Once established, biofilms can harbour pathogenic and antibiotic-resistant bacteria, potentially turning depuration systems into sources of recontamination if not properly maintained (Azeredo et al., 2017). In aquaculture systems, such biofilms may contribute to water quality deterioration and increased microbial loads. Therefore, regular sanitisation of depuration tanks after each cycle is essential to maintain system efficiency and biosecurity (Canadian Food Inspection Agency (CFIA)., 2017).

The present study aimed to evaluate the effectiveness of a sponge filter-based closed-water depuration system in reducing bacterial contamination in black clams collected from the Varapuzha region of the Cochin Estuary. Changes in THB, TC, FC, and *Vibrio* spp. during depuration were monitored, and the microbial load on tank-wall biofilms was furthermore assessed to evaluate the potential of recontamination.

2 Results

2.1 Bacterial load in initial shellfish samples

The initial bacteriological load of *Villorita cyprinoides* (black clam) was determined prior to depuration. The bacterial parameters assessed included Total Coliforms (TC), Faecal Coliforms (FC), Total Heterotrophic Bacteria (THB), and *Vibrio* spp. (Table 1).

Table 1 Initial bacteriological load in raw shellfish samples

Bacteriological Parameter	Load
Total Heterotrophic Bacteria (THB)	1.68×10^7 CFU/g
Total Coliforms (TC)	2.4×10^4 MPN/100 g tissue
Faecal Coliforms (FC)	1.5×10^4 MPN/100 g tissue
Vibrio spp.	5.1×10^5 CFU/g

2.2 Bacteriological changes in clams during depuration

Bacterial load in the *Villorita cyprinoides* (black clam) samples at different depuration time intervals (0 h, 6 h, 12 h, 24 h, 48h and 72 h) was recorded for all parameters (Table 2).

Table 2 Bacteriological load in shellfish samples at different depuration time intervals

Sample No	Depuration time intervals (Hours)	Total Heterotrophic Bacteria load (CFU/g)	Total Coliforms load (MPN index/100mL)	Faecal Coliforms load (MPN index/100mL)	*Vibrio* spp. load (CFU/g)
1	0.00	1.68×10^7	2.4×10^4	1.5×10^4	5.1×10^5
2	6.00	1.29×10^7	1.5×10^4	1.1×10^4	4.4×10^5
3	12.00	9×10^6	1.1×10^4	4.6×10^3	3×10^5
4	24.00	1.54×10^6	4.6×10^3	2.1×10^3	1.5×10^5
5	48.00	1.03×10^6	3.5×10^2	2.3×10^2	1.2×10^5
6	72.00	1.22×10^6	3.8×10^2	2.3×10^2	1.4×10^5

2.2.1 Reduction in THB (Total Heterotrophic Bacteria) count in *Villorita cyprinoides*

The initial THB count was found to be 1.68×10^7 cfu/g. A 1.21 logs reduction was obtained within the first 48 hours of depuration to a THB count of 1.03×10^6 cfu/g. After 48 hours of depuration around 93.86% (1.21 logs) of THB reduction was observed (Table 2 and Figure 1). However, complete depuration of total heterotrophic bacteria (THB) was not achieved. Total Heterotrophic Bacteria showed a moderate but statistically significant reduction ($F = 18.6$, $p < 0.05$).

2.2.2 Reduction in Total Coliform (TC) Count in *Villorita cyprinoides*

The initial TC count was found to be 2.4×10^4 MPN/100 mL (Table 2). Reduction of 0.72 log was obtained within the 24 hours of depuration, and a further 1.84 logs (98.54%) reduction was observed after 48 hours of depuration to a final TC count of 2.54 logs (Figure 2). Thus, during the entire 72 h depuration process using a sponge filter, a total reduction of nearly 1.8 logs to a final count of 3.8×10^2 MPN/100 mL could be accomplished. However, complete depuration of TC could not be attained, even after 72 h of depuration. TC load of 2.57 logs remained in shellfish.