Effects of Salinity and pH on the Growth of Blue-Green Algae, *Oscillatoria* sp. and *Microcystis* sp., Isolated from Pacific White Shrimp (*Litopenaeus vannamei*) Ponds

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ABSTRACT

Experiments were carried out to determine the effects of salinity and pH on the growth of *Oscillatoria* sp. and *Microcystis* sp. under laboratory conditions. *Oscillatoria* sp. and *Microcystis* sp. were isolated from Pacific white shrimp (*Litopenaeus vannamei*) low-salinity culture ponds. Mono-clonal culture of each species was kept in 250-ml BG 11 media in the laboratory for 10 days. For *Oscillatoria* sp. salinities of 0, 5, 10, 15, 20, 25 and 30 ppt were maintained throughout the experimental period, while for *Microcystis* sp. salinities of 0, 3, 6, 12, 15 and 18 ppt were maintained. The water pH was maintained at 3.0, 4.5, 6.0, 7.5, 9.0, 10.5 and 12.0 for both species. The algal growth was measured by the determination of chlorophyll-a every 2 days. Results under laboratory conditions showed that the optimal salinity levels for growth ranged from 0 to 10 ppt for *Oscillatoria* sp., and from 0 to 6 ppt for *Microcystis* sp. An increase of the salinity decreased growth of both species. Water pH levels from 7.5 to 9.0 were suitable for growth of both species, while a pH below or above these range caused significant decrease in growth of these blue-green algae.

Key words: *Oscillatoria* sp., *Microcystis* sp., *Litopenaeus vannamei*, salinity, pH

INTRODUCTION

Phytoplankton communities are considered beneficial because they maintain adequate environmental conditions for shrimp culture and when in moderate abundance supply dissolved oxygen and assimilate ammonia (Boyd and Tucker, 1992). On the other hand, most water quality problems in intensive shrimp culture ponds are the result of the unmanaged growth of phytoplankton communities, especially an excessive abundance of blue-green algae (*Oscillatoria* sp. and *Microcystis* sp.). These can lead to an imbalance in the pond dissolved oxygen budget, leading to periods of low dissolved oxygen concentrations that can stress shrimp and make them more susceptible to pathogenic infections. Most dominant blue -green algal blooms in the intensive culture of Pacific white shrimp (*Litopenaeus vannamei*) are composed of *Oscillatoria* sp. and *Microcystis* sp., particularly in low-salinity conditions. Both species can cause off-flavor (Schrader *et al*., 1998; Walker and Higginbotham, 2000) and massive die-offs of these algae are a primary cause of diseases in cultured fish and shrimp (Smith, 1996). In order to achieve good growth and yield for shrimp culture, properly

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managed water quality, pond bottom, and consistent phytoplankton abundance should be maintained (Limsuwan and Chanratchakool, 2004).

Phytoplankton growth and composition are affected by several environmental factors such as pH, light, temperature (Kallas and Castenholz, 1982) and salinity (Fu and Bell, 2003). The most important limiting factor for filament length and cell size of most phytoplankton was determined to be temperature (Alam *et al*., 2001). The pH level was related to nutrient dissolution which caused a change in the species composition and biomass of the phytoplankton (Celekli and Kulkoyluoglu, 2007). At a high pH, a consistent increase of cell division was differentially regulated in different species of phytoplankton (Alam *et al*., 2001).

Chlorophyll-a concentration has been widely used as a convenient correlation of biomass in estimations of phytoplankton growth (Reynolds, 1984). The purpose of this study was to investigate under laboratory conditions the effects of salinity and pH on the growth and chlorophyll-a biomass of *Oscillatoria* sp. and *Microcystis* sp. isolated from Pacific white shrimp low-salinity culture ponds.

MATERIALS AND METHODS

Experimental conditions

The experiments were carried out in a laboratory at the Department of Fisheries Science, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. Blue-green algae, *Oscillatoria* sp. and *Microcystis* sp., isolated from *L. vannamei* low-salinity culture ponds in Chachoengsao province were cultured for mono-algae. Subsequently, *Oscillatoria* sp. and *Microcystis* sp. were grown in 250 ml modified BG-11 medium at room temperature, (28.5±1.3°C) under fluorescent lights (1,250 ±32 lux) with a light/dark cycle (12 h/12 h) for 10 days. Three replicates were used for each treatment.

Effects of salinity and pH

The effects of salinity and pH were determined for the growth of mono-algae cultured under different salinity conditions of 0, 5, 10, 15, 20, 25 and 30 ppt for *Oscillatoria* sp., and 0, 3, 6, 9, 12, 15 and 18 ppt for *Microcystis* sp., respectively. Salinity concentrations were adjusted daily by using sea water and distilled water. For the pH study, the salinity was kept constant at 0 ppt and the pH levels were adjusted daily to desire levels of 3.0, 4.5, 6.0, 7.5, 9.0, 10.5 and 12.0 by using H_2SO_4 and NaOH solutions except for the control group. The growth of *Oscillatoria* sp. and *Microcystis* sp. was measured by the determination of chlorophyll-a every 2 days. The water samples were filtered immediately after sampling through a 47 mm Whatman GF/C glass fiber filter. Pigments on each filter were extracted with 10 ml of 90 % (v/v) acetone and optical densities of the supernatant then measured at 630, 645, 667 and, 750 nm by Miltonroy spectrophotometer (AHPA *et al*., 1995). The morphology of *Oscillatoria* sp. and *Microcystis* sp. was observed under a light microscope.

Statistical analysis

Analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) (Stell and Torrie, 1980) were used to test for significant differences among different salinities and pHs on the chlorophyll-a biomass of *Oscillatoria* sp. and *Microcystis* sp.

RESULTS AND DISCUSSION

Effects of salinity on the growth of *Oscillatoria* sp. and *Microcystis* sp.

The growth of *Oscillatoria* sp. and *Microcystis* sp. was observed and measured by determining the chlorophyll-a biomass. The results showed that at 5 ppt *Oscillatoria* sp. reached optimum growth with a chlorophyll-a of 4,455±435 µg/L; however, there were no significant differences among the 0, 5 and 10 ppt groups. Chlorophyll-a biomass of *Oscillatoria* sp. was lower at a salinity of 15 ppt and lowest at 30 ppt (Table 1 and Figure 1). For *Microcystis* sp. the optimum chlorophyll-a biomass was 5,244 ±1091 µg/L at 0 ppt (freshwater) and gradually decreased when the salinity increased and the lowest chlorophyll-a was observed at 18 ppt (Table 2 and Figure 2). There were no significant differences of chlorophyll-a ($P>0.05$) in the 0, 3, and 6 ppt groups. The salinity changes led to an aggregation of *Oscillatoria* sp., especially when the salinity was higher than 15 ppt (Figures 3 and 4). Salinity influences the physiology of blue-green algae and could disturb ion balance or induce nutrient deficiencies (Konopka, 1981). A decline in photosynthesis under hyperosmotic stresses could change the fine structure of the chloroplasts causing a disruption of energy transfer between the two photo systems (Konopka, 1981).

Table 1 Chlorophyll-a (µg/L) of *Oscillatoria* sp. cultured at different salinities for 10 days

salinity (ppt)	Time (days)					
	0	2	4	6	8	10
0	45±2^{a}	286±45^{a}	1,005±194^{a}	2,204±524^{a}	2,512±840^{a}	3,649±886^{a}
5	45±3^{a}	282±44^{a}	1,178±213^{a}	2,134±145^{a}	2,758±478^{a}	4,455±435^{a}
10	50±2^{a}	222±17^{ab}	750±48^{ab}	1,784±429^{a}	2,261±622^{a}	3,046±712^{a}
15	50±2^{a}	171±22^{b}	341±187^{b}	555±244^{b}	690±512^{b}	587±383^{b}
20	45±2^{a}	115±10^{c}	146±78^{c}	75±63^{c}	60±36^{c}	24±18^{c}
25	50±3^{a}	123±16^{c}	135±50^{c}	159±80^{c}	91±85^{c}	4±4^{c}
30	45±2^{a}	95±31^{c}	56±56^{d}	48±48^{c}	28±28^{c}	0±0^{c}

Different letters in the same column mean a significant difference ($P<0.05$)

Table 2 Chlorophyll-a (µg/L) of *Microcystis* sp. cultured at different salinities for 10 days

salinity (ppt)	Time (days)					
	0	**2**	**4**	**6**	**8**	**10**
0	55±3^{a}	595±48^{a}	908±56^{a}	2,051±100^{a}	4,133±431^{a}	5,244±1,091^{a}
3	55±3^{a}	619±54^{a}	1,801±75^{a}	1,714±143^{a}	3,475±545^{a}	3,943±73^{ab}
6	50±3^{a}	532±16^{a}	508±41^{b}	1,301±89^{b}	1,821±200^{b}	2,860±590^{ab}
9	55±3^{a}	543±51^{a}	563±24^{b}	1,012±41^{bc}	1,535±439^{b}	1,591±349^{b}
12	50±2^{a}	587±71^{a}	547±69^{b}	781±151^{c}	1,008±383^{bc}	841±324^{bc}
15	55±3^{a}	615±76^{a}	591±87^{b}	786±40^{c}	694±20^{c}	373±108^{c}
18	55±2^{a}	551±39^{a}	567±79^{b}	551±165^{c}	250±25^{c}	115±51^{c}

Different letters in the same column mean a significant difference (P<0.05)

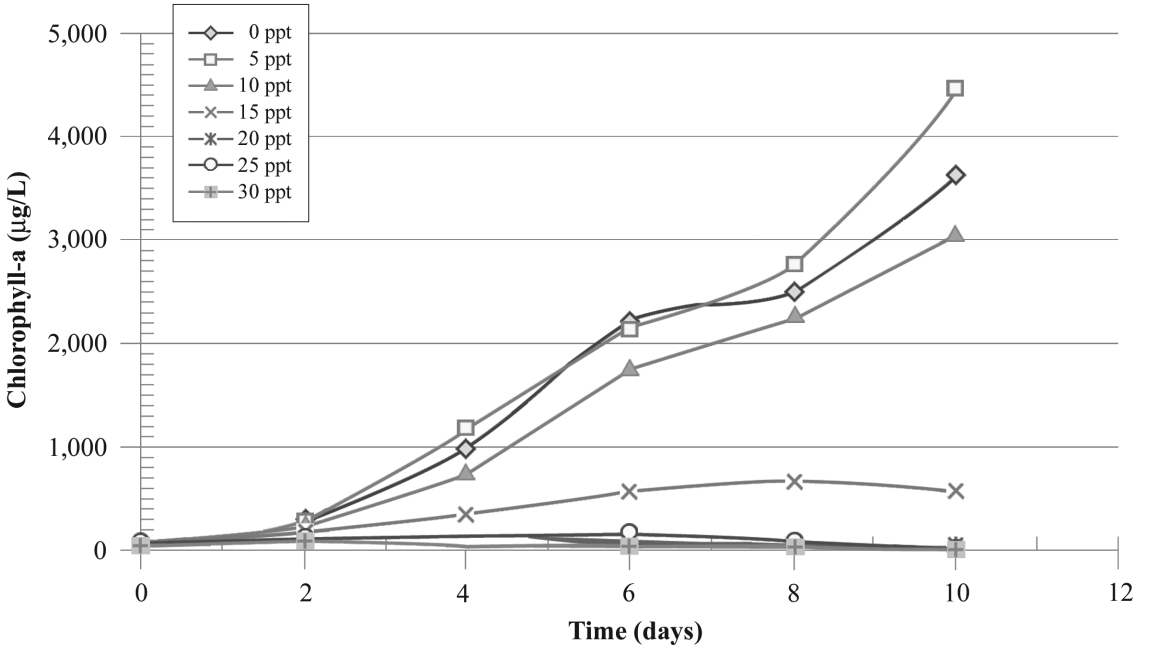

Figure 1 Chlorophyll-a of *Oscillatoria* sp. cultured at different salinities

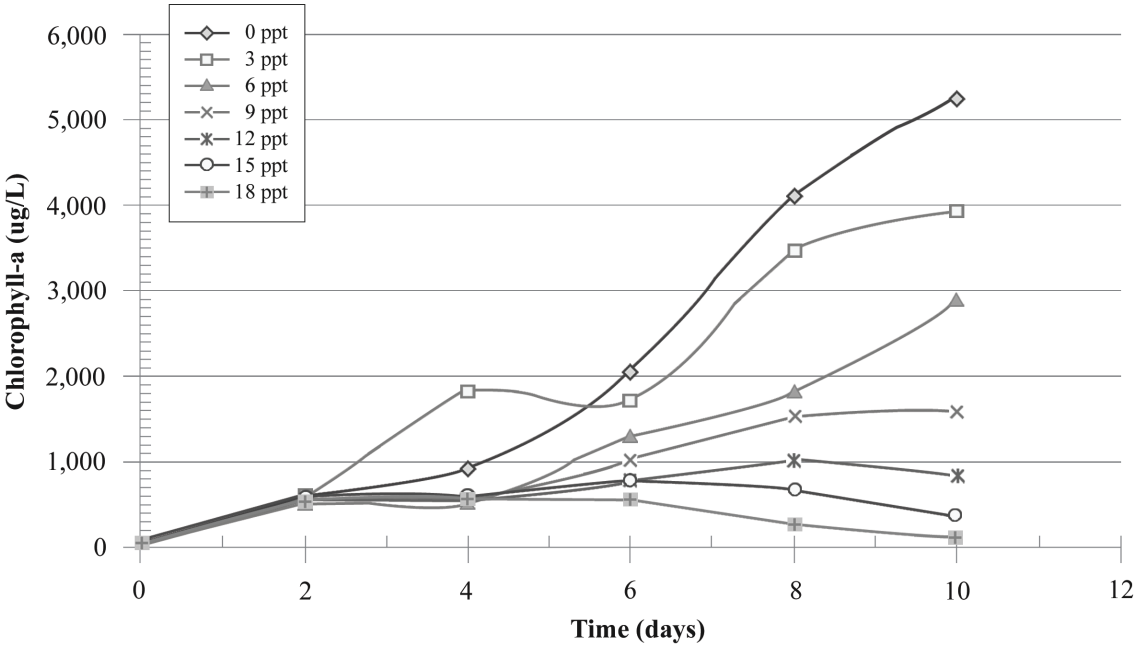

Figure 2 Chlorophyll-a of *Microcystis* sp. cultured at different salinities

Figure 3 The filaments of *Oscillatoria* sp. cultured at a salinity less than 10 ppt

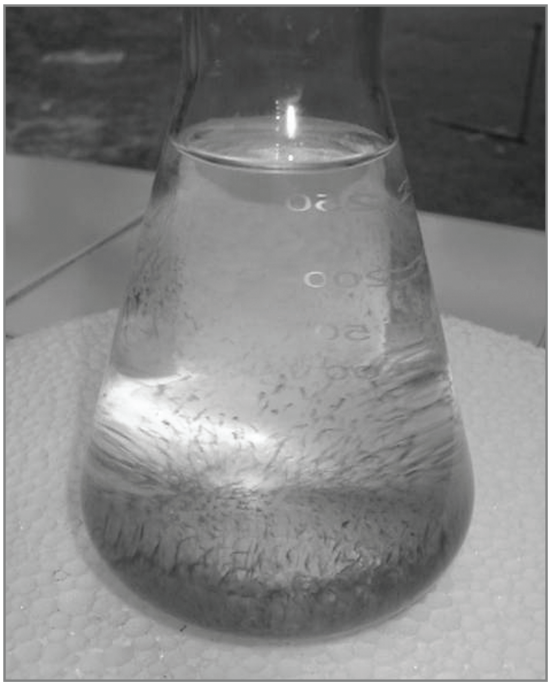

Figure 4 The filaments of *Oscillatoria* sp. aggregated at a salinity higher than 15 ppt

Effects of pH on growth of *Oscillatoria* sp. and *Microcystis* sp.

The optimal growth of *Oscillatoria* sp. and *Microcystis* sp. was observed in the pH 7.5 and 9.0 groups after 10 days and the highest chlorophyll-a was 6,012±716 µg/L for *Oscillatoria* sp, and 1,597±243 µg/L for *Microcystis* sp., respectively. The pH levels at lower and higher than these ranges caused adverse effect on the growth of both species (Tables 3, 4 and Figures 5, 6). Moreover, the filaments of *Oscillatoria* sp. were broken up into smaller filaments when water pH was lower than 6 (Figures 7). These results indicated that water pH influenced the growth of the blue-green algae, especially when the pH was higher than 9.0 or lower than 6.0; pH at these levels could inhibit photosynthesis and adversely affect the morphology of the blue-green algae. Similar results were reported by Brock (1973) who observed that blue-green algae were not found in natural habitats with a pH less than 5.0.

The highest photosynthetic rates for *O. rubescens* in natural populations were found between pH 6.5-8.5. If the pH was less than 6.0 or greater than 9, the photosynthetic rate was less than 50% of the rate at the optimal pH (Konopka, 1981) and the optimal growth of *M. aeruginosa* was found when water pH was between 7.5-9.0 (Wei *et al*., 2001).

Table 3 Chlorophyll-a (µg/L) of *Oscillatoria* sp. cultured at different pHs for 10 days

pH	Time (days)					
	0	**2**	**4**	**6**	**8**	**10**
control	64±7^{a}	2,287±220^{a}	3,513±657^{a}	5,012±1,312^{a}	6,293±1,511^{a}	7,102±819^{a}
3.0	64±7^{a}	235±197^{d}	6±0^{d}	13±0^{d}	10±0^{d}	0±0^{e}
4.5	65±7^{a}	618±426^{c}	412±134^{c}	124±91^{d}	31±4^{d}	56±7^{e}
6.0	64±6^{a}	1,548±295^{b}	2,513±870^{b}	3,849±618^{b}	4,523±766^{b}	5,043±1,044^{c}
7.5	65±7^{a}	1,938±179^{a}	3,015±411^{ab}	3,912±445^{b}	4,951±734^{ab}	5,817±648^{b}
9.0	65±6^{a}	1,713±674^{ab}	3,217±521^{a}	4,734±626^{a}	5,534±820^{a}	6,012±716^{b}
10.5	64±7^{a}	754±120^{c}	2,604±821^{b}	2,926±445^{c}	2,102±134^{c}	1,092±87^{d}
12.0	64±0^{a}	213±0^{d}	0±0^{d}	0±0^{d}	112±0^{d}	0±0^{e}

Different letters in the same column mean a significant difference (P<0.05)

Table 4 Chlorophyll-a (µg/L) of *Microcystis* sp. cultured at different pHs for 10 days

pH	Time (days)					
	0	2	4	6	8	10
control	47±2^{a}	378+109^{a}	534±134^{a}	620±137^{c}	1,111±187^{a}	1,651±195^{a}
3.0	47±3^{a}	145+4^{c}	0±0^{d}	0±0^{d}	0±0^{d}	0±0^{e}
4.5	48±3^{a}	323+48^{a}	11±1^{d}	0±1^{d}	78±3^{d}	95±2^{d}
6.0	47±2^{a}	356+86^{a}	422±123^{b}	511±129^{c}	878 ±132^{b}	1,340±211^{b}
7.5	47±3^{a}	334+94^{a}	562±137^{a}	645±154^{c}	928±165^{a}	1,597±243^{a}
9.0	48±3^{a}	334+128^{a}	556±93^{a}	790±94^{a}	1,090±153^{a}	1,485±137^{ab}
10.5	48±3^{a}	245+74^{b}	345±65^{c}	467±134^{c}	570±129^{c}	860±212^{c}
12.0	47±2^{a}	111+54^{c}	68±8^{d}	11±2^{d}	33±2^{d}	94±11^{d}

Different letters in the same column mean a significant difference (P<0.05)

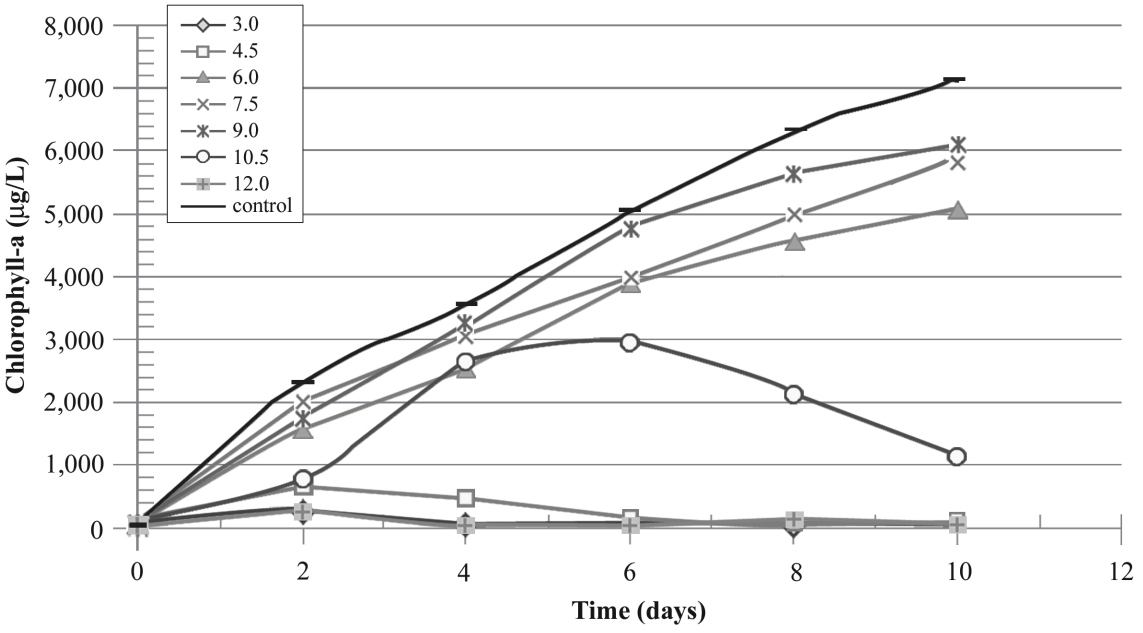

Figure 5 Chlorophyll-a of *Oscillatoria* sp. cultured at different pHs

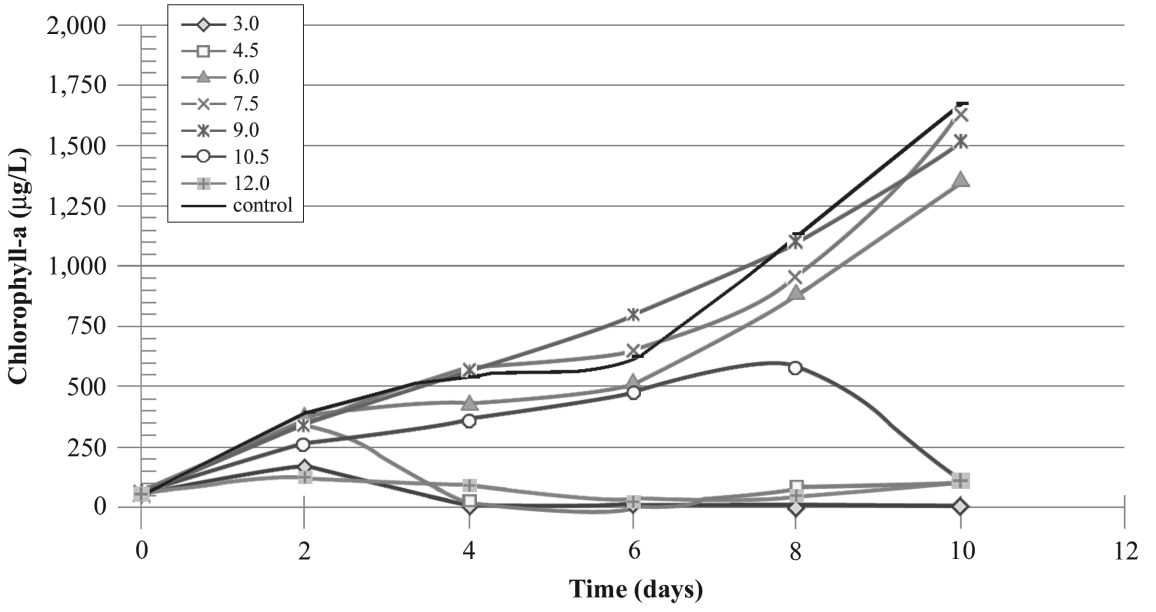

Figure 6 Chlorophyll-a of *Microcystis* sp. cultured at different pHs

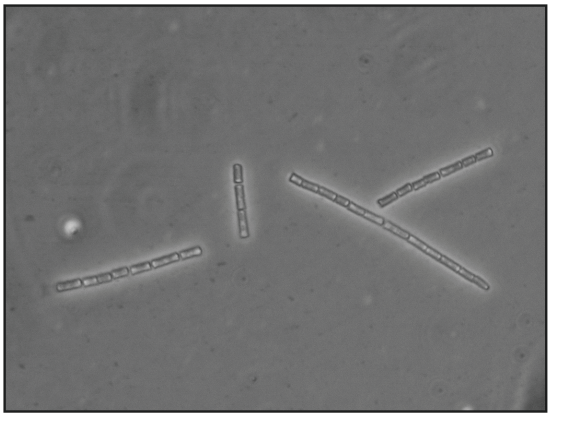

Figure 7 The broken up filaments of *Oscillatoria* sp. cultured at a pH lower than 6

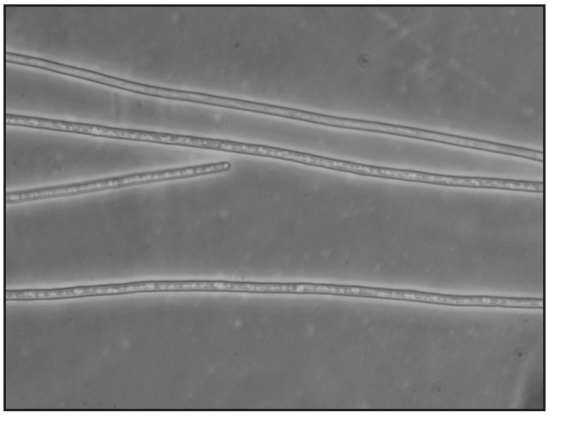

Figure 8 The normal filaments of *Oscillatoria* sp. cultured at a pH higher than 6

Results at the end of the study (day10) revealed that both *Oscillatoria* sp. and *Microcystis* sp. cultured at optimal levels of salinity and pH still had increased photosynthetic rates determined by measuring the concentration of chlorophyll-a. The recommended optimal water pH for *L.vannamei* culture ranges from 7.5 to 8.5 and is also suitable for the growth of both *Oscillatoria* sp. and *Microcystis* sp. The method of choice for controlling these algae at their optimal conditions was to control the nutrient budgets for these algae. A properly managed feeding program can reduce the nutrients for these algae, and additional amounts of suspended solids in the water column can also reduce algal growth by decreasing the photosynthetic rate (Limsuwan, 2000).

CONCLUSIONS

The optimal salinity levels for growth under laboratory conditions ranged from 0 to 10 ppt for *Oscillatoria* sp. and from 0 to 6 ppt for *Microcystis* sp. An increase in salinity led to a decreased growth of both species. Water pH levels from 7.5 to 9.0 were suitable for the growth of both species, while a pH lower or higher than this range was associated with a significantly decreased growth of these blue-green algae.

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