a progressive increase in microbial load on the tank walls over time, indicating the formation and accumulation of biofilm-associated bacteria during depuration.

Table 3 Microbial load in biofilm on depuration tank walls at different time intervals

Sample No	Depuration time intervals (Hours)	Total Heterotrophic Bacteria (CFU/g)	Total Coliforms load (MPN index/100mL)	Faecal Coliforms load (MPN index/100mL)	*Vibrio* spp. Load (CFU/g)
1	0.00	1.4×10^3	<3	<3	0
2	24.00	7.5×10^3	21	210	2.9×10^4
3	48.00	1.36×10^5	1100	>1100	2.48×10^5
4	72.00	2.83×10^5	>1100	>1100	2.85×10^6

3 Discussion

The high levels of faecal indicator bacteria detected in raw black clams collected from the Cochin Estuary indicate poor sanitary conditions in the harvesting environment and suggest a potential risk of enteric pathogen contamination. This is consistent with the filter-feeding habit of bivalves, which enables them to accumulate microorganisms from the surrounding water at levels often higher than those in the environment. Similar observations have been reported for shellfish harvested from polluted estuarine systems, where bacterial contamination has been associated with untreated sewage discharge, surface runoff, and other anthropogenic inputs. In the present study, the high initial counts of total coliforms, faecal coliforms, total heterotrophic bacteria, and *Vibrio* spp. confirm that clams collected from the study area may pose a food safety risk if consumed without adequate post-harvest treatment.

The results of the depuration trial demonstrated that the closed-water depuration system equipped with a sponge filter was effective in reducing bacterial contamination in *Villorita cyprinoides.* The most pronounced reduction occurred during the first 48 h of depuration, during which total coliforms, faecal coliforms, and total heterotrophic bacteria declined substantially. This pattern suggests that the early phase of depuration is the most efficient period for bacterial elimination under the present system conditions. Similar findings have been reported in previous studies, where the majority of bacterial reduction in bivalves occurred within the first 24~48 h of depuration. The marked decline observed in the present study indicates that the recirculating sponge filter system provided suitable conditions for effective microbial purging while maintaining clam survival.

Among the microbial indicators examined, faecal coliforms showed one of the highest rates of reduction and reached levels close to accepted regulatory limits after 48 h, further supporting the effectiveness of the system for improving the sanitary quality of the shellfish. In contrast, *Vibrio* spp. showed comparatively lower removal efficiency and persisted throughout the depuration period. This lower reduction may reflect the greater environmental resilience of *Vibrio* spp. and their ability to survive under depuration conditions more effectively than faecal indicator bacteria. The persistence of *Vibrio* spp. observed in this study is important from a food safety perspective, as it indicates that while the system is effective for reducing indicator bacteria, it may be less efficient in eliminating more robust or potentially pathogenic bacterial groups. Therefore, depuration alone may not always be sufficient to completely remove microbiological hazards from shellfish harvested from contaminated environments.

An additional practical finding of this study was the progressive increase in bacterial load on the inner walls of the depuration tank during the later stages of depuration. This trend suggests the formation of biofilms, which may act as reservoirs of microorganisms and contribute to secondary contamination of the system. The slight increase in bacterial counts observed after prolonged depuration may therefore be related to biofilm-associated recontamination. This finding highlights an important operational limitation of closed-water depuration systems: although they are simple, water-efficient, and suitable for small-scale use, their effectiveness depends strongly on proper cleaning and maintenance between depuration cycles. Overall, the present study demonstrates that a sponge filter-based closed-water depuration system can significantly improve the microbiological quality of black clams within a