Research Journal of Environmental Sciences

ISSN 1819-3412

Academic Journals Inc.

www.academicjournals.com

Research Journal of Environmental Sciences 4 (5): 452-459, 2010
ISSN 1819-3412

Assessment of Wastewater Purifying Efficiency by Vertical Polypropylene Belts in Light of Changing Ciliate Community Characteristics

[1]B. Zhang, [2]W.H. Lin, [2]Y.J. Wang and [1]R.L. Xu
[1]School of Life Sciences, Sun Yat-Sen University, Guangzhou 500275,
[2]Pearl River Fisheries Research Institute, Guangzhou 500380, PR China

Abstract: In order to evaluate the role of vertical polypropylene belts in wastewater treatment, changes in both water quality parameters and ciliate community characteristics were analyzed for 10 days. This experiment was carried out in four outdoor concrete tanks that were randomly assigned to two groups: the experimental group (with vertical polypropylene belts) and the control group (without vertical polypropylene belts). In the experimental group, the dissolved oxygen concentration increased quickly, the concentration of nitrogen compounds decreased sharply and polysaprobic ciliates were replaced by oligosaprobic ciliates. In the control group, however, the wastewater quality improved very little and polysaprobic ciliates remained as the predominant species throughout the course of the experiment. Analysis of ciliate community succession in wastewater showed that vertical polypropylene belts played a key role in water treatment by providing a temporary refuge for ciliates and serving as a colonization center, allowing for ciliate adaptation to their new surrounding niche. Additionally, this study suggests that the ciliate community on vertical polypropylene belts may possess a unique adjustment mechanism that allows it to cope with changes in the surrounding environment.

Key words: Bioindicator, ciliate, community characteristics, vertical polypropylene belts, wastewater

INTRODUCTION

Artificial substrates such as fiberglass window screens, plastic mesh, commercial artificial substrates (e.g., Aquamat™), strip aquatic mats, artificial aquatic mats and plant carpets, are widely applied in aquaculture and wastewater treatment because they attract a variety of microorganisms, which rapidly form biofilms as soon as the substrates are submerged into water (Bratvold and Browdy, 2001; Wu *et al*., 2005; Arnold *et al*., 2005, 2006; Feng *et al*., 2007). Moreover, strip aquatic mats, which are suspended vertically in water and receive varying intensities of light at different water depths, can support different microorganisms (especially photosynthetic microbes) that have a combined effect on wastewater treatment through formation of biofilms (Wu *et al*., 2005).

Ciliates have long been thought to play an important role in wastewater treatment. From an operational point of view, ciliates have been described as useful bioindicators of

Corresponding Author: Runlin Xu, School of Life Sciences, Sun Yat-Sen University, No. 135 Xingang West Road, Guangzhou 510275, PR China
Tel: +86-20-84113185

biological treatment performance and effluent quality in wastewater plants, due to their short life cycles and quick responses to environment changes, especially the introduction of pollutants (Salvado *et al*., 1995; Martin-Cereceda *et al*., 1996). Ciliates also serve as food sources for metazoans and are primary consumers of bacteria, fungi, algae and other protozoans. Therefore, they represent an essential trophic link in the food web and enhance the proliferation of microbes, which accelerates the degradation of organic material (Pernthaler *et al*., 1997; Martin-Cereceda *et al*., 2001; Degans *et al*., 2002). Ciliates may also improve water quality by directly grazing on suspended particles and dissolved organic matter (Salvado *et al*., 1995; Lee *et al*., 2004).

In this study, vertical polypropylene belts were used as artificial substrates for the treatment of aquaculture wastewater. To evaluate the wastewater purification processes that take place on vertical polypropylene belts, changes in wastewater quality and ciliate community succession were analyzed.

MATERIALS AND METHODS

Description of Vertical Polypropylene Belts

Vertical Polypropylene Belts (VPBs) used in this study were made of modified polypropylene, non-woven fabrics with large, rough surface area, loose, porous inner structure and superabsorbent capacity. Each VPBs consisted of 80 belts (70.0×5.0× 0.0 cm) arranged side by side. One end of each belt was fixed to a polyvinylchloride (PVC) pipe floating on the water's surface, while the other end was immersed. Prior to the experiment, all VPBs were submerged and suspended for 10 days in a natural body of water to collect ciliates.

Experimental Design

Aquaculture wastewater was taken from a greenhouse concrete pond used for the farming of European eels (*Anguilla anguilla*) in the Changting County, Longyan City, Fujian Province, China.

This experiment was carried out in four outdoor concrete tanks (length 4 m, width 2 m, depth 0.2 m) near the culture ponds, over a 10-day period (from June 8 to June 7, 2007). Each tank was filled with wastewater to a depth of 1 m and then randomly assigned to one of two groups: the experimental group (with VPBs) and the control group (without VPBs). Two VPBs were added to each experimental tank.

Sampling and Measurement of Water Quality Parameters

A water sample was taken from each tank before VPBs installation in the experimental group (called day 0) and at days 0, 3, 5, 7 and 9 after installation. Water samples were taken from a depth of 50 cm in each corner of each tank as well as from the tank's center. Subsamples of 1000 mL were fixed immediately in 2% (v/v) buffered formaldehyde. Fixed subsamples were allowed to settle via natural precipitation for 24 h. The resulting supernatant was gently discarded and the remaining samples (approx. 50 mL) were used for quantitative and qualitative analysis of ciliates. Meanwhile, the concentrations for Dissolved Oxygen (DO), ammonia, nitrite and nitrate were analyzed, according to the standard methods for the examination of water and wastewater.

Except for the first sample, taken before VPBs entry into the wastewater (day 0), all other VPBs samples were taken at the same time as the water samples (days 0, 3, 5, 7 and 9). Two belts of the VPBs from each tank were selected randomly. Ciliates were extracted by manually squeezing water from the units into a clean glass beaker. The water samples were measured for volume (approx. 200 mL) and fixed immediately in 2% (v/v) buffered formaldehyde. Fixed subsamples were allowed to settle via natural precipitation for 24 h. Supernatant liquid was gently discarded and the remaining samples (approx. 50 mL) were used for quantitative and qualitative analysis of ciliates. All sampled belts were replaced in their original positions, marked and excluded from subsequent sampling.

Biological and Statistical Analysis

Ciliates in samples were identified at various magnifications (1000x, 400x, 10000x), based on the size of species, using phase contrast microscopy (Eclipse, Model E800). Protargol stain (Berger, 1999) was used, as necessary, to aid species identification. Taxonomic classification of ciliates was based on Carey (1992) and Berger (1999). The ciliate abundance was estimated in terms of the most probable number according to the Stout's Table (Stout, 1962). Based on their preference for the quality of their habitat, ciliates were classified into four saprobic levels: polysaprobic, α-mesosaprobic, β-mesosaprobic and oligosaprobic species (Foissner *et al*., 1995; Yusindi and Taylor, 2006). Ciliates were also separated into four trophic categories (algivorous, bacterivorous, carnivorous and omnivorous) according to the descriptions of Madoni (2005) and Madoni and Braghiroli (2007).

The significance of differences ($p<0.05$) in the parameters of water quality and ciliate community characteristics between the experimental groups and control groups were assessed using the paired-samples t-test in the SPSS v.10.0 statistical package.

RESULTS

Over the course of the experiment, the rate of increase of DO concentration in the experimental group was faster than that in the control group (Fig. 1a-d). By day 0, the DO concentration in the experimental group had increased from 4.39 to 5.55 mg L^{-1} while the control group's DO level decreased to its minimum. Subsequently, both groups' DO concentrations increased steadily, but the maximum attained in the experimental group was significantly higher than that of the control group.

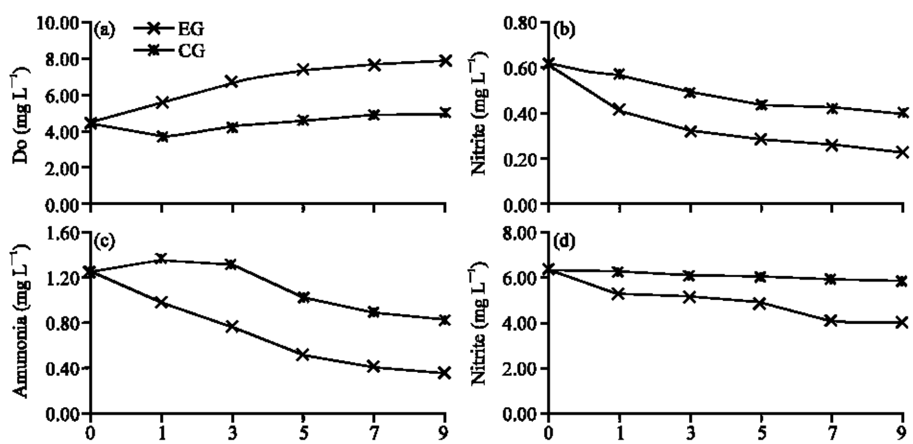

Fig. 1: (a-d) The change in water quality parameters over the 0-day period for the experimental group (EG) and control group (CG)

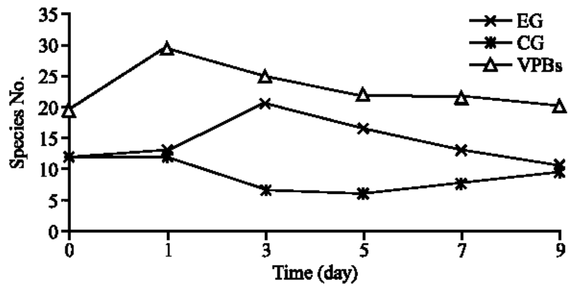

Fig. 2: The change in the number of ciliate species present on the VPBs and in the water of the experimental (EG) and control groups (CG) over the 10-day period

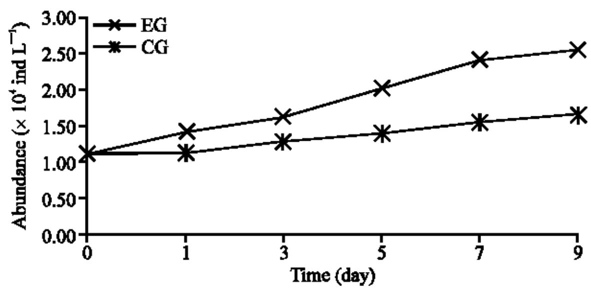

Fig. 3: The change in abundance of ciliates in the water of the experimental group (EG) and control group (CG) over the 10-day period

During the experiment course, the rates of decrease of ammonia, nitrite and nitrate concentration in the experimental group were faster than that in the control group (Fig. 1). The ammonia concentration in the experimental group continued to decrease over time and reached its lowest value at the end of the experiment. However, the ammonia concentration in the control group increased to its maximum by day 0 and then decreased continuously during the remainder of the experiment. Additionally, both the minimum and the rate of decrease of ammonia concentration in the experimental group were significantly higher than those in the control group. Both nitrite and nitrate concentrations decrease continually in all tanks during the experiment. However, the rates of decrease of nitrite and nitrate concentration in the experimental group were significantly higher than those in the control group.

The number of ciliate species in the experimental group, which was lower than that on the VPBs, was higher than that in the control group (Fig. 2). In the experimental group, the number of ciliate species presented in the water and on the VPBs increased first and then decreased, with maxima on days 3 and 0, respectively. Furthermore, in the experimental group, the number of species found on the VPBs was significantly higher than the number found in the water over the course of the experiment. In the control group, the number of ciliate species decreased first, reaching its minimum at day 5 and then increased.

The abundance of ciliates in all water tanks increased continually and reached a maximum at the end of the 10-day period (Fig. 3). However, the rate of increase in the number in the experimental group was significantly higher than that of the control group. On the VPBs, ciliate abundance decreased initially and reached a minimum at day 0, but then

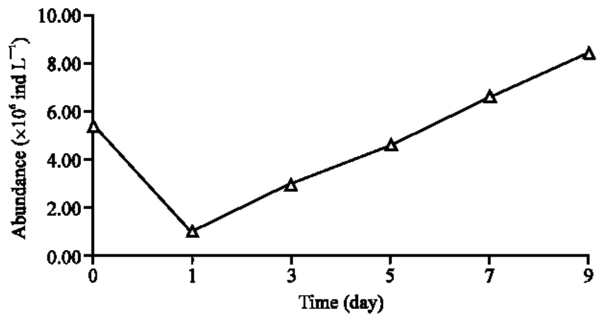

Fig. 4: The change in abundance of ciliates on VPBs over the 10-day period

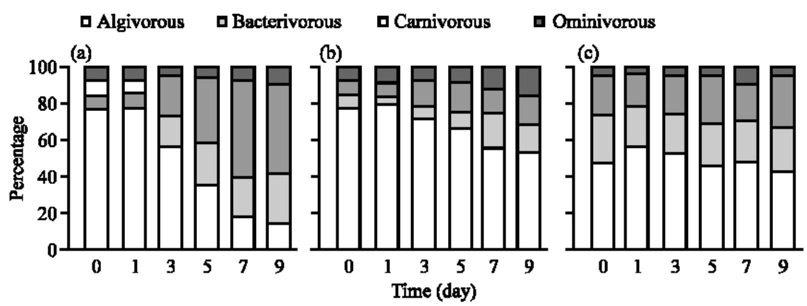

Fig. 5: The percentage contribution of algivorous, bacterivorous, carnivorous and omnivorous ciliates to the ciliate community in the water of the experimental group (a) and control group (b) and (c) on VPBs over the 10-day period

increased quickly and reached its maximum by day 9 (Fig. 4). Furthermore, in the experimental group, the number of ciliates on VPBs was significantly higher than the number in the water at all time points.

The contribution of ciliates from four trophic groups to the total community appeared diverse in different groups and time (Fig. 5a-c). In the experimental group, the percentage of bacterivorous ciliates increased slightly and reached its maximum at day 0, but then sharply decreased and reached its minimum at day 9. The percentages of both algivorous and carnivorous ciliates increased quickly and reached their maxima at day 9. In the water of the control group, the bacterivorous group was dominant in all samples, although, the percentages of other groups increased slightly over the course of the experiment. On the VPBs, the percentage of each group remained relatively stable throughout the experiment. Again, the bacterivorous group contributed most to the total population.

DISCUSSION

Water Quality Assessment by Ciliates

In this study, most of the ciliates examined in the wastewater on day 0 were polysaprobic species, such as *Colpoda reniformis*, *Vorticella microstoma*, *Vorticella campanula*, *Paramecium caudatum*, *Nassula ornate*, *Metopus es*, *Histriculus similes*, *Halteria grandinella*, *Didinium nasutum* and *Cyclidium glaucoma*. Their presence indicated that the aquaculture water was heavily polluted.

After the VPBs were added into the wastewater, polysaprobic species disappeared and other types, including oligosaprobic species (*Nassula gracilis*, *Dileptus anser*), α-mesosaprobic species (*Prorodon teres*) and β-mesosaprobic species (*Euplotes eurystomus*), appeared in their place. This change showed that the wastewater was being purified and the water quality was improving. In the control group, however, the water was dominated throughout the experiment by polysaprobic species, including *Vorticella microstoma*, *Vorticella campanula*, *Paramecium caudatum*, *Nassula ornate*, *Histriculus similes* and *Didinium nasutum*. These results suggest that the wastewater was still heavily polluted in the control tanks. Our studies also indicate that variation in the contribution of four functional-trophic classes of ciliates also correlated with the change in water quality (Madoni, 2005; Madoni and Braghiroli, 2007). The number of algivorous species and their abundance were higher in unpolluted areas, while bacterivorous ciliate levels were high in polluted areas (Madoni, 2005). Once wastewater was purified, the percentage of algivorous and carnivorous ciliates grew (Xu *et al*., 2002). At the beginning of this study, bacterivorous ciliates were the most highly represented class in the experimental group, indicating high level of organic compounds and bacteria. As treatment by the VPBs progressed, algivorous and carnivorous ciliates replaced bacterivorous ciliates as the predominant species, suggesting that the water quality was improving. However, in the control group, bacterivorous ciliates predominated throughout the entire experiment, indicating that the water quality remained poor.

Analysis of the VPBs Function in Wastewater Treatment

In the control group (without VPBs), both the decrease in the DO concentration and the increase in ammonia concentration indicated that the wastewater quality had deteriorated. These characteristics were consistent with the wastewater from European eels culture tanks, which often contains large amounts of uneaten food and feces. The decomposition of such organic matter consumes large amounts of DO and produces large amounts of ammonia. In the experimental group with VPBs, however, the DO concentration continually increased and the concentration of nitrogenous compounds decreased. It was suggested that VPBs could accelerate the rate of wastewater purification. On one hand, the superabsorbent capacity of VPBs may concentrate organic matter and microbes from the wastewater into biofilms. This process could accelerate the decomposition of concentrated organic matter by the combined effect of a variety of microbes present in a biofilm, in which cells are 100- to 1000-fold more abundant than in planktonic culture (Shanker and Mohan, 2001). This study also confirmed that the abundance of ciliates on VPBs was 100 times greater than in water.

Additionally, the method of installing VPBs appears to contribute to wastewater purification. In water lacking aquatic plants and external forces, most of the DO comes from algal photosynthesis. However, due to low water transparency, most algae, especially those that need to be attached to a substrate have difficulty growing in entrophic water, which lacks sunlight. The VPBs may provide such a surface for attached algal growth in sunlight (Azim *et al*., 2003). Thus, VPBs may increase the DO concentration in wastewater by improving the algal growth rate. Bratvold and Browdy (2001) also reported that an attached algal community on a vertical surface produced high water column nitrification rates, due to carbon fixation.

Analysis of the Function of a Vertical Surface in Ciliate Community Succession

The results of this study suggest that VPBs play a key role in water treatment by providing a temporary refuge for ciliates, allowing them to adapt to new niches. By day 0, the

VPBs contained the maximum number of ciliate species with all ciliates originating from species found in the wastewater or on VPBs at day 0. Specifically, 95.45% of the species found on VPBs and 84.62% of species found in wastewater on day 0 were still detected later in the experiment. Such results might be related to the characteristics of VPBs. When VPBs with loose, porous inner structures were added to the wastewater, ciliates and organic compounds adsorb onto them, forming many small, three-dimensional structures. These small niches could allow certain ciliate species to live in different microenvironments. Once those ciliates adapt to their niches, they would enlarge their living spaces by altering the environment around them. In the experimental group, the water reached a maximum number of ciliate species on day 3, with all species originating from those found on the VPBs at day 0. Thus, in the wastewater, the ciliate community is renewed quickly and the water purification rate is accelerated. Atilla *et al*. (2003) pointed out that artificial substrates contribute to meiofauna migration into water columns, possibly leading to a rapidly dispersing pool of meiofauna in estuaries. Hall-Stoodley *et al*. (2004) also confirmed that biofilms can expand by altering characteristics of the surrounding water.

Additionally, this study suggests that the ciliate community on the VPBs has some special adjustment mechanism to cope with changes to the surrounding environment. Prior to entry into the wastewater (day 0), the VPBs possessed four groups of ciliates, each of which favors a different pollution level: polysaprobic, α-mesosaprobic, β-mesosaprobic and oligosaprobic species. By the end of the experiment, there were still four groups of ciliates found. Moreover, although there was a drastic change in species composition, species number, total abundance of ciliates and water quality, the percentage of each of the functional-trophic classes of ciliates on the VPBs stayed relatively consistent.

ACKNOWLEDGMENTS

We are grateful to Mr. Lin Jinchang for providing facilities for carrying out the work. We would also like to thank Mrs. Lin Changyuan and Jiang Jianwen for their cooperation during the experiment. This research has been financially supported by China National Department Public Benefit Research Foundation (No. nyhyzx 07-0423 and No. 200803003).

REFERENCES

Arnold, S.J., M.J. Sellars, P.J. Crocos and G.J. Coman, 2005. Response of juvenile brown tiger shrimp (*Penaeus esculentus*) to intensive culture conditions in a flow through tank system with three-dimensional artificial substrate. Aquaculture, 246: 231-238.

Arnold, S.J., M.J. Sellars, P.J. Crocos and G.J. Coman, 2006. An evaluation of stocking density on the intensive production of juvenile tiger shrimp (*Penaeus monodon*). Aquaculture, 256: 174-179.

Atilla, N., M.A. Wetzel and J.W. Fleeger, 2003. Abundance and colonization potential of artificial hard substrate-associated meiofauna. J. Exp. Mar. Biol. Ecol., 287: 273-287.

Azim, M.E., A. Milsteinb, M.A. Wahabc and M.C.J. Verdegam, 2003. Periphyton-water quality relationships in fertilized fishponds with artificial substrates. Aquaculture, 228: 169-187.

Berger, H., 1999. Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). Kluwer Academic Publishers, Dordreche Boston London.

Bratvold, D. and C.L. Browdy, 2001. Effects of sand sediment and vertical surfaces (AquaMatsTM) on production, water quality and microbial ecology in an intensive *Litopenaeus vannamei* culture system. Aquaculture, 195: 81-94.

Carey, P.G., 1992. Marine Interstitial Ciliates. Natural History Museum Publications, UK.

Degans, H., E. Zollner, K.V. Gucht, L.D. Meester and K. Jurgens, 2002. Rapid Daphnia-mediated changes in microbial community structure: An experimental study. FEMS Microbiol. Ecol., 42: 137-149.

Feng, M.Y., T. Fang, J. Wu, Q. Mei, Y.H. Wu and J.T. Liu, 2007. Technology of algae-bacterium biofilm with different carries for improvement of water quality. Technol. Water Treatment, 33: 59-75.

Foissner, W., H. Berger, H. Blatterer and F. Kohmann, 1995. Taxonomische und ökologische Revision der Ciliten des Saprobiensystens. Band IV: Gymnostoatea, Loxodes, Suctoris. Informationsberichte des Bayer. Landesamtes für Wassersirtschaft, München.

Hall-Stoodley, L., J.W. Costerton and P. Stoodley, 2004. Bacterial biofilms: From the natural environment to infectious diseases. Nat. Microbiol. Rev., 2: 95-108.

Lee, S., S. Basu, C.W. Tyler and I.W. Wei, 2004. Ciliate populations as bio-indicators at deer island treatment plant. Adv. Environ. Res., 8: 371-378.

Madoni, P., 2005. Ciliated protozoan communities and saprobic evaluation of water quality in the hilly zone of some tributaries of the Po River (Northern Italy). Hydrobiologia, 541: 55-69.

Madoni, P. and S. Braghiroli, 2007. Change in the ciliate assemblage along a fluvial system related to physical, chemical and geomorphological characteristics. Eur. J. Protistol., 43: 67-75.

Martin-Cereceda, M., S. Serrano and A. Guinea, 1996. A comparative study of ciliated protozoa communities in activated-sludge plants. FEMS Microbiol. Ecol., 21: 267-276.

Martin-Cereceda, M., B. Perez-Uz, S. Serrano and A. Guinea, 2001. Dynamics of protozoan and metazoan communities in a full scale wastewater treatment plant by rotating biological contactors. Microbiol. Res., 156: 225-238.

Pernthaler, J., T. Posch, K. Simek, J. Vrba, R. Amann and R. Psenner, 1997. Contrasting bacterial strategies to coexist with a flagellate predator in an experimental microbial assemblage. Applied Environ. Microbiol., 63: 596-601.

Salvado, H., M.P. Gracia and J.M. Amigo, 1995. Capability of ciliated protozoa as indicators of effluent quality in activated sludge plants. Water Res., 29: 1041-1050.

Shanker, K.M. and C.V. Mohan, 2001. The potential of biofilm in aquaculture. J. World Aquac. Soc., 32: 62-67.

Stout, J.D., 1962. An estimation of microfaunal population in soils and forest litter. J. Soil Sci., 13: 314-320.

Wu, Y.T., T. Fang, C.Q. Qiu and J.T. Liu, 2005. Method of algae-bacterium biofilm to improve the water quality in eutrophic waters. Huan Jing Ke Xue, 26: 84-89.

Xu, K., J.K. Choi, E.J. Yang, K.C. Lee and Y. Lei, 2002. Biomonitoring of coastal pollution status using protozoan communities with a modified PFU method. Mar. Poll. Bull., 44: 877-886.

Yusindi, A.W. and W.D. Taylor, 2006. The trophic position of planktonic ciliate population in the food webs of some East African lakes. Afr. J. Aquat. Sci., 31: 53-62.