DEPURATION OF OYSTERS (*CRASSOSTREA* SPP.) USING ULTRA-VIOLET RADIATION

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ABSTRACT

The depuration of oysters (*Crassostrea belcheri* and *Crassostrea iredalei*) was studied using a recirculation system (3 exchanges / hour) with filtered sea water, sterilised by ultra-violet radiation. The flow rate of the system was 100 litres / min for a 2 m^3 set-up. Oysters were stocked in shallow trays with 50 oysters / tray or approximately 45 kg / set-up. The trays were designed to facilitate water circulation. The percentage reduction of coliform bacteria in the oyster meat was 98.6 % and 99.9 % after 24 and 48 hours respectively. The percentage reduction of faecal coliform bacteria was 99.9 % after 24 and 48 hours. Independent of the initial bacterial concentration, a 24-hour depuration period was sufficient to reduce oyster meat bacterial concentration to APHA standards (less than 230 MPN / 100 g meat of faecal coliforms). The meat quality of *C. belcheri* was not effected after depuration whereas the meat of *C. iredalei* became soft and watery since no feeding was provided during the depuration process.

INTRODUCTION

Oysters, like other filter feeding bivalves, tend to pump large volumes of water and may, as a result, depending on the pollution level of their environment, accumulate bacteria and other micro-organisms present in the surrounding water. These include pathogenic bacteria and virus. Human consumption of such contaminated shellfish has resulted in a number of gastrointestinal diseases (e.g. typhoid fever, paratyphoid fever, cholera and non specific gastro-enteritis) of bacterial (Wood 1976) and hepatitis-A of viral origin (Gerba 1988).

In Asia, transmission of bacterial and viral diseases through shellfish consumption is enhanced by prevailing eating habits namely the preferences for eating oysters, cockles and mussels raw or blanched, enhanced by improper sanitary practices of food producers and handlers.

In recent years, increased discharge of industrial and domestic wastes through rivers adversely affected the quality of water flowing through the oyster culture areas. While this is not necessarily bad for the growth of oysters, it has greatly increased the risk of contamination of the oysters produced.

Depuration is a process by which such bivalves could cleanse themselves. To effect this, the bivalves are placed in "clean" water that has been sterilised. Such water, passing through the body of the animal purges the bivalve within the allotted time period of depuration, normally 48 hours. By doing so, a wholesome product with an acceptable level of bacteria could be obtained.

Sterilisation of the sea water could be performed in different ways, the most commonly commercially used techniques are chlorination, ozonation and ultra-violet (UV) radiation. Due to the possibility of long-lived oxidant residues in the sea water produced by chlorination and ozonation that is sterilised (Mangum & McIlhenny 1975) and the resulting detrimental effect on marine life with large-scale discharges from depuration operations. This study was conducted to determine the suitability of ultra-violet radiation for depurating the local oysters (*Crassostrea iredalei* and *C. belcheri*).

MATERIALS AND METHODS

Oysters

C. iredalei and *C. belcheri* were used in this study because these oysters are the commercially harvested species for the "half-shell market". All oysters used in this study were obtained from rafts maintained by Muka Head Marine Research Station in Sungai Kuala Muda estuary, Kedah. The oysters were washed, then placed in shallow plastic trays, each measuring 60 cm x 40 cm x 20 cm depth.

Depuration System

50 oysters were placed in a tray, with a total of 6 trays being used in the set-up (approximately 45 kg / set-up). The trays were designed to facilitate the water flow. They were placed in a fibreglass trough (volume 200 L). The system consisted of 2 troughs, a reservoir tank and a header tank. Sea water filtered to 1 µm was continuously recirculated through the system for 48 hours. Sea water (flow rate 100 L/ min) was pumped from the reservoir tank through a UV sterilizer to a header tank, then allowed to flow into the depuration troughs by gravity.

With 2 m^3 water in the tank, a flow rate of 100 L / min would give 3 exchanges of water per hour for the system. Sterilisation of the water was obtained by an Aqua-UV sterilizer. The sterilizer contained 2 UV lamps of 40 W each and was capable of sterilising at the rate of 6-20 m^3 water/ hour (i.e. 100-300 L/min). At 6 m^3 / h, the manufacturer of the sterilizer, claims germicidal intensity emitted to be 28,000 mW sec/cm^2.

Totally 150 oysters were held in each troughs giving a total holding capacity of 300 oysters. For bacterial determination, 20 oysters were sampled at intervals of 0, 24, and 48 hours.

Bacterial Analysis

For determination of bacterial content, 20 oysters were individually washed in tap water, air dried, and shucked using a flamed knife. Shucked meat samples of the oysters were pooled. The oyster meat and liquid were aseptically transferred to a sterile beaker, 1:2 dilution was made with sterile phosphate buffered dilution water (pH 7), followed by blending for 90 seconds (APHA 1970).

Enumeration of faecal coliform and total coliform bacteria in oysters were carried out using the 5 tube Most Probable Number (MPN) technique. Presumptive total number of coliform bacteria were obtained using Lauryl Tryptose (LT) broth (incubated at 37 ^{o}C/ 24-48 hours), followed by confirmation with Brilliant Green Broth. To count faecal coliforms, positive LT cultures were tested in EC broth (incubated at 44.5 ^{o}C / 24-48 hours) and positive EC broth cultures were taken and streaked onto EMB (Eosin Methylene Blue) agar. A green sheen seen over the colonies would confirm the presence of *Escherichia coli.*

Aerobic (Total) plate counts were done using Nutrient Agar plus 1.5 % sodium chloride (37 ^{o}C, 48 hours), according to recommended procedures for a standard plate count for shellfish. This involved aliquots of oyster homogenates serially diluted tenfold from 10^{-1} to 10^{-2} using phosphate buffered dilution water (pH 7). Incubation occurred at 35 ^{o}C for 48 hours.

0.1 m aliquots of oysters homogenate were streaked on thiosulphate citrate bile salt sucrose (TCBS) agar for the isolation and counting of *Vibrio* spp. at 35 ^{o}C. Both green and yellow colonies were counted.

RESULTS

The results of this study are shown in Tables 1 and 2. The number of initial total and faecal coliform bacteria in oysters were 40,000 and 9,600 MPN per 100 g meat respectively. The total coliform bacteria count was reduced to acceptable levels of 86 MPN/100 g (99.78 % reduction) after 24 hours and 9 MPN/100 g meat (99.98 % reduction) at 48 hours. The corresponding number of faecal coliform bacteria was 8 MPN/100 g meat (99.92 % reduction) at 24 hours and 3 MPN/100 g meat (99.97 % reduction) at 48 hours, a reduction, which is far below the acceptable levels of 230 MPN/100 g meat.

Table 1. Changes in the bacterial levels in oysters during depuration from 0 hour to 48 hours.

	Bacterial count at a given time		
	0 hours	24 hours	48 hours
Total coliform (MPN/100 g meat)	40,000	86	9
Faecal coliform (MPN/100 g meat)	9,600	8	3
Total plate count (CFU/g meat)	56,250	680	12
Vibrio (CFU/g meat)	200	127	18

The initial total plate counts were 56,250 CFU/g (acceptable level = 500,000 CFU/g). The count was reduced to 680 CFU/g (98.79 % reduction) after 24 hours and 12 CFU/g (99.98 % reduction) after 48 hours. The number of *Vibrio* were reduced to a level of 127 CFU/g (36.5 % reduction) after 24 hours and 18 CFU/g (91.00 % reduction) after 48 hours from an initial count of 200 CFU/g. Generally, a percentage reduction between 98.79 % - 99.92 % occurred after 24 hours (except for Vibrio) while a further reduction after 48 h was minimal.

The number of dead oysters was monitored during depuration and 100 % survival was recorded for both *C. iredalei* and *C. belcheri* . Spawning occurred in *C. iredalei* at times during the first 2-3 hours after start of depuration. In tanks with water, eggs and sperms, the water was discarded, the oysters rinsed and the tanks refilled to restart depuration. Such incidents did not occur for *C. belcheri* .

The meat quality of *C. iredalei* became soft and watery after the depuration period compared to the firm meat of *C. belcheri* .

DISCUSSION

Ultraviolet (UV) light can eliminate bacterial pathogens from water by causing irreversible damage to the DNA of the pathogens (Blogoslawski & Stewart 1983). The results show that bacterial contaminated oysters can be effectively depurated in a recirculation system using ultra-violet radiation. Efficiency

Table 2. Percentage reduction in bacterial levels in oysters after 24 and 48 hours depuration.

	Reduction at a give time (%)		
	0 hours	24 hours	48 hours
Total coliform	-	99.78	99.98
Faecal coliform	-	99.92	99.97
Total plate count	-	98.79	99.98
Vibrio	-	36.50	91.00

of depuration was highest during the first 24 hours, with a bacterial reduction varying from 98.79-99.92 %. This agrees well with results reported for depuration efficiencies in other bivalves such as *Perna viridis* (Cheong & Syed 1982), *Crassostrea gigas* (Furfari 1966) and *Ostrea edulis* (Wood 1961), where UV depuration systems were used.

The reduction of *Vibrio* during the first 24 hours of depuration process was only 36.5% compared to 99 % of the coliform group, which means the depuration rates were not correlated between the vibrios and coliform bacteria. The slower rate of reduction in vibrios may be due to the existence of vibrios in other parts of the oysters body (e.g. adductor muscle) instead of in their alimentary tracts like the coliform group (Gerba 1988).

The maintenance of 3 exchanges of water per hour at the flow rate of 100 L/min through the system provided an effective depuration of the tropical oysters — a condition similar to that found by Souness *et al.* (1979) for the Sydney rock oyster *Crassostrea commercialis*.

UV treatment works well for relatively small-scale operations but has limitations when larger application is required. When using UV radiation, the sea water to be treated must be prefiltered to remove particulate matter up to 10 µm in size. This is done because suspended matter can shield pathogens from the UV light. It is also well known that UV penetration is directly related to water depth and turbidity (Blogoslawski & Stewart 1983).

A disadvantage of using UV depuration for *C. iredalei* is that it may induce spawning in *C. iredalei* and the oysters would become soft and watery, i.e. a lower meat quality. *C. belcheri* proved to be less sensitive to UV induction for

spawning.

According to the results obtained, a plant for depuration could be established in tropical regions with problems of contaminated shellfish culture areas, like Malaysia, post-harvest contamination (e.g. rinsing in polluted water) or a combination of the two. Initial evaluation suggests that it would be economically favourable to localise purification plants close to the point of final sale, where value of the product is high. However, certain factors need to be taken into account during the construction of the depuration plant (Ayres 1986):

A. The effect of time on the depuration
B. The effect of density on the depuration
C. The effect of pollution levels on purification rate
D. The effect of pre-treatment handling on purification
E. The effect of purification on keeping quality of live oysters

Much remains to be investigated with regard to depuration, particularly in the area of viral depuration or inactivation of biotoxins. The process does have limitations as existing depuration is ineffective in removal of heavy metals or pesticides within a commercially feasible period of time.

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