NOTES

Viability of *Vibrio cholerae* in Water Subjected to Different Levels of Chlorination

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For the past few years, Japan is the major buyer of Indian marine products particularly shrimps. However, a few consignments of frozen shrimps exported from India suffered rejection at Japan due to the detection of *Vibrio cholerae*. Detailed investigations revealed water as one of the major sources of contamination (Iyer *et al.*, 1988a). Hence it was considered essential to chlorinate the water used at all the stages of seafood processing. However, no data was available on the exact concentration of chlorine required to destroy effectively all the viable cells of *V. cholerae* present in the process water. Hence, studies were undertaken in this direction and the results obtained are presented in this paper.

Two strains of *V. cholerae* OI (Ogawa and Inaba) isolated by the authors from seafoods and another strain *V. cholerae* non OI isolated from shrimps were used in this study. These strains were separately inoculated into tubes containing sterile nutrient broth and incubated at 37°C for 20 h. The young cultures of the organisms were centrifuged for 15 min at the rate of 5000 r.p.m. and the liquid was aseptically decanted off. Ten ml of sterile isotonic saline was then added to the sediment and the contents of the tube were shaken so as to get a uniform suspension of the cells in the saline and this was centrifuged again for another 15 min. The sediment was once again washed with isotonic saline, centrifuged and the cells were suspended in 10 ml of fresh saline. Aliquots were added to 3l of sterile tap water in order to get varying numbers of bacterial cells and stirred well to get a uniform suspension of the organism in water. The exact number of bacterial cells were determined by direct plating on TCBS agar. Required quantity of sodium hypochlorite was added to the water containing *V. cholerae* to make the residual chlorine level 5, 10 and 15 ppm and stirred well. 900 ml of water was collected after 3, 5, 10 min. of adding chlorine. The samples were added to 100 ml each of sterile tenfold concentrated alkaline peptone broth and proceeded further for the detection of *V. cholerae* as per the method described by Iyer *et. al.* (1988b). Experiment was repeated again by changing the number of bacterial cells and by changing the serotype.

Results obtained are summarised in Table 1. From the results, it is clear that chlorination of process water to a residual level of 5 ppm is not sufficient to destroy the viable cells of *V. cholerae*. Similarly, it is also evident that contact time of 30 min is not sufficient to get full protection against *V. cholerae*. If the concentration of chlorine used for disinfection is 10 ppm even a contact time of 3 min is insufficient when the number of *V. cholerae* cells exceeds 70,000/ml.

Table 1. *Viability of V. cholerae in water during chlorination*

Treatment time, min	Cells/ml	*V. cholerae* ogawa			*V. cholerae* inaba			*V. cholerae* non 01		
		Growth after exposure to different chlorine levels								
		5 ppm	10 ppm	15 ppm	5 ppm	10 ppm	15 ppm	5 ppm	10 ppm	15 ppm
3		+	-	-	+	-	-	+	-	-
5	1,500	+	-	-	+	-	-	+	-	-
10		-	-	-	-	-	-	-	-	-
3		+	-	-	+	-	-	+	-	-
5	3,500	+	-	-	+	-	-	+	-	-
10		-	-	-	-	-	-	-	-	-
3		+	-	-	+	-	-	+	-	-
5	6,500	+	-	-	+	-	-	+	-	-
10		-	-	-	-	-	-	-	-	-
3		+	-	-	+	-	-	+	-	-
5	18,000	+	-	-	+	-	-	+	-	-
10		-	-	-	-	-	-	-	-	-
3		+	+	-	+	+	-	+	+	-
5	70,000	+	-	-	+	-	-	+	-	-
10		+	-	-	-	-	-	-	-	-
3		+	+	-	+	+	-	+	+	-
5	1,80,000	+	-	-	+	-	-	+	-	-
10		+	-	-	-	-	-	-	-	-

+ = growth present; - = growth absent

Results of the study indicates that a residual chlorine level of 10 ppm and contact time 5 min can give protection against *V. cholerae.*

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