Biofilms on the surface of gravels and aquatic plants in rivers and lakes with reusing reclaimed water

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Abstract Reuse of reclaimed water for the eco-environmental water has become an effective measure to resolve the urban water crisis. However, it still contains large amounts of contaminants which may significantly affect the aquatic ecosystems. Biofilm attached on the surface of multiple matrixes in rivers and lakes is one of the indicators for describing the health of aquatic ecosystem. In this article, we chose eight rivers and lakes as the sampling locations, including three kinds of reclaimed water with different treatment processes and natural water. The morphology, structure and component characteristics of biofilms attached on the surface of aquatic plants and gravels in eight water bodies were evaluated. The results showed that the biofilm on the matrix surface were typical porous media with basic skeleton composed of inorganic particles, microorganisms and algae. The pores were filled with the viscous extracellular polymeric substances (EPS) secreted by microorganisms. The biofilms contained calcite, quartz, illite, alkali feldspar, dolomite, clinocllore, goethite and inorganic salt, and approximately 50 % of these compositions are calcite and quartz. The concentration of EPS was 20.78–500.44 µg/mL, and that of the gravel biofilms was less than that in aquatic plants by 18.1–75.3 %. In these three reclaimed waters, 4–7 kinds of PLFA (phospholipid fatty acid) were detected in aquatic plants biofilms, more than one or two kinds than in gravel

biofilms. And the dominant PLFA were i15:0 and 18:0, the total contents of which were, respectively, 74.4, 62.9 and 85.9 % in the three kinds of reclaimed water. All biofilm samples contain major microorganisms such as *Pseudomonas*, *Acinetobacter* and *Comamonas*. This study is aimed to provide some support for assessing the health of river–lake ecosystem with biofilm indicator.

Keywords Reclaimed water · Rivers and lakes · Biofilm · Gravels · Aquatic plants

Introduction

Reclaimed water is usually reused for ecological maintenance of rivers and lakes in the world (Juanico and Friedler 1999; Huang and Wu 2008). Although the reclamation of water meets the discharge standard, it still contains a large amount of metal salts, organic pollutants, solid suspension particles, and microorganisms, which produce a serious impact on the aquatic ecosystems in the areas of reuse (Gan and Bai 2010). The most direct method for monitoring and evaluating the health status of the aquatic ecosystem is to monitor its biological status. The changes in biological communities in water can directly reflect on various chemical, physical, and biological factors and it also can reflect the health level of the aquatic ecosystem. Microorganisms in natural water contain bacteria, fungi, protozoa, metazoan and other species with characteristics of substantial amounts, various types, and fast propagation. It has become the important part of the aquatic ecosystem.

In fact, the majority of microbial organizations in water are not free. More than 90 % of them are in the state of biofilm attached on the bottom surface of solid-substrate (Costerton et al. 1999). Biofilms are composed of microbial

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population (bacteria, protozoa, and fungi), inorganic minerals, and organic polymer matrix (EPS and humus). Biofilms have high surface reactivity and large adsorption area on pollutants (Reichert and Wanner 1997). The process of coalescence, adsorption and degradation of pollutants in biofilms reflect the combination of physical, chemical and biological purification, which plays an important role in the process of water self-purification (Sergi et al. 2002). The structure and compositions of the biofilm reflect the community, structure and function of water microorganism in the long-term evolution. It could become the most effective strategy to monitor and evaluate the health status of aquatic ecosystem. However, a large number of investigations regarding biofilms are focused on sewage treatment (Picioreanu et al. 2001), drainage (Percival and Letal 1998) and other engineering fields. Due to natural biofilms living in the complex environment, the researches about the attached biofilms on the surface of pebbles and other sediments are limited to rivers (Qin 2008), lakes (Dong et al. 2005), wetlands and oceans (Baty and Metal 1996). The sampling methods of biofilm at present are usually focused on artificial inert matrix, and few reports with in on-situ sampling. Major water quality index of reclaimed water is between those of wastewater and natural water, which lead to special characteristics of attached biofilms in reclaimed water, but there are few reports about it so far (Liang et al. 2013). Now, some new methods, such as modern electron microscope, precise analysis and molecular biology have been developed quickly, and they make it possible to test the characteristics of biofilms.

Based on this study, the biofilms attached on aquatic plants and gravels in the natural and reclaimed water with different sewage treatment processes have been studied by the on-situ sampling. The morphology, structure and component characteristics of biofilms have been explored by environmental scanning electron microscope (ESEM), X-ray diffraction, PLFA biomarkers, polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE), and so on. It will provide a theoretical basis to assess the purification capacity and health status of aquatic systems.

Materials and methods

Sampling locations

The reclaimed water with three kinds of sewage treatment processes and natural reservoir bodies were chosen as the experimental subjects, including combinatorial process of deposition and sand filtration, traditionally activated sludge process and UF membrane filtration plus anaerobic-anoxic-

oxic process were chosen. Biofilms attached on the surface of gravels and aquatic plants were sampled and tested. The sampling locations and its information are shown in Fig. 1 and Table 1. The sampling points were all in the center of lakes or rivers; 500 mL water of each sample was collected and kept at constant temperature of -4°C .

Meanwhile, the water quality was tested based on water and exhausted water monitoring analysis method. The results in Table 2 revealed that the contents of nitrate, total nitrogen, total phosphorus, biochemical oxygen demand (BOD_5), chemical oxygen demand (COD_{Cr}), water-soluble phosphorus and bicarbonate in reclaimed water are approximately two times as high as that in surface water. Ammonia nitrogen is approximately five times higher than that in surface water; in contrast, the sulfate is only 1/5 of that in surface water. Therefore, the quality characteristics of municipal reclaimed water are among sewage water and natural water.

Collection and preparation of biofilm samples

Two kinds of biofilms samples were collected at each sampling location. One part of samples was used to examine surface morphology, and the samples were put in a ziplock and then transferred to the refrigerator in the laboratory as soon as possible. Ultrasonic vibration was used to detach the biofilms from the surface of the matrix. The suspension was divided into two parts. One part was used for PLFA and PCR-DGGE. Another part was used to analyze EPS compositions and inorganic chemical components.

SEM scanning

Biofilm samples were cut into small slices with the dimension of 0.6×0.6 cm, and then treated as following procedures: (1) fixation: the small slices of biofilms were fixed in 2.5 % glutaraldehyde solution, followed by the rinse with 0.1 M sodium phosphate buffer for three times (15 min for each rinse). After washing, the small biofilm slices were fixed in 1 % osmium tetroxide (OsO_4) for 3 h, and then rinsed with 0.1 M sodium phosphate buffer for three times (15 min for each). (2) Dehydration: the biofilm samples were dehydrated in ethanol at the concentrations of 30, 50, 70, 85 and 95 % for 5 min at each concentration condition, and then dehydrated in 100 % ethanol for three times (15 min for each). (3) Drying: carbon dioxide critical point drying was conducted through BAL-TEC CPD030. (4) Gold spray: gold spray-ion sputtering instrument BAL-TEC SCD005 was used to glide. (5) Photograph: scanning electron microscope FEIQUANTA200 was used for imaging. All images were acquired with magnification of $5,000\times$.

Fig. 1 Sampling locations. Labe ① is Yongdinghe Mencheng Lake; Labe ② is Yongdinghe Lianshi Lake; Labe ③ is Bahe River; Labe ④ is Honglingjin Park Lake; Labe ⑤ is South Hucheng River; Labe ⑥ is Taoranting Park Lake; Labe ⑦ is Qinghe River; Labe ⑧ is Aoyun Lake

X-ray diffractometer

Solid biofilms were ground uniformly and scanned by D8-Advance X-ray diffractometer. The polycrystal diffraction patterns were achieved. The basic scanning conditions were voltage of 40 kV, current of 40 mA, Cu as the target, and wavelength of 1.5406 Å. In order to determine the phase, the resultant maps were analyzed through Topas software.

EPS test

EPS is composed of proteins, polysaccharides, nucleic acids, uronic acids, lipids, humic acids and amino acids. Among them, extracellular proteins and extracellular polysaccharides are major components with the content of 70–80 % (Hong and Herbert 2002; Frølund et al. 1996). Phenol sulfuric acid method and Lowry method were used to test extracellular polysaccharides and extracellular proteins (Lowry et al. 1951; Nocker et al. 2007), respectively.

Diversity test of PLFA biomarkers

PLFA test was referred to the analytical methods described by Pennanen et al. (1999). The test processes included PLFA extraction from the biofilms, purification, methylation, gas chromatography–mass spectroscopic (GC–MS) analysis, biomass assessment, bacterial PLFA determination in biofilms and data analysis.

PCR-DGGE map test

The test processes were as follows (Xing et al. 2005): (1) microbial genomic DNA was extracted by chemical cleavage method after slight modifications, and the genomic DNA directly extracted from bacterial samples was purified according to previous descriptions (Sandhu et al. 2007). (2) The genomic DNA was purified using glass bead DNA gel extraction kit according to the manufacture's instructions (product number: SK111, Shanghai Sangon). (3) Genomic DNA was amplified by PCR. (4) DGGE maps of PCR amplified products were achieved by DGGE. (5)

Table 1 Sampling locations and reference

Water body type	Treatment process	Recharge water source	Names of river and lake	Area (m ²)	Matrix type	Reference	GPS
Natural water	–	Sanjiadian reservoir	Yongdinghe Mencheng Lake (SML)	0.67×10^6	Gravels	SMLS	N39°57′
					Aquatic plants	SMLP	E116°06′
			Yongdinghe Lianshi Lake (SLL)	10^6	Gravels	SLLS	N39°56′
					Aquatic plants	SLLP	E116°06′
Reclaimed water	Deposit and sand filtration process	Jiuxianqiao Reclaimed water works	Bahe (RJR)	158.4×10^6	Gravels	RJRS	N39°58′
					Aquatic plants	RJRP	E116°33′
		Honglingjin Park Lake (RJL)		0.16×10^6	Gravels	RJLS	N39°55′
					Aquatic plants	RJLP	E116°29′
	Conventionally activated sludge process	Gaobeidian Reclaimed water works	South Hucheng River (RGR)	0.77×10^6	Gravels	RGRS	N39°52′
					Aquatic plants	RGRP	E116°24′
		Taoranting Park Lake (RGL)		0.17×10^6	Gravels	RGLS	N39°53′
					Aquatic plants	RGLP	E116°22′
	Ultrafiltration ozone process (A ² /O)	Qinghe Reclaimed water works	Qinghe (RQR)	50.2×10^6	Gravels	RQRS	N40°01′
					Aquatic plants	RQRP	E116°21′
			Aoyun Lake (RQL)	1.2×10^6	Gravels	RQLS	N40°00′
					Aquatic plants	RQLP	E116°24′

SM Yongdinghe Mencheng Lake, *SL* Yongdinghe Lianshi Lake, *RJ* combinatorial process of deposit and sand filtration in Jiuxianqiao reclaimed water works, *RG* traditionally activated sludge process in Gaobeidian reclaimed water works, *RQ* ultrafiltration ozone process (A²/O) in Qinghe reclaimed water works, *R* rivers, *L* lakes, *S* gravels, and *P* aquatic plants

Table 2 Test results of water quality (mg/L)

Reference	NH ₄ -N	NO ₃ ⁻	TN	TP	BOD ₅	COD _{Cr}	SP	Ca ²	Mg ²	PO ₄ ³⁻	SO ₄ ²⁻	HCO ₃ ⁻	pH
SML	0.40	5.47	7.44	0.04	4.2	4	0.02	105	33.9	0.06	365	170	7.44
SLL	0.39	2.71	4.58	0.11	6.6	29.7	0.05	43.7	34	0.02	247	125	7.92
RJH	3.24	11.6	16.2	0.38	11.8	47.7	0.32	75	31.7	0.96	100	344	7.54
RJL	0.81	0.88	1.80	0.22	8.8	38.2	0.08	29.1	20.8	0.24	78.7	163	7.86
RGR	0.46	7.17	9.38	0.11	4	19.7	0.1	56.9	20.5	0.22	97.6	197	7.7
RGL	0.24	4.75	6.34	0.03	4.2	20.9	0.01	40.9	22.7	0.02	122	125	8.22
RQR	15.8	9.07	28.6	0.82	8.2	36.2	0.5	63.6	21.1	16.1	93.2	298	7.21
RQL	0.33	3.78	5.46	0.17	3.9	19.3	0.14	36.8	22	0.34	92.9	110	9.34

Recovery and sequencing of the bands; (6) Phylogenetic analysis: the sequences were input in the National Center for Biotechnology Information Webs (<http://www.ncbi.nih.gov>) when compared with the sequences in GenBank and the program, BLAST. The highest homology of the sequence and the high homology of the known species sequences as the references were downloaded. Phylogenetic analysis was completed by software Clustal X1.81 and Mega 3 through N-J method, and a phylogenetic tree was conducted (Kumar et al. 2004). Totally 1,000 duplicate

detections were used to validate the confidence of the phylogenetic tree by bootstrap (Felsenstein 1985).

Results and discussion

Biofilms surface formation characteristics

The formation of biofilms is highly correlated with hydrologic conditions, basement types, nutrition level, light

and other factors (Qin 2008). The proper basement types for the reproduction of bacteria in water include inert surface (gavels) and active surface (underwater part of aquatic plants). So the structural characteristics of biofilms were comprehensive performance under many factors. The structural characteristics of biofilms attached on the surface of gravels and aquatic plants are shown in Figs. 2 and 3, which exhibited that the biofilms attached on the surfaces of 16 kinds of matrix had obvious porosity as a kind of typical porous matrix, whose surface adsorbed a large number of particles including inorganic particles and organisms (rod-shaped bacteria, spherical bacteria, filamentous bacteria and ring bacteria) (Figs. 2, 3). The pores were filled with lots of EPS that was a viscous substance secreted by bacteria (Li et al. 2002). Because of the stickiness of EPS, the microbial communities and attachment were connected together to form stable network and heterogeneous porous structure. In addition, as shown in Fig. 3a–h, totally 2–5 kinds of fungi could be observed in the biofilms of aquatic plants and five kinds of fungi were from Aoyun Lake. Figure 2a–h reveals 1–2 kinds of fungi on the surface of gravels. When comparing (a), (c), (e), (g) with (b), (d), (f), (h) in Fig. 3, the content of algae in the biofilms of the lake is more than that of the river.

Biofilms inorganic components

Figure 4 shows the XRD maps (Aoyun Lake for example) of biofilms with X-ray, and Table 3 shows the results of quantitative analysis of inorganic components. It could be deduced that biofilms contain calcite, quartz, illite, alkali feldspar, dolomite, clinocllore, goethite, and inorganic salt. Calcite, quartz, illite, alkali feldspar, and dolomite were the major inorganic compositions in the biofilms, and the content of each substance was more than 10 %. Calcite and quartz revealed the highest contents and reached 50 %. Calcite was the most major component in the aquatic plant biofilms in the reclaimed and natural water with the contents of 31.30 and 43.21 %, respectively; while quartz was the most major component in the gravel biofilms with the contents of 23.85 and 35.54 %, respectively. Dolomite and clinocllore were the minor components of the biofilms, and the content of each substance was 5–10 %. Similarly, a small amount of goethite and inorganic salt was detected within 5 %.

Compared with the biofilms from the reclaimed water, natural water body and sewage, the major chemical elements in reclaimed water biofilms were similar to that in natural water biofilms and different from that in sewage, but the difference of biofilms between reclaimed water and natural water was minor elements and trace elements. The contents of chemical elements in biofilms from natural water body were determined by using inductively coupled

plasma emission spectrometer (ICO-AES) (Wang et al. 2002), and the result showed that Ca, Mg, K, Na, Al, Fe, and S were the major elements in biofilms; and Mn, Sr, Ti, P, and La were the minor elements; and Co, Cu, Pb, Li, B, Ni, Ce, V, Be, and Cr were the trace elements. Meanwhile, the content of iron oxide and aluminum oxide was higher than that of manganese oxide in biofilms in natural water (Dong et al. 2005, 2006). In addition, the contents of As, Ni and Zn in sewage biofilms were more than 30 % (Hu et al. 2005). This is mainly due to the degradation or absorption of heavy metal pollutants during sewage treatments and draining the reclaimed water into rivers and lakes, correspondingly resulting in the lower contents of As, Ni and Zn in reclaimed water than that in sewage (Hu et al. 2005). Furthermore, for the biofilms on the surface of gravels, there was no obvious rule for the content of calcite and quartz during the three treatment processes. However, for the biofilms on the surface of aquatic plants, the content of calcite in reclaimed water was lower than that in natural reservoir by 41.93, 9.05 and 32.84 %, respectively, while the content of quartz in reclaimed water was higher than that in natural reservoir by 33.77, 27.38 and 24.55 %, respectively.

Biomarker diversity of PLFA in biofilms

Different microorganisms contain different species and quantities of PLFA, especially some fatty acids in microbial cell membranes. Phospholipids can decompose rapidly after cell death and can represent the part activity of the cells. Therefore, PLFAs can be used as the biomarkers of microbial biomass and the indicators of the change in community structure (Pennanen et al. 1999). The results of PLFA biomarkers have shown in Fig. 5, which exhibited that the types and contents of the epiphytic microbial community in biofilms on the surface of different matrix from different water bodies were listed. The PLFA biomarkers in the biofilms contained i13:0, 14:0, i15:0, 15:0, 15:1 ω 1t, 16:1 ω 7c, 16:1 ω 13t, 18:2 ω 10t, 18:1 ω 9c, 18:1 ω 7t, 18:0, 18:2 ω 9t and 18:1 ω 5t. Generally, microbial species in the biofilms had 2–7 species. The species of PLFA in the biofilm on the aquatic plant surface were obviously more than those on the gravel surface. Similarly, the species on the aquatic plant surface generally also had 4–7 species; in contrast, the species on the gravel surface generally had 2–6 species. Compared with the biofilms microbial species in the sewage, the biofilms microbial species in the reclaimed water were obviously less. Similarly, 12 genera (species) of bacteria, 3 genera of fungi, 14 genera (species) of protozoa, 2 genera of metazoan, 2 genera of oligochaetes and crustaceans and 6 large groups among 35 species of microorganisms have been detected when the characteristics of biofilm microbial community in

Fig. 2 SEM images of biofilms on gravel surfaces. **a** SMLS, **b** SLLS, **c** RJRS, **d** RJLS, **e** RGRS, **f** RGLS, **g** RQRS, **h** RQLS. Labe ① is the bacilli in the shapes of the character; Labe ② is cocci; Labe ③ is algae; Labe ④ is the bacilli in the shapes of the word

Fig. 3 SEM images of biofilms on plant surfaces. **a** SMLP, **b** SLLP, **c** RJRP, **d** RJLP, **e** RGRP, **f** RGLP, **g** RQRP, **h** RQLP. Labe ① is the bacilli in the shapes of the character; Labe ② is cocci; Labe ③ is algae; Labe ④ is the bacilli in the shapes of the word; Labe ⑤ is mold fungi; Labe ⑥ is ring bacteria

Fig. 4 The XRD mapping of biofilm. **a** the gravel surface of RQL. **b** The plant surface of RQL

sewage were explored (Qiu and Ma 2005; Yu et al. 1990). The dominant bacteria in the sewage biofilm were ammonia-oxidized bacteria and nitrite-oxidized bacteria (FdZ-Polanco et al. 1996). The main reason of the above is that there are much more nutrients and pollutants in the

sewage than in the other two water bodies (Gan and Bai 2010). So the sewage can provide enough nutrients for microorganisms. Thus, fewer microorganisms in the reclaimed water biofilm were observed than in the sewage biofilm.

Table 3 Phase compositions and contents of attached biofilms from different water bodies and different matrix

Reference	Calcite	Quartz	Alkali feldspar	Illite	Clinocllore	Dolomite	Goethite	Inorganic salt
SMLS	20.82	27.97	23.67	14.57	7.31	5.25	0.41	0
SMLP	44.65	12.82	14.29	10.58	10.97	1.61	3.29	1.79
SLLS	19.88	35.1	24.14	10.16	8.68	1.38	0.11	0.55
SLLP	41.76	22.54	13.93	10.32	10.05	0.9	0	0.5
RJRS	19.72	15.32	30.17	10.91	8.55	12.86	1.56	0.93
RJRP	22.93	28.44	18.78	16.92	7.98	4.96	0	0
RJLS	32.18	23.43	16.97	15.77	9.3	1.44	0.25	0.65
RJLP	27.25	18.86	12.49	18.16	19.83	3.42	0	0
RGRS	25.46	18.57	14.63	7.25	6.47	26.45	0.64	0.53
RGRP	68.6	9.24	10.74	7.97	1.51	1.77	0.17	0
RGLS	28.48	19.29	20.38	11.88	7.07	12.23	0	0.67
RGLP	10	35.8	26.35	15.08	8.62	3.48	0	0.67
RQRS	5.21	38.33	29.97	11.15	11.24	2.25	1.14	0.72
RQRP	3.02	26.56	29.15	15.75	13.64	6.68	3.99	1.21
RQLS	20.91	28.14	30.52	10.17	6.53	2.92	0.14	0.68
RQLP	56.02	17.52	12.59	6.72	5.33	1.05	0.2	0.57

Fig. 5 The concentration of PLFAs from sixteen biofilms. *S* gravels, *P* aquatic plants

Besides, as shown in Fig. 5, i15:0 and 18:0 were the major PLFA in the biofilms from natural water and the reclaimed water with three kinds of wastewater treatment processes. The sum of both reached up to 74.4, 62.9, 85.9 and 72.8 %, respectively; while the biofilm from the reclaimed water with the combinatorial process of coagulation and sand filtration also contained the major PLFA 16:1ω7c with the content of 10.7 % and the biofilm of the reclaimed water with the traditionally activated sludge process contained the major PLFA 14:0 and i13:0 with the contents of 22.0 and 11.3 %, respectively. On the surface of gravels, the contents of i15:0 and 18:0 in the biofilm in the reclaimed water with the traditionally activated sludge process were lower by 51.8 and 26.3 % than those in the

biofilm in the natural reservoir water. However, no significant difference between other two kinds of reclaimed waters and natural reservoir water for the aquatic plant surface was observed. The contents of the i15:0 in three kinds of reclaimed water biofilms were higher by 24.9, 33.4 and 42.0 % than those in natural reservoir water. In contrast, the contents of 14:0 in three kinds of reclaimed water biofilms were lower by 66.3, 81.3 and 38.5 %, respectively. As for different kinds of wastewater treatment processes, a number of unique PLFA in the biofilm were detected. For example, 15:0 and 16:1ω13t were in the water biofilms with combinatorial process of coagulation and sand filtration. 15:1ω1t and 18:1ω5t were in water biofilms with the traditionally activated sludge process. However, 18:1ω7t was in the biofilm on the surface of aquatic plants.

Biofilms microbial community structure analysis with PCR-DGGE

Figure 6 showed the DGGE profiles after denaturing gradient gel electrophoresis for 16 kinds of DNA from the epiphytic microbial community in biofilms. It could find that each sample of epiphytic biofilms on the surface of different matrix was isolated with various electrophoresis strips after denaturing gradient gel electrophoresis analysis, DGGE bands of different biofilm samples revealed significant difference. The DGGE bands from the aquatic plant surface during the traditionally activated sludge process and the A²/O process were seven, and the DGGE bands from the gravel surface in natural reservoir water

Fig. 6 DGGE band pattern of 16S rDNA PCR from 16 biofilms. L1–L16 stand for SMLP, SMLS, SLLP, SLLS, RJLP, RJLS, RJRP, RJRS, RGLP, RGLS, RGRP, RGRS, RQLP, RQLS, RQRP, RQRS, respectively

from Yongdinghe Mencheng Lake were two. Figure 7 showed the phylogenetic tree of the dominant bacteria in different biofilm samples.

Moreover, as shown in Figs. 6 and 7, the number of microbial species in the biofilm of aquatic plants was more than that on the pebble surface. All biofilm samples had four kinds of strains such as *Pseudomonas* sp. CP1b, uncultured *Acinetobacter* sp. clone 3P-3-2-D21, *Acinetobacter* sp. 7-15 and *Comamonas* sp. 01xTSA12A_H10. Although different biofilm samples contained similar bands somewhat, the specificity of advantage bands existed. The dominant bacteria of the biofilm in reclaimed water during the combinatorial process of coagulation and sand filtration were *Acinetobacter* sp. 7-15 and uncultured *Cyanobacterium*; the dominant bacteria in water body biofilm during the traditionally activated sludge process were *Pseudomonas* sp. CP1b, *Pseudomonas fluorescens* strain CLW17, *Chryseobacterium taichungense* strain YNB68 and *Comamonas* sp. 01xTSA12A_H10. The dominant bacteria in the biofilm from water body during the UF membrane filtration plus anaerobic-anoxic-oxic process were uncultured *Acinetobacter* sp. clone SL35B-4, uncultured *Acinetobacter* sp. clone 402, uncultured *Acinetobacter* sp. clone SS38B062, uncultured *Acinetobacter* sp. clone JI71E112, and *Comamonas* sp. 01xTSA12A_H10. The dominant bacteria in the biofilms on the surface of different water bodies were also different. The dominant bacteria were uncultured *Acinetobacter* sp. clone 3P-3-2-D21, *Acinetobacter* sp. 2C56 and uncultured *Firmicutes bacterium* clone KWK6F.85. All of the above dominant bacteria are aerobic bacteria (Vestal

and White 1989), which suggested a quite consistent result between PCR-DGGE and PLFA. Meanwhile, the complexity of microbial community was much lower in the reclaimed water biofilm than in the sewage biofilm. Nitrification and degradation of organic materials were very important in the process of biological sewage treatments, which could result in the competition of dominant bacteria, ammonia-oxidized bacteria and nitrite-oxidized bacteria as well as the spatial distribution associated with short-range nitrification and denitrification, or simultaneous nitrification and denitrification processes (Fdiz-Polanco et al. 1996). This is mainly due to that the content of organics in sewage is higher and the organic compositions in sewage is more complex, which can provide sufficient nutrients to different microorganisms (Gan and Bai 2010). Therefore, there are much slower competition and much simpler spatial distribution between microorganisms in the reclaimed water biofilm than in the sewage biofilm. As a result, the types and content of microorganisms in the sewage biofilm are significantly more than those in the recycled water. Moreover, the longer history of natural water can result in a higher microbial evolution degree of water biofilm, thus leading to the production of protozoa in biofilms. On the other hand, there were no protozoa detected in the reclaimed water.

Biofilms organic components

The content of the extracellular proteins and extracellular polysaccharides in the attached biofilms from the surface of 16 kinds of matrix were shown in Fig. 8. First, the total content of EPS in the biofilms attached on the surface of gravels was 20.78–410.02 $\mu\text{g/mL}$, and that on aquatic plants was 77.63–500.44 $\mu\text{g/mL}$. So the former was less than the latter by 18.1–78.4 %. This may be due to that the portion surface of aquatic plants in water received more light that provided more energy for microbial photosynthesis and growth (Liang et al. 2013). Another possible reason should be that most of the particles in aquatic plants surface biofilm skeleton were large particles (Liang et al. 2013). So, the biofilm skeleton had a large number of pore channels that was beneficial to the transmission and absorption of nutrients and pollutants, which as a result accelerated the microbial decomposition of pollutants. In contrast, the microorganisms on gravel surfaces received less energy, and the dense pore structures were unfavorable to their growth and adsorption of pollutants. Therefore, the biofilms attached on aquatic plants had more EPS. Secondly, the total content of EPS of biofilms in reclaimed water treated by combinatorial process of coagulation and sand filtration was 121.89–339.13 $\mu\text{g/mL}$, and that by UF membrane filtration plus anaerobic-anoxic-oxic process was 84.02–500.44 $\mu\text{g/mL}$, and that by activated sludge

Fig. 7 Phylogenetic tree of predominant bacteria from 16 biofilms **Fig. 8** Histogram of EPS's concentration in the attached biofilms from different water. The upper part of the bar is extracellular proteins' concentration, the lower part of the bar is extracellular polysaccharides

process was 23.20–107.52 $\mu\text{g/mL}$. The total content of EPS of biofilms in nature water was 20.78–137.24 $\mu\text{g/mL}$. Meanwhile, on the surface of gravels, the total content of EPS in the biofilms was 111.2 % higher during the combinatorial process of coagulation and sand filtration, and 303.9 % higher during the UF membrane filtration plus anaerobic-anoxic-oxic process when compared with that in natural water; similarly, the total content of extracellular proteins was 32.8 % higher in the combinatorial process of coagulation and sand filtration and 11.3 % higher in the UF membrane filtration plus anaerobic-anoxic-oxic process when compared with that in natural water; the total content of extracellular polysaccharides was 243.4 % higher in the combinatorial process of coagulation and sand filtration and 798.6 % higher in the UF membrane filtration plus anaerobic-anoxic-oxic process when compared with that in natural reservoir water. In addition, on the surface of

aquatic plants, the total content of EPS in the attached biofilm was 174.2 % higher during the combinatorial process of coagulation and sand filtration and 260.0 % higher during the UF membrane filtration plus anaerobic-anoxic-oxic process when compared with that in natural water; the total content of extracellular proteins was 90.2 % higher during the combinatorial process of coagulation and sand filtration and 222.6 % higher during the UF membrane filtration plus anaerobic-anoxic-oxic process when compared with that in natural water; the total content of extracellular polysaccharides was 247.5 % higher in the combinatorial process of coagulation and sand filtration and 292.5 % higher in the UF membrane filtration plus anaerobic-anoxic-oxic process when compared with that in natural water. However, in the reclaimed water during the activated sludge process, the total content of EPS in the biofilms was lower than that in natural reservoir water. Generally, the contents of extracellular proteins, extracellular polysaccharides and EPS on the gravel surface revealed the reduction by 51.4, 18.8 and 39.3 %, respectively. Moreover, on the surface of aquatic plants, the contents of extracellular proteins, extracellular polysaccharides and EPS exhibited the reduction by 36.7, 6.8 and 20.7 %, respectively. This was mainly due to the reclaimed water treated with different processes. The combinatorial process of coagulation and sand filtration can improve the clearance rates of BOD and COD only up to 60 % and UF membrane filtration plus anaerobic-anoxic-oxic process can result in a removal rate of more than 80 % for BOD, the clearance rate of BOD of the activated sludge process was 90–95 % (Gan and Bai 2010). There are still much more nutrients and pollutants in the first two kinds of reclaimed water than the latter. So, two kinds of reclaimed water can provide enough nutrients for microorganisms that can secrete EPS. Thus, the total content of EPS of biofilms in reclaimed water treated by combinatorial process of coagulation and sand filtration and UF membrane filtration plus anaerobic-anoxic-oxic process both were higher than that of biofilms in reclaimed water treated by activated sludge process.

Besides, the content of 14:0 was decreased with the increase of total content of EPS in the river water biofilms and the aquatic plant surface biofilm. The content of 18:0 was increased with the increase in the content of extracellular polysaccharide and the content from 16:1 ω 7c was increased with the increase in the content of extracellular proteins in the lake water biofilm. However, whether other water bodies have similar change trend remain unknown. By comparing matrix surface characteristics of biofilms from different water bodies, the characteristics of the biofilm from the water subjected to the traditionally activated sludge treatment process have an obvious difference when compared with other two processes.

Conclusion

Biofilm was a typical porous matrix that absorbed a lot of particles such as inorganic particles, microbes (rod-shaped bacteria, spherical bacteria, filamentous bacteria and ring bacteria) and algae. The pores between these particles were filled with a lot of sticky materials such as EPS. The amount of algae in biofilm grown in lakes was larger than that in rivers. The visible microorganism in the biofilm attached on the surface of aquatic plants had 2–5 types, while 1–2 types were on the surface of gravels.

The proportion of calcite and quartz was approximately 50 % in the biofilm. The biofilm attached on aquatic plants in the reclaimed water and reservoir water had more calcite, which accounts for 31.30 and 43.21 %; while the biofilm on gravels had more quartz, which accounts for 23.85 and 35.54 %. The biofilms attached on the gravels had less EPS than those on aquatic plants, and so as extracellular polysaccharide and extracellular albumen.

The PLFA results have demonstrated that the biofilm microorganisms in the water were mostly bacteria; PLFA species in the biofilm were generally in the range of 2–7; PLFA in biofilm attached on aquatic plant was 4–7 types; and PLFA on gravel surface samples had 2–6 types. Dominant bacteria in the reclaimed water bodies were *Acinetobacter*, *Cyanobacteria aeruginosa*, *Pseudomonas fluorescens*, *Chryseobacterium* and *Comamonas*, while the dominant bacteria in natural water body were *Acinetobacter* and *Firmicutes*.

In conclusion, many advanced equipment and technology, such as ESEM, X-ray diffractometer, PLFA biomarkers and PCR-DGGE can make a fine test on the features of attached biofilms. Totally, the work has studied the morphology, structure and component characteristics of attached biofilms on the aquatic plant surface. Meanwhile, the gravel surface in eight kinds of water has also made meaningful research results. However, the work was only limited to the situ sampling to study the biofilm attached on the surface of water matrix, not the whole situation of the rivers and lakes ecosystem, which may be influenced by the background. Moreover, there is lack of the research methods for the treatments of reclaimed water. Whether the water is in a normal or excellent situation only can be judged by the attached biofilm. Therefore, it is necessary for us to make deeper research on the unique rivers and lakes.

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