Korean J. Limnol. 35 (4): 266~272 (2002)

Bacterial Abundances and Enzymatic Activities under Artificial Vegetation Island in Lake Paldang

Byeon, Myeong-Seop, Jae-Jun Yoo[1], Ok-Sun Kim[1], Seung-Ik Choi[2] and Tae-Seok Ahn[1,3]*

(*National Institute of Environmental Research, Inchon 404-170, Korea,* [1]*Department of Environmental Science, Kangwon National University, Chunchon 200-701, Korea,* [2]*Environmental Research Institute, Kangwon National University, Chunchon 200-701, Korea,* [3]*Kangwon Regional Environmental Technology Development Center, Chunchon, 200-701, Korea*)

For analyzing function of a microbial ecosystem which was created under the artificial vegetation island (AVI) installed at Lake Paldang, zooplankton and bacterial numbers and exoenzyme activities (β-glucosidase and phosphatase) were measured biweekly from 3 November 2001 to 20 April 2002 at AVI site and control site. Under the AVI, the water quality was worse than control site in term of comparing the environmental parameters. But, zooplankton number of AVI site was 25 times higher than that of control site. Respiratory active bacterial numbers were 3-8 times higher at AVI site. In addition, enzymatic activities were higher at AVI site than those of control site. These results suggest that the zooplankton-phytoplankton-bacteria relationships are closely coupled with each other and organic materials are eliminated by respiration of zooplankton and bacterial activities.

Key words : Lake Paldang, active bacteria, artificial vegetation island, enzyme activity, bacteria, water quality

INTRODUCTION

Artificial vegetation island (AVI) is regarded as one of ecotechnological methods which emply macrophytes to improve the water quality of natural small ponds and large artificial impoundment. There have been many reports on water quality improvement by using macrophytes, such as natural wetlands (Kim and Cho, 1996) and constructed wetlands (Ahn and Kong, 1998). Improvement of water quality was made through by symbiotic and synergistic activities between macrophytes and microbes. Root part of macrophytes plays as a medium and a food source to microorganisms. In addition, macrophytes deliver O_2 to underwater via oxygen transfer organs, which promotes the degradation of organic matters by microbes (Ahn and Kong, 1998). Nutrients produced by degradation of organic matters by microorganisms are used by plants and, organics and metabolites originated from macrophytes can be reused by microbes (Wolverton, 1987).

AVI, however, does not improve water quality efficiently such as TN, TP, COD and other general parameters because of limited installed area (Shimatani, 1996; Park *et al*., 2001). Moreover, water column can be easily mixed by physical factors, and hence the concentrations of dissolved matters at vegetation island area became similar to those of outer part. For water quality improvement, more than 20% of surface of lake and reservoir should be covered with AVI (Park *et al*., 2001). In spite of those uncertainties of

* Corresponding Author: Tel: 033) 250-8574, Fax: 033) 251-3991, E-mail: ahnts@kangwon.ac.kr

water quality improvement, AVI itself is an important place for aquatic organisms. In most of Korean artificial reservoirs, the littoral zones were disturbed by heavy fluctuation of water level and there is almostly no plant. This destroyed area could play no more ecological roles such as spawning place for fish such as crucian carp (*Carassius auratus*). As such, restoration of littoral zones in artificial reservoir is essential for sustaining aquatic ecosystem, and AVI could be a one of the restoration ecotechnology for creating spawning and hiding place for fish and zooplankton.

In Lake Paldang, an AVI (64.8 m × 41.5 m) was installed at 29 May 2000 with *Phragmites communis*, *P. japonica*, *Zizania latifolia* and *Typha angustata*, which are common plants around Lake Paldang. Like previous studies, water quality improvement was not clearly observed, but fish and zooplankton were more abundant at AVI than outer water column (Environmental Management Cooperation, 2000). Considering that there is no light under the AVI, the microorganisms probably have more important role under the AVI than other aquatic ecosystem. For analyzing the microbial ecosystem under the AVI, we measured the total bacterial numbers, active bacterial numbers and exoenzymatic activities of β−glucosidase and phosphatase.

MATERIALS AND METHODS

Water sampling

Water sampling and field measurements were carried out biweekly from 3 November 2001 to 20 April 2002 at artificial vegetation island (AVI) and control site in Lake Paldang. Water sample was collected at three points at each site and mixed in a large bottle. Every analysis was carried out in triplicates.

Environmental parameters

Chemical parameters of COD, SS, TN, TP and chlorophyll *a* were analyzed by Standard Method (APHA, 2000). Zooplankton density was measured by stereomicroscopy (SV−11, Zeiss).

Total bacterial number and respiratory active bacterial number

Water sample was mixed well and filtered on polycarbonate membrane (pore size 0.2 μm). Total bacterial numbers were counted with Acridine Orange staining method (Hobbie *et al*., 1977). To measure the respiratory active bacteria, 5 ml of water sample was mixed with 4 ml of R2A medium and 1 ml of 5−cyano−2, 3−ditolyl tetrazolium chloride (CTC, 5 mM) and incubated for 1 hour in dark chamber (Rodriguez *et al*., 1992). Counting was done by epifluorescence microscopy. For statistical performance, at least 20 stages were counted.

Activities of β−glucosidase and phosphatase

Enzyme activities were determined by modified method of Chróst (1989). Methylumbelliferyl (MUF) β−glucoside (10 mM) and MUFphosphate (10 mM) were used as substrates. The final concentrations of MUF substrate were 50, 100, 200, and 400 μM. After incubation for 1 hour *in situ* temperature, reaction was terminated by the addition of 0.5 ml of NaOH glycine buffer (0.2 M, pH 10.5), and then the concentration of MUF free acid produced was quantified by fluorescentphotometer (TD−360 Mini fluorometer, ex: 365 nm, em: 460 nm). Maximal degradation velocity (Vmax) was calculated by Lineweaver Burk equation. All enzyme activities were estimated in triplicates.

RESULTS

Environmental parameters

Most of the mean environmental parameters are higher at AVI site that at control site (Table 1). Also, zooplankton number of AVI site is about 25 times higher than that of control site (Fig. 1).

Table 1. The data ranges and mean values of environmental parameters in artificial vegetation island and control site.

	Range				Mean value	
	AVI		Control		AVI	Control
SS (mg/l)	7.8	34	3.6	22.4	21.1	12.7
COD (mg/l)	5.4	17.3	2.6	6.5	9.6	4.8
TN (mg/l)	3.7	12.6	3.3	7.8	9.2	5.7
TP (mg/l)	0.6	2.5	0.3	2.0	1.5	0.9
Chlorophyll *a* (μg/l)	3.7	99.5	5.1	261.4	36.0	50.6
Zooplankton (ind./l)	343	3906	2	405	1644	67

Fig. 1. Photograph of zooplankton abundance under the artificial vegetation island installed in Lake Paldang.

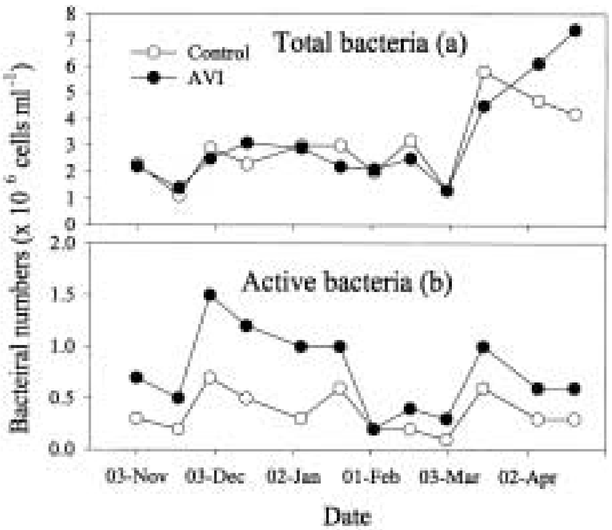

Fig. 2. The variations of total bacteria and respiratory active bacteria in an artificial vegetation island and a control site.

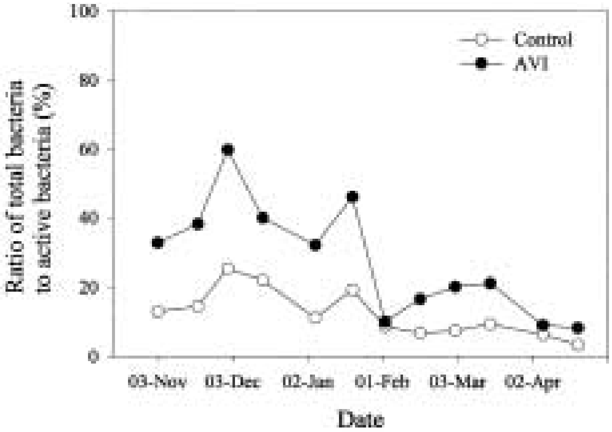

Fig. 3. The variations of ratio of total bacteria to respiratory active bacteria (%) of each sites.

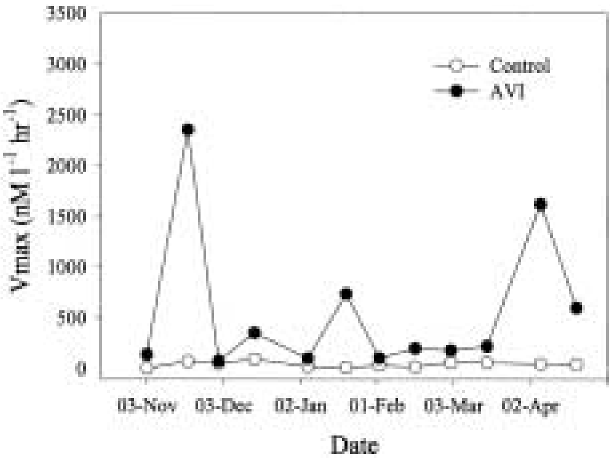

Fig. 4. The variations of β-glucosidase activities in an artificial vegetation island and a control site.

Total bacteria and respiratory active bacteria

As shown in Fig. 2a, the variations of total bacterial numbers have a similar pattern at both sites. Total bacterial numbers oscillated with a small variation from 3, Nov. 2001 to 3, March. 2002, but rapidly increased about 3-8 times in both sites after 3, March. There were no large differences in total bacterial numbers between two sites.

Respiratory active bacteria had a large temporal fluctuation, but have same patterns of variation in two sites (Fig. 2b). At AVI, the active bacterial numbers were more higher than those at control site. Particularly, in the cold season, the respiratory active bacterial numbers were higher than those of other periods. Due to the high values of respiratory active bacterial numbers, the ratios of active bacteria to bacterial number in AVI site were twice higher than in control site (Fig. 3).

Enzymatic activities

At AVI and control sites, Vmax of β-glucosidase was ranged 78-2358.7 nM l^{-1} hr^{-1} and 8.3-94.8 nM l^{-1} hr^{-1}, respectively and showed a distinct pattern between both sites (Fig. 4). At con-

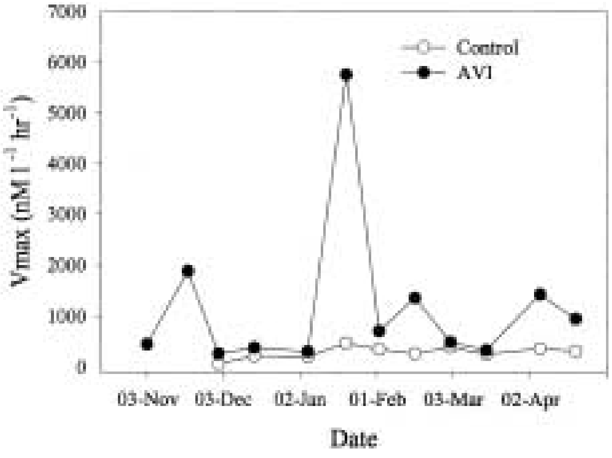

Fig. 5. The variations of phosphatase activities in an artificial vegetation island and a control site.

trol site, the β−glucosidase activity was lower than that of AVI site and stayed constant till the end of the investigation period. But at AVI site, β−glucosidase activity had irregular fluctuation.

The Vmax of phosphatase was ranged 70.8−480.7 nM l^{-1} hr^{-1} at control site and 271.9−5761.4 nM l^{-1} hr^{-1} at AVI site (Fig. 5). Like β−glucosidase activity, phosphatase activity of AVI site was higher than that of control site and exhibited irregular fluctuation.

DISCUSSION

AVI is composed of macrophytes, bed for sustaining macrophytes and submerged media for bacterial accumulation. Macrophytes are well known as a nutrient remover, and N and P could be eliminated by regular harvesting (Ahn and Kong, 1998). To uptake these nutrients, roots of macrophytes should bee spreading into water column adsorb nutrients. But in water column, the concentration of nutrients could be low due to dilution. Thus, the nutrient concentration in water column might not be sufficient for the growth of macrophyte. For overcoming this uncertainty for supplying N and P to macrophytes, the bacteria on media play an important role of accumulating low N and P into high state.

In aquatic ecosystems, bacteria can accumulate low concentration of nutrients into biomass of high concentration of nutrients (Chróst *et al*., 1994). This concentrated biomass could be transferred to higher trophic level by 'microbial loop' (Azam and Cho, 1987).

Because of the bacterial growth on media and roots of macrophytes, the concentrations of COD, SS, T N, T P were higher at AVI than those at control site. Which were already reported (Shimatani, 1996; Park *et al*., 2001). As such, a simple comparison of environmental parameters might not be proper evidence for water quality improvement. But, we have found out that there are higher numbers of zooplankton, active bacteria and enzymatic activities than outer water.

Zooplankton is a primary consumer of phytoplankton. For sustaining dense population under the AVI, there must be large amounts of organic materials. There are some possible sources of organic materials. The first one is phytoplankton. The mean values of chlorophyll *a* under AVI are less than half of outer part. Chlorophyll *a* concentrations under AVI just after AVI installation were half of outer water column. Moreover, under AVI, chlorophyll *a* concentration were higher at middle and lower layer than surface layer (Environmental Management Cooperation, 2000). Considering the factor that sunlight is blocked by bed and macrophytes under the AVI, large proportion of total phytoplankton came from outside of AVI by physical turbulence. Second one is organic material released from macrophytes. Most of the emergent macrophytes, biomass of belowground part is more than 40% of whole plant biomass (Wetzel, 1983). At AVI site, the particulate and dissolved organic materials were released into water directly because belowground part is submerged in water column. Thirdly, attached bacteria on media and roots of macrophytes could play a role of source for organic materials for zooplankton growth. The submerged solid surfaces such as roots of macrophytes are readily colonized by microorganisms, resulting in the development of biofilm containing a variety of bacteria, fungi, macromolecules and particulate materials (Hunik *et al*., 1993). In addition, between macrophyte and bacteria, there are some relationships such as symbiosis, commensalisms, and etc. Bacterial attachment requires hydrated polymer matrix (Fletcher, 1996), and attached bacteria synthesize the exopolysaccharide to prevent detachment of daughter cells (Vadevivere and Kirchman, 1993). Thus the microorganism itself and secreted polymers by attached bacteria could provide food source to zooplankton.

When zooplankton population run short of phytoplankton as a food source or increment of cyanobacteria in ambient water column occur, they shift from algaevore to bacterivore (Shim and Ahn, 1992). We could not observe the microscopic evidence of bacterivore of zooplankton. But, because the high ratios of active bacteria and enzymatic activities under AVI were observed even though total bacterial numbers varied similar at both sites, we may assume that the bacteria could be grazed by zooplankton, because grazing pressure makes bacteria active (Guede, 1988).

Degrading activities, or β-glucosidase and phosphatase activities, were higher at AVI than those at control site, and concentrations of water quality parameters such as particulate matters and nutrients were not high. However, the concentrations of COD, SS, TN and TP were higher in AVI area as appeared in Table 1. As such, improvement of water quality by AVI was not shown apparently in water quality parameters only.

β-Glucosidase is a broad specificity enzyme that catalyzes the hydrolysis of β-linked disaccharide of glucose, celluhexose, and carboxymethylcellulose (Barman, 1969) and it is very important and significant for glucose metabolism of microheterotrophs in aquatic ecosystems (Chróst, 1989). The Vmax values are higher at AVI than those at control site. According to Chróst (1991), addition of cellobiose strongly induced β-glucosidase synthesis. In addition, high molecular weight fraction of lake water was reported to stimulate the β-glucosidase activity (Kim *et al*., 1999). Thus, the higher activity in AVI was due to the diverse high molecule weight organic materials originated from macrophytes and microorganisms.

Phosphatase are originated from phytoplankton and bacteria, of which substrate affinities are different (Jansson *et al*., 1988). When chlorophyll *a* concentration reached at the concentrations of 40.6 μg/l, a 2.3 times higher than that of initial time, the phosphatase activity showed the highest value. Afterward, chlorophyll *a* concentration of AVI site showed slightly higher until late February. Phosphatase activity is induced when inorganic phosphate in the water is exhausted (Ahn *et al*., 1993) and/or content of cellular phosphate decreased (Chróst and Overbeck, 1987). Therfore, phosphatase activity indicates the concentration of available phosphate in water column and the phosphate deficit of the phytoplankton cell (Choi *et al*., 1992). But, the high TP values (Table 1) could be a source for sufficient phosphate under AVI. Therefore, high phosphatase activity in AVI indicates another source of phosphatase in the system. A possible source is membrane bound phosphatase in phytoplankton (Wetzel, 1991).

With above results, a hypothetical model could be suggested. Under AVI, zooplankton play an important role. Zooplanktons graze mainly on phytoplankton coming from outside of AVI and bacteria grown on media and roots of macrophytes. Zooplankton grazing pressure makes bacteria active. Also by 'sloppy feeding' of zooplankton, phytoplankton excretes phosphatase into water. By the succession of phytoplankton and zooplankton, the enzymatic activities are fluctuated (Fig. 4 and 5). Orthophosphate is generated by phosphatase and bacteria are responsible for uptake of this orthophosphate (Currie *et al*., 1986). The organic materials are eliminated by the respiration of zooplankton and bacteria attached to media and roots of macrophytes and fish grazing on zooplankton and phytoplankton.

AVI itself is a newly created complex ecosystem with macrophyte, zooplankton, phytoplankton, bacteria and fish. This AVI can be useful technique for restoration and sustaining aquatic ecosystem, and indirect water quality improvement.

ACKNOWLEDGMENTS

This subject is supported by Ministry of Environment as "The Eco technopia 21 project".

REFERENCE

Ahn, T.S. and D.S. Kong. 1998. Application of ecotechnology for nutrient removal. Frontier in biology: In Chou, C.H. and Shao, K.T. (eds). The challenges of Biodiversity, Biotechnology and Sustainable Agriculture, Academia Sinica, Taipei. pp. 209-216.

Ahn, T.S., S.I. Choi and K.S. Joh. 1993. Phosphatase activities in Lake Soyang, Korea. *Verh. Internat. Verein. Limnol*. **25**: 183-186.

APHA. 2000. Standard Methods for the examination of water and wastewater. 20th ed. APHA. N.Y.

Azam, F. and B.B. Cho. 1987. Bacterial utilization of organic matter in the sea. Ecology of microbial communities. In Fletcher, M., Grey, T.R.G and Jones, J.G (eds), "Ecology of Microbials Communities", Cambridge University, Cambridge. pp. 216-281.

Barman, T.E. 1969. Enzyme Handbook, vol. 2 Springer Verlag, Berlin. pp. 928.

Choi, S.I., T.S. Ahn and B.C. Kim. 1992. Degradation rates of organic phosphate in Lake Soyang. *Kor. J. Microbiol*. **30**: 113-118.

Chróst, R.J. 1989. Characterization and significance of β-glucosidase activity in lake water. *Limnol. Oceanogr*. **34**: 660-672.

Chróst, R.J. 1991. Environmental control of the synthesis and activity of aquatic microbial extoenzymes. In Chróst R.J. (ed.), Microbial enzymes in aquatic environments, Springer Verlag, N.Y. pp. 29-59.

Chróst, R.J. and H. Rai. 1994. Bacterial secondary production. In Chróst R.J. (ed.), Microbial ecology of Lake Plussee, Springer Verlag, N.Y. pp. 92-117.

Chróst, R.J. and J. Overbeck. 1987. Kinetics of alkaline phosphatase activity and phosphorus availability for phytoplankton and bacterioplankton in lake Plussee (north German eutrophic lake). *Microb. Ecol*. **13**: 229-248.

Currie, D.J., E. Bentzen and J. Kalff. 1986. Does alga bacteria phosphorus partitioning vary among lakes. A comparative study of orthophosphate uptake and alkaline phosphatase activity in freshwater. *Can. J. Fish. Aquat. Sci*. **43**: 311-318.

Environmental Management Cooperation. 2000. Report of artificial vegetation island management. EMC.

Fletcher, M. 1996. Bacterial attachment in aquatic environments: A diversity of surfaces and adhesion strategies. In Fletcher, M. (ed.), "Bacterial Adhesion". John wiley & Sons, N.Y., pp. 1-24.

Guede, H. 1988. Direct and indirect influence of crustacean zooplankton on bacterioplankton of Lake Constance. *Hydrobiologia*. **159**: 63-73.

Hobbie, J.E., R.J. Daley and S. Japer. 1977. Use of a nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol*. **33**: 225-1228.

Hunik, J.H., M.P. Hoogen, W. Boer, M. Smit and J. Tramper. 1993. Quantitative determination of the spatial distribution of *Nitrosomonas europaea* and *Nitrobacter agilis* cells immobilized in K Carrageenan gel beads by a specific fluorescent antibody labelling technique. *Appl. Environ. Microbiol*. **59**: 1951-1954.

Jansson, M., H. Olsson and K. Peterson. 1988. Phosphatase: Origin, characteristics and function in lakes. *Hydrobiologia* **170**: 157-176.

Kim, J.H. and K.H. Cho. 1996. Water quality improvement by aquatic macrophyte: A case study in Lake Paldangho. Proceeding of Korea-Japan Joint Symposium on Ecological Engineering. pp. 3-17.

Kim, K.K., S.H. Hong, D.J. Kim, S.I. Choi, and T.S. Ahn. 1999. The change of bacterial numbers and β-glucosidase activities by the size fraction of DOM in Lake Soyang. *Kor. J. Microbiol*. **35**: 35-40.

Park, H.J., O.B. Kwon and T.S. Ahn. 2001. Water quality improvement by artificial floating island. *J. Korean Env. Res. Reveg. Tech*. **4**: 90-97.

Rodriguez, G.G., D. Phipps, K. Ishiguro and H.F. Ridgway. 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. *Appl. Envriron. Microbiol*. **58**: 1801-1808.

Sim, D.S. and T.S. Ahn. 1992. On the feeding behavior of zooplankton in Lake Soyang. *Kor. Jour. Microbiol*. **30**: 129-133.

Shimatani Y. 1996. The effect and ecosystem of an artificial vegetated island, Ukishima, in Lake Kasumigaura. Proc. Korean-Japan Joint Symposium on Ecological Engineering. pp. 39-44.

Vandevivere, P. and D.L. Kirchman. 1993. Attachment simulates exopolysaccharide synthesis by a bacterium. *Appl. Envion. Microbiol*. **59**: 3280-3286.

Wetzel, R.G. 1983. Limnology, 2nd ed. CBS College Publishing. pp. 519-614.

Wetzel, R.G. 1991. Extracellular enzymatic interactions: Storage, redistribution, and interspecific communication. In Chróst, R.J. (eds) "Microbial enzymes in aquatic environments". Springer Verlag. 1991. pp. 6-28.

Wolverton, B.C. 1987. Aquatic plants for water treatment and resource recovery: An overview, In Reddy, K.R. and Smith, W.H. (eds) "Aquatic plants for water treatment and resource recovery". Magnolia Publishing Inc., Orlando, FL., pp. 141-152.

(Received 7 Oct. 2002, Manuscript accepted 13 Dec. 2002)

<국문적요>

팔당호에 설치된 인공식물섬에서의 세균 수와 체외효소 활성도의 변화

변명섭 · 유재준[1] · 김옥선[1] · 최승익[2] · 안태석[1,3]*

(국립환경연구원, [1]강원대학교 환경학과,
[2]강원대학교 환경연구소, [3]강원지역환경기술개발센터)

팔당호에 설치된 인공식물섬에서 미생물의 역할을 알아보기 위하여 동물플랑크톤 군집 크기, 총세균수, 활성세균수, β–glucosidase와 phosphatase의 체외효소활성도를 2001년 11월 3일부터 2002년 4월까지 격주로 인공식물섬이 설치된 지역과 바깥지역을 대상으로 조사 분석하였다. 인공식물섬 아래에서는 일반적으로 측정하는 환경요인들은 대조구보다 수질이 나쁜 것으로 나타났다. 그러나, 동물 플랑크톤의 수는 대조구보다 평균 25배, 활성세균의 수는 평균 3–8배, 그리고 체외효소활성도는 훨씬 높은 값을 보였다. 이러한 결과는 인공식물섬에서는 동물플랑크톤–식물플랑크톤–수초–세균의 밀접한 관계가 존재하고, 이 관계에 의하여 동물플랑크톤과 세균의 호흡, 분해작용으로 유기물이 제거되는 것으로 판단되었다.