4.3.3 Enumeration of Total Heterotrophic Bacteria (THB)

Ten grams of tissue was aseptically weighed and transferred into a tissue homogeniser (Masticator, Spain) and homogenised in 90 mL sterile distilled water for one minute. Serial dilutions were made from this homogenised sample by transferring 1 mL to 9 mL sterile distilled normal saline blank. Serial dilutions were made up to 10^{-5}. For enumeration of THB, 0.1 mL of appropriate dilutions (10^{-3} to 10^{-5}) were spread plated on sterile nutrient agar plates. The plates were then incubated at room temperature for 24 to 48 hours. Plates with colony number ranging from 25 - 250 were taken for counting and THB load is expressed as the number of colony forming units per gram of bivalve tissue (cfu/g).

4.3.4 Enumeration of *Vibrio* spp.

For enumeration of *Vibrio* spp., tissue samples were processed as described for THB analysis. Appropriate serial dilutions (10^{-3}~10^{-5}) were spread-plated onto Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar plates. The plates were incubated at room temperature for 24~48 h, and plates containing 25~250 colonies were counted. The results were expressed as CFU g^{-1} of bivalve tissue.

4.3.5 Biofilm sample analysis

Biofilm samples were collected from the inner walls of the depuration tank at 0, 24, 48, and 72 h using sterile cotton swabs. The swabs were transferred into sterile conical flasks containing 90 mL sterile distilled water and vortexed to ensure uniform suspension of biofilm-associated bacteria. The resulting suspension was analyzed for TC, FC, THB, and *Vibrio* spp. using the same procedures, media, and incubation conditions described for shellfish samples (Sections 4.3.1~4.3.4).

4.4 Depuration experiment

Prior to the experiment, the collected clams (*Villorita cyprinoides*) were examined to ensure that they were alive and actively filtering. The sampling site, characterized by fine silty-clay substrate rich in suspended particulate organic matter, provided favorable conditions for active siphonal filtration, which was further confirmed by visual observation of siphon extension, valve gaping, and rapid valve closure upon tactile stimulation. Clams showing no response, persistent shell opening, or foul odour were discarded prior to analysis.

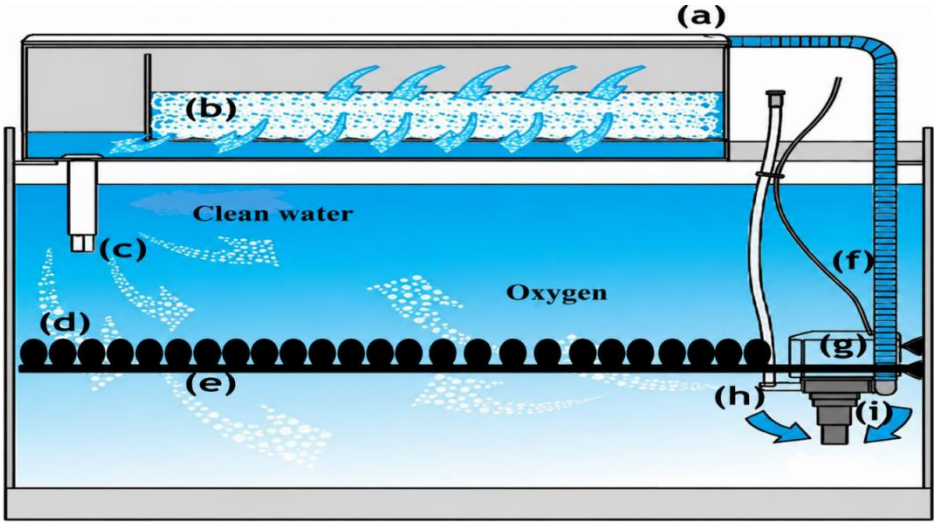

Figure 6 Schematic representation of the depuration tank

Figure caption: (a) Water outlet to bio filter (b) Sponge layer (c) Water outlet to tank after passing through the biofilter (d) Clam (e) Wire mesh (f) Water transporting pipe (g) Water pump (h) Air inlet (i) Water inlet

4.4.1 Design of depuration tank and depuration process

The depuration system consisted of a closed water holding glass tank with a capacity of 55 litres and dimensions 70 x 30 x 30 cm. A wall hung immersion water pump (Dophin P-708, China), placed 10 cm above the bottom of the tank, re-circulated (15 litres/min) the water in the depuration tank which was then passed through sponge filter held