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Disinfection of aquarium effluents by chlorination and UV treatment

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Abstract

The study was conducted to investigate the efficacy of chlorine and UV irradiation in disinfecting aquarium effluent. A non-agglutinating, avirulent strain of *Aeromonas salmonicida* (NCIMB 1102) was used as the test organism. Effluents from a fish tank were inoculated with a suspension of test organisms and subsequently treated with different concentrations of hypochlorite and UV irradiation separately and simultaneously. When used alone, 1.0 ppm hypochlorite reduced the viable cell count from 6.5 log to 3.0 log within 20 minutes of contact period. On the otherhand, when used in combination with UV irradiation only 0.5 ppm hypochlorite exerted the same bactericidal effect within the same contact period as was observed with 1.0 ppm hypochlorite alone. This result indicated that required dose of disinfectant for the disinfection of aquarium effluents can be considerably reduced when it is used in combination with UV irradiation.

Key words : Disinfection, Effluent, Chlorination, UV

Introduction

Effluents from aquacultural activities are a potential source of microorganisms pathogenic to fish and shellfish in the wider environment. Scientific laboratories working with the infected fish and pathogens in particular, are high risk activities since many of these species survive well in the aquatic environment. In order to prevent the possible spread of such pathogens in the environment, disinfection of waste water is of utmost importance (Austin and Austin 1993).

Chlorine is a strong oxidizing agent and rapidly penetrates microbial cells and kills the microorganism. Death results from the chemical reaction of hypochlorous acid with the enzyme triphosphate dehydrogenase which is essential to life process of the cell (White 1972). Chlorine, at a concentration of

1.0 to 0.2 mg/ml, has been demonstrated to efficiently remove bacterial pathogens like *Aeromonas salmonicida* and *Yersinia ruckeri* from natural lake waters within one minute (Wedmeyer and Nelson 1977) whereas infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV) were more resistant and required 0.7 to 1.0 /mg ml for inactivation.

Fish pathogenic bacteria, in general appear to be sensitive to UV light and unlike halogen disinfectants UV irradiation of water does produce undesirable by-products toxic to fish (Oliver and Carey 1976). Doses of 1.5 to 3.4 mWs/cm^2 have been reported to inactivate 99.9% of *Vibrio salmonicida*, *V. anguillarum*, *Yersinia ruckeri* and *A. salmonicida* (Sako and Sorimachi 1985). High intensities, *viz*, 4 to 10 mWs/cm^2 were required for human pathogenic and water quality indicator bacteria like *Salmonella*, *Escherichia coli* and *Vibrio* sp. (Chang *et al.* 1985 and Harris *et al.* 1987).

Combined application of a chemical disinfectant and UV irradiation may result in increased efficacy with lower levels of chemicals. Several investigators have expressed interest in the combined organic compounds in effluents and for enhanced disinfection (Venosa *et al.* 1984 and Glaze *et al.* 1991). Combining halogens and UV have not received the attention in water treatment studies. But in a recent studies it was shown that UV and chlorine based disinfectant had a synergistic effect (Liltvet and Landfald 1995, unpublished data).

The aim of this study was to investigate the disinfection efficiency of chlorine and UV irradiation in aquarium effluent and to determine if simultaneous application of chlorine/UV in these increased the efficacy.

Materials and methods

Test organism and growth procedure

A non-agglutinating, avirulent strain of *Aeromonas salmonicida* (NCIMB 1102) was used as a model bacterium in this study because virulent strain of this organism is highly pathogenic to salmonid fish causing septicemia and abundant in both fresh water and marine environment in the colder region. The bacteria were grown at 22°C for 3 days in tryptone soya agar (TSB, Unipath, Basingstoke). Cells were harvested by centrifugation at 2500 rpm for 10 minutes and the resultant pellet was resuspended in 0.85% sterile saline solution. The suspension was further diluted to give a concentration of approximately 10^8 cfu /ml.

Preparation of disinfectant and neutralizing solutions

Chlorine stock solutions were prepared from a sodium hypochlorite solution (14% w/v available chlorine). Appropriate volumes were added to distilled deionized water to obtain 10, 20, 30 and 40 ppm free chlorine. Stock solutions

(11, 22, 33 and 44 ppm) of a neutralizing solution for hypochlorite were prepared by dissolving appropriate quantities of sodium thiosulfate in distilled water. Four separate stock solutions of hypochlorite were prepared so that when 0.5 ml was added to 9.5 ml of the test sample the required concentration range of 0.5 to 2.0 ppm was attained. Similarly, since 1 gm of active chlorine is effectively removed by 2 gm of thiosulfate (Hom 1971), stock solutions of sodium thiosulfate from 11 to 44 ppm were prepared so that when 1 ml was added to the 10 ml test sample there will be a concentration ratio of 2:1.

Fish tank and UV assembly

Twenty five rainbow trout (100-150 gm each) were introduced in a tank of 100 gallon capacity and held for several days with standard feeding and husbandry. The tank was fitted with a pipe in the middle to discharge the effluent into sump tank (100 gallon plastic tank) mounted on the floor below the fish tank. The sump tank had two overflow pipes mounted near the top of the tank but below the level of the fish tank outlets. A submersible pump with integral float switch was mounted inside the sump tank to pump water through the UV unit (Aquarium sterilizer-Model 30) and then to waste. A side arm with valve was also present to control the rate at which water was pumped from the sump.

Water sample preparation

The effluent from the fish tank was collected from the sump tank in a sterile conical flask and brought to the laboratory. The water contained dissolved and suspended organic matter from feed pellets, faecal materials and other waste. The sample was heated at 80°C for 10 min in a water bath to kill the indigenous bacteria and was subsequently cooled to 7°C.

Hypochlorite treatment

Heat treated and cooled water sample was inoculated with the previously prepared bacterial suspension to obtain a suspension of approximately 10^7 cells/ml. From this suspension 9.5 ml aliquots were taken in 4.5 cm diameter sterile plastic disposable petri plates and 0.5 ml stock solutions of different concentrations of hypochlorite were added. Petri plates with the samples were immediately put in an ice bath (ice-water temperature 7°C) placed over a magnetic stirrer. After the required contact periods with stirring 1.0 ml of stock solution of different concentrations of neutralizing solution was added to each sample. After neutralizing for 1 min samples were subjected to total viable count according to drop count method (Miles and Misra 1938). The experimental procedure has been summarized in Fig. 1.

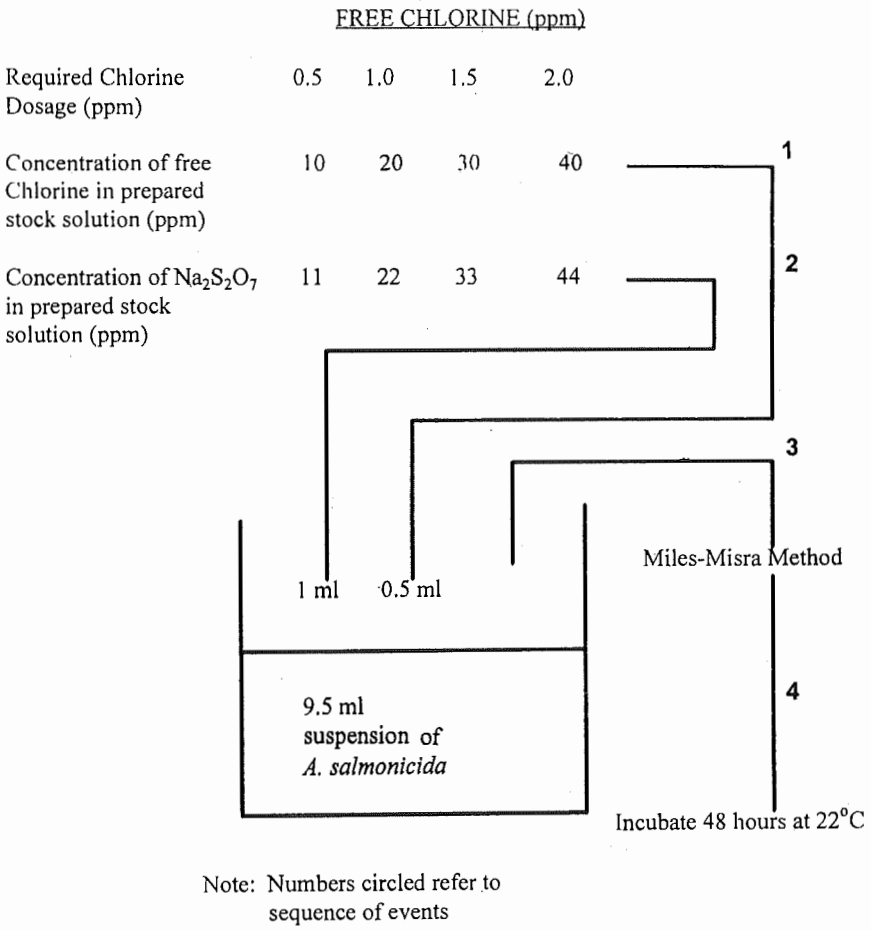

Fig. 1. Experimental procedure to assess bactericidal effect of sodium hypochlorite.

Combined UV-hypochlorite treatment

Effluent in the sump tank with indigenous and added bacterial inoculum was treated with calculated amount of hypochlorite solution to give the desired concentration of chlorine and mixed quickly and thoroughly by vigorous agitation. After selected time intervals (10, 20 and 30 min) the effluent water was passed through a UV unit by using a submersible pump. UV treated water sample was collected from the discharge point and quickly dechlorinated by sodium thiosulfate and subjected to total viable count (Miles and Mirsa 1938). Temperature of the aquarium effluent was around 7°C.

Results

Fig. 2 shows the logarithmic number of colony forming units (cfu) of bacterial cells/ml versus contact time of bacteria with different concentration of chlorine. A general trend in the loss of viability of the cells was observed with the

increase of contact time as well as concentration of chlorine. With 0.5 ppm chlorine, one log reduction was achieved within 5 minutes of contact period, thereafter the reduction was slow and gradual. There was a more rapid and massive reduction of bacterial number at the 1.0 ppm free chlorine concentration level. At this concentration two log reduction could be achieved within 5 minutes and three log reduction in 15 minutes, which is equal to 99.9% reduction in the viable count. The rate of reduction was rapid during the first 5 minutes with 0.5 ppm and the first 15 minutes with 1.0 ppm chlorine but after that, the slope of the curve levelled off. With 1.5 ppm and 2.0 ppm the number of viable cells fell below the level of detection within 5 minutes, which means the .99,9995% reduction in the cell number could be achieved in 5 minutes with 1.5 ppm chlorine under this experimental condition.

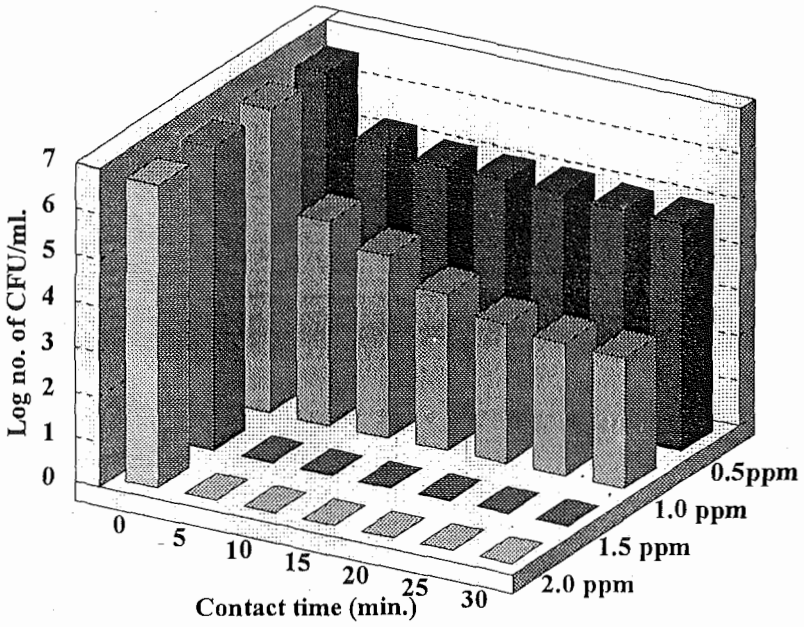

Fig. 2. Loss in viable count of chlorine exposed *Aeromonas salminicida* in aquarium effluent.

Fig. 3 shows the logarithmic number of colony forming units versus UV treatment and combined UV + hypochlorite treatment for different contact periods. With UV treatment alone a log value of 6.35 of starting suspension was reduced to 4.80 which means a reduction by more than 1.5 log. With a combined treatment of UV and 0.5 ppm chlorine log value was reduced to 3.0 within 20 minutes of contact time. This effect is identical with the effect of 1.0 ppm chlorine alone Showing that required chlorine alone showing that required chlorine. concentration is only 50% when it is used in combination with UV treatment. In the UV study test organisms were a mixed population because

indigenous bacterial flora of the aquarium effluent were not eliminated by treatment as was done when only hypochlorite was used. This was necessary to see the combined effect of UV and chlorine in the actual aquarium system.

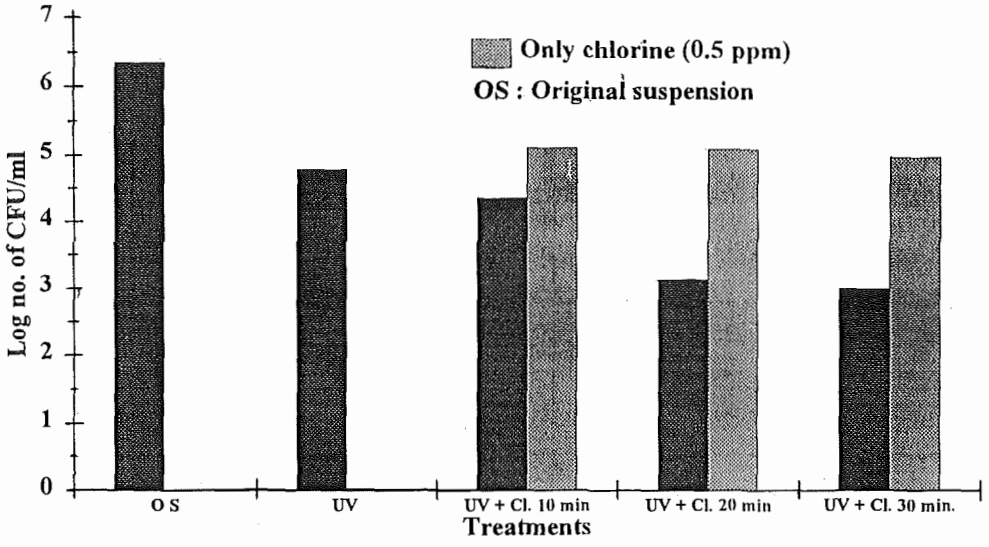

Fig. 3. Effect of UV, chlorine (0.5 ppm) and combined UV/chlorine (o.5 ppm) treatments on the viability of bacteria in aquarium effluent.

Discussion

From the experimental result of the present study it is apparent that freshwater effluents from laboratory based aquarium systems could be effectively disinfected with low doses of chlorine. The efficiency of disinfectant of a bacterial suspension in the effluent is proportional to the initial hypochlorite dose and contact time, therefore, complete destruction may be difficult with lower doses.

Incomplete oxidative destructivc of bacterial cells has been suggested to arise because of chlorine reacting with other substances (White 1972). Polprasert and Rajput (1984) demonstrated the reduction of chlorine residual and colicidal efficiency by increasing the suspended solid concentration in the chlorinated effluent.

The composition of effluents from different sources may vary considerably, depending on operational practice. Compared to commercial aquacultural activities the effluents used in this study had a low concentration of suspended matters. Doses and contact times used in this study can be compared to treated surface waters which are usually satisfactorily chlorinated with dose of 0.5 to 1.0 mg/l. Higher doses upto 5 mg/l are required for purification with high levels of

suspended matter or for the complete oxidation of ammonia and organic matter (Hom 1971). It is quite obvious then that chlorine demand will vary in different situations and depend on the nature of the organism in the effluent, nature and condition of water and temperature.

A combined treatment of UV and chlorine may increase the effectiveness of chlorination in destroying bacteria in the effluents. The present study showed that required chlorine concentration could be reduced by 50% when used in combination with UV treatment. Effectiveness of UV treatment of waste water is generally reduced due to protective effects to bacteria by particulate matter in the water (Qualls and Johnson 1983, Whitby and Palmater 1993). Such protection is a limiting factor in disinfection efficiency. Every effort should be made to reduce the quantity of suspended matter by filtration prior to disinfection.

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