Fishery Technology 58 (2021) : 160 - 165

Efficacy of a Closed Water Depuration System with Charcoal filter on the Bacteriological quality of *Villorita cyprinoides* var. *cochinensis* (Hanley, 1866)

Ally C. Antony[1], Reshma Silvester[3], P. A. Aneesa[2], Bini Francis[2], Mohamed Hatha Abdulla[2]*

[1] *MES College, Marampally, Aluva, Ernakulam - 683 107, India*

[2] *School of Marine Sciences, Cochin University of Science and Technology, Lakeside Campus, Cochin - 682 016, India*

[3] *School of Industrial Fisheries, Cochin University of Science and Technology, Lakeside Campus, Cochin - 682 016, India*

Abstract

Bivalves are good bio-indicators of the sanitary quality of the aquatic bodies in which they survive. The sanitary quality of the shellfish harvesting areas is assessed based on the faecal coliforms/*E. coli* levels of the harvesting water/shellfish tissue; on exceeding the regulatory limits depuration suggested. In the present study, the efficacy of a closed water depuration system attached with a charcoal filter to depurate Indian black clams (*Villorita cyprinoides* var. *cochinensis* (Hanley, 1866) was evaluated. The depuration system consisted of a closed water holding glass tank, with a wall-hung immersion water pump which re-circulated (18 l min^{-1}) the seawater [salinity – 10 ppt, pH -7.3, ambient temperature (29-30°C)] through a coconut shell based activated carbon (charcoal) filter. Clams were sampled at time intervals of 0, 6, 12, 24, 72, and 96 h to assess the reduction of total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS). Initial TC, FC, FS and *Salmonella* loads were assessed using standard microbiological methods. *Salmonella* was not detected in any of the clam samples. Complete removal of both FC and FS whose initial loads were 4.6 x 104 MPN 100 g^{-1} and 1.1 × 105 MPN 100 g^{-1} respectively was observed within 48 h. Maximum rate of depuration of TC, FC as well as FS were observed during the initial first 6 h. However, TC could not be fully depurated even after 96 h of depuration. The differences in the depuration rates of TC, FC and FS were found to be statistically significant ($p<0.05$). The FC load of the black clams selected for the study conformed only to the class C shellfish growing area of EU which could be reduced to acceptable regulatory limits of <230MPN 100 g^{-1} of depurated shellfish; which proved the system used is efficient. The system uses simple, cost-effective, easily available, natural, and renewable water treating agent such as coconut shell-based activated carbon and is suitable for household purposes. In India, black clams do not have much export value and is also not preferred to be eaten raw. Hence less stringent, cost-effective, and simple depuration measures as mentioned above may be sufficient enough to meet the required sanitary quality as it is consumed only after proper cooking.

Keywords: Shellfish; *Villorita cyprinoides*; Depuration; Faecal coliforms; Activated carbon; Cochin estuary

Introduction

Shellfish associated disease outbreaks have been reported worldwide and food safety is the major concern associated with bivalve consumption (Costa, 2013; Odeyemi, 2016). It is one of the delicacies preferred to be eaten raw by people in many parts of the world. Most of the shellfish growing areas are situated in shallow, nutrient rich, near-shore waters which often receive sewage discharges and being filter feeders bivalves accumulate all sorts of contaminants including pathogens in the surrounding waters (Pavoni et al., 2013). Realising the potential risk factor associated with consumption of bivalves, remedial measures are being implemented mainly at two stages- pre-harvest and post-harvest levels. At pre-harvest level, harvesting of shellfish is permitted only if the sanitary quality of the

Received 06 April 2021; Revised 26 July 2021; Accepted 27 July 2021

**E-mail: mohamedhatha@gmail.com*

growing area meets the standards prescribed by the regulatory agencies (NSSP, 2015), whereas at post-harvest level various strategies such as depuration in land-based plants or relaying in clean estuarine waters for longer periods is adopted. During depuration, harvested shellfish is placed in land-based plants containing clean estuarine water, to permit the purging of their gastrointestinal contents under controlled conditions (Oliveira et al., 2011; Sorio & Peralta, 2017). Contamination of shellfish growing waters with human faecal material, poses the significant health risk to consumers (Pavoni et al., 2013).

Various regulatory bodies such as National Shellfish Sanitation Programme (NSSP, 2015), European Union (EC No 854/2004) etc. have classified the harvesting areas based on the faecal coliform or *E. coli* levels either in shellfish harvesting waters (NSSP, 2015) or in bivalve tissue and intravalvular fluids (EC No 854/2004). Though depuration is not mandatory in India, there are a few published reports, on the depuration of oysters and clams from India (Balachandran & Surendran, 1984; Nambudiri et al., 1995; Chinnadurai et al., 2014).

Total coliforms, faecal coliforms and *E. coli* are the most commonly used indicators of faecal contamination in shellfish (Hackney & Pierson, 1994). However, of the three indicators listed, total coliforms may be present in other environmental sources also, hence provide least information regarding faecal contamination, whereas faecal coliforms and *E. coli* assays give reliable information on faecal pollution. Several researchers have attempted depuration of bivalves using various agents such as UV (Chinnadurai et al., 2014), chlorination (Pillai & Selvan, 1987) etc. No previous studies regarding use of activated carbon to disinfect process water in depuration systems is available, except that it was used to remove chlorine residues after disinfection of process water in a depuration plant in Italy (Casali et al., 1981; Rees et al., 2010). In present study, the efficacy of a simple home-based closed water depuration system, attached with coconut shell based activated charcoal filter to purify naturally contaminated *V. cyprinoides* was evaluated.

Materials and Methods

Live Indian black clams, *Villorita cyprinoides* var. *cochinensis* from Cochin estuary was used for the depuration experiments. Collections were made during the early morning hours with the help of local fishermen in sterile polythene bags and transported to the laboratory in live condition in an icebox. Bacteriological analysis was carried out within 1-2 hours of sample collection.

Approximately 15-20 medium sized shellfishes were surface cleaned, aseptically shucked and about 25g of meat and liquor was transferred to a sterile stomacher bag and blended with 225 mL of sterile peptone water in a stomacher (IUL Instruments, Spain). Total and faecal coliform levels were enumerated by three decimal dilution 3 tube most probable number (MPN) method using EC broth (Hi-media, India), as described in detail elsewhere (Hitchin et al., 1995; BAM, 2011) and the MPN index was calculated and expressed MPN index 100 g^{-1} of shellfish.

Faecal streptococci levels in shellfish, harvesting waters and sediments were enumerated by three decimal dilution 3 tube most probable number (MPN) method using Azide Dextrose Broth (ADB) (Hi-media, India), as described in detail elsewhere (APHA, 1992). From the presumptively positive tubes, the presence of faecal streptococci was confirmed by streaking onto Kenner Faecal (KF) Streptococcal Agar (Hi-media, India) and Bile Esculin Azide (BEA) agar (Hi-media, India); followed by growth on Brain Heart Infusion (BHI) broth with 6.5% NaCl. The results were expressed as MPN index 100 g^{-1} of shellfish.

Salmonella was detected by the pre-enrichment of the shellfish samples (processed as mentioned above) in buffered peptone water, selective enrichment in Tetrathionate Broth (TTB) and Rappaport-Vassiliadis Soybroth (RVS), followed by inoculation on Xylose Lysine Deoxycholate (XLD) agar and Hektoen Enteric Agar (HEA) as described elsewhere (Marceddu et al., 2017).

The depuration system consisted of closed water holding glass tank with nominal capacity of 53 litres and dimensions 51x36x34 cm (Fig. 1). A wall hung immersion water pump (Dophin P-708, China), placed 15 cm above the bottom of the tank (to avoid recirculation of settled faecal material) re-circulated (18 litres/min) the sea water in the depuration tank. It was then passed through an activated carbon filter (coconut shell based charcoal) held within a holder placed above the tank. Before the start of the experiment, activated charcoal was thoroughly backwashed to remove the fine carbon particles that

may be present. For every batch of depuration, 45 l of autoclaved natural seawater with salinity adjusted to 10 ppt and pH 7.3 was used. The experiment was carried out at ambient temperature (29-30°C). Prior to the depuration experiments, it was confirmed that the clams were alive and actively feeding. Approximately, 80 medium sized clams were arranged in monolayer on a plastic mesh tray as shown in Fig. 2. The tray was suspended 20 cm above, from the tank bottom to prevent re-contamination from the faecal material settled at the bottom. About 4-5 bivalves were taken out at various intervals of 0, 6, 12, 24, 72 and 96 h using a sterile spatula and total coliforms, faecal coliforms and faecal streptococci were enumerated as described above. Clams survived well throughout the experiment, however, any dead ones if found were removed from the system immediately. Clams were not fed during the entire period of depuration process.

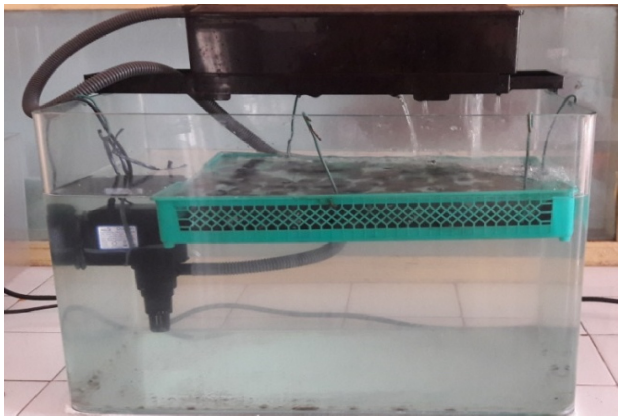

Fig. 1. Depuration tank with *Villorita cyprinoides* undergoing depuration

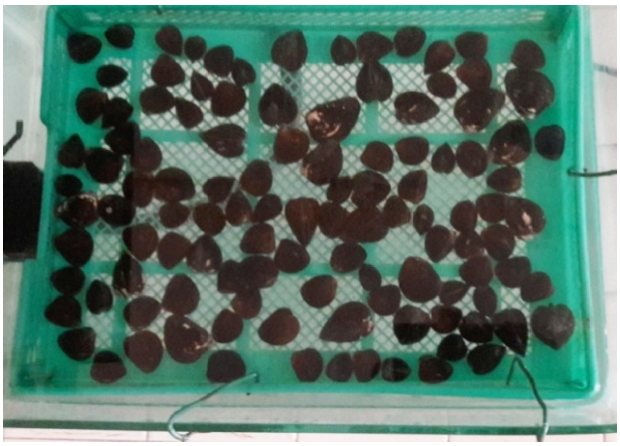

Fig. 2. *Villorita cyprinoides* arranged in monolayer on a plastic mesh tray for depuration

Statistical analysis of the results was performed using SPSS software 20 (Statistical Package for Social Science). The differences in the reduction of individual test organisms under varying time intervals and the relative reduction of various test organisms were analysed for significance using one-way analysis of variance (ANOVA) with Duncan's multiple range test. Significance level of the tests was set at ($p<0.05$).

Results and Discussion

The closed water depuration system attached with charcoal based filter used in the study was found to be effective for purification of naturally contaminated *V. cyprinoides* harvested from Cochin estuary. Activated carbon from coconut shell has predominantly pores in micro pore range (diameter in the range of less than 4 nm), which forms almost 85-90% surface area of coconut shell. Coconut shell-based charcoal has the advantages of being least dusty and derived from renewable resources. The removal of contaminants is believed to be due to two principal mechanisms; adsorption, and catalytic reduction, which involves the attraction of negatively-charged contaminant ions to the positively-charged activated carbon. Depuration and relaying have been shown to reduce faecal coliforms and other enteric bacteria to acceptable regulatory levels (Obadai et al., 2010) which will help to minimise the public health risk from faecal-borne bacterial pathogens in shellfish (Oliveira et al., 2011).

The initial TC count was found to be 5.04 logs (1.1×10^5 MPN 100 g^{-1}). One log reduction was obtained within the first 6 h of depuration, and further 2.5 logs reduction was observed after 96 h of depuration to a final TC count of 1.4 logs (Fig. 3). However, complete depuration of TC could not be attained and even after 96 h of depuration. The rates of reduction were found to be significant up to 12 h ($p=0.01$) whereas during the remaining intervals, reduction was not statistically significant.

The initial FC count of *V. cyprinoides* harvested from Cochin estuary was found to be 4.7 logs (4.6×10^4 MPN 100 g^{-1}) which conformed only to the microbiological standards of class C shellfish growing area of EU regulation. One log reduction was achieved within the first 6 h of depuration; later the rate of removal slowed down until the 12^{th} h (Fig. 4). Within 24 h, the FC load in shellfish could be reduced to <230MPN 100 g^{-1} (reduced by 2.5 logs

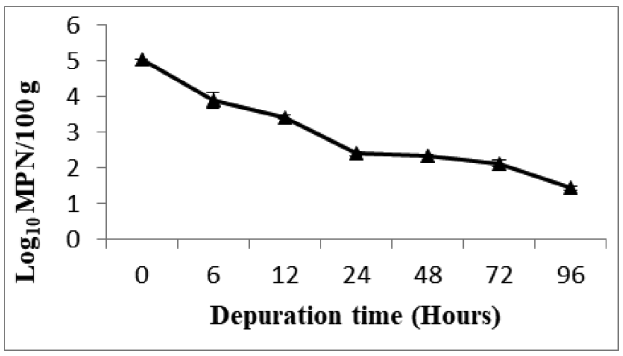

Fig. 3. Reduction of total coliforms from *Villorita cyprinoides* during depuration

i.e., 53% reduction), which falls in the acceptable FC regulatory limits of depurated shellfish. Total depuration of FC was observed within 48 h and the rates of reduction of FC was found to be statistically significant (p=0.01).

This is in agreement with the previous reports where shellfish were depurated to regulatory limits within 24 h (Power & Collins, 1989; Andritsos et al., 2016). In agreement to our findings Andritsos et al. (2016) also observed steep decline during the initial 10 hours of depuration. Similar finding was made by Nambudiri et al. (1995) where complete depuration of *V. cyprinoides* was accomplished in a UV based depuration system within 15 h of depuration.

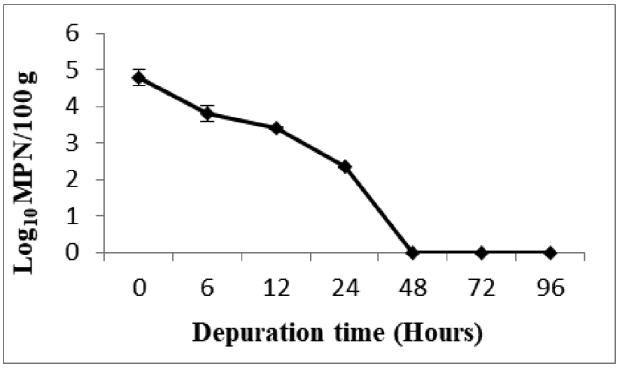

Fig. 4. Reduction of faecal coliforms from *Villorita cyprinoides* during depuration

Complete depuration of FS counts in *V. cyprinoides* samples from an initial count of 5.04 logs (1.1×10^5 MPN100 g^{-1}) was observed within 48 h (Fig. 5) out of which initial reduction of 1.4 logs was obtained within the first 6 h of depuration. The rates of reduction of FS at various time intervals was found to be statistically significant (p=0.01).

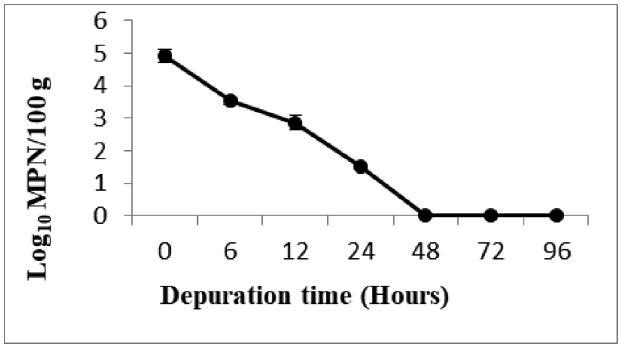

Fig. 5. Reduction of faecal streptococci from *Villorita cyprinoides* during depuration

Differential depuration rates were observed for TC, FC and FS from naturally contaminated *V. cyprinoides* (Fig. 6). While complete depuration of FC and FS was achieved within 48 h, TC could not be depurated completely even after 96 h. Nevertheless, the TC count was reduced by 3.64 logs to a final count of 1.4 logs (30 MPN 100 g^{-1}). Among FC and FS, the former was reduced by 2.4 logs within 24 h whereas the latter underwent a reduction by 3.4 logs. Maximum depuration rates of TC, FC as well as FS were observed during the initial 6 h. The rates of reduction between various test organisms were found to be statistically significant (p=0.01).

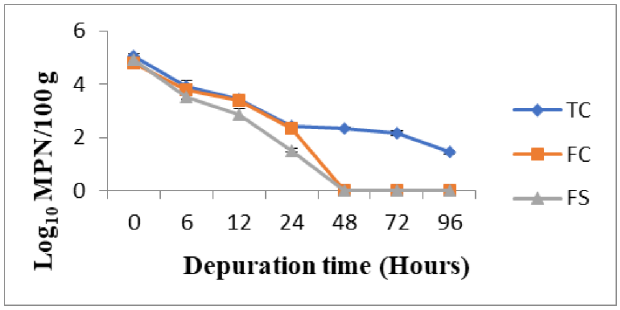

Fig. 6. Relative depuration of TC, FC and FS from *Villorita cyprinoides* during depuration

Among the three categories of sanitary quality indicators used (TC, FC and FS), removal of FC and FS demonstrated almost similar trends, whereas TC took comparatively longer depuration time. In contrary Love et al. (2010) demonstrated that *E. coli* depurated faster than *E. faecalis* from oysters (*Crassostrea virginica*) and hard-shell clams (*Mercinaria mercinaria*) in a flow-through depuration system. In present study, complete removal of FS as well as FC could be accomplished within 48 h depuration. This is in agreement with the findings of Casali et al. (1981) where 99% elimination of *Enterococcus* from

hen clams could be achieved within 48 h. Our results are in agreement with several previous studies where differential rates of purification were observed for total coliforms, faecal coliforms and faecal streptococci (Chinnadurai et al., 2014; Maffei et al., 2009). In agreement with our findings, Sorio & Peralta (2017) have reported that depuration in a recirculating flow water system was effective in reducing the *E. coli* levels to acceptable limits. *Salmonella* was not detected in the shellfish samples during the present study. *Salmonella* is not widely reported in bivalves and hence is not a threat to shellfish food safety (Marceddu et al., 2017).

The optimum parameters set during depuration such as temperature, pH, salinity, rate of water circulation etc. should be similar to the ambient conditions existing in the natural harvesting environment. This will help to minimise the stress to the oysters which would have otherwise impaired the whole depuration process (Power & Collins, 1989). Favourable tropical temperature is an important factor that influences depuration efficiency (Phuvasate et al., 2012), as lower temperatures reduced the efficiency compared to elevated temperatures (Buisson et al., 1981). However elevated temperatures may induce spawning which increases the water turbidity and reduce filtration rates which in turn impede depuration (Arakawa, 1990).

In conclusion, our closed water recirculation depuration system with activated charcoal made from locally available coconut shell waste was found to be effective for the depuration of naturally contaminated *V. cyprinoides*. Though it is effective in purifying moderate levels of microbial contamination, its efficiency in purifying higher levels of microbial contamination has to be thoroughly assessed. This is suitable for household purposes as it uses simple, cost effective, easily available, natural and renewable water treating agent such as coconut shell based activated carbon. However, for commercial purposes activated charcoal-based depuration could be coupled with a suitable antimicrobial agent that kills bacteria or viruses. Commercial establishments require much more accurate and efficient water treatment systems since the sanitary quality of the product to be marketed should meet global standards. However, in India, since the bivalve species under study does not have much export value, it is largely consumed in the domestic market itself and provide livelihood for thousands of fishermen community. Moreover, in India bivalve is not a commodity preferred to be eaten raw; hence less stringent, cost effective, simple depuration measures mentioned above may be sufficient enough to meet the required sanitary quality as it is consumed only after proper cooking.

Acknowledgements

The authors are thankful to the Department of Marine Biology, Microbiology and Biochemistry, Cochin university of Science and Technology for providing the facilities to carry out the research work. This research was conducted as a part of the doctoral work of the first author. The FIP-teacher fellowship granted by the University Grants Commission; Government of India is gratefully acknowledged.

References

Andritsos, N.D., Moschonas, G., Roukas, D., Ave, V.S., Athens, G. (2016) Elimination of *Escherichia coli* from mussels during treatment in a shellfish depuration system. Hydromedit. Conference

APHA (1992) American Public Health Association Standard methods for the examination of water and wastewater, 18th edn., American Public Health Association, American Water works Association, Water Environment Federation. 949 p

Arakawa, K.Y. (1990) Natural spat collecting in the Pacific oyster *Crassostrea gigas* (Thunberg.). Mar. Behav. Physiol. 17: 95-128

Balachandran, K.K. and Surendran, P.K. (1984) Studies on depuration of live clams (*Villoritta* sp.). Fish. Technol. 21: 65-69

BAM (2011) Bacteriological Analytical Manual. Chapter 4A, Diarrheagenic *Escherichia coli*. http://www.fda.gov/Food/FoodScienceResearch/Laboratory Methods/ucm070080.htm

Buisson, D.H., Fletcher, G.C., Begg, C.W. (1981) Bacterial depuration of the Pacific oyster (*Crassostrea gigas*) in New Zealand. New. Zeal. J. Sci. 24: 253-262

Casali, P., Marcucci, M.C., Canzonier. W.J. (1981) Depuration of the clam, *Venus gallilla*, in a commercial plant. L'Igiene Moderna 75: 669-692

Chinnadurai, S., Mohamed, K. S., Venkatesan, V., Sharma, J., Kripa, V. (2014) Depuration of bacterial populations in the Indian backwater oyster *Crassostrea madrasensis* (Preston, 1916): Effects on surface and bottom held oysters. J. Shellfish Res. 33(2): 409-414

Costa, R.A. (2013) *Escherichia coli* in seafood: A brief overview. Adv. Biosci. Biotechnol. 4: 450-454

EC (European Commission) (2004) Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules

for the organisation of official controls on products of animal origin intended for human consumption. Off. J. Eur. Communities, L 226, 25.6.04: 83-127

Hackney, C.R. and Pierson, M.D. (1994) Environmental Indicators and Shellfish Safety. Chapman and Hall, New York, 523 p

Hitchin, A.D., Feng, P., Watkins, W.D., Rippey, S.R., Chandler, L.A., Arlington, A. (1995) *Escherichia coli* and coliform bacteria. In: Food and drug administration bacteriological analytical manual (WATKINS, W. D., Ed) , 8th edn., pp 401-429

Love, D.C., Lovelace, G.L., Sobsey, M.D. (2010) Removal of *Escherichia coli*, *Enterococcus fecalis*, coliphage MS2, poliovirus, and hepatitis A virus from oysters (*Crassostrea virginica*) and hard-shell clams (*Mercinaria mercinaria*) by depuration. Int. J. Food Microbiol. 143: 211-217

Maffei, M., Vernocchi, P., Lanciotti, R., Guerzoni, M. E., Belletti, N., Gardini, F. (2009) Depuration of stripped Venus clam (*Chamelea gallina* L.): effects on microorganisms, sand content and mortality. J. Food Sci. 74: 1-7

Marceddu, M., Lamon, S., Simonetta, G., Consolati, Ciulli, S., Mazza, R., Mureddu, A., Meloni, D. (2017) Determination of *Salmonella* spp., *E. coli* VTEC, *Vibrio* spp., and Norovirus GI-GII in Bivalve Molluscs Collected from Growing Natural Beds in Sardinia (Italy). ID Foods, 6: 88

Nambudiri, D.D., Singh, I.S.B., George, S., Sherief, P.M.M.C. (1995) Design, fabrication and standardisation of a depuration system for bivalves. Fish. Technol. 32(2): 126-130

NSSP (National Shellfish Sanitation Program) (2015) Guide for the Control of Molluscan Shellfish 2015 Revision. US Food and Drug Administration. Retrieved from http://www.fda.gov/Food/Guidance Regulation/ Federal State Food Programs /ucm2006754.htm

Obadai, E.A., Nyarko, H.D., Amponsah, S.L. (2010) Effect of depuration on microbial content of mangrove oyster (*Crassostrea tulipa*) from Benya lagoon, Ghana. Ethiopian J. Environ. Stud. Manage. 3: 47-53

Odeyemi, O.A. (2016) Incidence and prevalence of *Vibrio parahaemolyticus* in seafood/ : a systematic review and meta-analysis. Springer Plus. 5: 464

Oliveira, J., Cunha, A., Castilho, F., Romalde, J.L., Pereira, M.J. (2011) Microbial contaminants and purification of bivalve shellfish: crucial aspects in monitoring and future perspectives: a mini-review. Food Cont. 22: 805-816

Pavoni, E., Consoli, M., Suffredini, E., Arcangeli, G., Serracca, L., Battistini, R., et al. (2013) Noroviruses in seafood: a 9-year monitoring in Italy. Foodborne Pathog. Dis. 10(6): 533-539

Phuvasate, S., Chen, M., Su, Y. (2012). Reduction of *Vibrio parahaemolyticus* in Pacific oyster (Crassostrea gigas) by depuration at various temperatures. Food Microbiol. 3: 51-56

Pillai, K.V. and Selvan, K. (1987) Study on bacterial quality on edible oyster *Crassostrea madrasensis* and its purification. Bull. Cent. Mar. Fish. Res. Inst. 42: 426-431

Power U.F. and Collins, J.K. (1989) Differential depuration of poliovirus, *Escherichia coli*, and a coliphage by the common mussel, *Mytilus edulis*. Appl. Environ. Microbiol. 55: 1386-1390

Rees, G., Pond, K., Kay, D., Bartram, J., Domingo S.J. (2010) Safe management of shellfish and harvest waters World Health Organization (WHO). IWA Publishing, London, UK

Sorio, J.C. and Peralta, J.P. (2017) Evaluation of a Small Scale UV-treated recirculating depuration system for oysters (*Crassostrea iredalei*). AJFST 5(4): 117-124