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Effects of filler height and mechanical ventilation on the treatment effect and bacterial community diversity of CRI

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ABSTRACT

This study was aimed at exploring the effect of filler height and mechanical ventilation on the treatment effect and bacterial community diversity of CRI (artificial rapid infiltration system), high-throughput sequencing technology was used to study the bacterial community diversity in three groups of CRI. The results showed that the average removal rates of chemical oxygen demand (COD), ammonia nitrogen, total nitrogen, total phosphorus and the concentration of nitrate nitrogen in the effluent of CRI were added by increasing the filler height. Adding mechanical ventilation could improve the removal rate of COD and total phosphorus and the concentration of effluent nitrate nitrogen, but reduced the removal rate of total nitrogen. The results of Chao1 index, observed species index, Simpson index and Shannon index all showed that increasing the height of filler increased the richness and diversity of bacterial communities in CRI, while increasing mechanical ventilation increased the richness and diversity of bacterial communities in the upper part of CRI, but decreased the richness and diversity of bacterial communities in the middle and lower parts of CRI. Increasing the filler height not only prolonged the contact time of wastewater with filler and its surface microorganisms, but also increased the average relative abundance of Nitrospirae at 0.8 m of CRI by 3.11%. Therefore, increasing the filler height is conducive to the removal of organic matter, phosphorus and nitrification in CRI. Increasing mechanical ventilation increased the average relative abundance of Proteobacteria by 4.81% and 13.86% at 0.3 and 0.8 m, and decreased the average relative abundance of Bacteroidetes by 2.34% and 4.59% at 0.8 and 1.3 m, respectively. Therefore, increasing mechanical ventilation is conducive to the removal of organic matter and phosphorus in CRI, but not conducive to denitrification.

Keywords: Filler height; Mechanical ventilation; Constructed rapid infiltration system; Nitrogen and phosphorus conservation; Bacterial diversity

1. Introduction

The shortage of irrigation water in arid areas of northwest China and the wanton discharge of rural domestic sewage have led to the gradual deterioration of water environment [1]. However, the contents of chemical oxygen demand (COD), suspended solids, some heavy metals and fecal coliform in the rural domestic sewage in this area exceed the standard, which cannot be directly used as agricultural irrigation water. Rural domestic sewage can be used as irrigation water after proper treatment, which can solve the problems of local agricultural irrigation resources shortage and water environment deterioration.

Anaerobic baffled reactor (ABR) has the characteristics of no aeration, low sludge yield, good removal of organic matter and suspended solids, and poor removal of nitrogen

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and phosphorus [2,3]. Taking ABR as the pretreatment process of rural domestic sewage irrigation and utilization can not only reduce the cost of sewage irrigation and utilization, but also eliminate the need for sludge disposal. However, the COD, suspended solids, some heavy metals and fecal coliform in the effluent of ABR sometimes exceed the irrigation water quality standard [4,5], so it is necessary to carry out follow-up treatment for the effluent of ABR. CRI has the characteristics of no aeration, no sludge discharge, less investment and good removal effect on organic matter and suspended solids [6,7]. By improving the CRI structure design, selecting the filler with poor adsorption effect on nitrogen and phosphorus and improving the operation conditions, the effluent can not only meet the water quality standard of farmland irrigation, but also fully retain nitrogen and phosphorus in the sewage, so as to reduce the amount of chemical fertilizer and improve the yield of crops.

At present, scholars at home and abroad have done a lot of research on the removal of organic matter and nitrogen and phosphorus by CRI [8–10], but there is no report on the removal of organic matter and nitrogen and phosphorus retention by CRI. In view of the above situation, three groups of CRI have been compared to study the effects of filler height and mechanical ventilation on CRI removal of organic matter, nitrogen and phosphorus retention and bacterial community diversity, so as to reveal the mechanism of filler height and mechanical ventilation enhancing CRI removal of organic pollutants and retention of nitrogen, phosphorus from a micro perspective.

2. Material and methods

2.1. Experimental device

Three groups of CRI were made of the same Plexiglass column with an inner diameter of 0.14 m; CRI1 was 1.1 m in height; The height of CRI2 and CRI3 was 1.6 m; Overflow ports were set at 0.05 m from the top of the column in three groups of CRI, 4 filling ports were set at 0.3 and 0.8 m from the top of the column in CRI1, and 4 filling ports were set at 0.3, 0.8 and 1.3 m from the top of the column in CRI2 and CRI3. The structure diagram and structure design of three groups of CRI system are shown in Table 1 and Fig. 1.

Table 1
CRI structure design

Height (m)	CRI1	CRI2	CRI3
0.1	Overflow height	Overflow height	Overflow height
0.4	Clay ceramsite	Clay ceramsite	Clay ceramsite
0.1	Gravel	Ventilation floor	Gravel
0.4	Coarse sand	Clay ceramsite	Clay ceramsite
0.1	Gravel	Ventilation floor	Gravel
0.4		Coarse sand	Coarse sand
0.1		Gravel	Gravel

2.2. Experimental water and methods

The experimental water was ABR effluent with elastic filler. The COD was 45.15~75.25 mg/L, the ammonia nitrogen was 29.12~41.30 mg/L, the nitrate nitrogen was 0.20~0.36 mg/L, the total nitrogen was 29.89~42.50 mg/L, and the total phosphorus was 2.45~3.76 mg/L. In the early stage, the start-up and operation parameters of the three groups of CRI were optimized. The results showed that the treatment effect of the three groups of CRI was the best when the hydraulic load was 1 m/d, CRI1 and CRI3 were intermittent influent (influent for 2 h, dry for 2 h) and CRI2 was continuous influent. After the operation of three groups of CRI was stable (the three groups of CRI had operated for 214 d), samples were taken from the water outlet and filler inlet of three groups of CRI for determination.

2.3. Analysis items and measurement methods

COD: potassium dichromate method; ammonia nitrogen: Nessler's reagent spectrophotometry; nitrate nitrogen: ultraviolet spectrophotometry; total nitrogen: ultraviolet spectrophotometry; total phosphorus: molybdenum antimony spectrophotometry.

2.4. High-throughput sequencing analysis method

(1) Sample collection

After the three groups of CRI operated stably, take three parallel samples from the filling port of the three groups of CRI. CRI1 (0.3 m, that is, 0.3 m from the top of the column, the same below), CRI1 (0.8 m), CRI2 (0.3 m), CRI2 (0.8 m), CRI2 (1.3 m), CRI3 (0.3 m), CRI3 (0.8 m) and CRI3 (1.3 m) were named as a(a1,a2,a3), b(b1,b2,b3), c(c1,c2,c3), d(d1,d2,d3), e(e1,e2,e3), f(f1,f2,f3), g(g1,g2,g3) and h(h1,h2,h3).

(2) DNA extraction and detection
(3) PCR amplification

The 16S rRNA gene of bacteria was amplified by universal primers. The name and sequence of primers were: upstream (338F and ACTCCTACGGGAGGCAGCA), downstream (806R and GGACTACHVGGGTWTCTAAT).

(4) PCR products were quantified and mixed
(5) Library construction
(6) Quality control and quantification of library
(7) Sequencing

The sequencing region was 16S V3-V4 region of standard bacteria.

3. Results and discussion

3.1. Removal effect of COD

The removal effect of three groups of CRI on COD is shown in Fig. 2. It can be seen from Fig. 2 that the influent COD did not meet the irrigation water quality standard of all crops (COD is 60 mg/L). The average COD removal rate

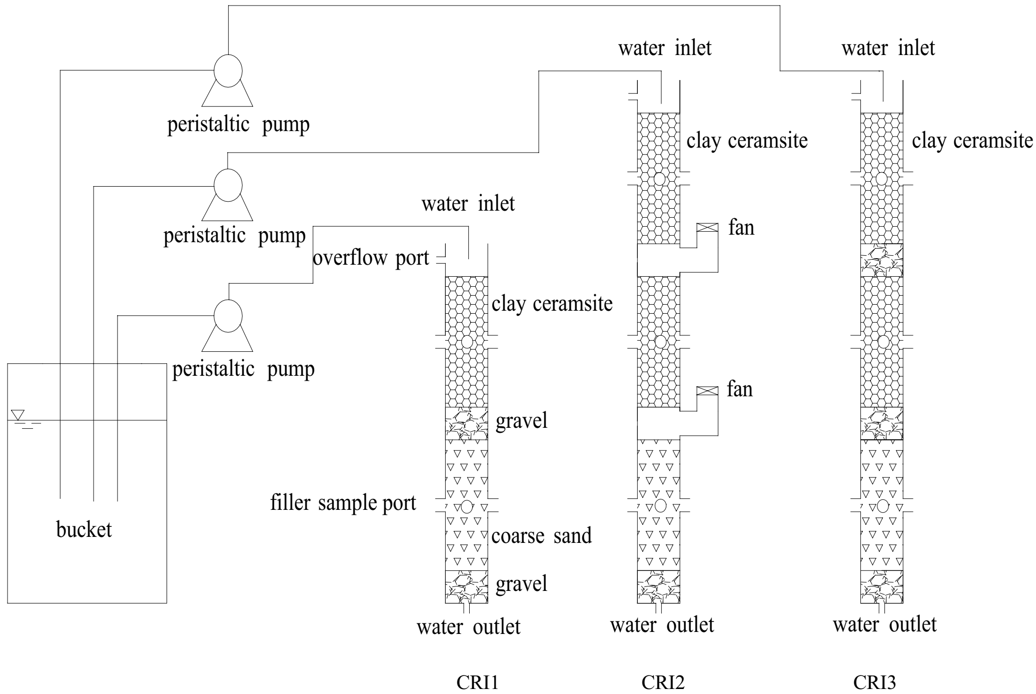

Fig. 1. CRI system structure diagram.

of three groups of CRI was as follows: CRI2 (55.12%) > CRI3 (51.91%) > CRI1 (48.14%). This was mainly due to the lowest effective height of CRI1, the shortest contact time between sewage and CRI1 filler, and the lowest COD removal rate because some organic matters flowed out of the system without filtration, adsorption and micro biodegradation. The effective height of CRI3 was higher than that of CRI1, the contact time between sewage and the filler in CRI3 was longer than that of CRI1, and more organic matters were filtered, intercepted, adsorbed and degraded by the filler, so the COD removal rate of CRI3 was higher than that of CRI1. The effective height of CRI2 was the same as that of CRI3, and two fans were installed in CRI2. The mechanical ventilation improved the dissolved oxygen in CRI2, so the COD removal rate of CRI2 was the highest, which indicated that increasing the filler height and mechanical ventilation was conducive to improving the COD removal rate of CRI.

3.2. Removal effect of ammonia nitrogen

The removal effect of three groups of CRI on ammonia nitrogen is shown in Fig. 3. It can be seen from Fig. 3 that the average ammonia nitrogen removal rate of three groups of CRI was as follows: CRI2 (98.72%) > CRI3 (97.90%) > CRI1 (63.60%). This was mainly because CRI1 had the lowest effective height, the shortest contact time between sewage

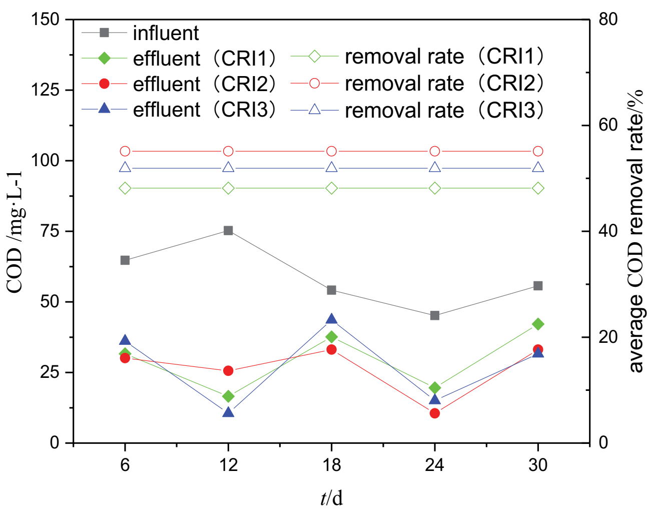

Fig. 2. Removal of COD by CRI.

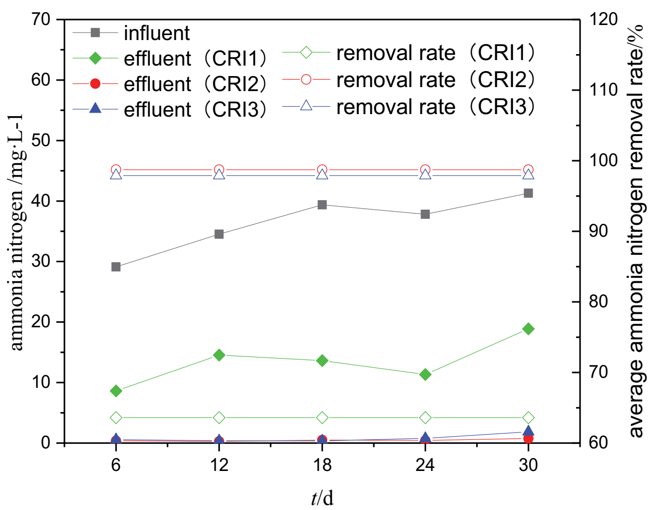

Fig. 3. Removal of ammonia nitrogen by CRI.

and CRI1 filler, and the ammonia nitrogen in the influent had flowed out of the system before being fully absorbed by the filler and utilized by nitrifying bacteria, so the ammonia nitrogen removal rate was the lowest. The effective height of CRI3 was higher than that of CRI1, the contact time between wastewater and the filler in CRI3 was longer, and the ammonia nitrogen in the influent could be fully absorbed by the filler and utilized by nitrifying bacteria, so the ammonia nitrogen removal rate of CRI3 was higher than that of CRI1. Mechanical ventilation created a good aerobic environment for CRI2, promoted the growth and reproduction of nitrifying bacteria, and make nitrification the strongest, so the removal rate of ammonia nitrogen was the highest.

In conclusion, increasing the height of filler and mechanical ventilation could improve the ammonia nitrogen removal rate of CRI, but the contribution of mechanical ventilation to improving the ammonia nitrogen removal rate of CRI was low, which was mainly because increasing the height of filler had greatly improved the ammonia nitrogen removal rate of CRI (the average ammonia nitrogen removal rate had increased from 63.60% to 97.90%), increasing mechanical ventilation has limited effects on improving the ammonia nitrogen removal rate of CRI.

Although increasing the height of filler could greatly improve the ammonia nitrogen removal rate of CRI, ammonia nitrogen was only converted into nitrate nitrogen by nitrification of nitrifying bacteria, which indicated that increasing the height of filler could only change the form of nitrogen in the influent, but cannot remove it. Plants mainly require ammonia nitrogen and nitrate nitrogen to grow, and the fertilizer takes effect faster and is more obvious when nitrate nitrogen is applied in dry land with cold climate (such as in arid areas of northwest China) [11]. Therefore, as long as further denitrification in CRI is prevented, the total nitrogen in wastewater can be retained.

3.3. Removal effect of nitrate nitrogen

The removal effect of three groups of CRI on nitrate nitrogen is shown in Fig. 4. It can be seen from Fig. 4 that the influent nitrate nitrogen was 0.20~0.36 mg/L. Compared with the influent, the effluent nitrate nitrogen of the three groups of CRI increased significantly, and the average nitrate nitrogen of the three groups of CRI from large to small was: CRI2 (32.64 mg/L) > CRI3 (31.67 mg/L) > CRI1 (20.09 mg/L), which was mainly because CRI1 had the lowest effective height, the shortest contact time between sewage and the filler in CRI1, and more ammonia nitrogen had already flowed out of the system before it was oxidized into nitrate nitrogen. Therefore, the effluent nitrate nitrogen of CRI1 was the lowest. The effective height of CRI3 was higher than that of CRI1, the contact time between wastewater and filler in CRI3 was longer, and the ammonia nitrogen in the influent could be fully transformed into nitrate nitrogen by nitrifying bacteria, so the nitrate nitrogen in the effluent was higher than that of CRI1. Mechanical ventilation created a good aerobic environment for CRI2, promoted the growth and reproduction of nitrifying bacteria, and made its nitrification the strongest. Moreover, mechanical ventilation destroyed the anaerobic denitrification environment in CRI2, making the denitrification degree in CRI2 lower than that in CRI3, so the effluent nitrate nitrogen was the highest.

In conclusion, increasing the height of filler and mechanical ventilation was beneficial to improve the effluent nitrate nitrogen of CRI, but the contribution of mechanical ventilation to improving the effluent nitrate nitrogen of CRI was low, which was mainly because increasing the height of filler had greatly improved the effluent nitrate nitrogen of CRI (the average effluent nitrate nitrogen was increased from 20.09 to 31.67 mg/L), Increasing mechanical ventilation was limited to improve the effluent nitrate nitrogen of CRI.

3.4. Removal effect of total nitrogen

The removal effect of three groups of CRI on total nitrogen is shown in Fig. 5. It can be seen from Fig. 5 that the influent total nitrogen was 29.89–42.50 mg/L. The average removal rate of total nitrogen from the three groups of CRI from large to small was: CRI3 (12.23%) > CRI2 (9.78%) > CRI1 (8.50%), which was mainly because the effective height of

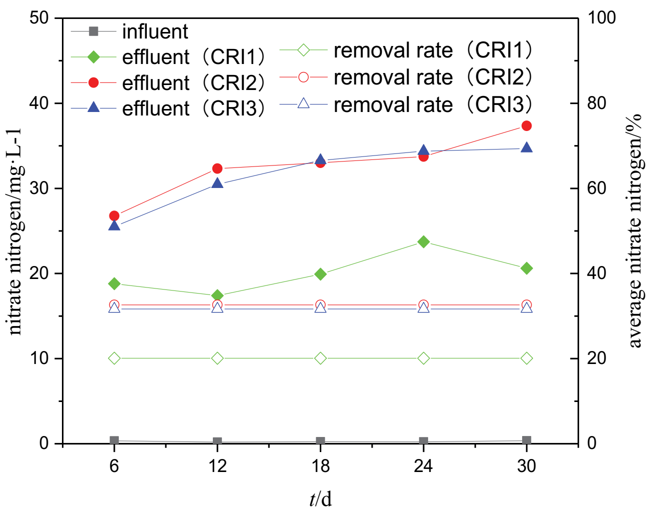

Fig. 4. Removal of nitrate nitrogen by CRI.

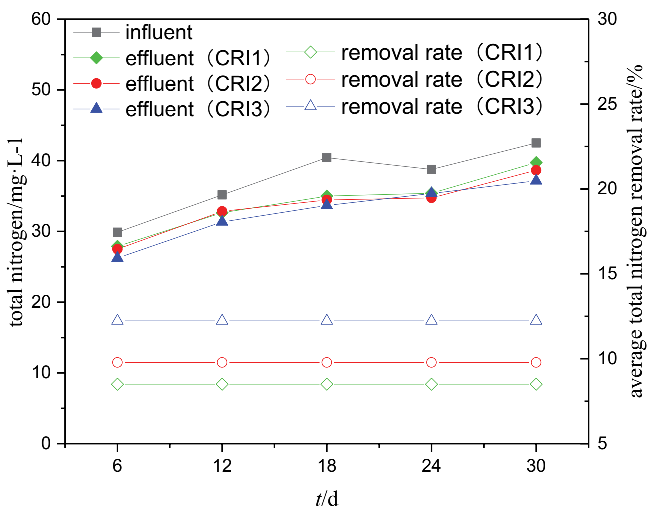

Fig. 5. Removal of total nitrogen by CRI.

CRI1 was the lowest, the contact time between sewage and the filler in CRI1 was the shortest, and the total nitrogen in the influent had not been fully absorbed by the filler and the micro-organisms were used, so the total nitrogen removal rate was the lowest. The effective height of CRI3 was higher than CRI1, the contact time between sewage and the filler in CRI3 was longer, and the total nitrogen in the influent could be fully absorbed by the filler and utilized by micro-organisms, so the removal rate of total nitrogen was higher than CRI1. Mechanical ventilation destroyed the anaerobic environment in CRI2, which led to the weak denitrification, so the total nitrogen removal rate was lower than CRI3.

In the whole operation stage, compared with the results of Zhang et al. [12], Song et al. [13] and Wang et al. [14] (the total nitrogen removal rate was 57.10%, 79.56% and 89.10%, respectively), the removal rate of total nitrogen of the three groups of CRI was very low, which was mainly because the effluent after the treatment was used for farmland irrigation, so in order to fully retain the nitrogen in the sewage, the total nitrogen removal rate of three groups of CRI was reduced, clay ceramsite and coarse sand with low saturated adsorption and high desorption rate of ammonia nitrogen were used as the fillers of three groups of CRI [7]. At this time, it was in July and August (the temperatures was higher), and the treatment effect of ABR with filler was better, which made the COD concentration of three groups of CRI lower (the average COD concentration of influent was 59.00 mg/L), and there was no additional carbon source, which led to the lack of denitrification carbon source. Mechanical ventilation destroyed the anaerobic environment in CRI2, which led to the weak denitrification.

In conclusion, the increase of filler height improved the total nitrogen removal rate of CRI, while mechanical ventilation was conducive to reducing the total nitrogen removal rate of CRI, but the contribution of mechanical ventilation to reducing the total nitrogen removal rate of CRI was relatively low (the average total nitrogen removal rate of CRI was only reduced by 2.45%), which was mainly due to the low COD concentration of the three groups of CRI (the average COD concentration of the influent was 59.00 mg/L). There was no additional carbon source, which led to the lack of denitrification carbon source. Therefore, the contribution of increasing mechanical ventilation to reducing the total nitrogen removal rate of CRI was low.

3.5. Removal effect of total phosphorus

The removal effect of three groups of CRI on total phosphorus is shown in Fig. 6. It can be seen from Fig. 6 that the influent total phosphorus was 2.45–3.76 mg/L. The average removal rate of total phosphorus from the three groups of CRI from large to small was:

CRI2 (61.60%) > CRI3 (58.55%) > CRI1 (32.70%), which was mainly because the effective height of CRI1 was the lowest, the contact time between sewage and the filler in CRI1 was the shortest, and the part of phosphorus in the influent could not be absorbed and precipitated by the filler, and it had been discharged from the system [15]. The effective height of CRI2 and CRI3 was higher, the contact time between sewage and the filler in CRI2 and CRI3 was longer, and the phosphorus in the influent could be absorbed and precipitated by the filler, so the average removal rate of total phosphorus was higher than CRI1. The average removal rate of total phosphorus in CRI2 was higher than that of CRI3, which may be due to the fact that the adsorption point and cation exchange capacity of CRI3 filler in this stage were less than CRI2, and the desorption phosphorus of filler in CRI3 was more than CRI2. Mechanical ventilation led to the rapid growth and reproduction of micro-organisms in CRI2, which led to the more demand for phosphorus.

In the whole operation stage, compared with the results of Guo et al. [16], Wang et al. [14] and Zhu et al. [17] (the total phosphorus removal rate reached more than 90%), the removal rate of total phosphorus of the three groups of CRI was lower, which was mainly because the effluent after the treatment was used for farmland irrigation, so in order to fully retain the phosphorus in the sewage and reduce the total phosphorus removal rate of three groups of CRI, clay ceramsite and coarse sand with small saturated adsorption and high desorption rate of phosphorus were used as the fillers for three groups of CRI [7]. Although the saturated adsorption of phosphorus by clay ceramsite and coarse sand was small, it still take a long time to reach the saturated adsorption of phosphorus by three groups of CRI due to the low phosphorus content in domestic sewage [18].

In conclusion, the increase of filler height and mechanical ventilation could improve the total phosphorus removal rate of CRI, but the contribution of mechanical ventilation to the improvement of the total phosphorus removal rate of CRI was relatively low (the average removal rate of total phosphorus of CRI was only 3.05%). CRI belonged to biofilm method, and its phosphorus removal mechanism was mainly adsorption, chemical precipitation and microbial synthesis and metabolism of fillers [14,17]. After long-term operation, the adsorption and chemical precipitation of phosphorus would reach saturation. When the biofilm on the filler reached a certain thickness, it would automatically fall off, resulting in the release of phosphorus to water, and CRI did not replace the filler and sludge discharge. Therefore, in the long term, the removal of total phosphorus by CRI could be ignored.

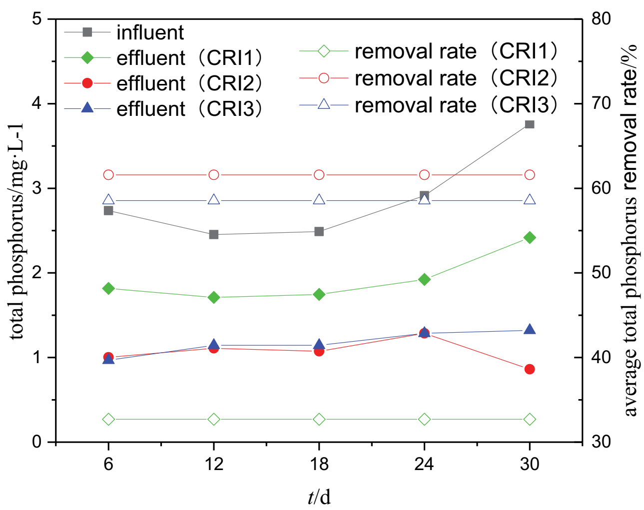

Fig. 6. Removal of total phosphorus by CRI.

3.6. OTU analysis in bacterial community

The petal diagram of bacterial community in three groups of CRI is shown in Fig. 7. It can be seen from Fig. 7 that the number of unique OTU in 1 increased gradually along the flow direction, while the number of unique OTU in CRI2 and CRI3 decreased first and then increased along the flow direction, indicating that the unique bacterial community in CRI1 increased gradually along the flow direction, while that in CRI2 and CRI3 decreased first and then increased along the flow direction. This may be due to the low concentration of organic matter and the surface characteristics of coarse sand in the lower part of CRI, which was conducive to the growth and reproduction of endemic bacterial community.

The number of unique OTU at the same filler height of three groups of CRI was different. At 0.3 m, the unique number of OTU in CRI was CRI2 > CRI3 > CRI1. At 0.8 m, the unique number of OTU in CRI was CRI1 > CRI2 > CRI3. At 1.3 m, the unique number of OTU in CRI was CRI3 > CRI2. This indicated that increasing the height of the filler increased the bacterial community in the upper part of CRI, but decreased the bacterial community in the middle part of CRI. This may be because increasing the height of the filler improved the oxygen environment in the upper part of CRI, and the improvement of the oxygen environment was conducive to the growth and reproduction of the bacterial community. The increase of mechanical ventilation increased the bacterial community in the upper and middle part of CRI, but decreased the bacterial community in the lower part of CRI. This was mainly because the increase of mechanical ventilation improved the oxygen environment in the upper and middle part of CRI.

3.7. Alpha diversity index

The changes of bacterial community richness, diversity and evenness in three groups of CRI are shown in Table 2. It can be seen from Table 2 that the values of Chao1 index, observed species index, Simpson index and Shannon index of samples with the same filler height in the three groups of CRI were 0.3 m: CRI2 > CRI3 > CRI1; 0.8 m: CRI3 > CRI1 > CRI2; 1.3 m: CRI3 > CRI2, which indicated that the increase of filler height increased the richness and diversity of bacterial community in CRI, while the increase of mechanical ventilation increased the richness and diversity of bacterial community in the upper part of CRI, but decreased the richness and diversity of bacterial community in the middle and lower part of CRI, mainly because the increase of filler height affected the natural reoxygenation process in CRI. Increasing mechanical ventilation changed the oxygen environment in CRI, and the change of oxygen environment had a greater impact on the bacterial community. The Pielou's evenness index of the samples with the same filler height in the three groups of CRI were 0.3 m: CRI3 > CRI2 > CRI1; 0.8 m: CRI3 > CRI1 > CRI2; 1.3 m: CRI3 > CRI2, which indicated that increasing the height of filler improved the evenness of bacterial community in CRI, while increasing mechanical ventilation reduced the evenness of bacterial community in CRI.

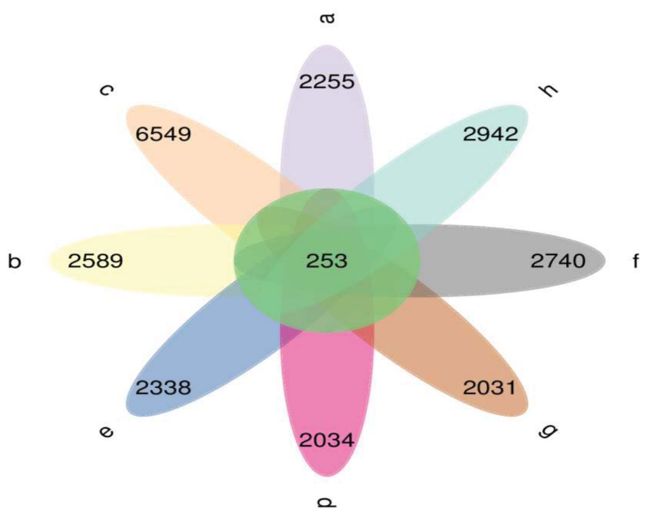

Fig. 7. Petal diagram of bacterial community.

3.8. PCoA analysis

The PCoA analysis of bacterial communities in three groups of CRI based on weighted UniFrac distance is shown in Fig. 8. It can be seen from Fig. 8 that several parallel

Table 2
Changes of richness, diversity and evenness of bacterial community

Sample	Chao1	Simpson	Shannon	Observed species	Pielou's evenness
a1	3,171.56	0.98558	8.94115	2,659.5	0.785902
a2	3,134.62	0.98931	9.04204	2,606.9	0.796788
a3	3,847.39	0.988642	9.13216	3,053.6	0.788868
b1	3,376.3	0.992044	9.16539	2,802.9	0.800283
b2	4,335.04	0.994207	9.56168	3,474.2	0.812899
b3	2,387.03	0.992451	9.10587	2,286.4	0.816021
c1	6,773.19	0.996003	10.0361	4,717	0.822385
c2	5,079.09	0.994513	9.70227	4,163.8	0.806931
c3	5,867.11	0.994726	9.85021	4,612.9	0.809288
d1	3,173.03	0.972508	8.58994	2,692.7	0.753847
d2	3,157.19	0.978781	8.69768	2,695.2	0.763212
d3	3,056.33	0.976005	8.64282	2,659.6	0.759678
e1	3,360.86	0.965687	8.3232	2,743.5	0.728714
e2	3,301.91	0.968065	8.40568	2,707.9	0.737154
e3	3,248.99	0.969926	8.40341	2,678.5	0.737971
f1	4,175.64	0.994855	9.76706	3,402.4	0.832492
f2	3,877.76	0.994302	9.71387	3,255.3	0.832482
f3	4,077.78	0.994792	9.75637	3,354.9	0.83302
g1	3,049.26	0.995961	9.66905	2,764.6	0.845728
g2	3,874.99	0.996327	9.80833	3,249.8	0.840754
g3	4,451.85	0.99618	9.91227	3,616.7	0.838571
h1	4,337.03	0.992443	9.557	3,474.1	0.812504
h2	4,282.07	0.992287	9.51548	3,409.4	0.810843
h3	3,541.71	0.994132	9.4157	2,924.7	0.817757

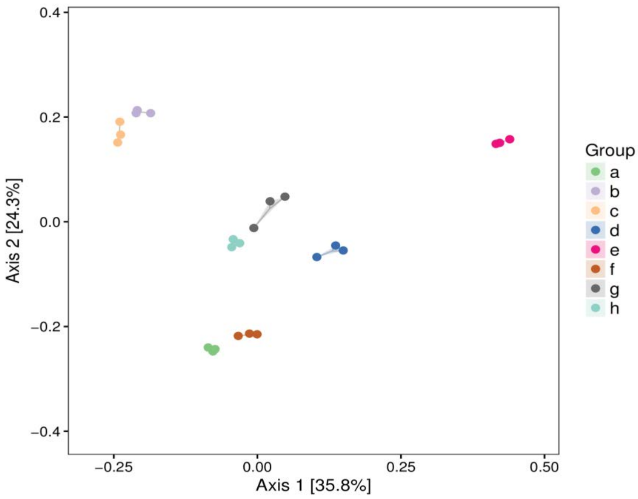

Fig. 8. PCoA analysis based on weighted UniFrac distance.

samples in all sample groups were very close, which indicated that the difference in the composition and abundance of bacterial community structure in all sample groups was small, and the genetic relationship was relatively close. In the PCoA analysis based on weighted UniFrac distance, the proportion of sample difference data explained by Axis1 and Axis2 was 35.8% and 24.3%. In the same CRI, the projection distance of the sample points at 0.8 m and 1.3 m on the Axis1 and Axis2 was relatively close, and the projection distance of the sample points at 0.3 m on the Axis1 and Axis2 was relatively far, which indicated that in the two dimensions of Axis1 and Axis2, the composition, abundance and genetic relationship of bacterial community in the three groups of CRI were constantly changing along the direction of water flow, the composition, abundance and genetic relationship of bacterial community in the middle part of CRI changed greatly along the flow direction, while the composition, abundance and genetic relationship of bacterial community in the middle and lower part of CRI changed little along the flow direction. This was mainly because the organic matter in the influent was mainly removed in the upper part of CRI, while the organic matter concentration difference between the middle and lower part of CRI was small.

The projection of the sample points with the same filler height in the three groups of CRI on the Axis1 and Axis2 also had a certain distance, which indicated that there were some differences in the composition and abundance of bacterial community with the same filler height in the three groups of CRI. Moreover, the projection distance of the sample points with the same filler height in CRI1 and CRI3 on the Axis1 and Axis2 was closer, while the projection distance of the sample points with the same filler height in CRI2 on the Axis1 and Axis2 was farther, which indicated that in the two dimensions of Axis1 and Axis2, increasing filler height and mechanical ventilation changed the structure, abundance and genetic relationship of bacterial community in CRI. The results showed that the increase of filler height had little effect on the bacterial community structure, abundance and genetic relationship in CRI, while the increase of mechanical ventilation had great effect on the bacterial community structure, abundance and genetic relationship in CRI. This was mainly because the increase of mechanical ventilation had a greater impact on the oxygen environment in CRI, and the change of oxygen environment had a greater impact on the bacterial community structure in CRI.

3.9. Bacterial community structure at phylum taxonomic level

The bacterial community structure at the phylum level in three groups of CRI is shown in Fig. 9. It can be seen from Fig. 9 that the average relative abundance of dominant bacterial communities in the three groups of CRI reached 45%–85%. Studies had shown that the presence of Firmicutes was conducive to nitrification and degradation of organic matter [19], the presence of Actinobacteria was conducive to the degradation of amino acids and other organic matter [20], the presence of Chloroflexi was conducive to the degradation of dissolved organic matter, EPS and microbial residues [21], the presence of Acidobacteria was conducive to the degradation of VFA, nitrate nitrogen and nitrite nitrogen [22], Nitrospirae was the main nitrite oxidizing bacteria in wastewater biological treatment, its presence was conducive to nitrification [23], the presence of Bacteroidetes was conducive to the degradation of denitrification, protein and organic matter [24], the presence of Proteobacteria was beneficial to nitrification and denitrification, biological phosphorus removal and organic matter degradation [25].

Among them, the average relative abundance of Proteobacteria in the three groups of CRI was the highest, and the relative abundance of Proteobacteria in the three groups of CRI gradually decreased along the flow direction, which indicated that Proteobacteria was the most dominant bacterial community in the three groups of CRI, and gradually decreased along the flow direction, mainly because the concentration of organic matter in the influent gradually decreased along the flow direction. Compared with CRI3, the average relative abundance of Proteobacteria at 0.3 and 0.8 m of CRI2 was 4.81% and 13.86% higher, respectively. The average relative abundance of Nitrospirae of CRI1 at 0.3 m was higher than that at 0.8 m, mainly because the natural ventilation effect at 0.3 m was better than that at 0.8 m. Compared with CRI1, the average relative abundance of Nitrospirae at 0.3 m of CRI3 was 5.90% lower, while that at 0.8 m was 3.11% higher. This may be because CRI3 attached more biofilms at 0.3 m, so its ventilation effect at 0.3 m was worse than CRI1, while CRI3 attached less biofilms at 0.8 m, and the air may enter CRI3 through the outlet pipe at the bottom of CRI3. Compared with CRI3, the average relative abundances of Nitrospirae at 0.3, 0.8 and 1.3 m of CRI2 were 4.39%, 2.09% and 8.44% lower, respectively. The average relative abundances of Bacteroidetes at 0.3 m were 4.98% higher, while the average relative abundances of Bacteroidetes at 0.8 and 1.3 m were 2.34% and 4.59% lower, respectively. This was mainly due to the two water distribution discs in CRI2 hindering natural ventilation into CRI2. In addition, there were more biofilms growing in the early stage near the CRI2 sampling port, which led to the water blocking phenomenon near the sampling port during the sampling period. Therefore, the ventilation effect near the sampling port was worse than that of CRI3 during the sampling period, and the concentration

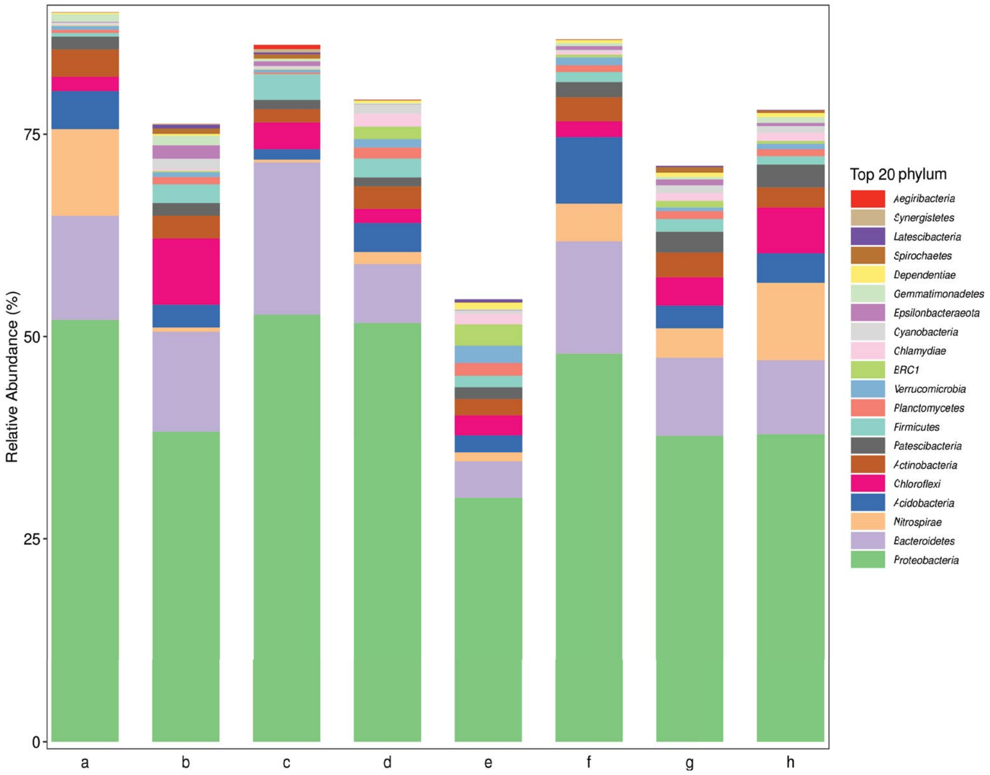

Fig. 9. Bacterial community structure at phylum taxonomic level.

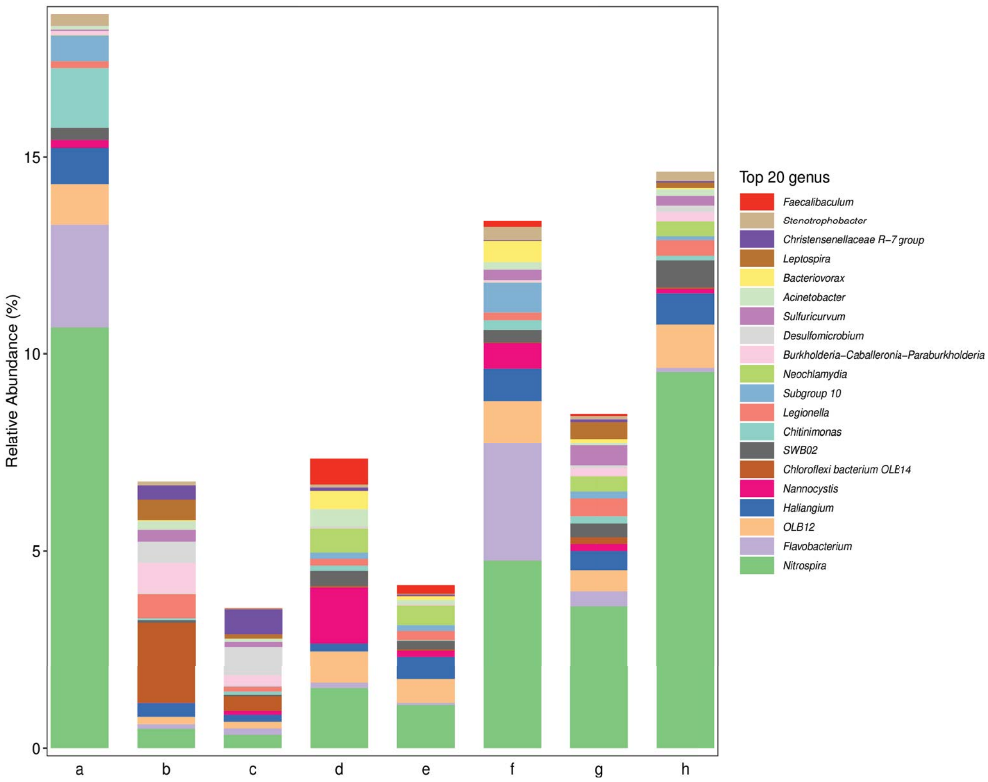

Fig. 10. Bacterial community structure at genus taxonomic level.

of organic matter entering the middle and lower part of CRI2 was lower than that of CRI3. The average relative abundances of Firmicutes, Patescibacteria, Actinobacteria, Chloroflexi and Acidobacteria in the three CRI groups were low, and the variation along the flow direction was small.

3.10. Bacterial community structure at genus taxonomic level

The bacterial community structure at the genus level of CRI is shown in Fig. 10. It could be seen from Fig. 10 that the sum of the average relative abundance of dominant bacterial communities in the three groups of CRI was 2%–20%. Studies had shown that Flavobacterium, as a kind of aerobic denitrifying bacteria, was beneficial to denitrification [26]. Nitrospira, as the main nitrite oxidizing bacteria in wastewater biological treatment, was beneficial to nitrification [23].

Among them, the average relative abundance of Nitrospira was the highest in the three groups of CRI, which indicated that Nitrospira was the most dominant bacterial community in the three groups of CRI, and Nitrospira belonged to Nitrospirae, and the variation trend of its average relative abundance in the three groups of CRI was consistent with that of nitrifying Spirillum.

4. Conclusion

- The average removal rates of COD, ammonia nitrogen, total nitrogen, total phosphorus and the concentration of nitrate nitrogen in the effluent of CRI were added by increasing the filler height. Adding mechanical ventilation could improve the removal rate of COD and total phosphorus and the concentration of effluent nitrate nitrogen, but reduced the removal rate of total nitrogen, which was conducive to the retention of nitrogen in wastewater. Since CRI did not discharge sludge, the removal of total phosphorus by CRI could be neglected in the long run.
- Adding the height of filler could increase the bacterial community in the upper part of CRI, but decreased the bacterial community in the middle part of CRI. Adding mechanical ventilation could increase the bacterial community in the upper and middle part of CRI, but decreased the bacterial community in the lower part of CRI.
- The richness, diversity and evenness of bacterial community in CRI were improved by increasing the height of filler. Increasing mechanical ventilation could increase the richness and diversity of bacterial community in the upper part of CRI, but decreased the richness and diversity of bacterial community in the middle and lower part of CRI, and reduced the evenness of bacterial community in CRI.
- Increasing the filler height not only prolonged the contact time of wastewater with filler and its surface microorganisms, but also increased the average relative abundance of Nitrospirae at 0.8 m of CRI by 3.11%, therefore, increasing the filler height is conducive to the removal of organic matter and phosphorus and nitrification in CRI. Increasing mechanical ventilation increased the average relative abundance of Proteobacteria by 4.81% and 13.86% at 0.3 and 0.8 m, and decreased the average relative abundance of Bacteroidetes by 2.34% and 4.59% at 0.8 and 1.3 m, respectively. Therefore, increasing mechanical ventilation is conducive to the removal of organic matter and phosphorus in CRI, but it is not conducive to denitrification.

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Conflict of interest

The authors declare no conflict of interest.

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