal in *Artemia* filterability.

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Table 1. The comparison of water qualities between control and treatment (The top line data and below line data are the average of control and treatment, respectively)

Water qualities	Exposure time (days)														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Water temperature (°C)	27.70 28.17	27.70 27.83	27.33 27.20	27.90 27.83	27.70 27.80	27.13 27.23	28.57 28.87	28.17 28.27	28.53 28.60	28.23 28.30	28.70 28.63	28.97 29.00	27.47 27.33	27.90 27.90	27.47 27.43
Salinity (ppt)	37.33 37.33	38.00 38.33	37.30 37.00	37.00 37.00	37.00 37.00	37.67 37.33	38.67 37.67	39.33 39.00	38.67 39.00	38.00 38.00	36.83 37.17	36.33 35.33	40.33 40.00	37.00 36.33	40.00 40.07
pH	8.05 8.23	8.04* 7.97*	8.33** 8.20**	8.06** 7.81**	7.82 7.82	8.28** 7.77**	8.12** 7.74**	8.12** 7.74**	8.17** 7.77**	8.41** 7.77**	9.09** 8.57**	8.99** 8.37**	8.53** 7.88**	8.53** 7.88**	8.57** 7.93**
Nitrite (mg/l)	0.021 0.020	0.017** 0.014**	0.017** 0.013**	0.016 0.012	0.012** 0.018**	0.028 0.021	0.043* 0.019*	0.056* 0.027*	0.116 0.066	0.162** 0.032**	0.280** 0.080**	0.410* 0.140*	0.670 0.740	0.440 0.250	0.330 0.410
Nitrate (mg/l)	0.065 0.161	0.118 0.032	0.055 0.049	0.048 0.046	0.085 0.076	0.060 0.076	0.078 0.054	0.210 0.086	0.174 0.111	0.298** 0.085**	0.370 0.160	0.680* 0.230*	0.730 0.400	0.700 0.530	0.560 0.610
Ammonia (mg/l)	2.89 2.99	3.60 3.69	2.92 3.12	2.11 1.86	3.27* 2.35*	2.27** 3.19**	1.84** 3.04**	1.960 2.542	1.940** 3.960**	1.630** 4.210**	1.200** 4.250**	0.510* 3.500*	0.530** 4.830**	0.220** 3.390**	0.540** 2.930**

Remark : **highly significant ($p < 0.01$), *significant ($p < 0.05$) and none label is not significant.

Table 1. (Continued)

Water qualities	Exposure time (days)														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Orthophosphate (mg/l)	0.048	0.029**	0.072	0.041	0.076**	0.042**	0.053**	0.058**	0.057**	0.046**	0.046**	0.037**	0.024**	0.024**	0.019**
	0.047	0.048**	0.089	0.050	0.046**	0.104**	0.126**	0.134**	0.153**	0.163**	0.159**	0.154**	0.154**	0.154**	0.159**
Total phosphate (mg/l)	0.058	0.081	0.072	0.045	0.064**	0.117	0.095**	0.104	0.141	0.108**	0.112	0.107**	0.074**	0.069**	0.074**
	0.090	0.090	0.096	0.066	0.046**	0.131	0.156**	0.173	0.153	0.188**	0.189	0.218**	0.185**	0.169**	0.204**
DO (mg/l)	0.66	3.13*	4.68*	3.59**	1.94	1.77	1.51	1.99**	2.00	4.15**	4.09**	5.03**	4.87*	5.17**	5.78**
	0.44	1.84*	3.18*	1.55**	1.33	1.70	0.98	1.12**	1.29	2.17**	2.18**	2.50**	3.04*	3.07**	3.25**
BOD (mg/l)	8.84	12.58	14.02	10.99	6.92	10.38	11.90**	7.84*	9.30**	5.30**	2.06**	11.85**	6.68**	5.78**	4.18**
	8.34	12.46	14.20	9.59	5.60	5.14	3.14**	1.53**	1.06**	0.00	0.54*	3.78**	0.02**	0.28**	0.72**
Suspended solids (mg/l)	249.68	172.33	195.68	260.68	246.00*	203.33	136.33	165.00**	190.67	196.33*	165.67*	196.00	134.00	221.00	205.67
	220.68	168.33	194.00	256.00	266.00*	258.67	123.67	133.33**	160.00	115.00*	120.67*	156.67	159.33	139.67	193.67
Chlorophyll a (µg/l)	441.30	371.30	699.50	497.27	268.50**	899.43**	396.77**	539.73*	506.67**	221.10	213.70*	335.63	382.38	437.37**	425.31
	467.60	359.13	499.00	345.87	456.47**	155.48**	91.60**	62.50*	53.10**	64.44	39.77**	9.70	20.81	13.07**	12.49
Chlorophyll b (µg/l)	77.37	66.03	136.30	99.97	60.30	154.33**	57.77*	95.27*	119.67*	83.47	64.13	90.13	98.77*	123.10**	109.15
	85.27	67.43	96.80	67.60	69.93	28.40**	27.77*	20.13**	19.03*	67.23	40.27	19.40	19.70*	9.70**	7.31
Chlorophyll c (µg/l)	131.02	112.47	246.63	150.43	62.90**	170.37**	113.77**	71.73*	90.93**	89.97	47.10	92.57**	94.46	69.27**	74.99**
	132.43	91.70	168.77	95.80	156.13**	26.10**	33.93**	40.83*	14.43**	57.00	87.45	22.92**	39.47	11.90**	12.41**

Remark : **highly significant ($p < 0.01$), *significant ($p < 0.05$) and none label is not significant.

Table 2. The plankton analysis of the control on the 1st step experiment

Taxa	The sequence of days in the experiment														
	Starting day	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day
Oscillatoria sp.	14,148	-	-	-	-	-	-	-	507	16,320	-	-	420	-	-
Trichodesmium sp.	265,687	870,982	3,717,466	3,094,757	3,958,013	913,680	144,183	44,213	701,300	112,757	133,448	40,717	-	-	518
Aphanocapsa sp.	20,552	2,377	-	221,427	393,767	1,531,807	2,106,747	726,067	870,860	2,279,537	2,445,627	1,253,883	246,436	48,116	-
Scenedesmus sp.	71,091	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cosmarium sp.	662	-	-	-	-	-	-	-	-	-	-	-	-	1,242	-
Spirogyra sp.	525	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlorella sp.	47,938	15,531	6,440	-	13,187	-	5,780	-	1,620	510	2,370	-	3,650	26,302	3,072
Melosira sp.	-	-	-	-	-	-	-	-	-	-	-	-	10,647	-	-
Cosinodiscus sp.	14,647	472,182	6,903	-	953	-	1,040	-	-	-	40,058	-	17,833	4,031	125
Skeletonema sp.	4,962	111,375	-	-	-	-	-	-	-	-	6,320	-	-	-	654
Cheatoceros sp.	1,575	73,867	-	-	-	-	-	-	-	-	-	-	-	-	-
Navicula sp.	-	1,665	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitzschia sp.	-	-	-	-	-	-	1,483	68,140	92,010	1,919,583	3,064,648	553,933	-	-	-
Euglena sp.	12,861	4,417	-	437,933	680,517	149,267	105,127	318,620	48,260	313,850	1,046,722	157,820	160,579	2,233	1,447
Gymnodinium lohmanni	189,444	719,882	774,380	224,337	458,727	587,037	2,378,038	1,495,307	1,619,210	1,682,563	487,792	845,000	5,172,013	8,052,646	3,910,195
Gymnodinium sp.	-	-	-	-	-	-	-	28,973	-	-	-	-	1,260	-	1,308
Summation of Phytoplankton	758,092	2,272,278	4,505,189	3,978,954	5,505,164	3,181,791	4,742,398	2,681,320	3,333,767	6,325,120	7,226,985	2,851,353	5,612,838	8,134,570	3,917,319
Globigerina sp.	63	-	-	-	-	-	-	-	-	-	-	-	129	-	-
Scyphidium sp.	-	-	-	-	-	-	-	-	-	-	-	2,800	-	-	-
Vorticella sp.	-	-	-	-	-	-	-	-	-	527	-	-	-	-	-
Zoothamnium sp.	-	-	-	-	-	-	-	-	-	5,100		-	-	-	-
unknown celiate	-	208	1,550	2,493	2,317	510	-	-	-	-	1,843	-	-	-	-
Leucosolenia sp.	-	422	-	-	-	-	-	-	-	-	-	-	-	-	-
Calanoid copepod	-	322	503	-	-	527	887	-	1,593	-	-	-	129	146	-
Unknown Ostracod	-	-	-	-	-	-	-	-	-	510	-	-	-	-	-
Daphnia sp.	-	-	1,087	-	-	-	-	-	-	-	-	-	-	-	-
Brachionus sp.	-	-	2,133	2,240	26,283	141,427	202,087	203,853	44,750	39,437	22,767	13,303	23,533	5,803	11,324
Summation of Zooplankton	63	952	5,273	4,733	28,600	142,464	202,974	203,853	46,343	45,574	24,610	16,103	23,791	5,949	11.324
Grand total of the plankton	758,155	2,273,230	4,510,462	3,983,687	5,533,764	3,324,255	4,945,372	2,885,173	3,380,110	6,370,694	7,251,595	2,867,456	5,636,629	8,140,519	3,928,643

Table 3. The plankton analysis of the treatment on the 1st step experiment

Taxa	The sequence of days in the experiment														
	Starting day	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day
Oscillatoria sp.	-	-	-	-	-	-	-	-	-	-	-	-	325	-	-
Trichodesmium sp.	167,338	157,035	2,227,526	695,557	3,680,907	206,637	125,467	25,030	18,363	6,447	21,143	9,963	108	-	-
Spirulina sp.	79	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aphanocapsa sp.	4,408	5,113	-	-	1,161,260	-	1,761,893	7,067	-	-	1,243,803	203,180	186,550	21,488	-
Scenedesmus sp.	29,661	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cosmarium sp.	158	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spirogyra sp.	1,583	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlorella sp.	81,155	5,368	1,340	543	-	2,717	-	-	-	-	-	-	13,802	8,687	1,865
Melosira sp.	-	168	-	-	-	1,630	-	-	-	-	-	-	4,528	-	-
Cosinodiscus sp.	28,732	49,592	14,060	-	3,307	-	-	-	-	-	-	-	5,547	1,650	-
Skeletonema sp.	15,258	-	-	-	-	-	-	-	-	-	-	-	-	-	277
Cheatoceros sp.	833	6,818	-	-	-	-	-	-	-	-	-	-	-	-	-
Gyrosigma sp.	-	84	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitzschia sp.	-	442	-	-	-	-	2,438	-	-	3,240	1,212,583	37,737	130	-	-
Euglena sp.	41,530	2,728	-	69,040	398,187	80,313	109,587	9,487	1,033	11,213	389,385	59,060	116,805	2,450	242
Gymnodinium lohmanni	280,425	233,838	173,787	59,810	530,593	199,683	2,592,713	56,683	299,467	96,027	81,005	248,500	3,857,029	3,892,580	2,129,753
Gymnodinium sp.	-	-	-	-	-	-	-	-	-	-	-	-	1,560	-	-
Summation of Phytoplankton	651,160	461,186	2,416,713	824,950	5,774,254	490,980	4,592,098	98,267	318,863	116,927	2,947,919	558,440	4,186,384	3,926,855	2,132,137
Globigerina sp.	79	106	-	-	-	-	-	-	-	-	-	-	368	-	-
Tintinnopsis sp.	-	-	-	-	-	-	-	-	-	-	-	-	108	-	-
Scyphidium sp.	-	-	-	-	-	-	-	-	-	1,080	-	1,013	-	-	-
Vorticella sp.	-	-	-	-	-	-	477	523	-	-	263	-	-	-	-
Zoothamnium sp.	-	-	-	-	-	-	-	-	-	-	-	-	260	-	-
Epistylis sp.	83	-	-	-	-	-	-	-	-	-	-	-	-	-	-
unknown celiate	-	-	447	-	-	-	-	-	-	-	-	-	-	-	-
Leucosolenia sp.	4,083	2,046	-	-	-	-	-	-	-	-	-	-	-	-	-
Actinosphaenium sp.	83	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Calanoid copepod	-	84	447	1,110	1,137	1,087	46	1,007	-	1,067	787	510	260	932	-
Brachionus sp.	-	-	960	6,207	40,550	47,593	177,930	110,713	68,920	65,947	20,603	5,713	2,102	2,813	3,158
Summation of Zooplankton	4,328	2,236	1,854	7,317	41,687	48,680	178,453	112,243	68,920	68,094	21,653	7,236	3,098	3,745	3,158
Grand total of the plankton	655,488	463,422	2,418,567	832,267	5,815,941	539,660	4,770,551	210,510	387,783	185,021	2,969,572	565,676	4,189,482	3,930,600	2,135,295

Table 4. The water qualities comparison before and after *Artemia* culture on the 2nd step experiment

Water quality	The order of waste water					The order of effluent				
	1i	2i	3i	4i	5i	1f	2f	3f	4f	5f
pH	7.48	7.81	7.4	7.63	7.21	7.52	7.7	7.47	7.38	7.37
Temperature (°C)	29.4	29.6	30	29.05	27.85	28.9	30.0	30.54	31.68	28.6
Salinity (ppt)	33.8	35	28.3	35.7	35.5	36.8	35.2	36.5	36.0	36.0
Nitrite (mg/l)	0.002	0.001	0.001	0.001	0.005	0.001	0.004	0.002	0.005	0.001
Nitrate (mg/l)	0.017	0.004	0.018	0.023	0.029	0.009	0.018	0.024	0.038	0.000
Total ammonia (mg/l)	0.141	0.109	0.187	0.185	1.074	0.936	0.628	0.903	1.556	0.613
Phosphate (mg/l)	0.004	0.002	0.007	0.016	0.008	0.026	0.024	0.056	0.028	0.004
Total phosphate (mg/l)	0.049	0.021	0.027	0.008	0.052	0.052	0.047	0.067	0.103	0.048
BOD 5day 20°C (mg/l)	6.41	4	9.48	6.22	8.86	0.37	0.2	1.43	0.04	8.74
Suspended solid (mg/l)	135.3	171	139.2	137.5	193.5	143.3	124.2	136.2	139.7	171.2
Chlorophyll a (μg/l)	66.8	143.8	157.8	79.1	165.2	6.2	11.7	19.2	6.4	50.09
Chlorophyll b (μg/l)	8.2	12.3	16.3	10.9	23.5	3.0	4.5	5.0	2.9	11.52
Chlorophyll c (μg/l)	32.4	72.6	53.9	24.6	64.4	7.7	12.6	10.2	7.6	28.5

Remark : i : before *Artemia* culture
f : after *Artemia* culture

Table 5. The plankton of both before and after *Artemia* culture in the various waste water exchange

Taxa	The sequence of water exchange (Batch of water changing)									
	1I	1F	2I	2F	3I	3F	4I	4F	5I	5F
Phylum Cyanophyta (Blue green algae)										
Family Oscillatoriaceae										
Oscillatoria sp.	377	-	147	-	-	-	1	53	572	-
Trichodesmium sp.	270,911	2,566	22,429	149	708,021	855	6,445	1,875	268,439	12,944
Family Chroococcaceae										
Chroococcus sp.	28,863,333	173,542	7,285,142	313,519	2,858,285	-	25,312	-	1,981,977	82
Aphanocapsa sp.	-	-	21	-	-	-	-	-	-	-
Phylum Chlorophyta (Green algae)										
Family Scenedesmaceae										
Scenedesmus sp.	547	-	-	-	-	-	-	-	-	-
Family Desmidiaceae										
Closterium sp.	309	-	-	-	-	-	-	-	-	-
Cosmarium sp.	485	-	-	-	-	-	-	-	-	-
Family Zygnemataceae										
Spirogyra sp.	-	-	-	-	4,812	-	-	64	-	-
Family Oocystaceae										
Chlorella sp.	47,807	328	1,617	593	-	58	-	87	-	-
Phylum Chrysophyta (Diatom)										
Family Merosiraceae										
Melosira sp.	316	-	-	-	-	-	-	-	-	-
Family Cosinodiscaceae										
Cosinodiscus sp.	94,879	318	9,996	32	-	-	-	-	-	-
Family Skeletonemaceae										
Skeletonema sp.	294,186	-	6,975	-	-	-	-	-	-	-
Family Chaetoceraceae										
Chaetoceros sp.	18,700	-	126	-	-	-	-	-	-	-
Family Tabellariaceae										
Climacosphenia sp.	75	-	21	60	-	-	-	-	-	-

Table 5. (Continue)

	The sequence of water exchange (Batch of water changing)									
	1I	1F	2I	2F	3I	3F	4I	4F	5I	5F
Family Naviculaceae										
Amphiprora sp.	678	-	65	-	-	-	-	-	-	-
Gyrosigma sp.	-	17	39	-	-	-		-	-	-
Navicula sp.	3,327	-	303	-	146	-	1	-	-	-
Family Nitzschiaceae										
Nitzschia sp.	170	166	1,515	1	15,576	48	-	180	1,475	-
Family Eucampiaceae										
Streptotheca sp.	-	-	271,112	61	20,393	-	7	-	3,763	-
Phylum Pyrrhophyta (Dinoflagellate)										
Family Euglenaceae										
Euglena sp.	-	-	71	-	1,032	222	6	118	1,189	31
Family Perdiidae										
Peridinium sp.	-	-	21	-	-	-	1	-	-	-
Gymnodinium lohmanni	-	-	105	-	4,731	600	18	382	6,975	560
Total (cell/l)	29,596,100	176,937	7,599,705	314,468	3,612,996	1,783	31,791	2,759	2,264,290	13,617
Phylum Protozoa										
Family Globigerinidae										
Globigerina sp.	110	-	-	-	-	-	-	-	-	-
Family Codonellinae										
Tintinnopsis sp.	-	-	19	-	-	-	-	-	-	-
Family Actinobolinidae										
Actinospaenium sp.	-	42	-	-	-	-	-	-	-	-
Family Scyphididae										
Scyphidium sp.	1,533	-	-	-	-	-	-	-	-	-

Table 5. (Continue)

	The sequence of water exchange (Batch of water changing)									
	1I	1F	2I	2F	3I	3F	4I	4F	5I	5F
Family Vorticellidae										
Vorticella sp.	-	171	-	654	-	-	-	1	-	-
Zoothamnium sp.	-	-	-	30	-	-	-	-	-	-
Family Celiat										
unknown celiate	92	-	-	-	-	-	3	-	-	-
Phylum Arthopoda										
Suborder Calanoida										
(Calanoid copepod)	3,031	1,516	405	698	292	54	1	474	1,290	-
Order Ostracod										
Unknown	-	26	699	59	-	-	-	-	-	-
Phylum Rotifera										
Brachionus sp. (rotifer)	-	2,120	-	1,050	202	106	2	147	129	32
Phylum Molusca										
Unknown Bivalvia	220	-	-	-	-	-	-	-	-	-
Total (ind/l)	4,986	3,875	1,123	2,491	494	160	6	621	1,420	32

Remark : I = influence, F = effluence

Table 6. The everage of plankton remaining after using *Artemia* feeding

Taxa	Waste water	Effluent	Lossing	% Loss	Remark
Oscillatoria sp.	219	11	208	95	
Trichodesmium sp.	255,249	3,678	251,571	99	
Chroococcus sp.	8,202,810	97,429	8,105,381	99	
Aphanocapsa sp.	4	0	4	100	
Scenedesmus sp.	109	0	109	100	
Closterium sp.	62	0	62	100	
Cosmarium sp.	97	0	97	100	
Spirogyra sp.	962	13	949	99	
Chlorella sp.	9,885	213	9,672	98	
Melosira sp.	63	0	63	100	
Cosinodiscus sp.	20,975	70	20,905	100	
Skeletonema sp.	60,232	0	60,232	100	
Cheatoceros sp.	3,765	0	3,765	100	
Climacosphenia sp.	19	12	7	37	
Amphiprora sp.	149	0	149	100	
Gyrosigma sp.	8	0	5	63	
Navicula sp.	750	0	750	100	
Nitzschia sp.	37,486	79	37,407	100	
Streptotheca sp.	98,423	12	98,411	100	
Euglena sp.	574	74	500	87	
Peridinium sp.	4	0	4	100	
Gymnodinium lohmanni	2,932	319	2,613	89	
Globigerina sp.	22	0	22	100	
Tintinnopsis sp.	4	0	4	100	
Scyphidium sp.	307	0	307	100	
Vorticella sp.	0	165	-	-	increased
Zoothamnium sp.	0	6	-	-	increased
Unknown celiate	19	0	19	100	
Actinosphaenium sp.	0	8	-	-	increased
Unknown copepod	1,290	548	742	58	
Unknown ostracod	140	17	123	88	
Brachionus sp.	67	691	-	-	increased
Unknown Bivalvia	44	0	44	100	
Summation	8,696,670	103,348	8,593,322	-	

Table 7. The Duncan's New Multiple Range Test of the pollutant removal rates in biological treatment

Batch number	The various removal rate			
	BOD (mg/ind/day)	Chlorophyll a (µg/ind/day)	Chlorophyll b (µg/ind/day)	Chlorophyll c (µg/ind/day)
1	0.0348a	0.3414a	0.0303a	0.1403a
2	0.0410a	1.6894a	0.0993a	0.7586ab
3	0.0651a	1.2076a	0.1001a	0.3868ab
4	0.1100a	1.3080a	0.0693a	0.3224b
5	0.0244b	4.8335b	0.5344b	1.5566c
Average	0.0550	1.8760	0.1667	0.6317

Remark : The pollutant removal rates response without letters in common are significantly different ($p < 0.05$)