Determination of Microbial Activities and Biomass in Biofilm Associated with Treatment Wetlands Compartments to Investigate Active Pollutant Processing Site

Adane Sirage Ali

Department of Environmental Science, Kotebe Metropolitan University, Addis Ababa, Ethiopia

Corresponding author
aliadane11@gmail.com

Rec date: **Acc date:** **Pub date:**

Copyright:

Abstract

I. psuedacorus *P. stratiotes*

Keywords: Floating treatment wetland; Macrophytes; Microbial activity; Biofilm

FTWs that incorporate common wetland plants growing in a hydroponic condition on floating rafts offer a potential solution to the major problems faced to ponds and conventional treatment wetlands [1]. FTW innovation can be practiced at all levels, with very low expense in all types of water body with ordinary engineering.

Despite the potential advantages of FTWs for the treatment of various wastewaters, there has been little information published to date about their design, construction and performance [2] and only few researchers assessed how the system functions [3,4].

Biofilms play the key roles in wastewater treatment systems including in conventional constructed wetlands and ponds. In FTWs, biofilm can effectively grow in the hanging roots, floating mat, sediment and in the free-water column between the sediment and the rhizosphere. Although, efficient removal of pollutants by FTW system is reported by few researchers, the location where the pollutants are actively processed and removed in the system has not been investigated yet.

Different zones of the system should be compared with respect to microbial parameters so that it gives clear information for the design and implementation of FTWs. Biofilm formations in the system need to be quantitatively explained. The active microbial cells distribution in the floating system should be also clearly identified so that the factors enhancing the microbial activity could be identified. This information is crucial in designing and implementing FTWs, which has no general design hitherto. Therefore, the objective of this study was to evaluate and compare microbial activities in different zones of the FTWs employing emergent macrophytes.

Six mesocosms were prepared from six buckets and one hundred liter influent tank was placed higher in the laboratory (Figure 1). The bottoms of every bucket were covered with gravels measuring about 2 Liters. Two pairs of suspending racks were prepared from white floater and several small holes were made to suspend the plants.

Two species of emergent macrophytes, *Iris pseudacorus* (IP) and *Phragmites australis* (PA), were selected. The macrophytes were placed on the suspending floater in such a way that the roots could grow suspended down to the water column. A pair of buckets was used as a control (without plant and floating mat). All of the mesocosms were prepared in duplicate. Twenty *I. pseudacorus*, twenty *P. australis* macrophytes were placed. The influent tank was filled with primary

adjusted to provide five days retention time for the influent. The set up was run for several months before biofilm sampling was done.

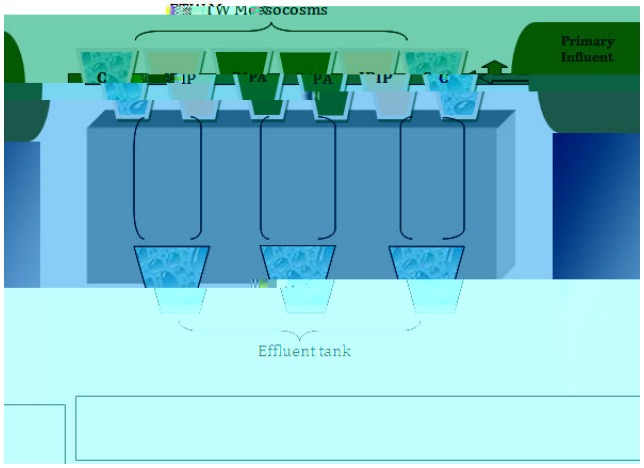

Figure 1: Schematic view of the experimental set up.

Nitrification denitrification

The microbial activity tests were done as suggested by Halsey [5]. Known volume of gravel and root containing biofilm were sampled from each FTWs. For biofilms in the water column, about 10 ml of water sample was taken from each FTW. All the samples were placed in 250 ml flask; and 180 ml of phosphate buffer and 0.4 ml of 25 g/L ammonium sulphate were added. The flasks were placed on a rotary shaker for 5 minutes with the speed of 150 rpm and allowed to stand for approximately 5 minutes after removing from the shaker. Approximately 10 ml aliquot was filtered and half of the sample was used to measure initial NO_3^--N concentration. The other half of the filtrated samples were placed in a refrigerator at 4°C to be used as reference. The remnant gravel, root and water sample in the flask was incubated for 72 hours at room temperature. 10 ml samples were taken at different time intervals and NO_3^--N concentration was measured.

Potential Denitrification Activity test was done similarly but the substrate was 2 ml of 9 g/L sodium nitrate and 2 ml of 12 g/L glucose were added and the test was done under anoxic conditions.

biofilm

Total microbial activity in the three compartments of the FTWs was estimated by Fluorescein diacetate (3',6'-diacetylfuorescein) hydrolysis [6,7].

For biofilm in the free-water column, 50-100 ml of water was filtered using 0.2 µm pore size polycarbonate membrane filter and carefully removed and placed into falcon tubes. Known volume of root and gravel were taken and placed in 50 ml of falcon tube in duplicate and then, 35 ml of 20 mM of phosphate

maximum FDA hydrolysis test that showed maximum microbial activity in the root associated biofilms in all of the wetlands.

Incubation Time (hr)	Nitrate production by compartments			
	Nitrate (µg/ml root)	Nitrate (µg/ml ravel)	Nitrate water)	(µg/ml

Table 1: Nitrate production through nitrifcation processes in the bioflms.

The slow nitrifcation rate and lesser nitrate accumulation is an indication that physiologically active nitrifying bacterial number in the water column was not enough to start nitirifcation rapidly in all of the wetlands and could nitrify. The delayed nitrifcation process in the gravel bioflm is an indication that the gravel contains less physiologically active microbes than the other compartments.

Denitrifcation activity: Average denitrifcation rate in the root, gravel and water for the FTWs over 72 hours of incubation varied between 0.09 and 0.19; 0.03 and 0.1; 0.02 and 0.24 µg NO_3^--N/ml/hour respectively. The variation among the compartments was tested by One-way ANOVA (unstacked) with post hoc comparison. The analysis showed that denitrifcation associated with the root bioflm was signifcantly varied ($P<0.05$) from the other two compartments (Table 2).

Nitrate concentration declined from 9.56 µg/ml to 0.05 µg/ml root surface within 72 hours of incubation (Table 2). The high denitirifcation rate and nitrate removal in the root zone of the FTW scan be associated with plant attributes to denitrifers. Although denitrifcation predominantly takes place in the sediments of wetlands [12], recent studies showed signifcant contributions of denitrifcation taking place on periphytic communities attached to submerged macrophytes [13]. Decaying parts of the macrophytes provide suitable condition for denitrifying bacterial growth [13]. Although macrophytes oxygen supply through the roots can be inhibitory, the net efect depends on plant species, growth rate and total biomass.

Nitrate concentration declined from 8.75 µg/ml to 1.04 µg/ml gravel surface within 72 hours of incubation. The nitrate removal in gravel within 24 hours of incubation was very slow which was similar to denitrifcation in the free-water bioflm, but unlike the denitrifcation by free-water bioflm, the concentration rapidly declined and reached limiting concentration in 9 more hours of incubation. Compared to denitrifcation due to free-water bioflm, it was rapid and much better in terms of nitrate transformation capacity.

The low denitrifcation rate in the water column bioflm shows that physiologically active denitrifying bacteria suspended in the free-water column was low at the beginning and hence, until the denitrifers multiply and become physiologically active, denitrifcation rate was low. This could be due to the influence of continuous aeration from the atmosphere ins µg/mh atmo [illegible] mo la y,v d [illegible]

emergent macrophytes since the floating mat hampers light entrance and this enhances the growth of heterotrophic and anaerobic microbial-dominated biofilm community. There was higher fluorocein hydrolysis in the control than the FTWs and this may be due to the fact that light can reach to the surface so that it gives favorable conditions for diversified microbial community growth.

Microbial activity in the bulk water was low compared to the gravel and the root zone may be due to the rarity of suitable attachment site in the free-water zone.

Viable microbial biomass in the biofilm taken from different FTW compartments was measured as a concentration of ATP (Table 3). Viable (physiologically active) microbial community in the three compartments varied significantly ($P<0.05$). Viable microbial community varied between 3.2×10^8(ATP=9.66 ng/ml) (PA) and 4.5×10^8(ATP=35.99 ng/ml) (IP) cells/ml of root surface, 2.5×10^7 (ATP=2.5 ng/ml) (IP) and 4.9×10^7(ATP=3.9 ng/ml) (PA) cells/ml of water; and 1×10^7(ATP=0.8 ng/ml) (IP) and 1.6×10^7(ATP=1.3 ng/ml) (PA) cells per ml of gravel (Table 3). Physiologically active microbial number in the control was much lower than both FTWs.