Microbial mechanism of organic contaminant removal of sodium bentonite/clay (BC) mixtures in high-permeability regions utilizing reclaimed wastewater

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Abstract

In high-permeability regions, utilizing reclaimed wastewater, sodium bentonite/natural clay (BC) mixes may be used to effectively reduce reclaimed wastewater riverbed infiltration; however, the removal efficiency of pollutants in riverbed media dramatically reduced. Microorganisms play vital roles in contamination transportation and transformation. Accordingly, the current study sought to investigate the microbial community of a riverbed medium and the removal of contamination using phospholipid fatty acid (PLFA) biomarkers. Results indicate that the local hydrological condition of the riverbed medium significantly changed after infiltration reduction using a mixture of sodium bentonite and clay (BC): saturation and non-saturation regions. It was difficult for nutrients and microorganisms to penetrate the infiltration reduction layer due to the high densification of the infiltration reduction layer, and the concentration of PLFA on the lower surface of the infiltration reduction layer was 82.0% less than that on the upper surface, and the species number of PLFA decreased by 6. The number and diversity of microorganisms also reduced in the riverbed medium due to the above reasons. Comparing with natural riverbed, in the non-saturation region of BC riverbed, the amount of PLFA and nitrifying bacteria was decreased by 49.0%, 19.1%, respectively. As to the saturation region of BC riverbed, the amount of PLFA and nitrifying bacteria was decreased by 39.8%, 31.4%, respectively. Besides, the amount of PLFA in both the non-saturation and saturation regions of the BC riverbed decreased in concurrence with an increasing sodium bentonite ratio. The findings may be used to explain the effect mechanism of infiltration reduction materials on the contamination removal efficiency of riverbed media.

Keywords Infiltration · Microbial mechanism · Groundwater contaminant removal · Reclaimed wastewater

1 Introduction

With faster development of urbanization, water requirements of living and industry in megacities grow fast and continuously, resulting in groundwater overuse, decreasing water tables, and increasing surface water infiltration rates, causing lack of eco-environmental water in most of

the urban rivers and lakes. Reusing reclaimed water as an unconventional water resource would be one effective and available solution to resolve this problem because the amount of reclaimed water is large and stable.

But for special locations, certain surface water landscape should be ensured, and damage of urban underground engineering should be prevented from enhancement of

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groundwater level. So, it is required that reducing water infiltration should be treated to urban rivers and lakes reused reclaimed water. Many researches showed that bentonite could not only reduce infiltration well, but also is good at purification of contaminants. Due to the expansivity and large positive ion-exchange capacity (CEC) of bentonite layer, bentonite and its modified productions own good adsorption ability to contaminants [21, 27, 32].

However, the removal rate of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, COD_{Cr} and BOD_5 in the riverbed medium after infiltration reduction using a mixture of sodium bentonite and clay was lower than that in the natural riverbed medium [28]. According to previous reports, the removal of contamination in soil mainly occurs via biodegradation [25]. Microorganisms (nitrifying bacteria, denitrifying bacteria, actinomyces, fungi) play an important role in removing N, COD_{Cr} and BOD_5 [13]. However, recent studies of stratified soil microbial communities were limited to the fields of farmland [6, 9, 10], rangeland [2], deserts [3, 15], and dry riverbeds [16, 17]. It is difficult to sample natural riverbed media in situ because the overlying water will damage the integrity and accuracy of natural riverbed media samples. Recent studies of riverbed media have primarily focused on removal of contamination from the soil column [12]. Although studies regarding the contamination removal mechanism have focused on the biological and abiological mechanism (precipitation, filtration, adsorption, ion-exchange, and redox) [4, 25], there are few reports on the contamination removal of riverbed media based on infiltration reduction using a mixture of sodium bentonite and clay, and studies related to the biological mechanism of contamination removal in riverbed media after infiltration reduction using a mixture of sodium bentonite and clay are rare. The development of modern molecular methods in biology makes it possible to systematically test the effect of infiltration reduction in the biological mechanism of the contamination removal in riverbed media.

Therefore, the microbial community in a riverbed medium after infiltration reduction was evaluated using PLFA biomarkers [26]. The effect of infiltration reduction, which was performed using sodium bentonite and clay, on the microbial community of the riverbed medium was analyzed via the ecology parameter. This study is of great significance for explaining the mechanism of infiltration reduction materials on the contamination removal efficiency of riverbed media.

2 Materials and methods

2.1 Experimental apparatus

Four 500 mm (inner diameter) poly-methyl methacrylate (PMMA) simulation columns were constructed for data

collection, i.e., one column per soil treatment approach. During testing, the experimental system was isolated from sunshine and sources of external heat, with a constant hydraulic head maintained in order to effectively simulate a natural riverbed. As shown in Fig. 1a, the constructed simulation system comprised four primary component parts, in addition to auxiliary materials as follows: Support System (Steel supporting frame, steel supporting base, waterproof rubber washer) and Water Supply System (5000-L storage tank, PVC water supply pipeline (2 cm inner diameter), water pump, 60-L Mariotte bottle) and Soil Column System (300 × 50 (ID) cm plexiglass soil column, tensiometers, porous clay pipes), and Outflow System (PVC protection box, soil water bottles, groundwater bottle). In order to simulate and elucidate “real-world” conditions more appropriately, a multi-aperture lysimeter (bore diameter, $d = 25$ mm) approach was employed to examine the effects of subsurface depth on contaminant concentrations.

2.2 Experimental materials and treatments

Riverbed media were collected from the dry riverbed at Zhongmen Temple Channel (N149°39'53"29.0" E 116°09'59.5") at a sampling depth of 1–3 m. The sampling location is immediately downstream from the Yongding River and is equivalent to the larger river in terms of media type, depth, and stratification. Large particles were removed by sieving (2 cm). Natural sodium bentonite was acquired from the Xuanhua bentonite mine (Miyun Yunfeng Earth and Stone Plant) in the Miyun prefecture, while clays were sourced from the Yan Village Soil Plant in the Mentougou district, Beijing. Nature sediment was sampled from Lianshi Lake (39°56'44"N 116°6'53"E), which is located on the Yongding River. Reclaimed wastewater was attained from the Qingyuan Water Reclamation Plant and conformed to current standards for environmental usage [11]. The primary physical characteristic of experimental materials is shown in Table 1.

Based upon results of a previous bench-scale study [31], BC treatment mixture ratios of 12% bentonite + 88% clay (BC12), 16% bentonite + 84% clay (BC16), and 20% bentonite + 80% clay (BC20) were employed as experimental treatments, with these experiments carried out at a constant hydraulic head height of 300 mm. Therefore, four soil treatment (BC12, BC16, BC20, and Nature sediment) approaches were undertaken (Table 2).

2.3 Collection of samples

Samples were collected from the soil column. As the system had run for 360 days, plexiglass device was removed.

Fig. 1 a Schematic of experimental soil columns with inset photograph of laboratory setup used in the current study (all units in mm); 1—storage tank, 2—PVC pipeline for water supplying, 3—water pump, 4—Mariotte bottle, 5—PVC protection box, 6—soil

water sample bottle, 7—groundwater collector, 8—porous clay pipe, 9—plexiglass soil column, 10—rubber washer, 11—steel supporting base, 12—tensiometer, 13—steel supporting frame; b structural composition of simulation column filling

Table 1 Primary physical characteristic of experimental materials

Materia	Coarse (%)	Medium (%)	Fine (%)	Super fine (%)	Coarse silt (%)	Dry weight (g/cm ³)	Initial moisture content (%)
Riverbed media	8.6	37.3	24.2	22.8	7.1	2.1	4.0
River mud	7.5	28.1	30.4	16.0	14.0	1.2	158.9
Bentonite	0.5	2.6	18.9	15.0	63.0	0.8	12.0
Clay	—	2.5	10.5	63.0	24.0	1.8	16.7

Then, samples were scrapped off by knife from the upper

Table 2 Soil treatment approaches

Infiltration reduction materials	Components of soil column	Hydraulic head (cm)
Nature sediment	6 cm Nature sediment +200 cm Original sand from riverbeds + 25 cm reverse filter	30
Bentonite–clay mixture	2 cm 12% bentonite-88% clay mixture +200 cm Original sand from riverbeds +25 cm reverse filter	30
	2 cm 16% bentonite-84% clay mixture +200 cm Original sand from riverbeds +25 cm reverse filter	30
	2 cm 20% bentonite-80% clay mixture +200 cm Original sand from riverbeds +25 cm reverse filter	30

Fig. 2 Sampling locations in soil column

and lower surface of the infiltration reduction layer, the non-saturation and the saturation region of riverbed (Fig. 2). Each soil sample (100 g) was placed in a Ziploc bag and transferred to a refrigerator (-20°C) as soon as possible. The number of samples is shown in Table 3.

2.4 PLFA biomarkers

PLFA biomarker tests were performed according to the analytical methods described by Pennanen et al. [18]. 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.

2.5 Ecological parameters of the microbial community

The test results of the PLFA biomarkers were used for calculating the microbial community polymorph sediment infiltration reduction layer ism parameters of the biofilms. The parameters were calculated using Shannon–Wiener's diversity index H [7, 14], Pielou's evenness index J [29, 30], and Simpson's dominance index D [23].

3 Results and analysis

3.1 Effects of reducing infiltration on the microbial community in the non-saturation region

The concentration and distribution of PLFA in the non-saturation region of the riverbed medium are shown in Figs. 3 and 4. In addition, the ecological parameters of the microbial community are shown in Table 4. As shown in Fig. 3, the concentration of PLFA in the non-saturation region of the riverbed medium was between 254.80 and 771.29 mg/kg, whereas after infiltration reduction using a mixture of sodium bentonite and clay, it was dramatically reduced compared to that in the natural riverbed. In particular, the concentration of PLFA in the non-saturation region of the riverbed medium was 30.6–67.0% lower than that in the natural riverbed when the bentonite content reached 12–20%. Moreover, the concentration of PLFA in the non-saturation region of the riverbed medium decreased with increasing bentonite content. The concentration of PLFA in medium containing 20% bentonite decreased by 34.7% and 52.4% compared with media containing 16% and 12% bentonite, respectively.

In Fig. 4, there were a total eight species of PLFA in the non-saturation region of four different riverbed media,

Table 3 Sampling locations and reference

Infiltration reduction materials	Sampling locations	Sampling depth in soil column	Reference
Nature sediment (NS)	Infiltration reduction layer		
	Upper surface (US)	0–0.3 cm	NS-US
	Lower surface (LS)	6–7 cm	NS-LS
	Riverbed		
	Non-saturation (NS)	56–57 cm	NS-NS
Bentonite–clay mixture (BC)	Saturation (SS)	186–187 cm	NS-SS
	Infiltration reduction layer		
	Upper surface (US)	0–0.3 cm	BC-US
	Lower surface (LS)	2–3 cm	BC-LS
	Riverbed		
	Non-saturation (NS)	52–53 cm	BC-NS
	Saturation (SS)	186–187 cm	BC-SS

Fig. 3 The concentration of PLFA in non-saturation region of riverbed. Note: BC12, 12% bentonite +88% clay; BC16, 16% bentonite +84% clay; BC20, 20% bentonite +80% clay

including *Gram-positive bacteria* i15:0, *pseudomonas* 16:0, *heavy pyrolysis hydrogen bacillus* 18:0, *actinomyces* 10 Me18:0, general bacteria 14:0, and fungi 16:2w4,6t, 18:2w6,9c, and 18:1w9t. The *heavy pyrolysis hydrogen bacillus* 18:0 was completely distributed in the non-saturation region of the four different riverbed media, and it was the dominant bacteria. In addition, the *Gram-positive bacteria*, *pseudomonas*, and *actinomyces* exert nitrification activity and are nitrifying bacteria [5, 22]. The content of nitrifying bacteria was 69.4%. There were less types of PLFAs in the non-saturation region of the riverbed medium that underwent infiltration reduction compared to the natural condition (three and four types, respectively). The nitrifying bacteria content exhibited the same pattern, with values that were 7.6–37.2% higher in the non-saturation region of the natural riverbed medium compared to that with 12–20% bentonite. Moreover, the content of nitrifying bacteria in the non-saturation region of the riverbed medium increased with increasing bentonite content.

Fig. 4 The distribution of PLFA in non-saturation region of riverbed. Note: BC12, 12% bentonite +88% clay; BC16, 16% bentonite +84% clay; BC20, 20% bentonite +80% clay**Table 4** Ecological parameters of microbial community in non-saturation region of riverbed

Reference	S	H	J	D
NS-NS	4	1.11	0.80	0.64
BC12-NS	4	1.00	0.91	0.61
BC16-NS	3	0.72	0.66	0.43
BC20-NS	3	1.02	0.93	0.61

S, number of species; H, Shannon–Wiener's diversity index; J, Pielou's evenness index; D, Simpson's dominance index; BC12, 12% bentonite +88% clay; BC16, 16% bentonite +84% clay; BC20, 20% bentonite +80% clay

The concentration of nitrifying bacteria in the medium containing 20% bentonite increased by 5.5% and 47.0% compared to those containing 16% and 12% bentonite.

As shown in Table 4, under different infiltration reduction conditions, the three ecological parameters (the Shannon–Wiener diversity index, the Pielou's evenness index, and the Simpson dominance index) in the non-saturation region of the riverbed medium exhibited various changes in range 0.72–1.11, 0.66–0.93, and 0.43–0.64, respectively. The diversity index and the dominance index of the microbial community in the non-saturation region of the riverbed medium that underwent infiltration reduction were lower compared to the natural condition, whereas the evenness index was greater. With increasing bentonite content, the three ecological parameters in the non-saturation region of the riverbed medium all initially decreased and then increased. Particularly, the three parameters were maximum when the bentonite content was 20%.

3.2 Effects of reducing infiltration on the microbial community in the saturation region

The concentration and distribution of PLFA in the saturation region of the riverbed medium are shown in Figs. 5 and 6. In addition, the ecological parameters of the microbial community are shown in Table 5. As shown in Fig. 5, the concentration of PLFA in the saturation region of the riverbed medium was between 93.97 and 250.47 mg/kg, whereas after infiltration reduction using a mixture of sodium bentonite and clay, it was dramatically less than that in the natural riverbed. In particular, the concentration of PLFA in the saturation region of the riverbed medium was 3.1–62.5% lower than that in the natural riverbed when the bentonite content reached 12–20%. Moreover, the concentration of PLFA in the saturation region of the

Fig. 5 The concentration of PLFA in saturation region of riverbed. Note: BC12, 12% bentonite +88% clay; BC16, 16% bentonite +84% clay; BC20, 20% bentonite +80% clay

Fig. 6 The distribution of PLFA in saturation region of riverbed medium. Note: BC12, 12% bentonite +88% clay; BC16, 16% bentonite +84% clay; BC20, 20% bentonite +80% clay

riverbed medium decreased with increasing bentonite content. The concentration of PLFAs in the medium containing 20% bentonite decreased by 18.7% and 61.3% compared to those containing 16% and 12% bentonite.

In Fig. 6, there were a total of two PLFA species in the non-saturation region of the four different riverbed media, which included *pseudomonas* 16:0 and *heavy pyrolysis hydrogen bacillus* 18:0. The *pseudomonas* is denitrifying bacteria due to exerting denitrification activity in hypoxic conditions [20]. The content of denitrifying bacteria was 76.4%. There were more types of PLFAs in the saturation region of the riverbed medium that underwent infiltration reduction compared to the natural condition (two and one types, respectively). However, there were less denitrifying bacteria in the saturation region of the riverbed medium that underwent infiltration reduction

Table 5 Ecological parameters of microbial community in saturation region of riverbed

Reference	S	H	J	D
NS-SS	1	0.00	–	0.00
BC12-SS	2	0.60	0.86	0.41
BC16-SS	2	0.66	0.96	0.47
BC19-SS	2	0.60	0.86	0.41

S, number of species; H, Shannon–Wiener's diversity index; J, Pielou's evenness index; D, Simpson's dominance index; BC12, 12% bentonite +88% clay; BC16, 16% bentonite +84% clay; BC20, 20% bentonite +80% clay

compared to the natural condition. The content of denitrifying bacteria in the saturation region of the riverbed medium was 28.3–37.7% lower compared to the natural riverbed when the bentonite content reached 12–20%. Moreover, the content of denitrifying bacteria in the saturation region of the riverbed medium initially decreased and then increased with increasing bentonite content. The content of denitrifying bacteria in the medium containing 16% bentonite decreased by 13.1% and 13.1% compared to those containing 12% and 16% bentonite.

As shown in Table 5, the diversity index and the dominance index of the microbial community in the saturation region of the riverbed medium that underwent infiltration reduction were higher compared to the natural condition because there was only one species of PLFA in the nature riverbed. In addition, under different infiltration reduction conditions, the three ecological parameters (the Shannon–Wiener diversity index, the Pielou's evenness index, and the Simpson dominance index) in the saturation region of the riverbed medium exhibited different changes in range 0.60–0.66, 0.86–0.96, and 0.41–0.47, respectively. With increasing bentonite content, the three ecological parameters in the saturation region of the riverbed medium all initially increased and then decreased. Particularly, the three ecological parameters in the medium containing 12% bentonite remained unchanged compared to the medium containing 20% bentonite.

3.3 Microbial communities in different surfaces of the infiltration reduction layer

The concentration and distribution of PLFA in the different infiltration reduction layers are shown in Figs. 7 and 8. In addition, the ecological parameters of the microbial communities are shown in Table 6. As shown in Fig. 7, the concentration of PLFA in the lower surface of the infiltration reduction layer was dramatically less than that in the upper surface of the infiltration reduction layer. In particular, the concentration of PLFA in the lower surface of the infiltration reduction layer (117.92–335.52 mg/kg) was 79.2–84.1% lower than that in the upper surface of the infiltration reduction layer (726.53–2102.89 mg/kg). After infiltration reduction, the remaining concentration of PLFA in the bentonite infiltration reduction layer was less than that in the sediment infiltration reduction layer. The remaining concentration of PLFA in the bentonite infiltration reduction layer was 7.4–64.8% lower than that in the sediment infiltration reduction layer when the bentonite content reached 12–20%. Moreover, the concentration of PLFA in the lower surface of the infiltration reduction layer decreased with increasing bentonite content. The remaining concentration of PLFAs in the medium containing 20%

Fig. 7 The concentration of PLFA in different infiltration reduction layers. Note: BC12, 12% bentonite +88% clay; BC16, 16% bentonite +84% clay; BC20, 20% bentonite +80% clay

Fig. 8 The distribution of PLFA in different infiltration reduction layers. Note: BC12, 12% bentonite +88% clay; BC16, 16% bentonite +84% clay; BC20, 20% bentonite +80% clay

bentonite decreased by 24.4% and 62.1% compared to those containing 16% and 12% bentonite.

As shown in Fig. 8, there were a total of 14 PLFA species in PLFA different infiltration reduction layers, including *Gram-positive bacteria* i15:0 and i16:0; *Gram-negative bacteria* 16:1w14t; *pseudomonas* 16:0; *heavy pyrolysis hydrogen bacillus* 18:0; general bacteria 14:0, 15:0, 16:1w7c, and 18:1w10t; and fungi 18:1w9c, 18:1w9t, 18:2w6,9c, 18:2w7,9c, and 18:2w3,9t. *Pseudomonas* 16:0 and *heavy pyrolysis hydrogen bacillus* 18:0 were the dominant bacteria. After infiltration reduction, there were less types of

Table 6 Ecological parameters of microbial community in different infiltration reduction layers

Reference	S	H	J	D
NS				
US	9	1.65	0.75	0.74
LS	4	1.16	0.84	0.64
BC12				
US	6	1.30	0.73	0.66
LS	2	0.67	0.97	0.48
BC16				
US	3	0.81	0.74	0.49
LS	2	0.66	0.96	0.47
BC20				
US	4	1.04	0.75	0.58
LS	2	0.50	0.72	0.32

S, number of species; H, Shannon–Wiener's diversity index; J, Pielou's evenness index; D, Simpson's dominance index; BC12, 12% bentonite +88% clay; BC16, 16% bentonite +84% clay; BC20, 20% bentonite +80% clay

PLFAs in the lower surface of the infiltration reduction layer than that in the upper surface of the infiltration reduction layer (2–4 and 3–9 types, respectively). In addition, for the lower surface of the infiltration reduction layer, there were less types of PLFAs in the bentonite infiltration reduction layer than that in the sediment infiltration reduction layer (two and four types, respectively). In addition, the *pseudomonas* content in the lower surface was 49.2% more than that in upper surface. The general bacteria content in the lower surface was 65.0% less than that in the upper surface. The fungi content in the lower surface was 94.5% less than that in the upper surface. Due to the decrease in the concentration and number of species, the diversity index and the dominance index of the microbial communities in the lower surface were less compared to the upper surface (diversity index of 1.06 and 0.88, dominance index of 0.55 and 0.54, for the upper and lower surfaces, respectively), whereas the evenness index was larger (0.73 and 0.88, for the upper and lower surfaces, respectively).

4 Discussion

Partial hydrological conditions might change significantly due to the use of sodium bentonite and clay as the infiltration reduction materials. Because the permeability coefficient of the infiltration reduction material is far lower than that of the riverbed medium, there exists an unsaturated zone under the infiltration reduction layer that is saturated on the bottom of the column [24]. This partial change in hydrological conditions may influence the microbial community of the riverbed medium, and further influence the

removal efficiency of contamination. Therefore, the paper studied the effect of infiltration reduction, which was performed using a mixture of sodium bentonite and clay, on the microbial community of a riverbed medium and the removal of contamination using phospholipid fatty acid (PLFA) biomarkers.

The results showed that after the disposal of reducing infiltration, both the concentration and types of PLFAs in the non-saturation region of the riverbed medium were less than that in the natural riverbed. Moreover, the microorganisms were mainly aerobic bacteria, facultative bacteria, and fungi. Because the medium was taken from a dry riverbed, the living conditions of the microorganisms in the non-saturation region did not significantly change despite the infiltration reduction. The non-saturation region provides an environment with sufficient oxygen for the growth of microorganisms [16, 17]. Aerobic microorganisms develop quickly by accumulating biomass and producing EPS, which leads to the enrichment of aerobic microorganisms at the surface of riverbed porous medium [19]. In addition, the sediments taken from the natural riverbed contain high amounts of TN, TP, TOC, and organic matter that provides nutrition for the growth of microorganisms, allowing for abundant microorganisms in the sediments. Nutrients and microorganisms are able to easily enter the riverbed medium. The infiltration reducing material is very dense, and the pores act as a filter due to the porosity of the medium. The main composition of bentonite is montmorillonite mineral, whose surface has an obvious sorption to the microorganism colloid and the necessary nutrients. Therefore, it is difficult for particulates and microorganisms in the upper recycled water to pass through the reducing infiltration layer, and few microorganisms attach to and grow in the riverbed medium [1, 8]. This study showed that after reducing infiltration, the PLFA concentration dropped to 117.92–335.52 mg/kg in the lower surface from 726.53–2102.89 mg/kg in the upper surface, which corresponds to a 79.2–84.1% decrease. There were six fewer types of PLFAs in lower surface compared to the upper surface. Because nutrients are adsorbed and intercepted by the reducing infiltration layer of the bentonite and it is difficult for upper microorganisms to pass through the reducing infiltration layer to reach the riverbed medium, after disposal of the reducing infiltration material, both the concentration and species of PLFA in the non-saturation region of the riverbed medium were less than that in the natural riverbed.

In addition, the PLFA concentration in the saturation region was also lower than that in the natural riverbed, which was made up of primarily facultative bacteria. In the natural riverbed, the non-saturation region is as thick as 4.9–35.5 m. However, after the reducing infiltration material is laid, the thickness reduces to less than 2 m because

the saturation region has come out, so part of the non-saturation region in the lower layer becomes part of the saturation region. This partial change in the hydrological condition results in changes in the moisture distribution. Water completely fills the space of the medium in the saturation region, which causes an apparent variation to the microorganism-living environment, corresponding to total oxygen to an extreme lack of oxygen, which makes it difficult for aerobic bacteria to grow. Therefore, instead of aerobic bacteria in the non-saturation region, facultative bacteria dominate the saturation region. The non-saturation region is characterized by an aerobic environment, which helps microorganism survival. With increasing depth of the medium, the nutrients are gradually consumed, and the nutrient content will become higher in the non-saturation region compared to the saturation region. This provides the possibility to form diversified microbial communities in the non-saturation region of the riverbed medium and causes all three ecological parameters of non-saturation region to increase compared to the saturation region.

The contents of microorganisms in the non-saturation region and the saturation region of the riverbed medium that underwent bentonite reducing infiltration disposal are both lower compared to the natural riverbed. In addition, there is a higher content of bacteria compared to fungi, so the bacteria content in the disposed medium is lower than that in the natural riverbed. The riverbed medium can eliminate COD_{Cr} and BOD_5 due to biodegradation by the bacteria [29, 30]. When recycled water passes the medium, the bacteria attached to the medium surface make complete contact with COD_{Cr} and BOD_5 , and the bacteria consume a large amount of COD_{Cr} and BOD_5 . When disposed by bentonite, the bacteria in the disposed medium are less than that in the natural riverbed, which leads to a smaller consumption of COD_{Cr} and BOD_5 . Therefore, the medium's capacity of eliminating COD_{Cr} and BOD_5 declines.

The elimination to N corresponds to $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$. The nitrobacterium transfers $\text{NH}_4^+\text{-N}$ to NO_2^- and then resolves NO_2^- to NO_3^- , via nitrification [5, 22]. The *Gram-positive bacteria*, *Gram-negative bacteria*, *pseudomonas*, and *actinomyces* all exert nitrification activity. Due to the partial change in the hydrological conditions after the infiltration reduction and the infiltration reduction layer's interception of microorganisms, the number of nitrobacterium in the non-saturation region of the disposed riverbed medium was smaller than that of the natural riverbed. In this situation, the nitrification in the medium is weakened, which causes a decrease in the elimination of $\text{NH}_4^+\text{-N}$ in the medium. The medium eliminates $\text{NO}_3^-\text{-N}$ mainly via denitrification. Due to denitrifying bacteria, some $\text{NO}_3^-\text{-N}$ is converted to organonitrogen compounds, the component of thallus. The

remaining part becomes N_2 , which is released. The facultative *pseudomonas* in the medium exerts denitrification activity [20]. Also due to the partial change in the hydrological conditions after the infiltration reduction and the infiltration reduction layer's interceptions of microorganisms, the number of facultative *pseudomonas* in the disposed riverbed medium is smaller than that in the natural riverbed, which reduces denitrification in the riverbed medium. However, during the elimination process, nitrification converts $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$, which enhances the difficulty of $\text{NO}_3^-\text{-N}$'s elimination. These two factors allow the riverbed medium following the infiltration reducing of bentonite to efficiently eliminate $\text{NH}_4^+\text{-N}$ while being unable to efficiently eliminate $\text{NO}_3^-\text{-N}$.

5 Conclusions

- The concentrations of PLFA in both the non-saturation and the saturation region of the riverbed medium were 30.6–67.0% and 3.1–62.5% lower, respectively, compared to those in natural riverbed when bentonite content reaches 12–20%. Moreover, the concentration of PLFA in both the non-saturation and saturation region of the riverbed medium decreased with increasing bentonite content. The concentration of PLFAs in the non-saturation region of the riverbed medium containing 20% bentonite decreased by 34.7% and 52.4% compared with those containing 16% and 12% bentonite. The concentration of PLFAs in the saturation region of the riverbed medium containing 20% bentonite decreased by 18.7% and 61.3% compared with those containing 16% and 12% bentonite.
- Due to the interception of microorganisms by the bentonite reducing infiltration layer and the partial change in the hydrological conditions after infiltration reducing, the concentration and species of microorganism decreased dramatically in the disposed riverbed medium. Therefore, the ability of the medium to eliminate COD_{Cr} and BOD_5 declined.
- The content of nitrifying bacteria in the non-saturation region of the dry riverbed was 19.1% less than that in the natural riverbed after bentonite reducing infiltration. The content of denitrifying bacteria in the saturation region of the dry riverbed was 31.4% less than that in the natural riverbed after bentonite reducing infiltration. Therefore, the removal efficiency of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ dramatically reduced in the disposed riverbed medium. In addition, the removal ratio of $\text{NO}_3^-\text{-N}$ was lower due to the $\text{NO}_3^-\text{-N}$ surplus from converting $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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