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Comparative study of microbial community structure in different filter media of constructed wetland

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Abstract

Comparisons of microbial community structure, in eight filter media of zeolites, anthracite, shale, vermiculite, ceramic filter media, gravel, steel slag and bio-ceramic, were undertaken by analyzing the phospholipid fatty acid (PLFA) composition. A total of 20 fatty acids in the range of C_{11} to C_{20} were determined but only 13 PLFAs were detected in steel slag. They consist of saturated fatty acids, branched fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. The variation of fatty acids was revealed in the relative proportions of these fatty acids in different media. The aerobic prokaryotes were the predominant group in all media. The PLFA composition showed significant differences among the eight different media by Tukey's honestly test. It was found that steel slag was significantly different in the microbial community as compared to other filter media, probably due to its alkaline effluent. Steel slag alone is probably not a good choice of substratum in constructed wetlands. The principle components analysis (PCA) showed that zeolites, bio-ceramic, shale and vermiculite had a similar microbial community structure while steel slag and ceramic filter media were distinct from other media.

Key words: microbial community structure; phospholipid; filter media; biomarkers; constructed wetland; steel slag
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Introduction

In recent years, constructed wetlands have been widely used in the treatment of wastewater due to its simplicity, low cost and maintenance requirement (Haberl, 1999; Kivaisi, 2001). Filter media is one of the three essential parts of constructed wetland. In addition to providing physical support for plant growth and surfaces for biofilm growth, media promotes the sedimentation and filtration of pollutants. By choosing suitable filter media, high purification efficiency of constructed wetlands can be achieved. Laboratory column and batch experiments have been performed with zeolites to assess their sorption of ammonium nitrogen (Papadopoulos et al., 1996; Demir et al., 2002). Moreover, attention has been paid to the potential use of industrial byproducts in constructed wetlands and other wastewater-treatment systems. Drizo et al. (1997) performed studies on shale, a byproduct from the coal-mining industry, and reported that this material showed high P-sorption capacity as well as an adequate hydraulic conductivity. Another material is steel slag, which may also constitute a solid waste problem for the steel industry. Steel slag has been tested in batch and column experiments. These investigations showed that the material has strong potential for P-removal (Baker et al., 1998; Johansson, 1999).

Microorganisms play a vital role in the mineralization of organic matter. A diverse mixture of both aerobic and anaerobic bacteria is involved in the degradation of organic matter and cycling of nutrients in the constructed wetland system. Therefore, understanding the microbial communities in filter media of constructed wetland would be important. However, studies on filter media have been focused on removing the organic load and other contaminants from wastewater (Kietlińska, 2003; Korkusuz et al., 2005; Zhang, 2007). The information available on the microbial communities in different filter media is rather limited.

As an alternative to cultivation-based techniques, phospholipids fatty acid (PLFA) analysis is increasingly common in studies of microbial communities (Malik et al., 2008). Phospholipids are essential membrane components of all living cells. They are not found in storage products or in dead cells. Hence, PLFA can provide an estimate of the viable microbial biomass contained in a sample. PLFA analyses is based on the fact that different subsets of microbial communities differ in their fatty acid composition though few PLFAs can be considered to be

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absolute signature substances for a single species or even a specific group of organisms. They can provide indications of overall changes in major groups, such as fungi, actinomycetes, and other Gram-positive and Gram-negative bacteria (Amir et al., 2008). In a recent contribution, Jin and Kelley (2007) combined total PLFA identification of eukaryotes and prokaryotes with PCR-denaturing gradient gel electrophoresis (DGGE) to assess the microbial community diversity and composition in different types of constructed wetlands. The objective of this study was to investigate and compare the microbial communities in different filter media (zeolites, anthracite, shale, vermiculite, ceramic filter media, gravel, steel slag and bio-ceramic) of non-planted vertical flow constructed wetland using PLFA analysis. We hope to provide a reference to assist with the choosing of suitable media in constructed wetland.

1 Materials and methods

1.1 Set up of experiment

Eight PVC columns (diameter of 25 cm and height of 110 cm) were filled with filter media to a height of 100 cm to mimic non-planted vertical constructed wetlands. Influent wastewater was the mixture of water from East Lake, Wuhan and the sewage from the septic tank in front of the office building of Institute of Hydrobiology, Chinese Academy of Sciences. In order to supply the media surfaces in the columns homogeneously with the wastewater, perforated circular tubes for the inflow were mounted on the top. Treated wastewater was collected at the bottom of the column.

Each PVC column was filled with one kind of filter media. The media were as follows: gravel from Wuhan, Hubei Province; zeolites and anthracite from Gongyi, Henan Province; shale from Yichang, Hubei Province; vermiculite from Dalian, Liaoning Province; ceramic filter media from Zibo, Shandong Province; steel slag provided by Wuhan Iron and Steel Company and bio-ceramic from Jiangxi Province. The size of the granulated media was approximately 8–12 mm.

1.2 Operation of the experimental set-up

The PVC columns were operated from April 1, 2006 to May 30, 2008. The influent wastewater was pumped into the columns by a peristaltic pump at an inflow rate of 5–7 L/hr. The hydraulic loading rate was about 1000–2000 mm/day. The influent and effluent water of the columns were sampled once a week to investigate the treatment performance of the filter media (Zhang, 2007). The parameters COD, BOD_5, TN and TP were tested according to the Methods for Examination of Water and Wastewater (SEPAC, 2002), and the characteristics of influent wastewater are as follows (mg/L): COD 84.5, BOD_5 21.26, TN 12.53, TP 0.92.

1.3 Sampling

After 24 months operation period of the systems, the media samples were collected using a cylindrical corer. The media surfaces (0–10 cm) were divided into quadrants and the sampling points within each quadrant were randomly selected. Triplicate media were taken per quadrant, combined in a polyethylene bag and transported to the lab using cooler filled with ice. In the laboratory, samples were manually homogenized. From these homogenized samples, triplicate subsamples of 5 g were taken and placed in sterile 50 mL tubes for PLFA analysis.

1.4 PLFA analysis

Lipids were extracted according to the procedure of Bligh and Dyer (1959). Briefly, subsamples were extracted for 2 hrs in 19 mL of a one-phase extraction mixture containing chloroform:methanol:phosphate buffer (1:2:0.8, *V/V/V*). After centrifugation for 20 min at 2500 r/min, 5 mL chloroform was added, as was 5 mL buffer, the mixture was then swirled and left to separate overnight. Lipids were extracted the following day by centrifuging and filtering the supernatant through Whatman No. 2 filter papers. The chloroform layer was decanted and dried under N_2. Phospholipids were separated from neutral lipids and glycolipids on solid phase extraction columns, 0.50 g silica (Agilent Technologies, Inc., UK). The column was conditioned with 3 mL $CHCl_3$, and lipids were transferred to the column with four 150 μL aliquots of $CHCl_3$. Neutral lipids and glycolipids were eluted with 5 mL $CHCl_3$, followed by 5 mL acetone. Polar lipids were eluted with 5 mL methanol, and dried under N_2. Samples were then subjected to mild alkaline methanolysis by dissolving them into 1 mL of methanol: toluene (1:1) and 1 mL of 0.2 mol/L KOH, and then heating at 37°C for 15 min. Resulting fatty-acid methyl esters were extracted with two 2-mL aliquots of hexane after adding 2 mL of H_2O and 0.3 mL of 1.0 mol/L acetic acid. Hexane aliquots were combined and dried under N_2 at room temperature. Samples were then dissolved into 150 μL hexane. Samples were analyzed using a Shimadzu GC-2014C Gas Chromatograph equipped with a flame ionization detector, a 30-m WondaCAP 5 column (Japan). A 1-μL injection was analyzed at an initial temperature of 80°C, ramped to 160°C at 20°C/min, then ramped to 250°C at 4°C/min. Peaks were identified based on retention time using standards (Supelco Bacterial Acid Methyl Ester Mix 47080-U, Sigma-Aldrich, US) and the software (GCSolution) provided by the Shimadzu Company (Japan). The fatty acid nomenclature used is as follows: total number of carbon atoms: number of double bonds, followed by the position of the double bond from the methyl end of the molecule; *cis* and *trans* geometry are indicated by the suffixes *c* and *t*. The prefixes *a* and *i* refer to *anteiso*- and *iso*-branching position of hydroxyl (OH) groups are noted; numbers preceded by ω indicate the position of OH groups from the aliphatic end of the fatty acids; and *cy* indicates cyclopropane fatty acids.

1.5 Data analysis

SPSS 13.0 for windows was used to do one-way ANOVA and principle components analysis (PCA). For PLFA profiles, the mole percent of individual fatty acids was used in the analysis.

2 Results and discussion

2.1 Composition and relative content of PLFAs

PLFA analysis identified a total of 20 different fatty acids in samples, including saturated fatty acid (SATFA), branched-chain fatty acid, cyclopropane fatty acid, monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). The relative amounts of PLFA groups are shown in Table 1. High percentages of 16:0 fatty acids were determined in all samples. The even-numbered saturated PLFAs (31.31%–58.77%) and MUFAs (27.37%–45.70%) were present in greater amounts followed by the branched-chain PLFAs (5.97%–14.75%). The most dominant were saturated fatty acids 12:0, 16:0 and 18:0, branched-chain fatty acids i15:0, MUFAs 18:1ω9c, 18:1ω9t and 16:1ω9. The relative content of odd-numbered fatty acids (3.55%–7.50%) were low while the PUFAs and cyclopropane fatty acids were the lowest (< 5%). The PLFAs detected in steel slag were the fewest of only 13. More were detected in bio-ceramic (18), gravel and ceramic filter media (19). Others were detected 20 PLFAs. In general, the numbers of fatty acids present in the media showed variation, indicating the differences in the lipid contributing communities.

2.2 Community structure of eight different media

PLFA profiles can provide insight into the microbial community structure because of a relative abundance of certain PLFAs which differ considerably among specific groups of microorganisms. For example, although MUFAs can occur in both Gram-negative and Gram-positive bacteria, their relative contribution to the total PLFA content in Gram-positive bacteria is typically very small (e.g., < 20%). Thus, MUFAs can be used as general biomarkers for Gram-negative bacteria (Ratledge and Wilkinson, 1988). Characteristic fatty acids are usually biomarkers indicating different groups of microorganisms. For example, fatty acid 16:0 which is widespread in various types of microorganisms is often used to indicate the total microbial biomass (Salomonová, 2003). Cyclopropane fatty acids were suggested to be more indicative of aerobic bacteria than of anaerobic bacteria (Parkes and Taylor, 1983).

On the basis of the presence of biomarker fatty acids, Findlay et al. (1993) classified the microorganisms into four distinct functional groups, namely, microeukaryotes (polyunsaturated fatty acids), aerobic prokaryotes (monounsaturated fatty acids), Gram-positive and other anaerobic bacteria (branched fatty acids in the range of C_{14} to C_{16}), and SRB and other anaerobic bacteria (cyclopropane and branched fatty acids in the range of C_{17} to C_{19}). The relative distributions of different microbial groups in the media of the present study are shown in Fig. 1.

The media were found to contain different proportions of these four microbial groups. The aerobic prokaryotes were the predominant group in all media. The microbial group, microeukaryotes, varied from 2.63% (zeolites) to 4.46% (ceramic filter media). The proportions of aerobic prokaryotes group were found highest in shale (45.70%) and the lowest in steel slag (27.37%). The biomarker fatty acids of the microbial group, Gram-positive and other anaerobic bacteria, were 13.19% in anthracite of the highest and 5.97% in steel slag of the lowest. The signature fatty acids of group, SRB and other anaerobic bacteria, varied from 2.26% in steel slag to 6.90% in vermiculite. Groups aerobic prokaryotes, Gram-positive and other anaerobic bacteria, and SRB and other anaerobic bacteria in steel slag were all the lowest.

The mean values of the PLFA groups in these eight media were tested by Tukey's honestly significant difference (HSD) test. The differences between the means were found to be significantly different (Table 2), indicating the

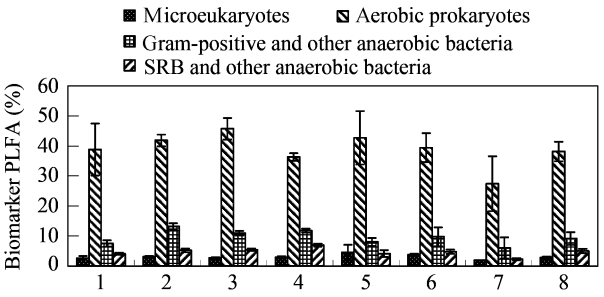

Fig. 1 Relative proportions of different microbial groups in media. (1) zeolites; (2) anthracite; (3) shale; (4) vermiculite; (5) ceramic filter media; (6) gravel; (7) steel slag; (8) bio-ceramic. Data are presented as means ± SD.

Table 1 Mean percentage and standard deviation of identified PLFA groups in eight filter media

PLFA group	Zeolites	Anthracite	Shale	Vermiculite	Ceramic filter media	Gravel	Steel slag	Bio-ceramic
Even numbered saturated	44.45 (11.44)	31.73 (1.73)	31.31 (2.86)	36.25 (2.98)	34.34 (6.29)	38.32 (5.88)	58.77 (15.31)	39.61 (4.08)
Odd numbered saturated	3.55 (0.78)	5.37 (1.47)	4.08 (0.71)	6.01 (0.68)	6.94 (2.32)	4.90 (1.25)	7.90 (5.91)	5.60 (1.12)
Branched	8.30 (1.07)	14.75 (1.12)	12.04 (0.83)	13.60 (0.77)	8.85 (1.67)	10.72 (3.24)	5.97 (3.52)	10.02 (2.20)
MUFA	38.76 (8.68)	41.80 (1.95)	45.70 (3.60)	36.26 (1.27)	42.68 (8.91)	39.39 (4.81)	27.37 (9.15)	38.13 (3.24)
PUFA	2.63 (0.65)	3.07 (0.21)	2.67 (0.20)	2.86 (0.33)	4.46 (2.57)	3.76 (0.32)	3.02 (0.00)	2.78 (0.34)
Cyclopropane	3.17 (0.35)	3.61 (0.51)	4.20 (0.33)	5.02 (0.36)	3.20 (0.83)	3.69 (0.63)	2.27 (0.34)	4.05 (0.59)
Number of PLFAs	20	20	20	20	19	19	13	18

Values are the means of three subsamples, with standard deviations given in parentheses.

Table 2 Analysis of significant difference for the different PLFA groups and microbial groups in eight media

	Low ⟶ High							
PLFA group								
Even numbered saturated	3^{a}	2^{a}	5^{ab}	4^{ab}	6^{ab}	8^{ab}	1^{b}	7^{c}
Odd numbered saturated	1^{a}	3^{a}	6^{a}	2^{a}	8^{a}	4^{a}	5^{a}	7^{a}
Branched	7^{a}	1^{ab}	5^{abc}	8^{bc}	6^{bcd}	3^{cde}	4^{de}	2^{e}
MUFA	7^{a}	4^{ab}	8^{ab}	1^{b}	6^{b}	2^{b}	5^{b}	3^{b}
Cyclopropane	7^{a}	1^{ab}	5^{ab}	2^{b}	6^{b}	8^{bc}	3^{bc}	4^{c}
Microbial group								
Microeukaryotes	1^{a}	3^{a}	8^{a}	4^{a}	7^{a}	2^{a}	6^{a}	5^{a}
Aerobic prokaryotes	7^{a}	4^{ab}	8^{ab}	1^{b}	6^{b}	2^{b}	5^{b}	3^{b}
Gram-positive and other anaerobic bacteria	7^{a}	1^{ab}	5^{ab}	8^{bc}	6^{bc}	3^{bc}	4^{c}	2^{d}
SRB and other anaerobic bacteria	7^{a}	1^{b}	5^{b}	6^{b}	8^{b}	2^{b}	3^{b}	4^{c}

1–8 are the same as that in Fig. 1. Values increased from left to right, superscripted with different letters (a–d) are significant difference at $p = 0.05$ level.

presence of significant differences in the microbial community structure. The odd numbered saturated PLFA did not have any difference among the media studied. The even numbered saturated PLFA were present in significantly high proportions in steel slag while MUFA, branched and cyclopropane PLFA were in significantly low proportions.

Similarly, the relative proportions of the microbial groups were also significantly different in the media (Table 2). The biomarker fatty acids of microeukaryotes were higher in ceramic filter media than other media but the differences were not significant, indicating the absence of a significant difference in the proportion of mecroeukaryotes. Biomarker fatty acids of aerobic prokaryotes, Gram-positive and other anaerobic bacteria and group SRB and other anaerobic bacteria were all significantly lower in steel slag, and the latter two groups showed almost the same trends among the eight media (Table 2). Steel slag, zeolites and ceramic filter media were of the lowest proportion while anthracite, shale and vermiculite were of the highest proportion. Gravel and bio-ceramic were in the middle.

These differences in the PLFA groups and the microbial groups revealed the significant variation in the microbial community structure of the eight filter media. Lehtola et al. (2004) found that the microbial community structure of copper biofilms was different from plastic (polyethylene, PE) by PLFA analysis in a pilot-scale water distribution system. The proportion of cyclopropane fatty acids was higher in copper than PE biofilms suggesting that in copper biofilms there were more Gram-negative bacteria in the stationary growth phase than in the PE biofilms. The authors suggested the reason was probably that phosphorus was released from the plastic pipes. Similar results were found in our study that microbial community structure was affected by the media type. As all the media were supplied with the same influent, such a significant variation in the microbial community structure is probably due to the differences in the media characteristics (Table 3). Media characteristics such as pH and water content are expected to control microbial community composition (Bååth and Anderson, 2003; Drenovsky et al., 2004). But it is difficult to correlate PLFA profiles to any of the media characteristics perhaps due to the implicitly confounding nature of PLFA data, as different microbial groups share common

Table 3 Characteristics of the eight media (Zhang, 2007)

Media	Porosity (%)	Permeability coefficient (m/day)	Water content (%)	Organic matter content (%)
Zeolites	41.8	13.7	10.1	5.32
Anthracite	32.6	135.3	7.57	15.66
Shale	50.7	9.2	14.99	0.81
Vermiculite	37.6	8.0	67.04	4.88
Ceramic filter media	40.7	113.4	8.78	7.21
Gravel	38.6	2.3	4.33	1.74
Steel slag	40.6	90.8	5.07	0.80
Bio-ceramic	38.9	99.5	19.62	0.41

fatty acids (Lacombe et al., 2009). Steel slag was especially different from other media. It had the fewest PLFAs and lowest proportion of three microbial groups. It was reported to have alkaline effluent of pH 11–12 while other media's effluents were 6.5–8.0 (Zhang, 2007). Previous studies found that several PLFAs were strongly correlated to soil pH and the fungal/bacterial biomass index tended to increase slightly with increasing soil pH (3.0–7.2) (Bååth and Anderson, 2003). Not only does pH appear to have a profound effect on the microbial community composition, but the changes due to pH were similar in very different soils and when very different causes of the pH gradient were studied. Most of the materials that trap P efficiently (Johansson, 1999; Agyei et al., 2002) were characterized by high pH values (9–12), which created an unfavourable environment for bacteria (Renman et al., 2004). Perhaps the alkaline environment in steel slag was not suitable for bacteria to survive, thereby the microbial community was significantly different from the other media. It was found that steel slag had strong potential for P-removal, but was not very good at removing other contaminants. It had the lowest removal efficiency of ammonia nitrogen (Zhang, 2007).

2.3 Ratios of characteristic fatty acid

Table 4 shows the ratios of characteristic fatty acids in different media. The sum of *iso* and *anteiso* fatty acids of 15:0 to 16:0 will give an indication of the proportions of the bacteria (Mancuso et al., 1990). These ratios ranged from 0.071 (steel slag) to 0.546 (anthracite), indicating an approximately 7.7-fold difference in the proportion of

Table 4 Ratios of characteristic fatty acids in substrate of constructed wetland

Media	(i+a15:0)/ 16:0	18:1ω9c/ 18:1ω9t	MUFA/branched PLFA
Zeolites	0.247 (0.033)	0.592 (0.045)	4.664 (0.935)
Anthracite	0.546 (0.035)	0.636 (0.016)	2.840 (0.154)
Shale	0.412 (0.020)	0.466 (0.046)	3.794 (0.118)
Vermiculite	0.419 (0.033)	0.628 (0.005)	2.669 (0.171)
Ceramic filter media	0.244 (0.040)	0.914 (0.908)	4.849 (0.819)
Gravel	0.272 (0.064)	0.583 (0.020)	3.762 (0.943)
Steel slag	0.071 (0.049)	0.312 (0.252)	5.615 (2.831)
Bio-ceramic	0.306 (0.079)	0.445 (0.027)	3.864 (0.635)

Values are the means of three subsamples, with standard deviations given in parentheses.

bacteria signatures. The PLFA profiles provide not only information on the community structure but also information on the metabolic status of microorganisms. The *trans* to *cis* ratios appear to indicate the stress experienced by the microorganisms. The ratios in most bacterial and sediment samples were found to be less than 0.1 (Gillan and Hogg, 1984; Guckert et al., 1985), but the ratio was greater than 1 during starvation (Mancuso et al., 1990). It has been suggested that the *trans/cis* ratio of MUFA may be useful as an index of stress for the determination of the nutritional status of bacteria in aquatic environments. In the present study, the *trans*/*cis* ratio of fatty acid in media ranged from 0.312 (steel slag) to 0.914 (ceramic filter media), suggesting that microbes are likely to be exposed to some physiological stress like starvation. From the results of PLFA composition in bacterial isolates in the reported results, the branched PLFA and MUFA can be used as the characteristic fatty acids of Gram-positive and other anaerobic bacteria, and aerobic prokaryotes, respectively. A ratio less than 1 will indicate the dominance of anaerobic bacteria, while a ratio above 1 will indicate the predominance of aerobic bacteria. These ratios were more than 1 in all the media indicating the relative dominance of aerobic prokaryotes. This is in agreement with earlier studies of integrated vertical constructed wetland (Wu et al., 2006). Physiological indicators are often used in studies of contaminated sites to provide insight into the potential for biodegradation. The method is valuable in such cases, because physiological information can be linked to changes in chemical parameters that may reflect microbial activities. Pfiffner et al. (1997) observed increased biomass with increasing concentrations of hydrocarbon contamination; they reported higher *trans/cis* and cyclopropane/MUFA stress ratios associated with the increased biomass.

The PLFA profiles (based on PLFA mole percentages) were analyzed using PCA (Fig. 2). The PCA plots of the first two principal components (PCs) accounted for 52.85% and 20.05% of the total variance. SATFA (11:0, 12:0, 13:0, 15:0, 18:0), branched PLFA (a15:0, i15:0, i16:0, i17:0) and cyclopropane PLFA (cy17:0, cy19:0) correlated more with PC1 ($r > 0.85$) while PUFA (18:2ω9) and MUFA (18:1ω9c) correlated more with PC2 ($r > 0.85$). Zeolites and bio-ceramic, shale and vermiculite had similar microbial community structure while steel slag and ceramic filter media were distinct from other media (Fig. 3).

In vertical subsurface flow constructed wetlands with intermittent hydraulic loading, the bulk of the microbial productivity (> 80%) takes place within the first 10 cm of the filter body (Tietz et al., 2008). Therefore, although the samples were collected from the surface of the media, they could represent the whole columns to some extent.

The purification performance of constructed wetlands is based on combined action between microbes and filter material, which may be complemented by plants. The elimination of easily degradable organic wastewater compounds, as well as nitrogen and phosphorus processes, is a consequence of a combination of chemical, physical and biological processes (Truu et al., 2009). Zhang (2007) has

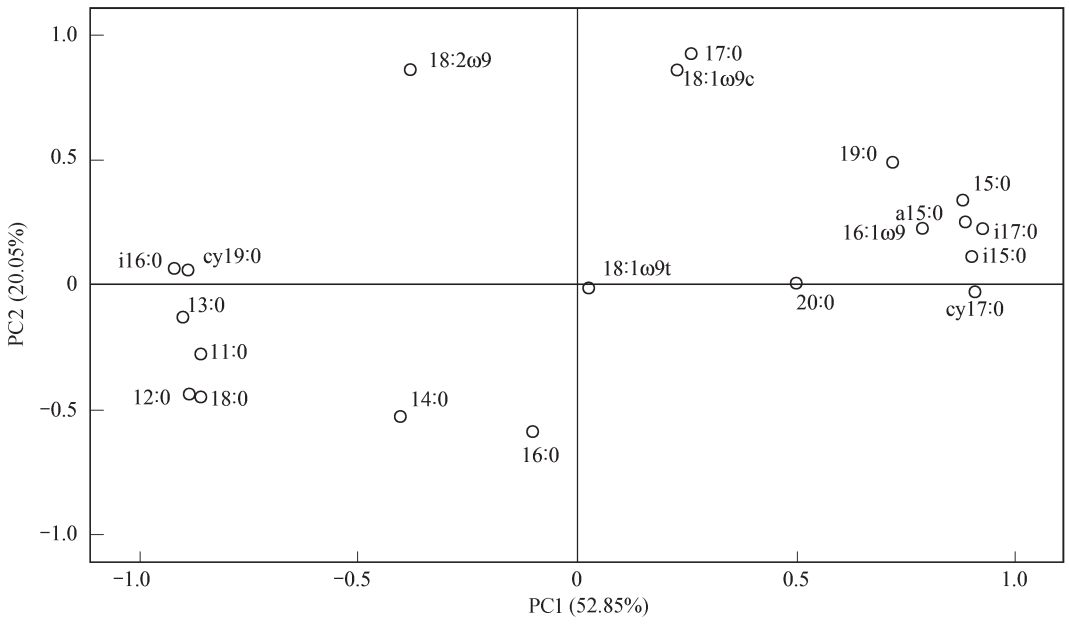

Fig. 2 Principal component analysis showing loading values for individual PLFAs.

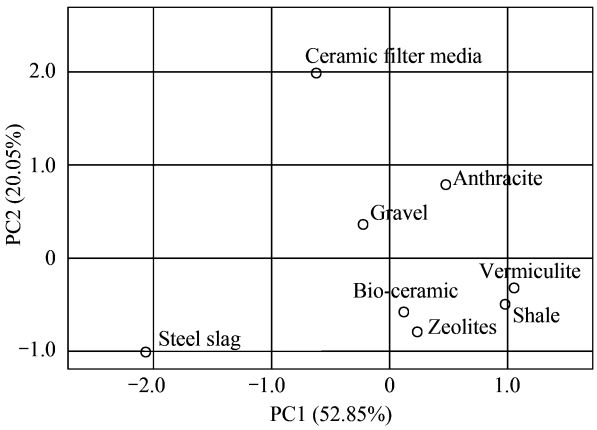

Fig. 3 Principal components analysis of PLFAs structures in the eight media.

studied the treatment performances of eight media. It was found that zeolite and ceramic filter media had the highest removal efficiencies of total nitrogen. However, our results were not in agreement with his findings. The purification capacities of the media did not correlate well with the microbial community structure. Moreover, the removal efficiencies of total nitrogen decreased over time (unpublished data) indicating the saturation of media. Therefore, it was probable that chemical and physical processes like absorption and sedimentation played a dominant role in purification in the systems, while the role of biological process was very limited.

In this study, phospholipid analyses have been performed in eight different filter media. Phospholipid fatty acid profiles provide information on the viable microorganism and insight into the structure of the microbial community. However, individual fatty acid cannot be used to represent specific species (a single microorganism can have numerous fatty acids and the same fatty acids can occur in more than one species) (Kirk et al., 2004). PLFA is a microbial community profiling tool that produces profiles of limited complexity, thus PLFA is often used for conjunction with other molecular profiling methods to assess microbial diversity in soil and water (Jin and Kelly, 2007). More approaches must be included to improve our understanding of microbial community in the future. This will allow for the identification of potential bottlenecks and problems, the development of novel high-rate treatment systems and ultimately, the increased efficiency of constructed wetland.

3 Conclusions

In summary, a total of 20 fatty acids in the range of C_{11} to C_{20} were determined but only 13 PLFAs were detected in steel slag. They consist of saturated fatty acids, branched fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids, and variation was revealed in the relative proportions of these fatty acids in different media. The aerobic prokaryotes were the predominant group in all media. The PLFA composition showed significant differences among the eight different media. The results of Tukey's honestly significant difference test further confirmed that steel slag was significantly different in microbial community when compared with other filter media, probably due to its alkaline effluent. As the transformation and mineralization of degradable organic pollutants is mainly performed by microorganisms, steel slag alone probably is not a good choice of substratum in constructed wetlands. It was found that zeolites, bio-ceramic, shale and vermiculite had similar microbial community structure while steel slag and ceramic filter media were distinct from other media by PCA analysis.

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