

Design, Fabrication and Standardisation of a Depuration System for Bivalves

D. Damodaran Nambudiri*, I.S. Bright Singh¹, Sajan George,
P.M. Sherief, C. Maniappan², T.M. Ayoob³

Processing Technology Department, College of Fisheries
Panangad, Cochin - 682 506, India

A depuration system was designed and fabricated for cleaning of bivalves. The unit consisted of a depuration chamber, biological filter and UV sterilizer. The system was evaluated based on trial studies conducted using *Villorita cyprinoides*. *Escherichia coli* was used as the indicator bacteria. Seeding of the animals by bacteria was carried out using sea water of 10‰ salinity and a bacterial concentration of 1×10^9 cfu ml⁻¹. On 6 h exposure of clams to the *E. coli* cell suspension, seeding was achieved to the desired level (6.69×10^8 cfu g⁻¹). On depuration of seeded *V. cyprinoides* for a period of 15 h using the depuration system complete elimination of *E. coli* could be achieved.

Key words : Depuration system, bivalves, *Villorita cyprinoides*, *Escherichia coli*.

Shallow waters near the coast or estuaries, provide good environmental conditions for bivalve growth. However, coastal waters are also the areas often subjected to pollution from different sources. By virtue of their filter feeding system shellfish accumulate human pathogenic bacteria and viruses when grown in polluted waters (Souness, 1990; Kueh & Kwong, 1985).

Depuration is the process of purification of shellfish in which the animals are placed in disinfected recirculating or running sea water and allowed to actively filter feed. This process leads to elimination of bacteria from the bivalves (Jones *et al.*, 1991; Timoney & Abston, 1984). Disinfection of circulated sea water can be achieved by use of UV radiation, ozone treatment, irradiation, etc (Schneider *et al.*, 1991; Beghian & Mallet, 1989). The objective of this study was to develop a suitable depuration system for bivalves

and to evaluate the efficiency of the system for removal of bacteria by depurating animals seeded with indicator bacteria.

Materials and Methods

Clams (*Villorita cyprinoides*) used for this study were collected from the brackish water fish farm of the College of Fisheries, Panangad, Cochin. The clams (length: 3-4 cm) were brought alive to the laboratory.

For determining optimum salinity of depuration, clams were maintained in troughs of water with varying salinities viz., tap water, 5, 10, 15 and 20‰ sea water. Each tray contained 3 l water with 15 animals. They were observed for irritability, appearance of faecal matter and the protrusion of siphon at intervals of 15, 20, 30, 45 & 60 min. Irritability was taken as the capacity to respond to stimuli. This was tested by prodding the animals by a glass rod and noting the animal's response to it by closing the valves with a jerk.

* Corresponding author

Present address: 1. School of Environmental Studies, Cochin University of Science & Technology, Cochin - 682 016, India

2. Ice & Freezing Plant, Matsyafed, Cochin - 682 005, India

3. Saudi Fisheries Co., P.O. 6535, Dammam, Saudi Arabia

Accumulation of *Escherichia coli* by the clams (*V. cyprinoides*) was monitored by keeping 10 clams each in 3 l portions of 10‰ sea water seeded with *E. coli* to a level of 10^9 cfu g⁻¹. The sea water used was passed through a biological filter for a week, prior to seeding. A set of 10 clams were drawn at 6 h intervals for estimation of *E. coli* count following the most probable number method using EC broth at 44.5°C for 24 h.

The depuration system consisted of a depuration chamber, biological filter and UV steriliser (Fig. 1). The depuration chamber was made of fibre glass in which 8 mesh platforms could be placed one above the other vertically. The biological filter consisted of a chamber made of fibre glass provided with a perforated platform at the bottom to support sand and gravel. The latter served as the substratum for the attachment and colonisation of nitrifying bacteria. Water from the biological filter was pumped to the UV steriliser using compressed air from an air compressor.

The flow rate of air was adjusted to get uniform pumping of water. In order to maintain the biological filter when the equipment was not in use, a by-pass chamber was provided.

To prepare the system for depuration process, sea water of 10‰ salinity was circulated for a week or two. This facilitated the settling down and colonisation of nitrifying bacteria which was indicated by the marked reduction in the level of ammonia in water. The UV light was switched on for a desired period so as to ensure zero *E. coli* count in water. The clams used for evaluation had an initial *E. coli* count of 6.69×10^8 which was attained by keeping the animals in 10‰ sea water seeded with *E. coli* to a level of 10^9 , for 6 h. The seeded clams were spread on the mesh platforms in the depuration tank. Efficiency of depuration was tested by determining *E. coli* count periodically. Depuration was carried out at ambient temperature.

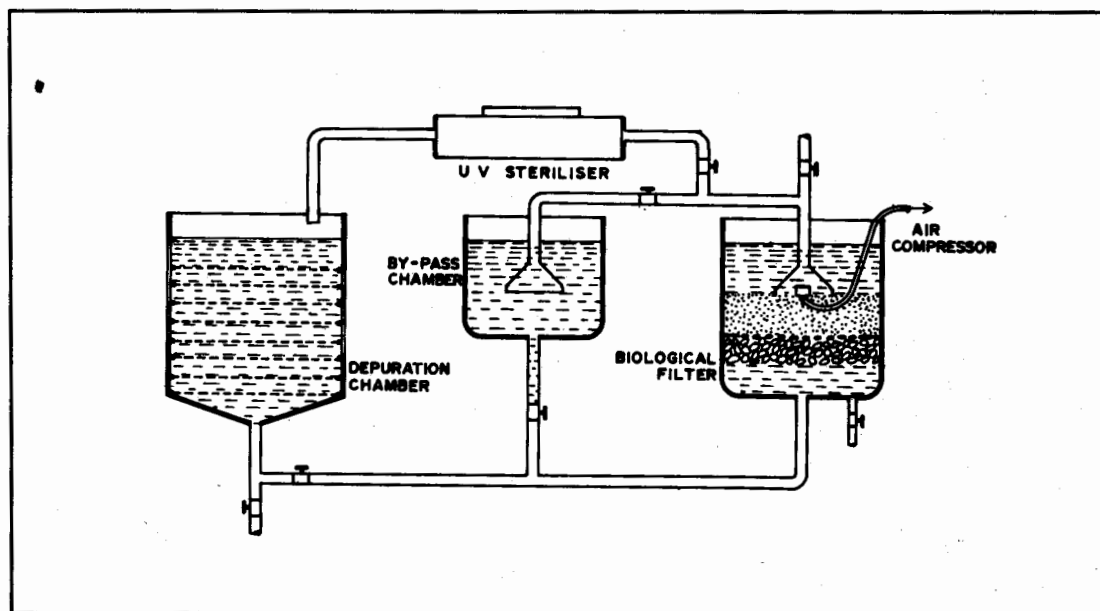


Fig. 1. Experimental depuration system

Data showing the influence of salinity on survival was analysed using ANOVA technique. Wherever the influence was found significant ($p < 0.05$), pair-wise comparison was done using critical difference.

Results and Discussion

Irritability response of *V. cyprinoides* maintained at various salinities are shown in Table 1. All the animals at salinities 10, 14 & 20‰ showed positive irritability response at the end of 60 min exposure. Analysis of variance indicated that these salinities had similar effect on animals as far as irritability response was concerned.

Table 1. Irritability response of *Villorita cyprinoides* exposed to different salinities (No. out of 15 animals)

Time, min	Tap water	5‰	10‰	15‰	20‰
15	0	0	3	3	3
20	0	0	6	9	6
30	0	3	6	9	9
45	0	9	12	14	14
60	0	12	15	15	15
Mean*	-	4.8 ^b	8.4 ^a	10 ^a	9.4 ^a

* Same superscripts indicate that the means are not significantly different ($p > 0.05$); Critical difference: 2.7

Effect of different salinities on appearance of faecal matter is shown in Table 2. On exposure for a duration of 150 min at salinities 10, 15 & 20‰ all the animals responded positively. ANOVA revealed that the salinities 5 and 10‰ were critically different from the others. Protrusion of siphon as a response to different salinities were studied and the results are shown in Table 3. Among 15 animals tested, 13 responded positively to 10‰ salinity. ANOVA indicated that treatment means of 10‰ salinity is critically different from other salinities. Based on the above results, 10‰ sea water, at which *V. cyprinoides*

appeared to be active, was chosen for further studies involving seeding of *E. coli*. Habitat salinity varied from 10 to 14‰ during the period of these experiments.

Table 2. Appearance of faecal matter in *Villorita cyprinoides* exposed to different salinities. (No. out of 15 animals)

Time, min	Tap water	5‰	10‰	15‰	20‰
15	2	0	1	2	2
30	3	1	10	5	8
45	3	6	11	9	12
60	5	9	14	11	12
90	6	11	15	14	14
150	6	13	15	15	15
Mean*	4.2 ^a	6.6 ^b	10.0 ^c	9.3 ^c	10.5 ^c

* Same superscripts indicate that the means are not significantly different ($p > 0.05$); Critical difference: 1.93

The trend in accumulation of *E. coli* by *V. cyprinoides* is shown in Table 4. Mesquita *et al.* (1991) employed *E. coli* as one of the faecal indicator bacteria for determining depuration period for the mussel, *Mytilus edulis*. Table 5 shows the effect of depuration of *E. coli* count of samples of seeded clams. The animals were found free of *E. coli* on 15 h depuration. The rate of bacterial elimination achieved here is much more rapid than

Table 3. Protrusion of siphon of *Villorita cyprinoides* exposed to different salinities (No. out of 15 animals)

Time, min	Tap water	5‰	10‰	15‰	20‰
20	0	0	3	0	2
30	0	0	2	0	2
45	0	1	2	3	2
60	0	3	9	4	4
90	0	3	13	4	3
150	0	7	13	9	6
Mean*	-	2.3 ^a	7.0 ^b	3.3 ^a	3.1 ^a

* Same superscripts indicate that the means are not significantly different ($p > 0.05$); Critical difference: 2.59

Table 4. Accumulation of *Escherichia coli* by *Villorita cyprinoides*

Duration, h	<i>E. coli</i> , cfu g ⁻¹
0	Nil
6	6.69x10 ⁸
12	9.12x10 ⁸
18	1.66x10 ⁹
24	4.06x10 ⁹

in studies reported earlier (Putro *et al.*, 1990). Increased siphoning rate contributed by the optimum salinity can be one of the reasons for this rapid rate. Mesquita *et al.* (1991) reported that when mussels (*M. edulis*) were depurated at conditions similar to those experienced in commercial shellfish industries in UK, there was a rapid rate of elimination of *E. coli* as compared to bacteriophages. Putro *et al.* (1990) found on experiments conducted with ark, blood cockles and green mussels that after 3 day depuration on a semi-commercial level at 28°C, *E. coli* count of the animals could be practically brought to zero. However, *Vibrio parahaemolyticus* count remained constant for all the samples. On depurating bivalves, in running water Power & Collins (1989) showed that *E. coli* number reduced by 2.9 log cycles within 52 h of depuration. Son &

Table 5. *Escherichia coli* content of ambient water and *Villorita cyprinoides* during depuration

Duration, h	Water cfu ml ⁻¹	<i>V. cyprinoides</i> cfu g ⁻¹
0	Nil	6.69x10 ⁸
3	5.5x10 ⁶	1.9x10 ⁷
6	1.3x10 ⁴	1.5x10 ⁵
9	2.4x10 ²	5.3x10 ⁴
15	Nil	Nil
18	Nil	Nil
21	Nil	Nil
24	Nil	Nil

Number of animals used for each experiment: 30;
Values are average of 3 readings.

Fleet (1990) had reported that cleaning of *E. coli* on depuration could be used to indicate the cleaning of other pathogenic bacteria like *Salmonella* sp., *Bacillus cereus*, *Clostridium perfringens* and *Vibrio parahaemolyticus*.

The depuration system designed in this study has proved efficient in eliminating accumulated indicator bacteria in less than 15 h. Level of initial contamination was one of the major factors influencing success of depuration of bivalves. The mechanism of microbial elimination seems to be initially of a mechanical nature which is followed by another phase that depends upon the microbial species and their accumulation. (Corre *et al.*, 1990). The recirculating type depuration system designed here not only overcomes the limitations of operating a flowing water depuration system in sea food processing units but also reduces the time required for completing the depuration process.

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