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BACTERIAL DEPURATION OF GROSSLY CONTAMINATED EDIBLE OYSTER, *CRASSOSTREA MADRASENSIS* (PRESTON)

ABSTRACT

Microbial quality of edible oyster, *Crassostrea madrasensis* (Preston) from the natural beds of Tuticorin coast and the elimination of microbes by depuration were investigated. Oysters were grossly contaminated by human pathogens such as Salmonellae, Vibrios, *Staphylococcus aureus Escherichia coli,* and *Enterococcus faecalis.* Depuration of oysters in chlorinated flow-through saline water reduced the levels of faecal coliform by > 99.97% within 24 h. Counts of *E. faecalis* and *S. aureus* were reduced by 99.40% and 39.23% respectively, in 48 h. Salmonellae and Vibrios remained persistantly even after 48 h of depuration. Mortality of oyster was 2.21% during depuration.

INDIA has a 6,100 km coastline, numerous estuaries, backwaters and creeks, and abundant bivalve resources. Unfortunately, the shallow coastal waters are the areas often subject to pollution from different sources. Oysters, being filter feeders and sedantary, are more vulnerable to bacteriological and chemical pollution than other animals and transmit many diseases to man when consumed (Richards, 1988). Between 1986 and 1990 in the US, there were 12,376 documented cases of bivalve shellfishborne diseases, not including cases of paralytic shellfish poisoning (Cook, 1991). Infections with fatality rates of over 50% are associated with eating contaminated raw oysters where *Vibrio vulnificus* becomes concentrated through filter feeding (Anon, 1997). To overcome this predicament, the process of depuration is used. Oyster depuration has been successfully practiced in many countries. In India, the bivalves including oysters collected from wild are currently sold undepurated immediately after collection. This calls for appropriate measures to make the bivalve safe for human consumption before they are marketed. This paper reports on the bioaccumulation of human pathogens in wild oysters and the result of the depuration trial of grossly contaminated edible oyster, *Crassostrea madrasensis* harvested from polluted coastal water in Tuticorin, India.

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Live edible oysters, *Crassostrea madrasensis* (Preston) were collected from the feral population of oysters along the Tuticorin coast. Water samples from the harvest site were collected in 300 ml capacity sterile glass containers. Both oyster and water samples were brought to the laboratory within an hour and assessed for microbial quality by APHA (1976) methods. For depuration, oysters were sorted into a desirable grade (8.44 ± 1.22 cm in length and 5.72 ± 0.74 cm in breadth) and rinsed off weed and silt thoroughly with freshwater by means of a jet spray hose. Adhering barnacles and other fouling organisms were removed with a sharp scalpel. Oysters, after cleaning, were placed in chlorinated seawater (3.0 ppm) for 30 min for conditioning. They were then arranged individually on meshed circular trays and loaded into circular cement tanks of 3.45 m^3 capacity. The process water used for depuration was drawn from a saline borewell and filtered through sand filter. The filtered saline water, before being added to the depuration tank, was chlorinated to 5.0 ppm level and dechlorinated by the addition of sodium thiosulphate (12.0 ppm) and vigorous aeration in a reservior. The optimum conditions provided for depuration of oysters are presented in Table 1. Water level in the tank was kept constant by a pipe which drained from the bottom and which was held at the same level as that in the tank. At every 12 h interval, oysters alongwith the tray were removed from the tank and washed well with chlorinated saline water (5.0 ppm) by means of a jet spray hose. Depuration tanks were also cleaned with chlorinated water to remove the faeces and pseudofaeces. After washing, the trays with oysters were reloaded into the tanks and the depuration continued. Tanks were checked frequently for oyster mortality. Dead ones were removed immediately and the numbers noted. Microbial load of the oyster and process water were monitored at regular intervals during depuration. Process water samples were taken at the tank outlet aseptically in sterile glass containers. APHA (1976) methods were followed for the enumeration of total plate counts (TPC), total coliforms (TC), faecal coliform (FC), *Enterococcus faecalis* counts (EFC) and *Staphylococcus* spp, and isolation and identification of *S. aureus*, Salmonellae and Vibrios.

TABLE 1. *Conditions provided for bacterial depuration of oyster*

Parameters	Value
Water temperature (°C)	30.00-31.67
Salinity (ppt)	20.08-21.90
Dissolved oxygen (% saturation)	52.42-88.51
pH	7.30-7.60
Water flow rate (ml/min/oyster)	39.70
Oyster density (number/1)	0.73
Water depth (cm)	15.0
Tank size (m^3)	3.45

TABLE 2. *Bacterial quality of oyster and shellfish water of Tuticorin coast*

Parameters	Oyster	Shellfish water
Log Total plate count/g or ml	3.15-4.55	2.96-2.97
Log Total coliforms/g or ml	2.63-3.38	1.66-2.04
Log Faecal coliform/g or ml	2.36-3.04	1.04-3.04
Log *Enterococcus faecalis* count/g or ml	1.85-3.23	1.00-1.81
Log Staphylococcal count/g or ml	2.29-2.96	1.00-2.24

Salmonellae and Vibrios were present in both oyster and water samples

Microbiological data on shellfish waters revealed that the levels of total and faecal coliforms in Tuticorin coast far exceeded the limits (TC MPN ≤ 70/100 ml and FC MPN ≤ 14/100 ml) recommended for shellfish waters (USPHS, 1986). Shellfish from 'approved' waters may be consumed without depuration; however, shellfish from 'restricted' waters (containing a mean MPN ≤ 700 TC or ≤ 88 FC/100 ml) must be depurated. Shellfish in 'prohibited' waters (contaminating an MPN of > 700 TC or > 88 FC/100 ml) may not be harvested or depurated in United States (USPHS, 1986). Oysters collected from Tuticorin coast were grossly contaminated judging from the initial TC (log 2.63-log

3.38 g), FC (log 2.36-log 3. 04/g) and other faecal bacteria due to bioaccumulation (Table 2). Presence of pathogens such as *S. aureus,* Salmonellae and Vibrios in oysters and shellfish waters possibly indicate the contamination of the coastal water by domestic sewage.

During depuration with a flow rate of 39.70 ml/min/oyster and an oyster density of 0.73/1 of process water, the MPN of FC was reduced by > 99.73% in first 12 h and then to > 99.97% within 24 h. These results are in accordance with Gacutan *et al.* (1986) who observed a reduction of 98.27.99.91% after 24 h in grossly contaminated *C. iredalei.* Similarly, the TC were reduced by 99.90% in 48 h. The counts of *E. faecalis* were reduced steadily by 99.40% in 48 h (Table 3). Similar reduction was recorded by Maddan *et al.* (1986). Data concerning rates of depuration of human pathogenic bacteria are conflicting. It has been demonstrated that contaminated bivalve molluscs with high level of *E. coli, Salmonella, Clostridium perfringens* and *Vibrio parahaemolyticus* can be purified, so that these organisms are undetectable after 48 h of depuration (Son and Fleet, 1980). However, detailed studies on the elimination of *E. coli* and *Salmonella* from oysters carried out by Janssen (1974) and Rowse and Fleet (1982) showed that low levels of bacteria may still be present in oyster after 48 h. In the present study, pathogens like *S. aureus*, Salmonellae and Vibrios remained persistantly even after 48 h, thus confirming the earlier reports (Janssen, 1974; Rowse and Fleet, 1982). Presence of trace amounts of bacterial pathogens in depurated shellfish does not generally cause illness to consumers because threshold levels required to cause illness are seldom reached (Richards, 1991). Although the disinfection of process water by chlorination resulted in a reduction of TPC by 2.5 folds in 24 h, the final count was higher after depuration than initially (Table 3). Several workers reported ten fold reduction in TPC during depuration. However, reduction to fewer than log 4.0/g are seldom seen for some shellfish species, perhaps because of the presence of an indigenous gut flora (Son and Fleet, 1980; Richards, 1991).

TABLE 3. *Effect of depuration on the bacterial quality of oyster and process water*

Parameters	Hours of Deputation				
	0	12	24	36	48
OYSTER*					
Log Total plate count/g	4.55	Spr	4.15	4.20	4.72
Log Total coliforms/g	3.38	1.36 (99.04%)	0.60 (99.83%)	0.63 (99.82%)	0.36 (99.90%)
Log Faecal coliform/g	3.04	< 0.48 (99.73%)	< 0.05 (99.97%)	< 0.05 (99.97%)	< 0.05 (99.97%)
Log *Enterococus faecalis* count/g	3.23	3.004 (39.88%)	2.33 (87.20%)	1.48 (98.21%)	1.00 (99.40%)
Log Staphylococcal count/g	2.96	2.88 (16.57%)	2.85 (21.55%)	2.82 (27.62%)	2.74 (39.23%)
PROCESS WATER					
Log Total plate count/ml	2.09	3.74	4.20	4.18	4.16
Log total coliforms/100 ml	1.18	2.66	2.66	1.36	1.59
Log Faecal coliform/100 ml	<0.30	0.60	0.48	<0.30	<0.30
Log *Enterococcus faecalis* count/ml	<1.00	2.53	2.11	1.48	1.18

* both Salmonellae and Vibrios remained persistently even after 48 h of depuration. Spr. Spreader, Values in parenthesis are percentage reduction

Hydrological parameters of the process water did not vary much during depuration and even did not affect the depuration. Dissolved oxygen was maintained above 50% saturation. The mortality of oysters was only 2.21%. Oysters remained physiologically active and were effective in purging out the microbes to an acceptable level of FC ≤ 2.3/g (APHA, 1976). Disruption in the depuration process rightly after collection of oyster for sampling was done to flush away biodeposits, faeces and pseudofaeces which sedimented on the tank bottom or attached to the tank sides or on the trays and to avoid any drastic change in rank condition. It was recommended that during depuration tanks be drained 24 h intervals, the oysters taken out to remove biodeposits (Furfari, 1966). However, it was argued by Delvin and Eng (1973) that draining and flushing of tanks periodically may resuspend the detritus during the refilling, leading to increase in bacterial levels in both process water and oysters. During depuration, there was an increase in the levels of TPC and TC in process water (Table 3) probably due to the multiplication of bacteria and/or resuspension of discharged oyster faeces from the tank bottom during flushing or refilling. The results of this study provide and additional supportive evidence to the earlier observation (Delvin and Eng. 1973).

In summery, oysters harvested from the Tuticorin coast were grossly contaminated and were cleansed to an acceptable level of FC ≤ 2.3/g within 24 h. The total cost for depuration procedures was Rs. 5/- per kg oyster (shell on) and it can be sold in the retail market @ Rs. 50/- per kg picked meat. The process of depuration, therefore, would enable marketing of safe product popularisation of this underutilized species for human consumption.

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