CHLORINE DEMAND AND BACTERIAL ABUNDANCE OF SHRIMP POND WATER UNDER DIFFERENT SUSPENDED SOLID CONCENTRATIONS

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ABSTRACT

An experiment has been conducted to evaluate the chlorine demand and efficacy of 6 chlorine concentrations in brackish pond water, to control indigenous bacteria in shrimps pond. Pond water (salinity: 3-4 ppt) at three different levels of total suspended solids (TSS) (26, 69, and 114 mg/L) were collected in glass jars and treated with chlorine concentrations of 0, 5, 10, 20, 30, and 50 mg/L, respectively. Residual chlorine concentration was determined at 0, 12, 24 hours, and subsequently every day for 7 days. The chlorine demand of the water was then calculated. Total organic carbon and total ammonia nitrogen were measured in the beginning and at the end of the experiment. Chlorine demand of the shrimp pond water was significantly affected by initial TSS concentration of 114 mg/L, completed in 24 hours, as compared to 7 days in pond water with TSS concentrations of 69 and 29 mg/L. Complete bacterial inactivation in pond water with TSS concentration of 114 mg/L was detected, immediately after the application of chlorine concentration of 50 mg/L. In pond water with TSS levels of 69 mg/L and 29 mg/L, the inactivation of the bacteria was observed 48 hours after the application of chlorine at 50 mg/L and 30 mg/L, respectively. Chlorine concentration of 50 mg/L could deactivate bacteria in pond water with TSS level of 29 mg/L for up to 96 hours.

KEYWORDS: **residual chlorine, chlorine demand, total organic carbon, suspended solid, bacteria, shrimp pond**

INTRODUCTION

Chlorination has been recently practiced in shrimp farming directly to grow-out ponds or reservoir ponds (Kongkeo, 1995; Boyd, 1996). Shrimp farmers apply chlorine at a dose rate of 180 to 300 kg/ha or 18 to 30 mg/L to eliminate pathogenic microorganisms and their carriers in the pond (Kongkeo, 1995; Hedge *et al*., 1996), and at 0.1 mg/L for controlling algal bloom (Boyd & Massaut, 1999). When chlorine dissolves in pond water, it forms free residual chlorine. Part of this free chlorine which reacts with organic and oxidizable substances is referred as chlorine demand. The residual oxidize damage nucleic acid and/or protein of microorganisms and cause lethal effects (Archer *et al*., 1997; Chanratchakool, 1995).

High concentrations of organic matter and suspended solid which often occur in water and accumulate in the bottom sediment of shrimp ponds (Hopkins *et al*, 1993; Dierberg & Kiattisimkul, 1996), cause high chlorine demand and reduce the efficacy of chlorine. As a result, more chlorine is required to disinfect target organisms. It has been shown that the chlorine dose required to inactivate bacteria in water increase 100 times in the presence of 50 mg/L organic matter at neutral pH condition (Harakeh, 1986).

Little information is available on chlorine demand and its effect on aquatic organisms of shrimp ponds, despite the fact that chlorine is applied routinely to most shrimp ponds in Thailand. The present study aims to determine chlorine demand of pond water, and the effective chlorine dose required to inactivate those organisms.

MATERIALS AND METHODS

Water samples were collected from shrimp ponds with different TSS concentrations of i.e., 29, 69, and 114 mg/L which were obtained at 16-day, 2.5-month, and 3.5-month cultured period, respectively. The ponds were located at Samutprakan province, in Thailand and were treated using similar methods. Before starting the culture period, ponds bottom was dried for one to two months till the soil was cracked and then filled with freshwater coming from a canal nearby. The pond water was then mixed with brine water (100-120 ppt) to get 5 ppt water and then stocked with 15-day-old (PL15) shrimp larvae of black giant tiger shrimp (*Penaeus monodon*) at stocking density of 43 shrimp/m^2. The shrimps were cultured for three to five months. During the culture period, concentration of total suspended solids and TAN ranged from 29 mg/L and 1.04 mg/L (at the start of the culture) to 140 mg/L and 1.48

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mg/L (closed to the harvest time) respectively. Two hundred liters water sample were taken from the whole column of the pond by using column water sampler made from PVC with 0.1 m diameter connected to hydraulic -pump. The water samples were then kept separately in 250-L plastic container, brought to the laboratory, and aerated.

The water sample was mixed homogeneously and distributed to 12 glass jars with water volume of 9 L each, and aerated overnight to increase the dissolved oxygen (DO) lend up to at least 5 mg/L. The water was then treated with different chlorine concentrations by dissolving alloted concentrations of calcium hypochlorite or High Test Hypochlorite (HTH) (with 65% active ingredient) with 25-mL distilled water. All jars were placed out doors in a roofed place with temperature ranging from 27.9 to 28.0 °C.

The experiment was set up in a factorial design with 6 levels of active chlorine concentrations (0, 5, 10, 20, 30 and 50 mg/L), 3 levels of TSS concentrations (29, 69 and 114 mg/L), in duplicate.

Water pH was measured before and 12, 24, 48, 96, and 168 hours after chlorine application using a digital pH-meter (Hanna HI8424). Total organic carbon (TOC) and total ammonia nitrogen (TAN) were measured at 0 and 168 hours (7 days) after chlorine application by taking 150-mL of water sample from each jar. Total organic carbon and total ammonia nitrogen were analyzed by using Combustion Infra Red and Phenate methods (APHA, 1992).

About 100-mL water sample was collected from each treatment at 0, 12, 24 hours and subsequently every day until the total residual chlorine in the water was zero, which was about 7 days resulting a preliminary study, for free and total residual chlorine (FRC and TRC) analysis. Total residual chlorine is the sum of free residual chlorine and the combined residual chlorine, amount of free chlorine reacts with nitrogen compound. The analysis was done by using *N,N*-diethyl-*p*-phenylenediamine (DPD) ferrous titrimetric method. Bacterial abundance was measured by taking 20-mL water sample from each treatment, kept in 20-mL sterilized vials and then directly analyzed by using plate count method with droplet modification (APHA, 1992). Bacteria abundance was measured at 0, 48, 96, and 168 hours.

Chlorine demand was calculated by subtracting the amount of chlorine applied and the total residual chlorine. Chlorine demand and bacteria abundance were analyzed by two-way analysis of variance at a=0.05 (Box *et al.*, 1978). Significant difference among treatments were analyzed with Tuckey HSD method, using a statistical software (Statistical version 5.0)

RESULTS

Chlorine demand

Free and total residual chlorine (FRC and TRC) increased a chlorine concentrations were applied and decreased with TSS concentrations and time of observation (Figure 1 & 2). FRC and TRC concentrations of pond water with TSS concentration of 29 mg/L (16-day old pond), were higher than those with TSS concentration of 69 and 114 mg/L (2.5 and 3.5-month old ponds) respectively ($P<0.01$). FRC concentration immediately after chlorine application (0 h) in pond water with TSS concentration of 29, 69, and 114 mg/L were 0.16 to 34.41 mg/L, 0.08 to 26.04 mg/L, and 0.8 to 20.93, respectively. FRC concentration reduced to less than 0.1 mg/L 24 hours after chlorine application in all treatments, except at chlorine treatment of 50 mg/L and 29 mg/L TSS. FRC concentration in chlorine treatment of 50 mg/L, could not be detected in pond water with TSS concentration of 114 mg/L and was recorded 69 mg/L, 48, and 168 hours after chlorine exposure, and about 0.32 mg/L in pond water with TSS of 29 mg/L.

Consumption of chlorine was affected by initial TSS concentration (Figure 3). Chlorine consumption in pond water with TSS concentration of 114 mg/L was faster than that in pond water with TSS concentration of 69 and 29 mg/L, and the consumption was completed within 24 hours exposure. While in pond water with TSS concentration of 69 and 29 mg/L, the consumption was completed within 168 hours. Figure 3 also shows that chlorine consumption in pond water with TSS of 69 mg/L was faster than in pond water with TSS of 29 mg/L. Except at 168 hours measurement, chlorine consumption in pond water with TSS of 114 mg/L was significantly higher than in pond water with TSS of 69 mg/L and 29 mg/L ($P<0.01$).

Two-way analysis of variance reveales a significant difference in chlorine consumption among chlorine concentration treatments during the experimental period. The chlorine consumption increased with increasing the chlorine concentrations ($P<0.01$).

Bacterial abundance

Concentration of TSS and FRC affected the inactivation of bacteria. Increasing TSS and decreasing chlorine concentration, reduced bacterial inactivation (Figure 4). Immediately after chlorine application (0 h) bacterial inactivation in pond water with TSS concentration of 29 mg/L was higher than in pond water with TSS of 69 and 114 mg/L ($P<0.01$). Chlorine concentrations of 30 and 50 mg/L produced higher bacterial inactivation than other chlorine concentrations. At the TSS concentration of 29 mg/L, a chlorine concentration as low as 10 mg/L with FRC concentration of 1.16 mg/L, could completely inactivate bacteria. To achieve the same result in pond water with TSS concentration of 69 and 114 mg/L, at least 30 mg/L active chlorine should be applied with FRC concentration of 5.58 and 9.92 mg/L, respectively. After 48 hours of chlorine exposure, bacteria development could be noted in all treatments except at chlorine concentration of 50 mg/L (FRC 4 mg/L) in pond water with TSS of 69 mg/L and chlorine concentration of 30 to 50 mg/L (FRC 1.58 and 12.28 mg/L) in pond water with TSS of 29 mg/L. The number of bacteria number in pond water with TSS of 29 mg/L was significantly lower than in pond water with TSS of 69 and 114 mg/L ($P<0.01$). Complete bacterial inactivation could still be noted in chlorine concentration of 50 mg/L (FRC 4.56 mg/L) in pond water with TSS of 29 mg/L after 96 hours exposure time.

Measurement of other water quality parameters (Figure 5) revealed that total organic carbon (TOC) concentration in TSS 69 and 114 mg/L immediately after chlorine application was about 15.80 mg/L and

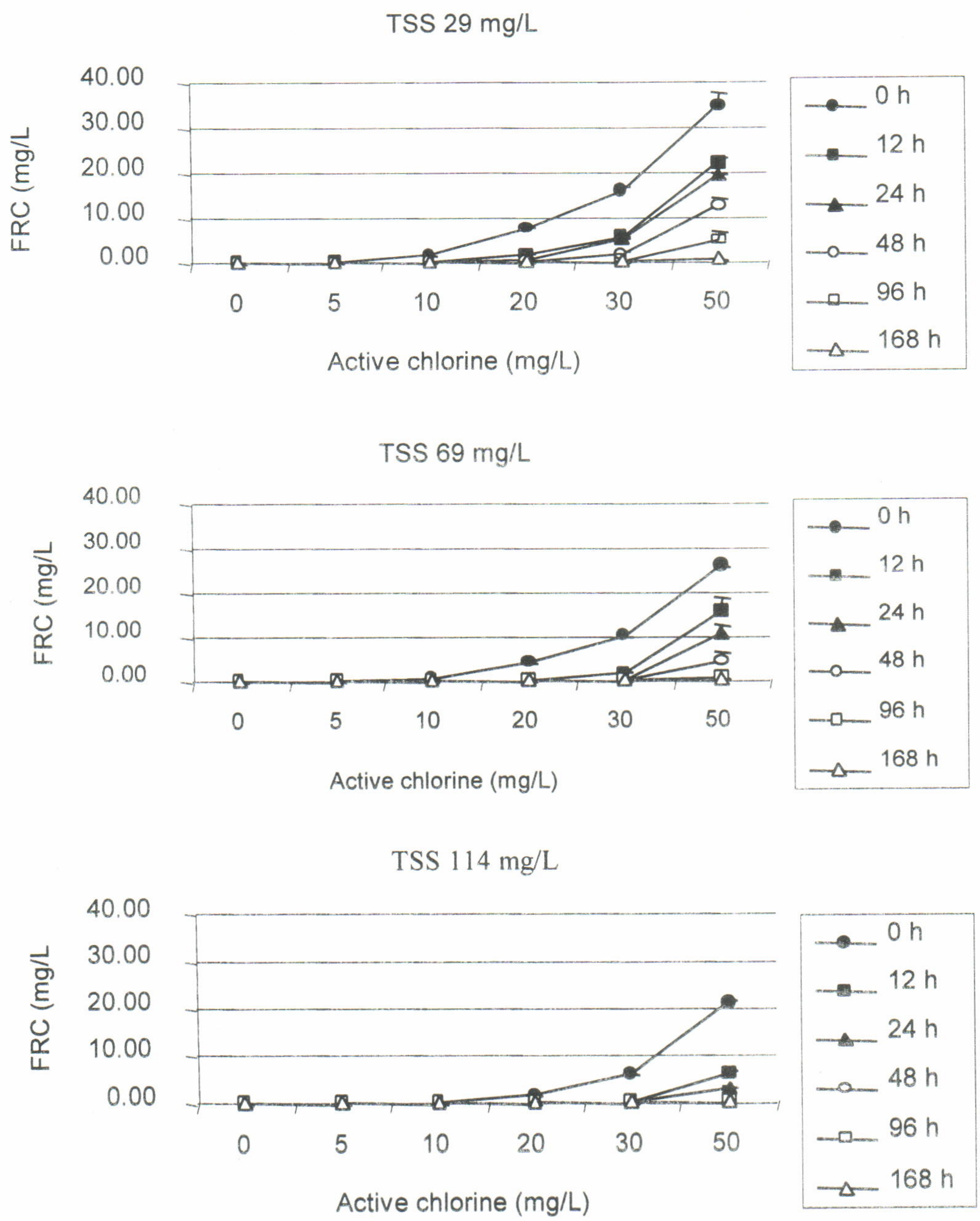

Figure 1. Free residual chlorine (FRC) of different total suspended solid (TSS) concentrations of shrimp pond water treated with different chlorine concentrations. Bars represent standard error

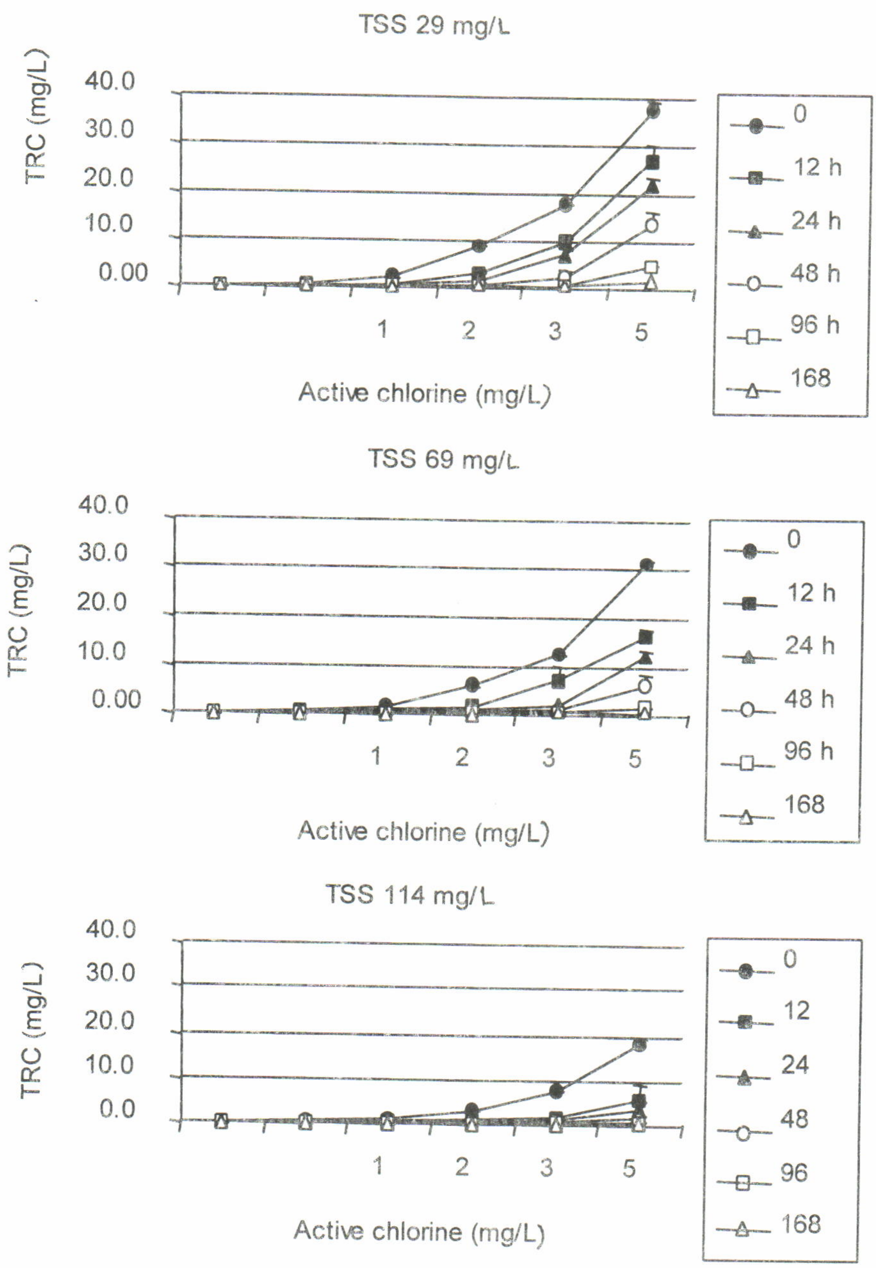

Figure 2. Total residual chlorine (TRC) of different total suspended solid (TSS) concentrations of shrimp pond water treated with different chlorine concentrations. Bars represent standard error

significantly higher than in TSS of 29 mg/L ($P<0.01$) which was 9.25 mg/L. On the other hand, no significant difference was observed among chlorine concentrations both immediately and 168 hours after chlorine application.

A significant difference was also observed in total ammonia nitrogen content. Total ammonia concentration immediately after chlorine was applied in pond water with TSS of 29 mg/L was significantly lower than in pond water with TSS of 69 and 114 mg/L ($P<0.01$), and was lower in high chlorine concentration (50 mg/L) than in the lower concentration of 20 mg/L. After 168 hours of chlorine exposure, total ammonia in pond water with TSS of 69 mg/L was higher than in pond water with TSS of 29 mg/L and 114 mg/L. No total ammonia variation was detected among chlorine concentrations. This might relate to no difference in bacterial density among the chlorine treat-

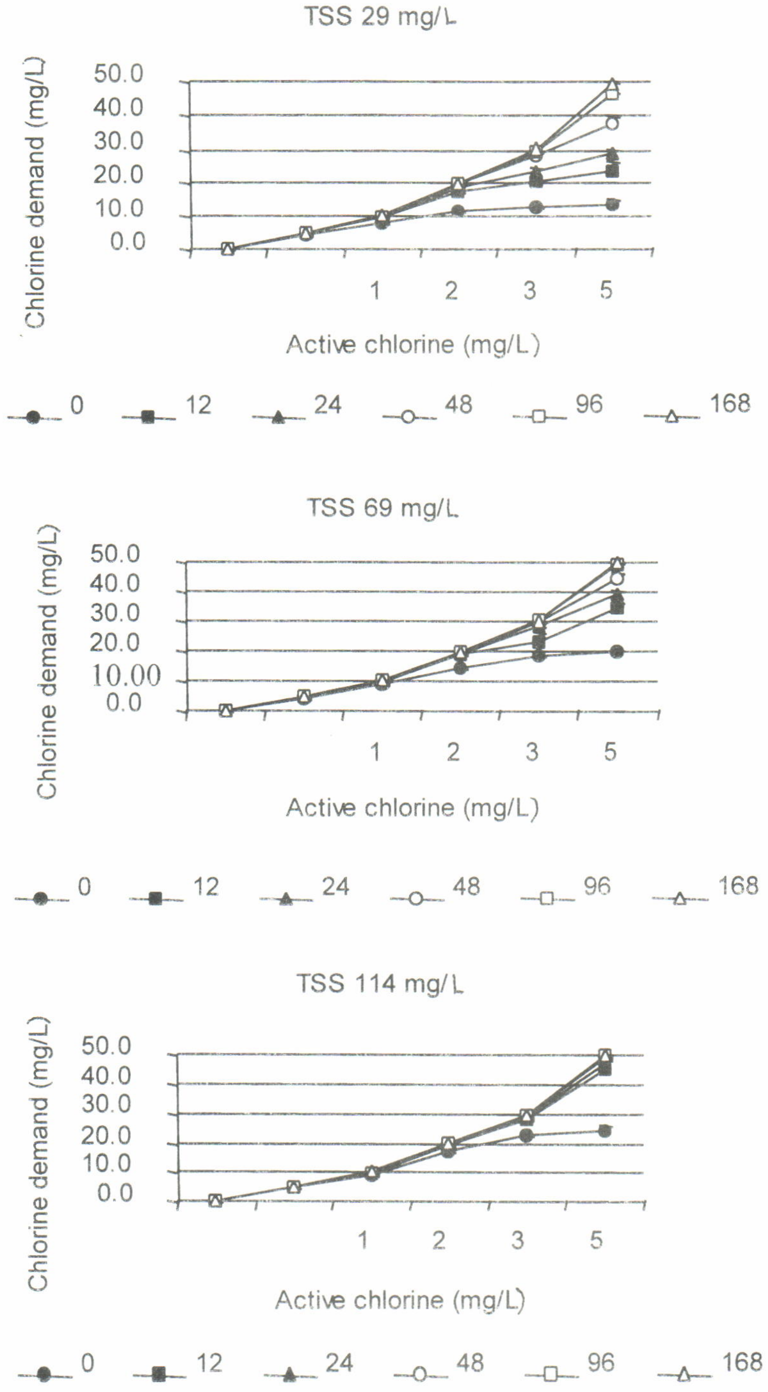

Figure 3. Chlorine demand of different total suspended solid (TSS) concentrations of shrimp pond water treated with different chlorine concentrations. Bars represent standard error

ments as a result of very low free residual chlorine concentration 168 hours after chlorine exposure.

A clear pattern was observed in pH value (Figure 6). Water pH in pond water with TSS of 114 mg/L immediately and after 168 hours chlorine application was lower than in pond water with TSS of 69 and 29 mg/L ($P<0.01$). Among chlorine concentrations, chlorine at a concentration of 20 to 50 mg/L after 0-hour chlorine exposure had higher water pH than in control and in chlorine concentrations of 5 to 10 mg/L. After 168 hours of chlorine exposure, a significantly higher water pH was found in high chlorine concentration compared to in chlorine concentration of 5 mg/L.

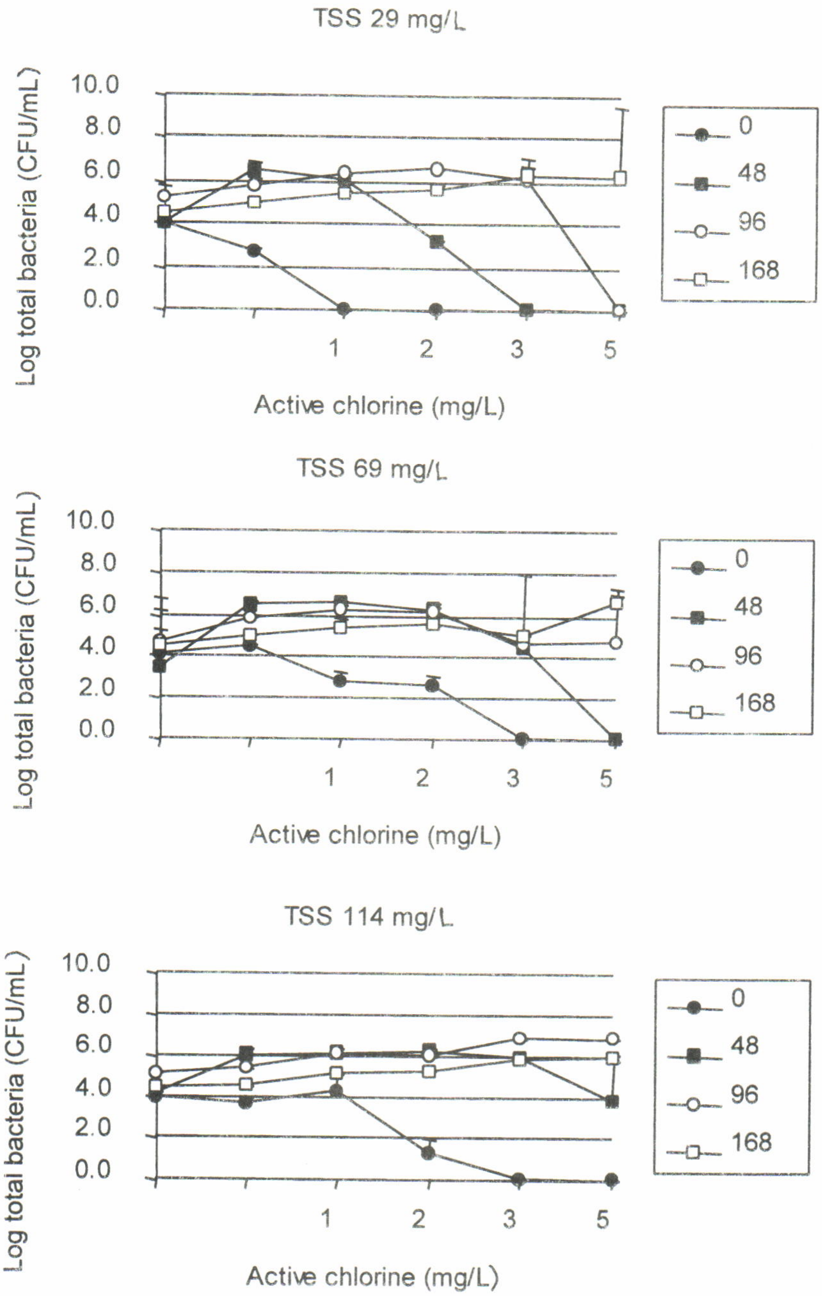

Figure 4. Bacterial number of different total suspended solid (TSS) concentrations of shrimp pond water treated with different chlorine concentrations. Bars represent standard error

DISCUSSION

To obtain effective concentration of free residual chlorine for inactivating pathogenic organisms, in the range of 0.1 to 1.0 mg/L (Boyd, 1996), factors affecting chlorine consumption (chlorine demand) such as concentration of organic and suspended matters, reduced substances present in shrimp pond water, and time of exposure (contact time) have to be considered. Increased TSS concentration with period of shrimp culture, resulted in an increasing concentration of active chlorine required need to occur immediate (less than 1 hour) for bacterial inactivation. Active chlorine as low as 5 mg/L, already fulfilled the chlorine demand and produced free residual chlorine needed to inactivate the bacteria in 16-day-old pond water with TSS concentrations of 29 mg/L. For 2.5 and 3.5-month-old ponds with TSS concentration of 69 and 114 mg/L respectively, another 5 mg/L active chlorine was needed to get free residual chlorine within the suggested concentrations. If bacterial inactivation is needed for a much longer period, for example 24

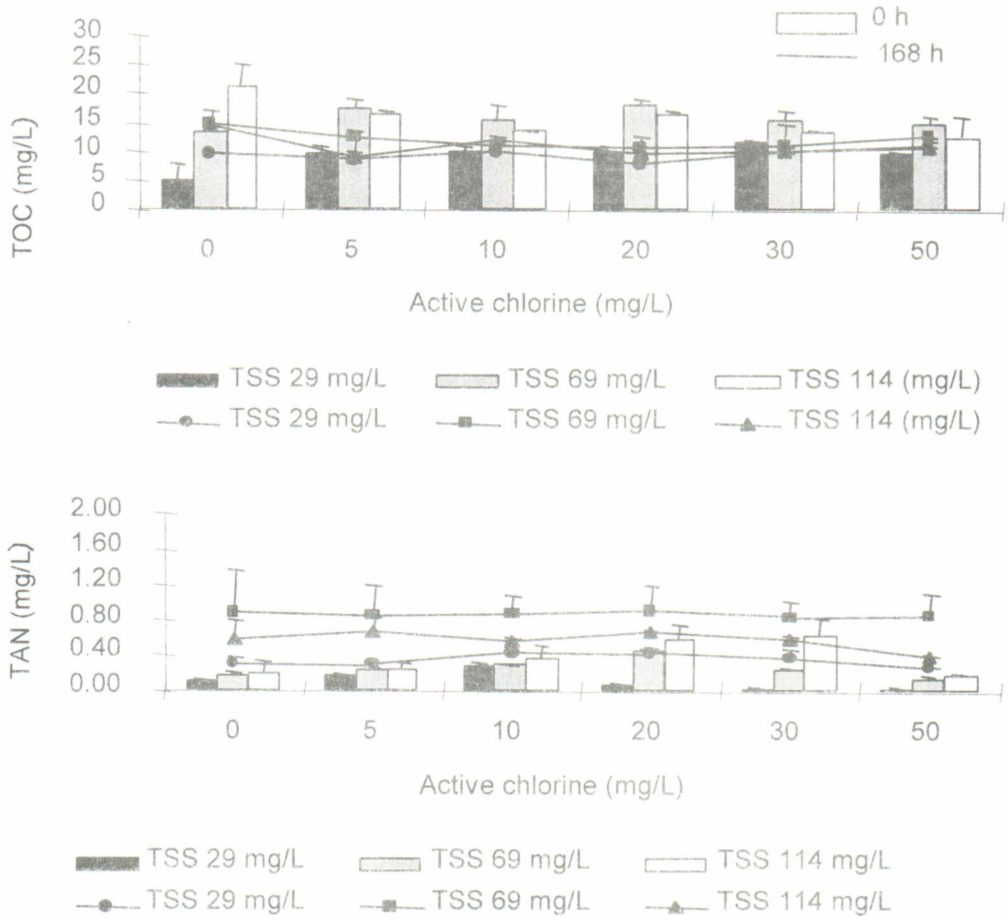

Figure 5. Total organic carbon (TOC) and total ammonia nitrogen (TAN) of different total suspended solid (TSS) concentrations of shrimp pond water treated with different chlorine concentrations. Bars represent standard error

hours, the concentration of active chlorine applied has to be more than 10 to 30 mg/L for pond water with TSS concentration of 29 and 69 mg/L respectively, and 50 mg/L for pond water with TSS of 114 mg/L. Compared to pond water with TSS of 69 and 114 mg/L, application of active chlorine at concentration of 50 mg/L in pond water with TSS concentration of 29 mg/L could maintain the free residual chlorine at concentration recommended for a longer period of 96 hours.

Present study shows that bacteria inactivation correlates with the presence of free and total residual chlorine concentration rather than with the concentration of active chlorine applied. In order to get a complete bacteria inactivation, concentrations of free and total residual chlorine in water have to be more than 1.0 and 2.17 mg/L, respectively, which are higher than those suggested by Boyd (1996). The experiment also indicated that although complete bacterial inactivation was reached, bacterial growth would resume when concentrations of free and total residual chlorine decreased below the concentration mentioned earlier.

In addition to free and total residual chlorine, bacterial inactivation also depended on the presence of TSS concentration. TSS not only increases the demand on chlorine but may also protect the bacteria from the disinfectant (Harakeh, 1986). This notion is in accordance with the results of this experiment. Higher concentrations of free and residual chlorine were needed to inactivate bacteria in 2.5 and 3.5-month-old ponds with TSS concentration of 69 and 114 mg/L than in 16-day-old pond water with TSS of 29 mg/L. Observations immediately after chlorine application (0 h) showed that free and total residual chlorine at concentrations of 1.16 and 2.40 mg/L completely inactivated bacteria in 16-day-old pond water but it did not produce the same effects in 2.5 and 3.5-month-old ponds.

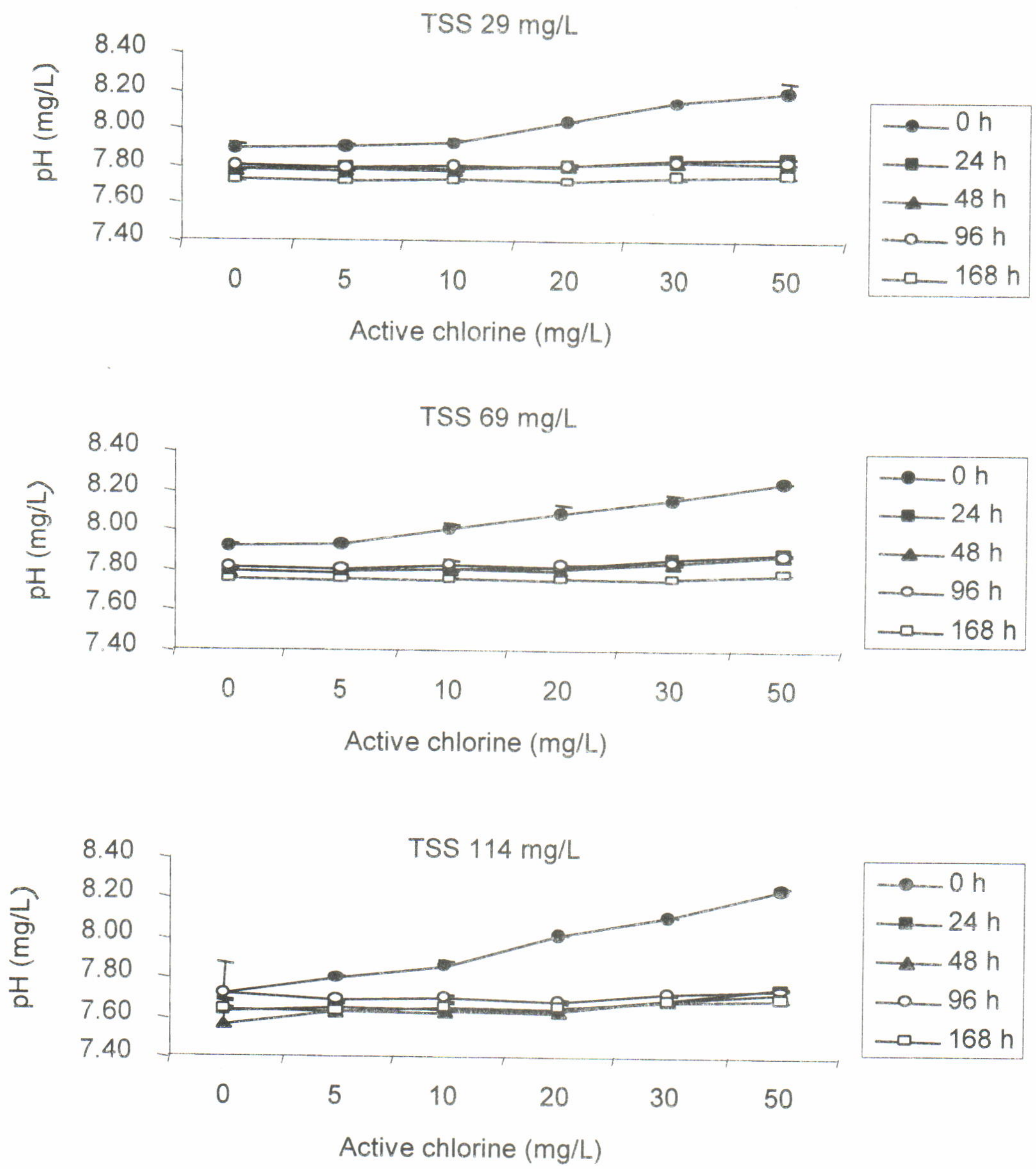

Figure 6. pH of different total suspended solid (TSS) concentrations of shrimp pond water treated with different chlorine concentrations. Bars represent standard error

Chlorine demand and bacterial inactivation of shrimp pond water were significantly affected by the initial chlorine concentration, TSS concentration and duration of chlorine exposure. To obtain FRC within the concentration which effectively inactivates total bacteria in 24 hours, the initial chlorine concentration applied has to be more than 10 and 30 mg/L for shrimp pond water with TSS concentration of 29 and 69 mg/L, respectively. While for shrimp pond water with TSS concentration of 114 mg/L, higher concentration of 50 mg/L should be used.

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