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Response of Freshwater Biofilm to pollution and ecosystem in Baiyangdian Lake of China

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Abstract

An experimental study was undertaken to highlight the potential applicability of biofilms as biomonitors forming simultaneously on natural and artificial substrata in Baiyngdian Lake(China).We investigated the responses of freshwater biofilm in 8 site of Baiyngdian Lake and compared with control site (a reservoir) to assess the relative health of water. Exposure to pollution and its impact on biofilms were assessed by measuring the biomass production, Chlorophyll concentration, the algal composition, extracellular enzyme activity of bacterial communities and Polysaccharide content. This relation between the biological characters of biofilms and water quality were discussed, and the relative health of regions were demonstrated by the degree of deviation based on bioflim indicator in the following order: Fu river (S4) < Duan cun (S8) < Nan Liuzhuang (S5) < Wang jiazai (S1) < Cai putai (S7) < Zao lingzhuang (S2)< Shao Chedian (S3).. The result indicated that biofilm can provide information for pollution detection and ecological health assessment of water, and biofilm on aritificial substrata was recommended for biomonitoring in the Baiyangdian Lake.

Keywords: Freshwater biofilm, community analyses, Biomonitoring, Substrata

1. Introduction

Effect of toxic pollution on aquatic communities is one of major environmental concerns. Toxicity assessment is generally based on single species testing which requires extrapolation routines to estimate community-level effects(Schmitt-Jansen et al. 2007). Community-level testing is a feasible tool in ecotoxicology because it provides a relatively high ecological realism and reliability when assessing the ecological consequences of contaminant exposure(Landner et al. 1989).The application of rapid and easy-to-handle tests employing multi-species level investigations and multiple endpoints is therefore a challengable but meanigful task .

Biofilms are complex communities, composed mainly of photoautotrophic (algae) and heterotrophic microorganisms (bacteria, fungi, protozoa), which accumulate at surfaces of artificial or natural substrata and are typically surrounded by a matrix of extracellular polymeric substances (EPS)(Denkhaus et al. 2007; Kr pfl et al. 2006). Biofilm exist for more than 3.8 billion years and today are ubiquitous on Earth, playing a fundamental role in the various biogeochemical cycles and dynamics of the aquatic ecosystems(Denkhaus et al. 2007). Biofilms can be regarded as community-level monitoring systems for detecting the effects of toxicants on aquatic systems, because they can accumulate pollutants and their characteristic response to major changes in water quality(Kr pfl et al. 2006; Sabater et al. 2007). Biofilms possess many of the attributes required for community-level monitoring systems (Kr pfl et al. 2006; McCormick et al. 1994; Nocker et al. 2007; Porsbring et al. 2007): (1) they are widely distributed; (2) they are sessile, thus can reflect the real conditions of the habitat; (3) they respond more rapidly to environmental changes because of their short life cycle than higher level organisms; (4) these communities are composed of many biological taxa with various environmental tolerances; (5) biofilm samples can be relatively easily collected.

A lot of methods are used for detecting the effects of toxicants by use of biofilms, varying from structurally-based to functionally-based and from in vitro-based to systemic approaches(Sabater et al. 2007). The effects of toxicants on the biofilms can be expressed as variations of community composition(Barranguet et al. 2003; Dorigo et al. 2008; Massieux et al. 2004;

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Morin et al. 2008), growth (biomass)(Barranguet et al. 2003; Guasch et al. 2007; Lawrence et al. 2008; Morin et al. 2008), and physiological properties such as photosynthesis, respiration, or use of the dissolved organic materials(Guasch et al. 2007; Ricart et al. 2009; Schmitt-Jansen and Altenburger 2007). For example, Tlili et al(Tlili et al. 2008) assessed the ecotoxicological impact of the herbicide diuron on biofilms by measuring pesticide concentrations in biofilms, biomass parameters (chl a, AFDW), community structure (using 18S and 16S rDNA gene analysis by DGGE, and HPLC pigment analysis to target eukaryotes, bacteria and photoautotrophs, respectively). Different artificial and natural substrate were useded to collect the biofilms, such as glass(Dorigo et al. 2008; Morin et al. 2008) , plexi-glass (Kr pfl et al. 2006),prepex(Ricart et al. 2009) and stone substrata(Tlili et al. 2008).Some researchers made comparisons of biofilms grown on artificial and natural substrata, however, their biological conclusions are contradictory(Kr pfl et al. 2006). The contradictions may be related to the differences in the goals of their study, and the environment studied and the methods used (Cattaneo et al. 1992).

We conducted a study in Baiyangdian Lake, the largest freshwater body in the North China Plain. In recent decades, the water quality of this lake has been degraded due to sewage discharge and aquaculture, and industrial contaminants form factories in nearby city of Baoding(Chen et al. 2008). Previous studies showed that organic contaminants such as DDT (dichloro-diphenyltrichloroethane) and lindane are bioaccumulated and biomagnified in the food webs of the aquatic ecosystems of lake (Dou et al. 1998). At the same time, fisheries yields and benthic and planktonic diversity have been decreased (Xu et al. 1999).These caused a serious ecological problems, which require that the urgent actions need to be taken to monitor the pollutants and their ecological consequences on Baiyangdian Lake.

Considering the necessity of monitoring of pollutants and their ecological consequences in the Baiyangdian Lake, we investigated the applicability of biofilms as biomonitors forming simultaneously on natural and artificial substrata by analyzing the relationship between the biological characters of biofilms and water quality . In order to evaluate the response of biofilm to pollutants in freshwater, the biomass production, Chlorophyll concentration, the algal composition, extracellular enzyme activity of bacterial communities and Polysaccharide content were determined in present study. The results of this study can provide a scientific basis for selecting a substratum for bio-monitoring the Baiyangdian Lake in Northern China and the similar lakes in other region.

2. Materials and Methods

2.1 Sampling site

Baiyangdian lake (38° 44′ ～ 38° 59′ N , 115° 45′ ～ 116° 06 E) is located in the eastern part of Baoding, Hebei province in China, about 8 m above sea level, with average depth 2-4 m, and an area of 366 km2. The lake has a great recreational value and also supports local agriculture, tourism, and fisheries. Urban pollution causing degradation of water quality comes from the city of Baoding (population about 100 000), situated on the western of the lake. Moreover, agricultural effluents of several smaller settlements and light industrial waste from the surrounding area flow into the lake and cause the pollution. Sampling was conducted at nine national monitoring sites (Fig. 1), including one reservoir which used as control in this study in Baingyangdian Drainage Basin.

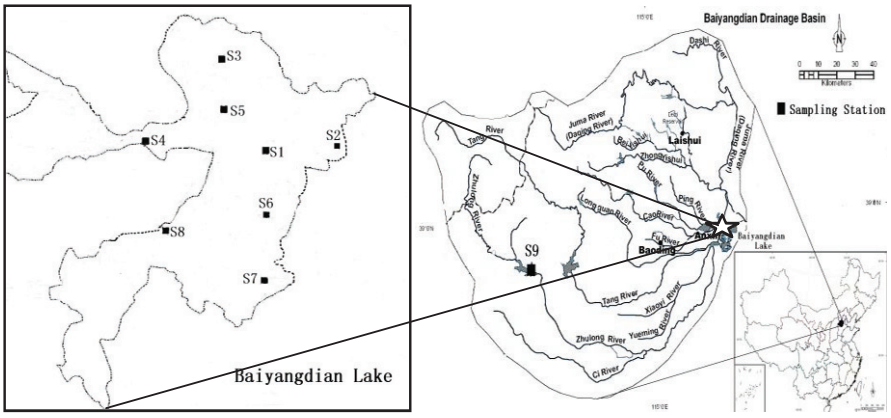

Fig.1 Map of sampling site in Baiyangdian Drainage Basin, China

S1:Wang jiazhai; S2:Zao lingzhuang; S3:Shao chedian; S4:Entrance of Fu river; S5:Nan liuzhuang; S6:Quan tou; S7: Cai putai; S8:Duan cun; S9: Wangkuai reservoir (control site)

2.2 Sampling procedure of biofilm

The natural biofilms were developed in the Baiyangdian Lake between September and October of 2008. The biofilms on artificial substrata were grown on carbon-fiber membrane submerged into the water (at depth of 20cm) in plastic holders fixed to a plastic plate which was connected to a landing-stage at 6 m distance from the riverside. Each plastic plate contained 10 pieces of carbon-fiber membrane with a surface area of 40 cm2. The position of the plastic plate was parallel to the water-stream. After 10 days the carbon-fiber membrane were removed from the plastic plate and the biofilm were scraped by a knife. Simultaneously, the biofilms on natural substrate were scraped from reed stems surface (under water at depth of 20cm) per sampling site. The biofilms were divided into two parts and suspended into sterile plastic tube using 0.2 μm-Nuclepore filtered water from the reference site. For physical and chemical analysis, one part of the biofilms was deep-frozen (-25℃) after centrifuged in lab and the other part of the biofilms was put into prefiltered stream water with formaldehyde solution for biological investigations. Samples were transported to the laboratory in a cold box. The water parameters, temperature (T), PH, dissolved oxygen (DO), were directly determined in site. And triple water samples were collected for chemical analyse in the laboratory, including CODMn and concentration of Fluoride, Arsenide, Hg and Cu.

2.3 Characters of biofilm analysis

2.3.1Total biomass

The organic matter content was evaluated by calculating the ash -free dry matter (AFDM). Three subsamples of each biofilm suspension (2ml) were flittered through 50mm glass fiber filter (0.2-μm pore size). Each filter was weighed, after drying for 24h at 105 °C in order to calculate the mineral matter. The filter were then combusted in an oven at 450°C (SX-4-10 Fiber Muffle，Test China) for 1h, and weighed again to calculate the mineral matter(Tlili et al. 2008). The AFDM was caculated by subtracting the mineral matter from the total dry matter. Result are expressed as g·m-2.

2.3.2 Chlorophy Ⅱconcentration

Three subsamples of each biofilm suspension (2ml) were flittered through 50mm glass fiber filter (0.45-μm pore size). Chlorophy Ⅱ was extracted from frozen filter membranes using 90% hot ethanol (80℃) after freeze-thawed for 3-5 cycles and kept for 12h in the dark at -20℃. Chl a, b and c were determined spectrophotometrically (UNIC 2100 spectrophotometer) following hot ethanol extraction and incubation at 4℃ in the dark for 6h. To ensure complete extraction of chlorophyll, samples were sonicated (Selecta, 40W power, 40 kHz frequency) for 4 min at the end of the extraction period, then centrifuged 10 min at 3000 rpm. The wavelengths measured were 630 nm, 647 nm, 664 nm and 750 nm. The concentration of Chl a, b and c were calculated as following formulas(Huang T L et al. 2002). Final concentrations were given as μg·cm−2.

chl a=[12.12(D664-D750)-1.58(D647-D750)-0.08(D630-D750)]VE / S·d

chl b=[-5.55(D664-D750)+21.5(D647-D750)-2.72(D630-D750)]VE / S·d

chl c=[-1.71(D664-D750)-7.77(D647-D750)+25.08(D630-D750)]VE / S·d

where chl a,chl b and chl c are the concentration of Chla, b and c，respectively, μg·cm−2; D664, D647, D630 and D750 are the absorbance of extraction at 630nm, 647nm, 664nm and 750nm, respectively; VE is the volume of final extraction, ml; S is the area of biofilm subsample, cm−2; d is optical path curette, cm.

2.3.3 The algal abundance and composition

To estimate the abundance and composition of biofilm algae, biofilms scraped from substrate were kept in 15 mL sterile plastic tubes containing 12mL prefiltered stream water（0.2 μm pore size) and 5% formaldehyde solution. To ensure accuracy, samples were sonicated (Selecta, 40W power, 40 kHz frequency) for 20s before counting. The abundance of total alga in the biofilm samples, expressed as number of alga cell per square centimete, was measured using blood cell count board. The classification of algae was followed the direction of "The freshwater algae of China: systematic, taxonomy and ecology"(Hu H J et al. 2006) and the proportion of different species was measured under the microscope (OLYMPUS BX41).

2.3.4 Extracellular enzyme activity

The extracellular enzyme activities of β-glucosidase (BETA) and phosphatase (PHOS) were measured by using fluorescent-linked substrates. BETA activities were measured using 5 mM p-nitrophenyl-β-D-glucopyranoside (Wako) in citrate–phosphate buffer pH 5.0, based on the appearance of p-nitrophenol in the solution according to (Terra et al. 1979). The activity of PHOS was determined using 5 mM p-nitrophenyl phosphate as substrate in citrate–phosphate buffer pH 4.5. Enzyme activity assays were based on the release of a chromophore (p-nitrophenyl or p-nitroaniline) after the hydrolysis of the corresponding artificial substrates. In all assays reaction volumes were incubated at 30 °C for periods of 0, 10min, 30min, 1h, 2h,

and 4h and initial rates of hydrolysis were calculated. And hydrolysis was stopped by 0.1M NaOH solution. Comparing the absorbance of p-nitrophenol (λ = 410 nm) for different incubation period, the incubation period of the maximum absorption for BETA and PHOS is 1h. Two types of blanks (substrates plus buffer or water alone) were run with each set of assays to follow any changes in fluorescence not due to biofilm suspension. The absorbance of p-nitrophenol (λ = 410 nm) was measured after incubation for all samples. The results were given as the amounts of hydrolysis per square centimeter of biofilm within an hour (nmol·cm-2·h-1).

2.3.5 Polysaccharides content

Polysaccharide content was determined using Phenol-sulfuric acid method proposed by (Dubois et al. 1951) and modified by (Liu et al. 1973). The biofilm from one fiber was detached into thick walled glass tubes filled with distilled water (1ml). After vortex mixing, 5% phenol solution (1ml) was added and mixed again. Sulfuric acid (5ml) was then added and mixed. Solutions were cooled down and centrifuged (3000rpm, 10min). Absorption measurements were taken using a spectrophotometer set at 488 nm wavelength. The polysaccharide content representing as glucose equivalents (mg·cm-2) was calculated using glucose for the standard curve (0-200ug mL-1).

3. Results and discussion

3.1 Biomass of biofilm

As shown in Fig. 2, biomass of biofilm (abundance of alga and AFDW) were different ($p<0.05$, T test) among the sites in Baiyangdian Lake. The biomass at site 4 was the highest among all the sites. The AFDW of biofilm on natural substrata changed in the range of 0.13-10.82 mg/cm2, different from 0.15-2.2 mg/cm2 on the artificial substrata. The difference of range also can be found in abundance of alga between natural substrata and artificial substrata. This difference can be attributed to the growing time of biofilm and substrata. However, the Biomass parameters of biofilm on Natural substrata were significantly correlated with these of biofilm on artificial substrata as shown in table 1. It can be concluded that the biomass parameters of biofilm on different substrata reflect the same dynamics among regions. Biofilm biomass may express the long-term effect of toxicants on communities(Sabater et al. 2007), and the biomass parameters of biofilm on different substrata can be used to reflect the effect of toxicants in this study.

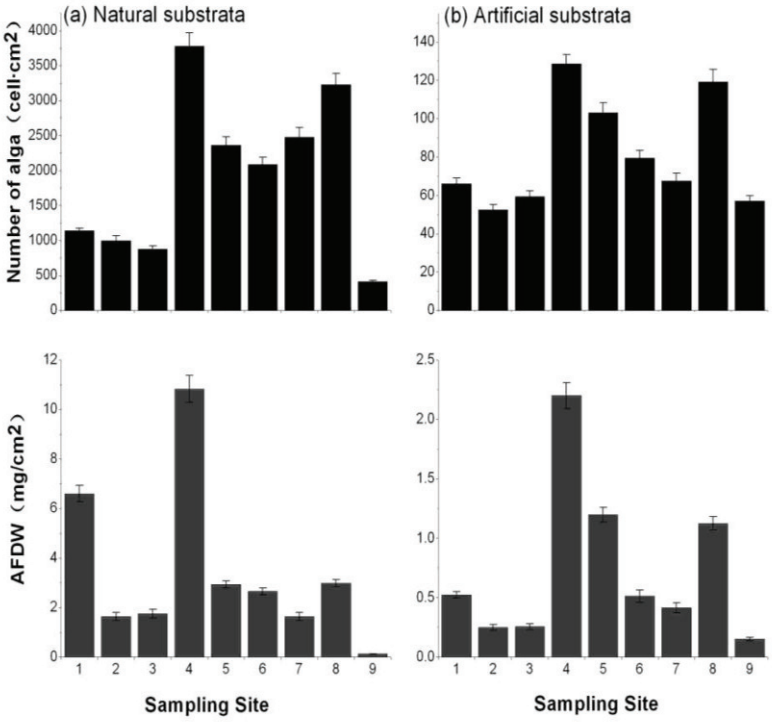

Fig.2 Biomass of biofilms formed on different substrata

Table1. Biomass parameters Pearson Correlations

	AFDW（a）	AFDW（b）	Number of alga（a）	Number of alga（b）
AFDW（a）	1	.811**	.589	.627
AFDW（b）		1	.858*	.933**
Number of alga（a）			1	.908**
Number of alga（b）				1

(a): Natural substrata; (b): artificial substrata.

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

3.2 Concentration of Chlorophy Ⅱ

Significant differences in Chlorophy Ⅱ concentrations were observed between biofilms formed on natural and artificial substrata ($p<0.05$, Paired-T test) with more phytoplankton in biofilms on natural substrata. But the significant relationships of Chlorophy Ⅱ concentrations between two substrata(Chla, r2=0.822; Chlb, r2=0.811; Chlc, r2=0.728; $p<0.05$) showed that the concentrations reveal the same trend in pigment level, as the Chlorophy Ⅱ concentrations of site 4 was the highest. The proportions of pigment show the contribution of diatoms, green algae, and cyanophyta, and indicate that that the green algae were dominant in the community. As pigment composition changes after brief exposure and can be used as a biochemical marker of toxic effects(Sabater et al. 2007). Fig.3 shows the details about the effects of toxicants at different site with various pigment level in Baiyangdian Lake.

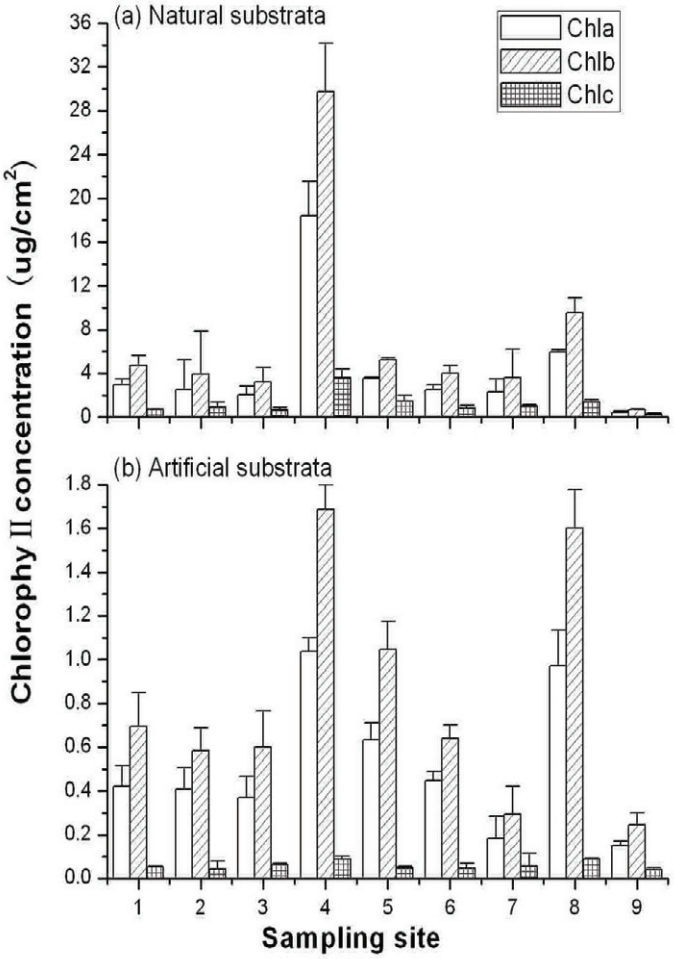

Fig.3 Chlorophy a, b and c concentration in biofilms formed on different substrata

3.3 Composition of Algal

Studying the alga composition it was pointed out that the community of biofilm is a diverse community, and more over, the green algae are dominant in Baiyangdian Lake while the diatoms are dominant in control site. Comparing the relative abundance of alga in Fig. 4, it is clear that the algological compositions were similar between the biofilms formed on artificial and natural substrata ($p>0.05$, Paired-T test), however, the compositions among site showed totally different ($p<0.05$, T test). In case of artificial substrate the presence of diatoms was 40% higher at the site 7 and 10% lower at the site 8. It can be concluded that the alga composition was strongly influenced by the sampling site. Communities changed their compositions to cope with toxicants, and to favor the growth of the most tolerant taxa. This effect might be detected at the large group level and sometimes at the species level(Sabater et al. 2007). So the difference of alga composition in biofilm both formed on artificial and natural substrata can be used to reflect the toxicant effects in Baiyangdian Lake.

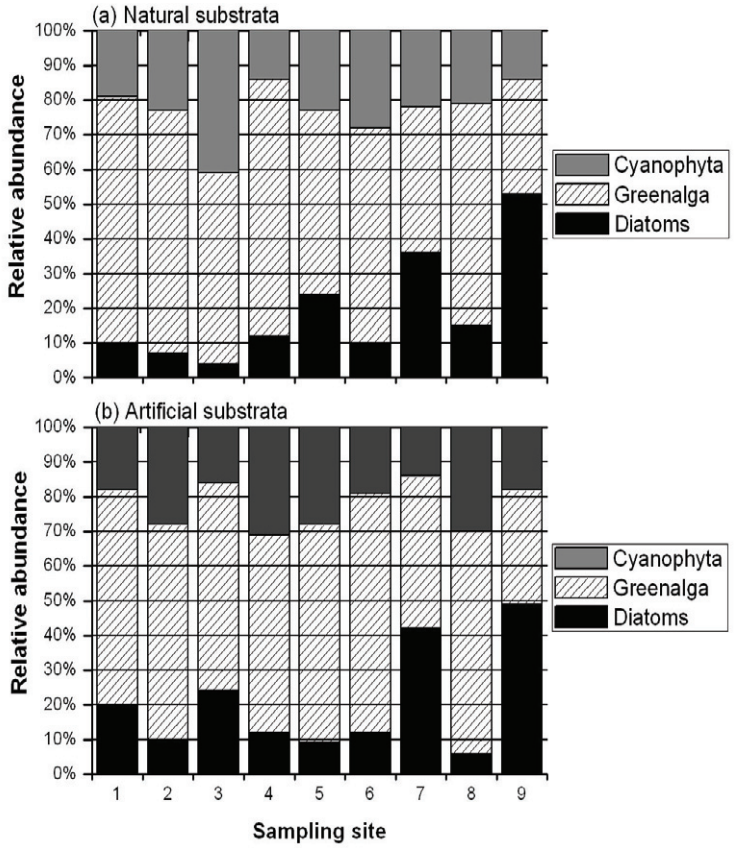

Fig.4 Relative abundance of alga species in biofilms formed on different substrata

3.4 Activities of Extracellular Enzyme

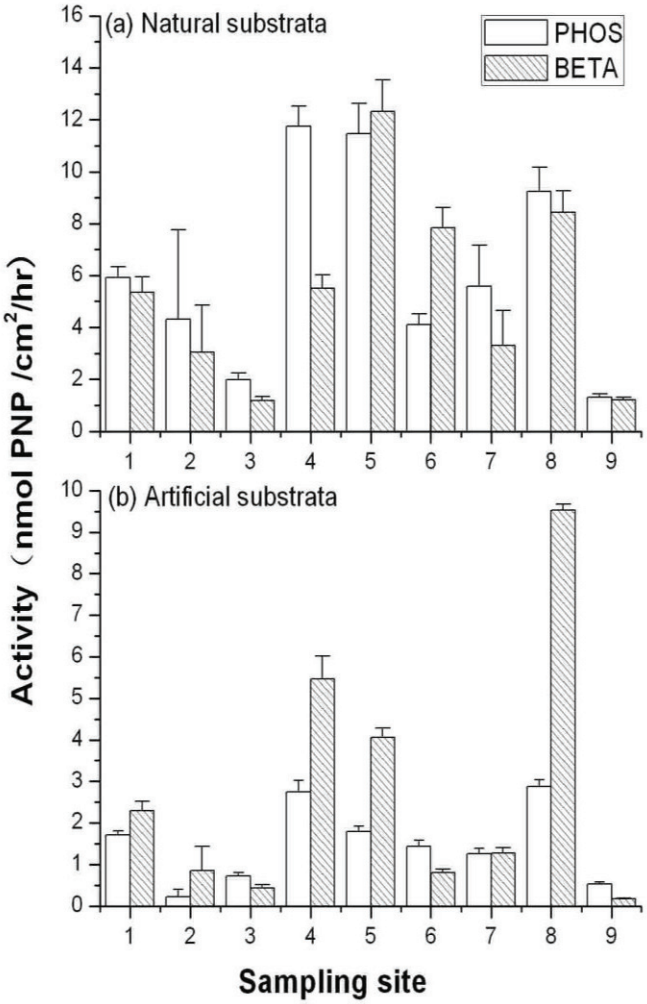

Fig.5 Extracellular enzyme activity in biofilms formed on different substrata

The extracellular enzyme activities shown in Fig.5 reveal that the bacteria communities between various sampling sites were significantly different. The activity of β-glucosidase (BETA) was higher than phosphatase (PHOS) in the biofilm on natural substrata except for site 6, and there was the highest activity of BETA at site 5 while the highest activity of PHOS at site 4. Conversely, the activity of PHOS was higher in the biofilm on artificial substrata, and both enzymes had highest activity at site 8. It can conclude that different substrata had significantly different influences on the composition of bacteria community. The activities of enzyme can be considered as indicators of the microbial potential of polymer degradation and metabolism in their respective environments(Denkhaus et al. 2007). It has been revealed that variations in hydrology and water chemistry at both regional and local scales are important controls on heterotrophic microbial activity(Webster et al. 1997).Differences among regions in extracellular enzyme activity imply that different classes of compounds mixture are being degraded and the pollution among regions are concentrated at the different levels.

3.5 Contents of Polysaccharides

Fig.6 display the concentration of polysaccharide content changing with different sampling site and biofilm substrata. The polysaccharide in biofilm on natural substrata was varying from 0.03 to 1.21 mg glu-eq/cm2, compared with artificial substrate varying from 0.003 to 0.14 mg glu-eq/cm2. The difference may be connected with difference of microorganism compositions and growing time on two substrata.

In contrast to planktonic microorganisms, biofilms secrete multiple EPS, which can make up about 50-90% of the total organic matter of biofilms. Polysaccharides are characteristic components of the EPS(Sutherland 2001).EPS determine the structural and functional integrity of microbial biofilms, and contribute significantly to the organization of the biofilm community(Branda et al. 2005).So it is necessary to study the polysaccharide contents of biofilm and the change of EPS. Polysaccharide content can be used as an indicator for monitoring pollutants' effects on biofilm.

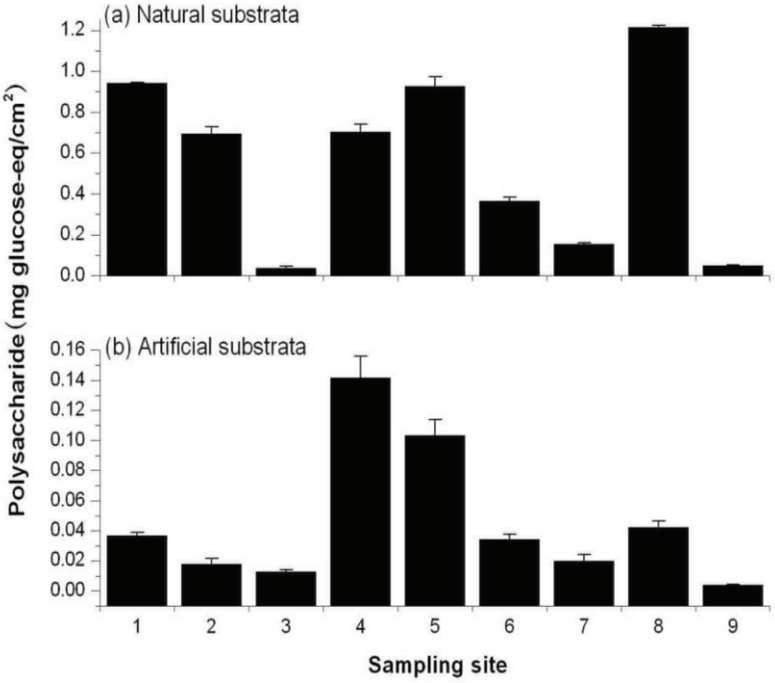

Fig.6 Polysaccharides content in biofilms formed on different substrata

3.6 Water quality and relative health assessment

Physico-chemical parameters found from the biofilm at different sampling sites are shown in Table 3. The heavy pollution was detected in the entrance of Fu River because of the input of sewage and industrial contaminants from the Baoding city. Wangkuai reservoir was the drinking water source and conservation area, so its water quality was much better and the reservoir was regarded as control site. On the basis of the water quality data and biofilm index data obtained for the investigated sites, Pairwise Pearson correlation analysis was carried out. Significant correlations were obtained between NH4-N and biofilm indicator, CODMn and biofilm indicator (Table 4). However, the biofilm indicators for water quality showed slight difference between the biofilm on artificial and that on nature substrata. It can be concluded that the nutrients and organic pollutants influenced the composition and function of biofilm, and the changes of biofilm indicators can be used to reflect the effects of pollutants.

Table2. Water quality parameters of sampling site

	Sampling site	T	PH	OD mg/L	NH4-N mg/L	CODMn mg/L	Fluoride mg/L	Arsenide mg/L	Hg mg/L	Cu mg/L
1	Wang jiazai	18.0	8.1	10.4	0.15	6.8	0.80	0.0084	0.000077	<DL
2	Zhao linzhuang	18.2	8.0	8.2	0.13	6.7	0.87	0.0021	<DL	<DL
3	Shao Chedian	17.6	8.4	8.2	0.12	6.5	0.91	0.0032	<DL	<DL
4	Fu river	19.5	8.0	4.9	16.70	8.9	0.97	0.0068	0.001947	<DL
5	Nan Liuzhuang	18.0	8.4	10.9	0.40	7.1	0.84	0.0067	<DL	<DL
6	Quan tou	18.1	7.9	5.4	0.09	7.1	1.06	0.0063	<DL	<DL
7	Cai putai	17.6	8.4	8.2	0.12	6.5	0.91	0.0032	<DL	<DL
8	Duan cun	18.5	8.1	9.5	0.13	7.4	0.93	0.0076	<DL	0.008
9	Wang kuai	18	8.1	9	0.05	2.1	0.33	<DL	<DL	<DL

Table3. Water parameters and Biofilm Indicator Pearson Correlations

Biofilm Index / Water Parameter / substrata	AFDW	Abundance of alge	Chla	Chlb	Chlc	PHOS activity	BETA activity	Polysaccharide

NH4-N	Artificial	.835**	.628	.647	.640	.623	.030	.332	.845*
	Nature	.845**	.610	.965**	.965**	.929**	.555	.524	.661
CODMn	Artificial	.661	.615	.754*	.728*	.613	.496	.509	.789*
	Nature	.662	.729*	.631	.627	.689*	.695*	.628	.638

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

In light of biofilms indicators (AFDW, abundance of alge, Chla, Chlb, Chlc, alga composition, extracellular enzyme activity and Polysaccharides content) on the different substrata, degree of deviation were calculated (Table 4)for different sampling sites in Baiyangdian Lake and compared with control site. The degree of deviation(D) was calculated as following formula.

$$Dj=\left(\sum \frac{Iji-Ci}{Ci}\right)/n$$

Where j is the sampling site number ; i is the biofilm parameter number; Dj is the degree of deviation site j; Iji is the biofilm parameter i of site j; Ci is the biofilm parameter i of control site; n is the biofilm index number.

Table4. Degree of deviation of Sampling site in Baiyangdian

Sampling Site / Substrata of Biofilm	1 Wang jiazai	2 Zhao linzhuang	3 Shao Chedian	4 Fu river	5 Nan Liuzhuang	6 Quan tou	7 Cai putai	8 Duan cun
Artificial	3.726	1.351	1.086	12.287	8.157	2.509	2.177	11.035
Nature	10.516	3.246	2.613	21.235	9.166	5.686	3.928	10.217

Comparisons among the degree of deviation of regions in Baiyangdian Lake, demonstrated that the relative water health of sites was in the following order: Fu river (S4) < Duan cun (S8) < Nan Liuzhuang (S5) < Wang jiazai (S1) < Cai putai (S7) < Zao lingzhuang (S2)< Shao Chedian (S3). The results of water health of Baiyangdian Lake obtained in this study was similar to those obtained in previous studies by using different methods(T Zhang 2010). As S4 and S2 were the pollutants input and output sites, respectively, the relative healths of S4 was the worst due to pollutant accumulation and S2 was better for self-purification of lake. S8, S5, S1, S7 were in main area of human activity and influenced by different levels of pollution, while S3 was protected as conservation area and showed a better health. Biofilm on both substrata can be used to reflect the relative health in Baiyangdian Lake, but the results from artificial substrata was more stable than those from nature substrata.

4. Conclusions

Biofilm biomass express the long-term effect of toxicants on communities; Chlorophyll concentration shows the details about the effects of toxicants in pigment level; the algal composition detected the effect at the large group level; extracellular enzyme activity of bacterial communities shown influences on the composition of bacteria community; and Polysaccharide content reflected EPS of biofilm which contribute significantly to the organization of the biofilm community. Simultaneously, significant correlations were obtained between water quality and biofilm indicator in our study. The indicators of biofilm measured in this study changed among site and can reflect toxic effects of different site. In this paper, it used the degree of deviation on basis of biofilm indictors to assess the relative water health of sites and obtained similar results to other methods in previous studies. The results indicate that the biofilm can provide information for pollution detection and ecological health assessment of water in the lake.

Small differences were detected in the biological characters of biofilm formed on different substrata, owing to the growing time and substrata surface, but biofilm on artificial and natural substrata reflected the similar situation of pollution in Baiyangdian Lake. Therefore, both substrata would be suitable for biomonitoring the pollution in Baiyangdian basin. However, if we want to extend our investigations to a large-scale detection about the effect of pollutants on the communities of the biofilm, the artificial substratum seems more advantageous since it is much easier to form standardized methods and to be extended. Therefore, the biofilm on artificial substrata is recommended for further biomonitoring investigations. Moreover, more studies should be done to: 1) further investigate the indicators of biofilm and select sensitive indicators at the different level; 2) study the methods to combine information from biofilm index to an integrated index; 3) select acceptable control site for health assessment; 4) modify and apply biofilm for monitoring at a lager scale.

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