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Depuration of a freshwater clam (*Batissa violacea*) from Rewa River in Fiji using a bio-filter set-up in closed and open water circulatory system

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Abstract

The effectiveness of the freshwater bivalve *Batissa violacea* depuration was tested in closed and open water circulatory system over a 48 h period. The closed circulatory system included a sand biofilter. Microbial levels were assessed every 4 h using Total Aerobic Plate Count (TPC) for heterotrophs and Most Probable Number (MPN) for coliforms. TPC and coliform loads in bivalve tissue reduced rapidly to low and undetectable levels in a closed circulatory system while open system showed a slower reduction. Both TPC and coliform loads remained above detectable levels throughout the depuration period. Closed system showed similar patterns of logarithmic reduction of TPC and coliforms in all cases with R^2>0.95 and p<0.001. Similar results were observed for tank water however, reduction of TPC and coliforms were slower. Biofilm formation was observed in the interior walls of the aquarium tanks over 48 h in all cases. Physicochemical parameters did not show any significant change. The reduction in TPC and coliform load in *B. violacea* suggests that biofilter in a closed water circulatory system is a simple, cost-effective, water conserving and effective way to significantly reduce the spoilage and coliform bacterial load that is accumulated in the clams.

Introduction

Pacific Island Countries (PICs) have a large proportion of populations residing near natural water bodies including the coastal and river systems (Andrew *et al*. 2019). These populations generally have heavy socio-economic and subsistence dependence on these coastal resources from these sources including Fiji (Waqalevu 2015; Andrew *et al*. 2019). Bivalves are known as a rich source of protein and are highly perishable in untreated form which hinders the process of transport and storage (Frazier and WestHoff 2000; Udoh *et al*. 2017).

Shellfish-borne bacterial and viral diseases are one of the major concerns for public health (Botana 2014). Bivalves accumulate heavy metal toxins and various pathogens from fecally contaminated natural water during their filter feeding behaviour (Hatha *et al*. 2005a; Botana 2014; Waqalevu 2015). Therefore, inappropriate disposal of raw and partially treated sewage into the natural water

bodies is a major factor contributing to the increasing incidence of shellfish-borne illness (Hatha *et al*. 2005a). *Escherichia coli* is considered as a common fecal indicator bacterium (coliform) which is used to measure microbial contamination of bivalve molluscs (Murchie *et al*. 2005) and is directly related to the degree of anthropogenic impact. Since specific pathogen detection is always time-consuming and costly, *E. coli* is used as a surrogate organism, the numbers of which provide an estimate of degree of pollution and possible presence of pathogens that it contains (Oliveira *et al*. 2011).

Batissa violacea or Kai, as known locally, is a freshwater bivalve that serves as a popular food item in Fiji (Richards *et al*. 1994). Fishermen around Rewa River in Fiji catch bivalves (Waqalevu 2015) for subsistence consumption or to sell it in supermarkets (frozen and without shells) and in live condition in local market and roadside stalls. During sale, the bivalves are normally stored at ambient temperature that is conducive for proliferation of pathogenic and mesophilic bacteria (Hatha *et al*. 2005a; Waqalevu 2015). They are generally less processed and are mostly known to be consumed raw (Oliveira *et al*. 2011). *B. violacea* is a filter feeder targeting suspended matter including sediments, detritus, diatoms and algae (Mayor *et al*. 2018). Filter feeding exposes the bivalve to high levels of varied bacterial flora including pathogenic and non-pathogenic bacteria (Hatha *et al*. 2005a; Waqalevu 2015; Oranusi *et al*. 2018).

Depuration is a technique used worldwide within the shellfish industry. Depuration of bivalves typically takes place for 48 h and has proven to reduce the number of bacterial load in shellfish significantly (Amadi 2016; Waqalevu 2015). During the depuration, shellfish continue to filter feed in clean running water, and previously ingested microorganisms are eventually expelled and entrapped in a thread like faeces coated with layer of mucous. When bivalve molluscs are held in clean water in tanks for several hours it maximizes the expulsion of intestinal contents. Waqalevu (2015) used a UV filter system for depuration of *B. violacea* from Rewa River of Fiji over a three-day period. Reduction of coliform numbers were observed however, coliforms still showed significant presence in the bivalve and tank water even after 72 h of depuration. Also, the depuration system was water intensive and not as economical for local use. A more efficient and economical depuration system is required for *B. violacea.* Hatha *et al*. (2005a) investigated the gut contents of *B. violacea* from Rewa River and found a varied flora of spoilage and pathogenic bacterial load. A suitable technique is required to reduce bacterial load from the bivalves to make it safe for consumption. Both Waqalevu (2015) and Hatha *et al*. (2005a) recommended an effective and economical depuration system for *B. violacea* in Fiji. An economical depuration system based on recirculating water through a biofilter could greatly conserve water and achieve depuration of the bivalve clams to safe levels of bacterial load for human consumption.

This study was aimed at: (1) determining the effect of depuration on total aerobic bacteria and coliform levels in *B. violacea* tissue; (2) comparing the effectiveness of closed water circulatory system having a biofilter set-up with an open water system having continuous flow of water on total aerobic bacteria and coliform load in *B. violacea* tissue over a 48 h depuration period.

Experimental

Sample collection and site

Bivalve samples were collected from a sandy freshwater river ecosystem called Rewa River, located in the south-eastern region of the island of Viti Levu in Fiji (178°31'14.3"E and 178°31'14.5"E longitude; 17°59'12.2"S and 18°00'51.0"S latitude) (Fig. 1). Samples were hand collected by divers from depths of 1 to 3 m using polythene bags.

A water sample was taken at the site by dipping a sterile airtight glass bottle about 1 m below the surface and holding it faced opposite the current flow for 1 min. This sample was taken back to the lab for pH, salinity, dissolved oxygen (DO) and bacteriological analysis. Water temperature was taken of the river at the collection site using alcohol bulb thermometer (Thomas Scientific 1173H09). Sediment samples were taken aseptically by scooping out from the river bottom into sterile polythene bags. More than 400 live clams were

collected by scooping them with hand from the sediment at the bottom of the river and placed in sterile polythene bags. A sample of two clams was immediately taken in sterile polyethylene bag and

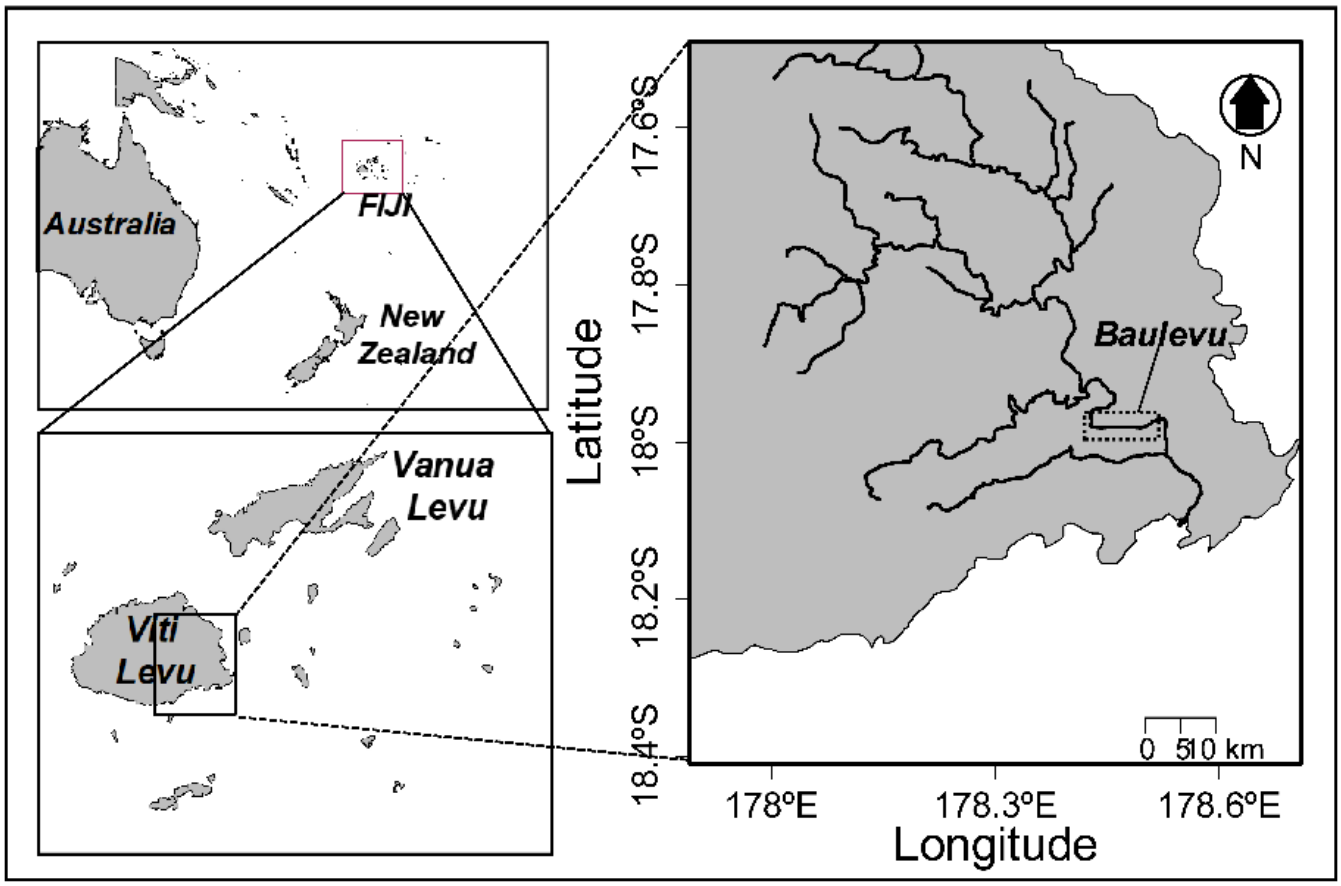

Fig. 1. Location of the sample collection site in the Southeastern region of the island of Viti Levu in Fiji. Rewa River watershed is shown. Sample collection area within Rewa River (Baulevu) is marked in black box (dotted line). Inset shows Fiji and its location within the South Pacific region.

transferred into a cooler with ice for total aerobic plate count (TPC) and coliform count. The time interval for transporting of Kai from site to depuration tanks was approximately 30 min. More than 400 clams were transported to the laboratory in live condition maintained at around 20 ºC in a sterile container. Upon reaching the lab the clams were transferred into two aquarium tanks (made of silica glass) in equal numbers (200 bivalves each). The time between collection of samples and transferring the bivalves in the aquarium tanks was approximately 1 h. The first tank was maintained with closed water circulatory system with a biofilter set-up while the second tank was maintained under open water system without a biofilter. Both set-ups are shown in Fig. 2.

Experimental set-up and sampling

Bivalves were placed in the tanks on a mesh platform below 20 cm depth from the water surface and at least 20 cm from the bottom surface (Fig. 2). After placing the bivalves in each aquarium tank, two bivalves were removed and placed in a homogenizing sterile bag. This was noted as sampling at zero h. Tap water (chlorinated) was used for the depuration tanks. The temperature of water in the aquarium tank was taken before placing the clams inside the aquarium tank. Tank water and swab sample of the aquarium tank were taken. 10 mL of tank water was taken from below the water surface aseptically in sterile vials. A water sample was also taken prior to introduction of bivalves in the tanks. For swab samples, a sterile cotton swab was taken and dipped in a tube of

sterile distilled water (SDW). Excess water was driven out by pressing against the walls of the tube. The inside walls of the aquarium tank were swabbed in a square of ~5 cm × 5 cm (25 cm^2). The tip of the swab was then cut off in a 10 mL dilution blank of SDW. Bivalve, water, swab samples and temperature were taken at every 4 h interval for up to 48 h. pH, salinity and, DO were also measured every 4 h. Water, bivalve and swab samples were analyzed for TPC and coliform load.

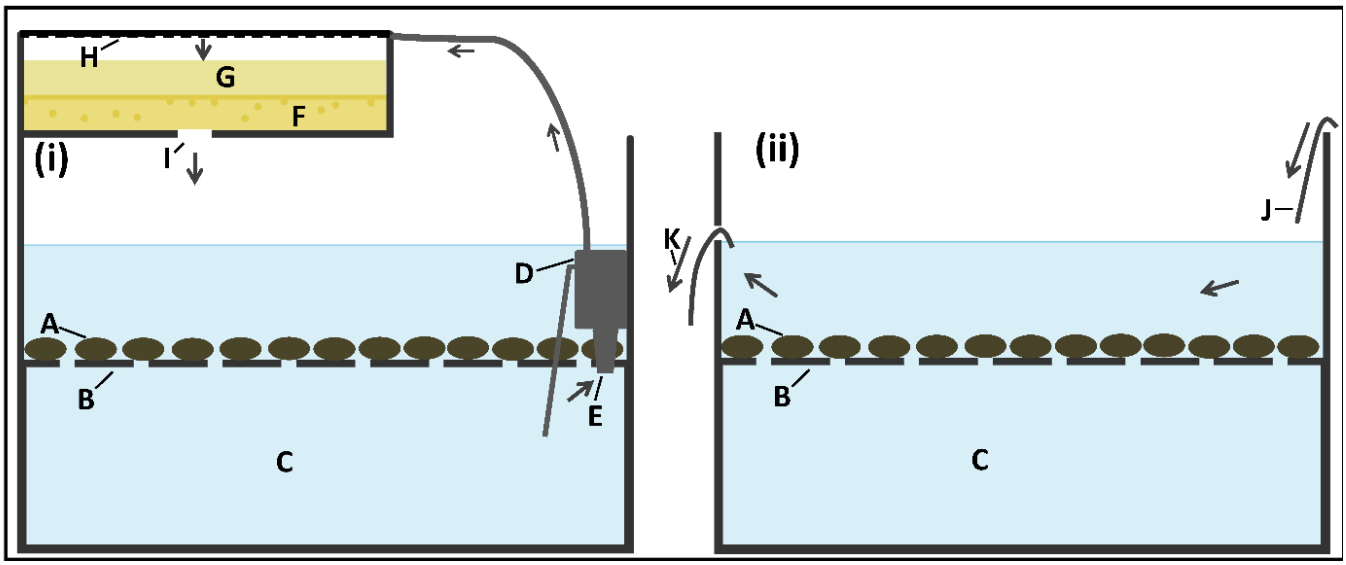

Fig. 2. Experimental Set-up, (**i**) Closed water circulatory system; (**ii**) Open water system; (**A**) bivalves; (**B**) wire mesh; (**C**) tap water; (**D**) water pump; (**E**) water inlet; (**F**) sponge; (**G**) sediment (sand) layer; (**H**) perforated pipe; (**I**) water outlet back in the tank; (**J**) water (tap water) inlet source; (**K**) water outlet leading out of the tank. Arrows show water pathway.

Bacteriological analysis

Bivalve shells were thoroughly washed with SDW. The shells were aseptically opened with a sterile scalpel and the meat was transferred to sterile homogenizing bags. The meat from two bivalves was weighed and diluted 10 fold accordingly with SDW. For example, if the weight of meat from two bivalves was 10 g, 90 mL of sterile distilled water was poured into the bag. The meat was homogenized using a digester for 5 min. Each sample was serially diluted up to 10^{-5} using 9 mL SDW blanks. For tank and swab samples, 1ml of the water was taken and serially diluted up to 10^{-5} using 9 mL SDW blanks. 1mL and 0.1 mL of each dilution was plated using pour plate technique (Clark 1967) in standard plate count agar and incubated at 37 ºC for 48 h. For each sample, three replicates were done. After incubation, the colonies were counted and the TPC load of the undiluted sample was calculated. TPC load was expressed as colony forming units per gram of sample ($cfu.g^{-1}$) for bivalve samples and colony forming units per ml of sample ($cfu.mL^{-1}$) for tank water and swab samples.

For coliform analysis, 10^{-2} dilution of each sample was used. Standard most probable number (MPN) method using 3 tube dilution (West 1989) was used for coliform enumeration. 1 mL of the 10^{-2} dilution was pipetted out into 99 ml of SDW giving a 10^{-4} dilution. 3 × 10 mL volumes of this dilution were transferred into 3 tubes of 10 mL sterile double strength lactose broth. 3 × 1 mL and 3 × 0.1 mL volumes of the samples were transferred to 10 mL sterile single strength lactose broth. Each tube contained an inverted Durham's tube. The tubes were incubated at 37 ºC for 24 – 48 h. The inverted Durham's tubes were checked for gas production. The numbers of positive tubes were recorded in each sample and referred to MPN table to find out the MPN index of coliform load.

Physicochemical analysis of water

Temperature, salinity, DO and the pH measurements were taken at every 4 h interval for 48 h. pH and temperature were measured using

digital pH electrode with temperature sensor (pH meter, Hanna Instruments HI2002), salinity was measured using digital conductivity probe (EC meter, Hanna Instruments HI2030), DO was measured using digital polarographic dissolved oxygen probe (DO meter, Hanna Instruments HI2040). All measurements were performed according to the manufacturer's instructions manual.

The whole experimental set-up and procedure was repeated three times (three trials) from samples collection to physicochemical analysis of water and results were presented as mean of multiple replicates within each trial.

Statistical analysis

Least squares regression models with polynomial functions were used to determine the trends of bacterial load reduction patterns. Models at $p<0.05$ were used to develop suitable trend reproduction models. Models were subjected to t-tests, F-tests, and selections for best fit models were made using Akaike Information Criterion (AIC) (Akaike 1981), R^2 and p-values. ANOVA was used for comparison of bacterial load and load reduction patterns. For this study all statistical analysis was performed using the "R" software, version 3.6.1 (The R Core Development Team 2019).

Results

Bacteriological analysis

Text Bacteriological analysis of water and sediment sample of the site showed high level of TPC (cfu.ml^{-1}) and coliform load (MPN.mL^{-1}) for each of the three experimental trials (Fig. 3). TPC and coliform loads were much higher for trial 3 compared to trials 1 and 2. Also, TPC and coliform load of sediment was significantly higher than water from the site for all three trials ($p<0.001$). Other parameters of sampling site are shown in Table 1.

Analysis of the bivalves showed high initial loads detectable levels and slightly above FAO standard requirement of <300 cfu.100 g^{-1} (Lee *et al*. 2008) for all three trials throughout the test.

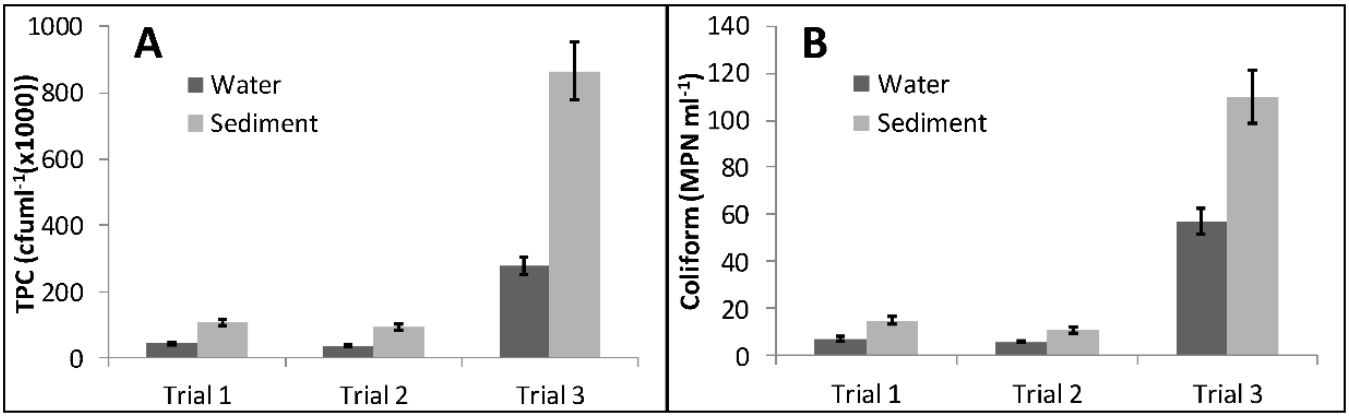

Fig. 3. Total Plate Count Bacteria (TPC) (cfu.mL^{-1}) and coliform load (MPN.mL^{-1}) of water and sediment samples obtained from the sampling site in Rewa River, Rewa, Fiji. The graphs show (**A**) TPC and (**B**) coliform load for three experiment trials (Trial 1, Trial 2, Trial 3) from the site of bivalve sample collections. Each bar is shown as mean ± SE of three replicates.

of TPC and coliform at 0 h (Fig. 4) for each of the three trials. For closed circulatory system, both TPC and coliform loads reduced rapidly to low and undetectable levels within 16 h which persisted for up to the 48 h mark. In all closed system trials fecal coliform load reached below FAO standard requirement of <300 cfu.100 g^{-1} (Lee *et al*. 2008). Open system showed slower reduction of both TPC and coliform loads. Bacterial loads remained above

Both, the TPC and coliform load reduction trends for closed system were significantly different ($p<0.05$) from open system in all cases. All three trials for closed system showed similar patterns of growth reduction. In all three cases, the growth reduction of TPC and coliforms in the bivalve and tank water can be determined using the equations as outlined below with $R^2>0.95$ and $p<0.001$. The growth reduction trend of TPC in *B. violacea* over

the depuration period for closed system follows the general trend represented by Eq. 1:

$$T_t = a_{t(0)} \times e^{-0.259 \times t} + \varepsilon \quad (1)$$

where *T* is the population of TPC in the bivalve, *t* is the depuration time, *a* is the TPC population at depuration time *t* of zero (0) h and ε is an undefined error term.

The growth reduction trend of coliforms in *B. violacea* over the depuration period for closed system follows the general trend represented by Eq. 2:

$$C_t = b_{t(0)} \times e^{-0.228 \times t} + \varepsilon \quad (2)$$

where *C* is the population of coliforms in the bivalve and *b* is the coliform population at depuration time *t* of zero (0) h.

Table 1. Physical and bacteriological parameters of the sampling site in Rewa River. Values are shown as mean ± SE of 5 replicates.

Parameter	Value
Water Temperature [°C]	27.40 ± 1.42
pH	6.94 ± 0.24
Salinity [ppt]	0.03 ± 0.01
Dissolved oxygen [mg.L^{-1}]	7.06 ± 0.04
Turbidity [m]	1.24 ± 0.34
Total Plate Count [cfu.mL^{-1}] – Bivalve	$9.95 \times 10^6 \pm 9.81 \times 10^2$
Coliform load [MPN.mL^{-1}] – Bivalve	904.34 ± 18.5

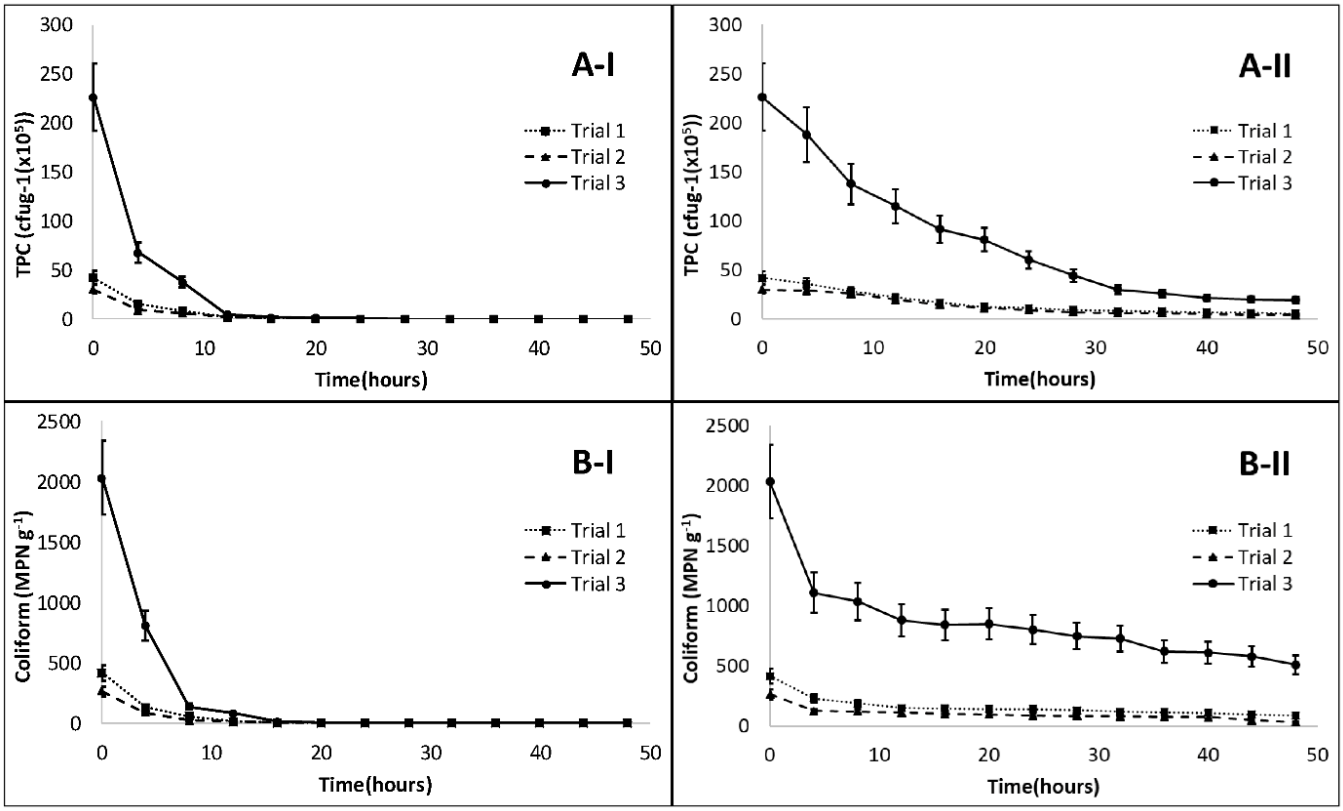

Fig. 4. Variation in bacterial load of bivalve (*Batissa violacea*) over a 48 h depuration period for three different trials. (**A**) Total Plate Count Bacteria (TPC) (cfu.g^{-1}); (**B**) coliform load (MPN.mL^{-1}). (**I**) closed water circulatory system; (**II**) open water system. Each point is shown as mean ± SE of three replicates.

Prior to introduction of bivalves, aquarium tank water samples showed negligible TPC and undetectable coliform load for all three trials (Fig. 5). Water samples taken 4 h after introduction of bivalves showed high level of both TPC and coliform load. This could have been due to the release of microbes in mucous threads into the water by the clams. For closed circulatory system,

TPC reduced to very low levels within 28 h while coliforms reduced to undetectable levels in 24 h. The microbial populations remained at these levels up to the 48 h period. For the open system, TPC continued to increase for up to 20 h followed by reduction in number beyond this point. Coliforms increased in number from 4 to 8 h followed by reduction. TPC remained above detectable levels at 48 h. Coliforms reduced significantly but remained above detectable levels for trial 3 and reached undetectable levels for trials 1 and 2. The difference may be due to the initial higher coliform load for trial 3. For closed circulatory system, the population reduction pattern for TPC and coliforms were similar for all three trials. The growth reduction trend of TPC in tank water for closed system over the depuration period from the 4 h mark follows the general trend represented by Eq. 3:

$$W_t = d_{t(4)} \times e^{-0.143 \times t} + \varepsilon \quad (3)$$

where W is the population of TPC in tank water and d is the TPC population at depuration time t of zero (4) h. The growth reduction trend of coliforms in tank water for closed system over the depuration period from the 4 h mark follows the general trend represented by Eq. 4:

$$F_t = g_{t(4)} \times e^{-0.848 \times t} + \varepsilon \quad (4)$$

where F is the population of coliforms in tank water and g is the coliform population at depuration time t of zero (0) h.

Table 2 shows statistical information for the fitness of Eqs. 1 – 4 with actual values.

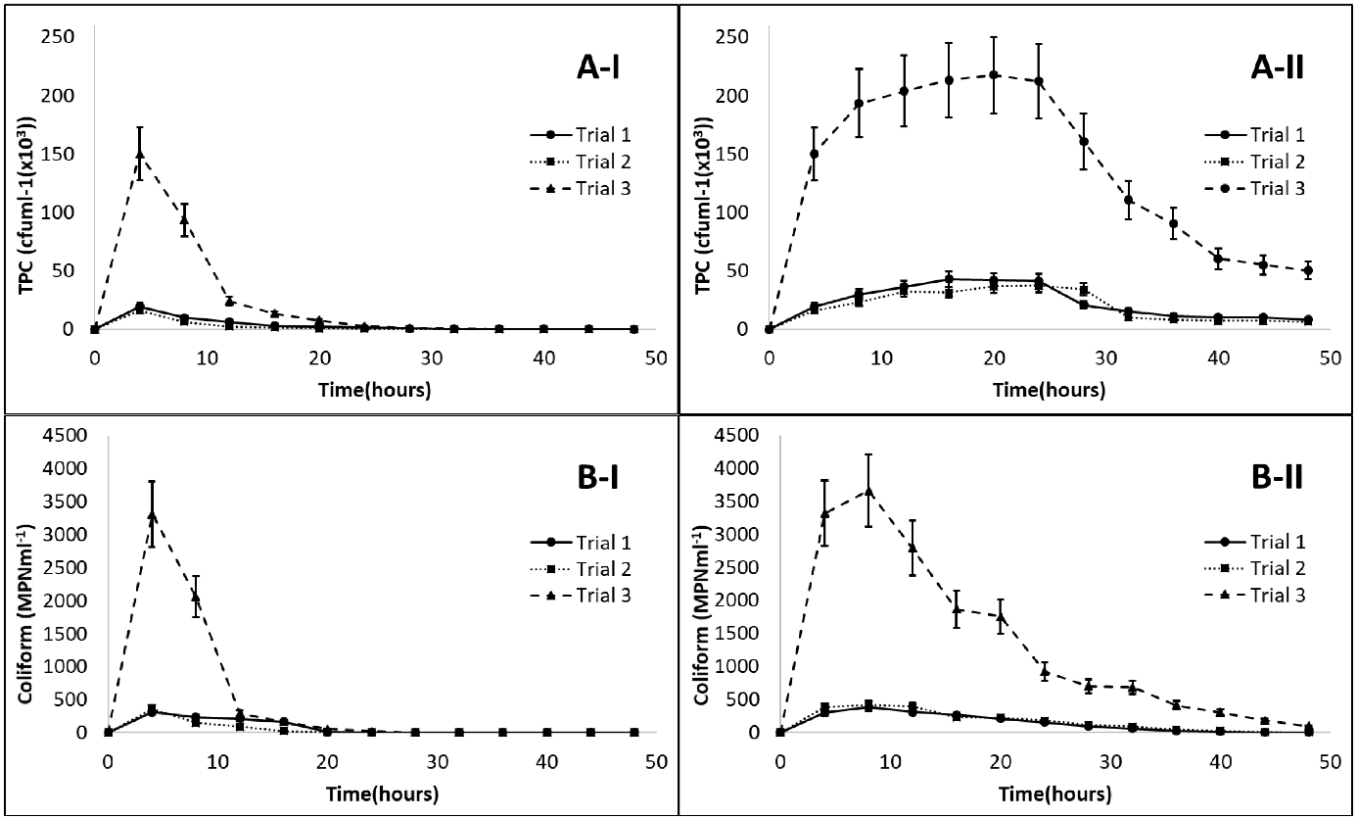

Fig. 5. Variation in bacterial load of tank water over a 48 h bivalve depuration period for three different trials. (**A**) Total Plate Count Bacteria (TPC) (cfu.mL^{-1}); (**B**) coliform load (MPN.mL^{-1}). (**I**) closed water circulatory system; (**II**) open water system. Each point is shown as mean ± SE of three replicates.

Bacteriological analysis of the swab samples showed that at zero h there was undetectable TPC and coliform count for all three trials. As shown in Fig. 6, both the TPC and coliform load increased through progression of the depuration period in all cases. TPC and coliform loads reached higher values for open system compared to closed circulatory system. These results confirmed the formation of biofilm in the interior tank walls of the glass aquarium over the 48 h depuration period.

Table 2. Statistical parameters for Equations fitness to the trajectories for different experimental trials.

Equation fit		R^2	*p*-value	DF	t-value	F statistic	AIC
Equation 1	trial 1	0.977	1.10×10^{-09}	10	21.41	458.30	30.57
	trial 2	0.957	5.20×10^{-07}	10	11.28	127.30	32.55
	trial 3	0.960	1.69×10^{-08}	10	16.17	261.50	72.84
Equation 2	trial 1	0.987	5.51×10^{-11}	10	29.02	842.10	73.85
	trial 2	0.975	1.46×10^{-09}	10	20.81	433.10	70.09
	trial 3	0.954	8.35×10^{-08}	10	13.70	187.60	133.92
Equation 3	trial 1	0.996	1.17×10^{-13}	10	53.90	2905.00	13.02
	trial 2	0.951	4.28×10^{-08}	10	14.69	215.70	38.91
	trial 3	0.968	5.18×10^{-09}	10	18.27	333.80	89.28
Equation 4	trial 1	0.974	4.13×10^{-10}	10	27.38	687.30	71.53
	trial 2	0.964	4.36×10^{-09}	10	24.28	383.40	65.23
	trial 3	0.967	8.35×10^{-09}	10	25.70	407.36	67.25

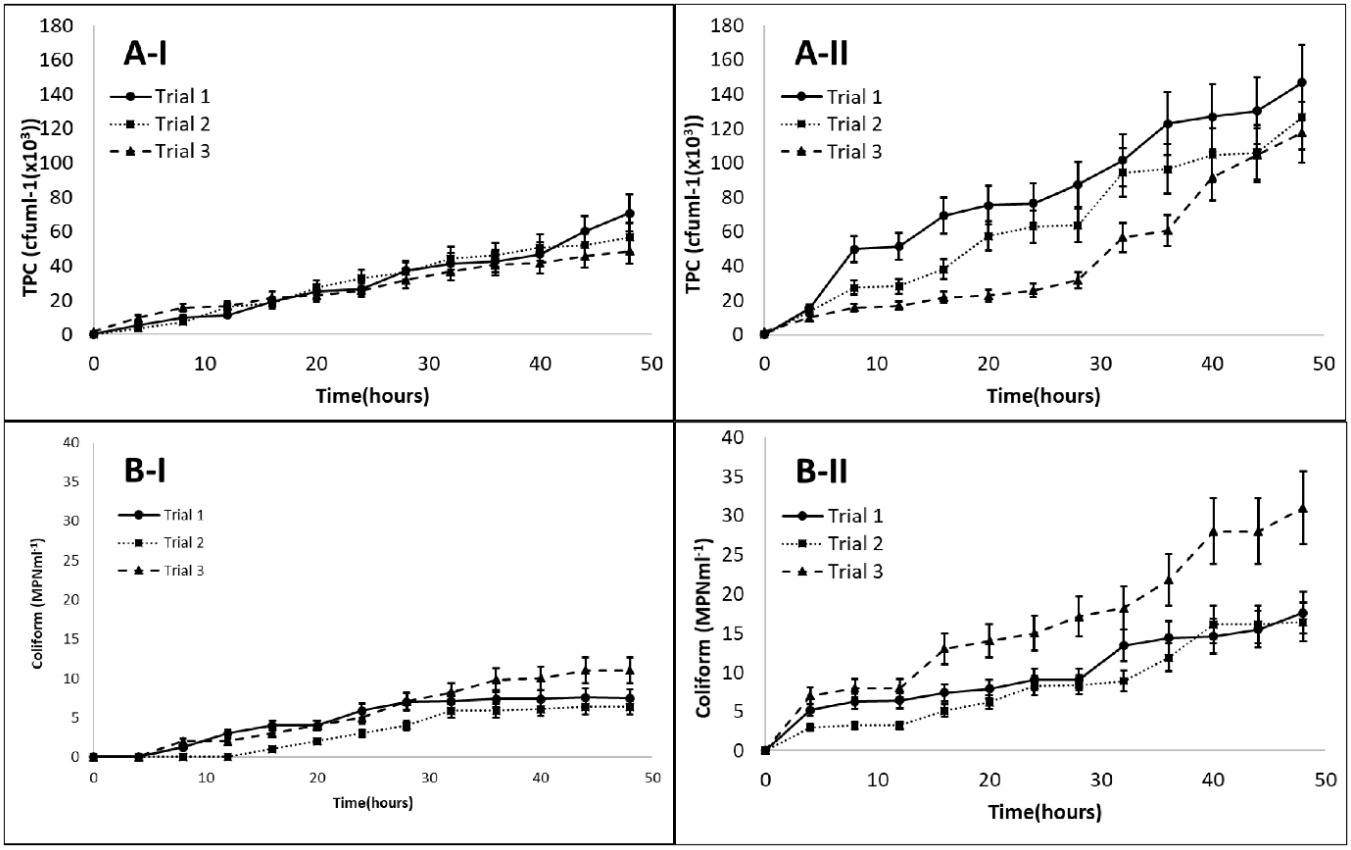

Fig. 6. Variation in bacterial load of the biofilm developed on tank interior wall over a 48 h bivalve depuration period for three different trials. (**A**) Total Plate Count Bacteria (TPC) (cfu.mL^{-1}); (**B**) coliform load (MPN.mL^{-1}). (**I**) closed water circulatory system; (**II**) open water system. Each point is shown as mean ± SE of three replicates.

The trajectory of physiochemical parameters of aquarium tank water for the three experimental trials over the depuration period of 48 h is shown in Fig. 7. Temperature varied within a difference of 1 – 3 ^{o}C but remained relatively constant. For the open system, a slight dip in temperature was observed between 20 and 36 h range. This may be due to the cooling of the sourced tap water at late night and morning hours. Dissolved oxygen remained fairly constant over the depuration period except for a dip at the 8 h mark for closed circulatory system. This was observed for all three experimental trials. This may be due to the use of oxygen by high microbe load upon first

introduction followed by recovery which may have been due to the mixing of oxygen from dripping water from the pump and tap. The pH value did not show any significant change and salinity remained at 0 throughout the experiment.

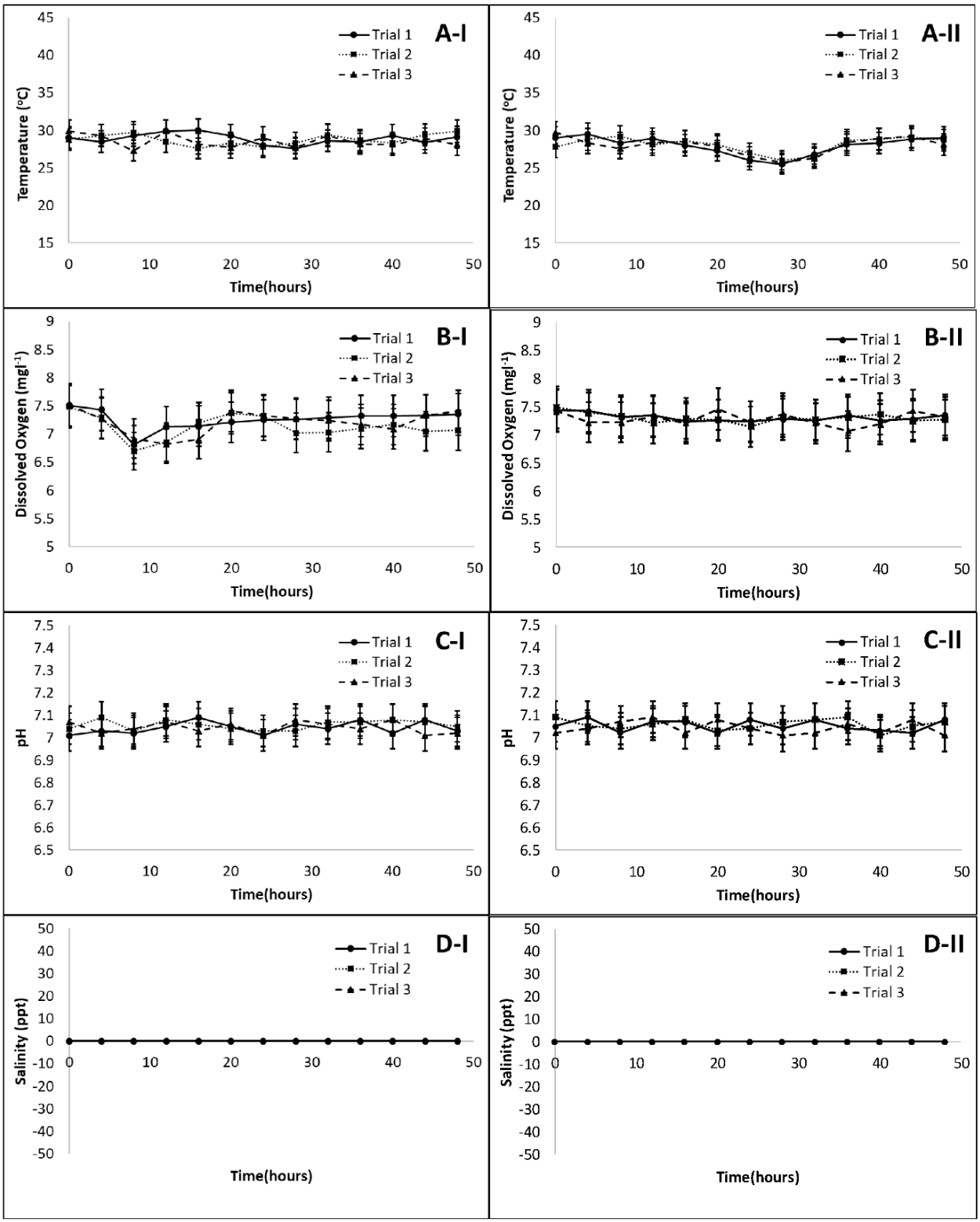

Fig. 7. Variation in physicochemical properties of aquarium tank water over the 48 h depuration period for three different trials. (**A**) temperature (°C); (**B**) dissolved oxygen (mg.L^{-1}); (**C**) pH; (**D**) salinity (ppt). (**I**) closed water circulatory system; (**II**) open water circulatory system. Each point is shown as mean ± SE of three replicates.

Discussion

B. violacea is a filter feeder exposed to high bacterial load in the aquatic environment (Mayor *et al.* 2018). As such, high bacterial load is expected in the gut contents of *B. violacea* as shown by Baker (2016). Elevated levels of pathogens and spoilage bacteria in bivalves pose a health hazard for consumers. While spoilage bacteria do not necessarily pose a direct health risk, they increase the rate of food spoilage which can affect consumer health. TPC of *B. violacea* from Rewa River has been shown to contain bacterial community from predominantly Micrococcus and Bacillus genera, as well as *Aeromonas*, *Pseudomonas*, *Vibrio*, *Acinetobacter*, *Streptococcus* and *Alcaligenes* (Hatha *et al*. 2005a). *Aeromonas* and *Vibrio* contain potential pathogen species of importance in seafood (Hatha *et al*. 2005b; Vivekanandan *et al*. 2005). Depuration of bivalves is necessary to ensure that bacterial load in *B. violacea* reaches low and acceptable levels for consumption (Hatha *et al*. 2005a; Lee *et al*. 2008; Waqalevu 2015). This work was aimed at determining the effectiveness of depuration in closed and open water systems for reduction bacterial load in *B. violacea* to safe levels for consumption. The set-up was aimed to be both economical and effective to be feasible at both industrial and small scale levels.

The results showed that using a sand biofilter in closed water circulatory system was highly effective in reducing TPC and coliform load in *B. violacea* to undetectable levels within 16 h of depuration. The biofilter seemed to have trapped the microbes over time. This system proved to be much better in comparison to the open circulatory system where TPC and coliform loads reduced at much slower rate and remained above detectable levels throughout the depuration period. Similar results were observed in the Dore and Lees (1995) where open system of depuration took much longer to reduce TPC and coliform loads to acceptable levels in comparison to closed water system for oysters (*Crassostrea gigas*) and mussels (*Mytilus edulis*).

B. violacea with high initial bacterial load had a much slower reduction trend in open water depuration system. For closed circulatory system, initial bacterial load in *B. violacea* had little impact on bacterial reduction trend in contrast to open water system. Previous works have shown depuration of bivalves to be effective in reducing TPC and coliform loads to undetectable and safe levels for human consumption (Schweimanns and Felbeck 1985; Dore and Lees 1995; Sreenath and Hatha 2005). Waqalevu (2015) used a closed system of *B. violacea* depuration with a UV filter system in a 5,000 L tank. Although reduction of coliforms was observed in the bivalves, significant numbers were still present even at 72 h of depuration. Also, coliform load in tank water initially reduced followed by an increase over the depuration period. The depuration set up was also highly water intensive and costlier than the simple closed water depuration system designed in this study.

For closed water circulatory system, the growth reduction of TPC and coliforms in bivalve and aquarium water follows an exponential reduction trend in all cases as represented by Eqs. 1 – 4. Eqs. 1 and 2 can be used to determine the depuration period for reduction TPC and coliform load in *B. violacea* to safe levels for human consumption and/or in food industries and market areas where the bivalve is sold or packaged fresh. The initial bacterial load needs to be known for the equations. Eqs. 3 and 4 can be used to estimate when the aquarium water will have low bacterial load and is safe for release into the environment.

Significant loads of coliforms were present in the bivalves as well as site water and sediment samples in all cases. This indicates organic pollution from household sources including faecal pollution (Martinez and Oliveira 2010) in the shellfish growing waters. Waqalevu (2015) showed the presence of *E. coli* and *Enterobacter* in Rewa River water, indicating anthropogenic fecal pollution. Bivalves tend to bioaccumulate pathogenic microorganisms within their tissues over time (Martinez and Oliveira 2010). While there are stringent standards in developed countries for shellfish growing waters, such regulatory controls are non-existent/not followed in many of the developing countries. Large number of fishermen harvest shellfish from the rich shellfish beds of natural waters and sell it for human consumption without proper depuration. Development of a simple, cost-effective and water conserving

depuration system would be of great use to local fishermen and even for domestic use. In this study depuration using a biofilter in a closed water circulatory system was effective in reducing coliform and TPC load in the tissues of *B. violacea* to low and safe levels in a short period of time. The reduction in the TPC and coliform load in the aquarium tank water with biofilter probably had been due to the biofilter system. When bacteria and faecal waste laden mucus from shellfish passed through white sand in the biofilter, they could have been trapped while allowing water to pass through back into the tank.

Formation of biofilm on the interior tank walls was observed in all cases. Both TPC and coliform levels increased in approximately a linear pattern over the depuration period. Biofilms formation is a survival strategy for microorganisms (Prakash *et al*. 2003; Johnson 2008; Kumar *et al*. 2017). The aquarium walls were made of silica glass, which provides a suitable substrate for microorganisms which would likely be the reason for biofilm formation. However, the biofilm did not affect the bacterial load in the bivalve or tank water samples.

From a practical point of view, in an industrial set-up, open system has an advantage over closed system. In closed system, the sand in the biofilter needs to be replaced and the tank walls cleaned between each batch. In open system only tank walls need to be cleaned between batches. This saves some time. However, the overall advantage is higher for closed system in terms of the economic cost and conservation of water. The closed water circulatory system with biofilter assembly conserves a lot of water as it does not have additional water input or output. It uses a fixed amount of water which circulates with assistance of a water pump. Water being the most precious resource, there is a pressing need to conserve the same in all the systems that use water. Closed system uses sand as a biofilter where the mucus laden with microorganisms get trapped. This can be later treated to remove the bacterial load. This is another advantage over the open water system where there is a continuous flow of water and microorganisms released by the bivalves get released into the environment. The collection and treatment of this large volume of water will not be cost effective or feasible. The limitation of the closed water circulatory system with biofilter set-up is that it is ineffective in preventing biofilm formation. However, the flaking off of the biofilm associated bacteria will take much more duration that the required depuration time. Hence the reintroduction of the biofilm associated bacterial load does not pose a threat to the efficacy of closed water system evaluated in this study. With every depuration cycle, there is a need to clean the depuration tank as not doing so may affect depuration time and flaking off of the biofilm can cause recontamination of the bivalves.

Conclusion

The reduction in TPC and coliform load in freshwater clams (*B. violacea*) suggests that the biofilter in a closed water circulatory system is a simple, cheap and effective way to significantly reduce the bacterial load that is accumulated in the clams. It can be concluded that proper use of the closed water circulatory system can reduce the risk or bacterial related health problems from consumption of *B. violacea*. Aseptic methods and proper handling need to be used to prevent post-depuration contamination of the bivalves. Hatha *et al*. (2005a) recommended usage of ice made from potable water for packing *B. violacea* to control post-harvest proliferation of pathogens and spoilage bacteria. Proper sanitation and cooking will also help in reducing the risks from pathogens. Depuration has negligible effect on the taste of *B. violacea* (Waqalevu 2015) and does not pose any threat of losing its taste popularity among consumers. Although depuration is also known to reduce metal toxicity from shellfish (Anacleto *et al*. 2015), this was not assessed in the current study. The two set-ups tested here have only been tested against bacterial loads. It should be noted that there are other forms of health risks from bivalve consumption such as heavy metal, toxins and viruses which have not been tested here. Further work is needed in these areas.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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