Research on the effects of salinity on the microbial community of novel constructed wetland

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Abstract. In this paper, a novel patent intermittent aeration vertical flow constructed wetland (IA-VFCW) was developed, and the influences of salinity on the microbial community of IA-VFCW were investigated. The experimental results showed that bacterial communities at the genus level were transited along with the salinity varied from 0‰ to 20‰. *Ferritrophicum* and *Psychrobacter* show an increasing tendency and the maximum abundance was 14.8% and 9.8%, respectively. As salt concentration was increased, *Cyanobacteria* was gradually disappeared. A total of 8 samples were carried out by microbiology, ten different genera were associated with the removal of nitrogen which included as follows: *Halomonas*, *Thiobacillus*, *Cyanobacteria*, *Denitratisoma*, *Arcobacter*, *Acinetobacter*, *Sulfurimonas*, *Nitrosomonas*, *Dechloromonas* and *Hydrogenophaga*.

1. Introduction

Constructed wetland is an environment-friendly and cost-effective wastewater treatment technology, which are artificially established by simulating natural or semi-natural wetland purification system. It is composed of gravel, cinder, sand, soil and aquatic plants which totally constitute a microecological system. Compared with traditional sewage treatment technology, constructed wetland takes the advantages of lower investment and operating cost, less management requirement, excellent pollutant removal efficiency, and better ecological and economic benefits. In recent years, it has been widespread exploited and applied for the treatment of domestic sewage and industrial wastewater [1-2].

The pollutant removal performance of constructed wetland was mainly affected by matrix, plant, microorganism and environment conditions, in which microbial degradation was especially the main contributor[3]. Microorganisms play a crucial role in the nutrient’s conversion[4-5]. In aerobic zones, functional microorganisms convert most organic pollutants to CO₂ and NH₄⁺ to NO₂⁻, subsequently to NO₃⁻. Eventually, anaerobic microorganisms, such as fermenting bacteria, methanogens, denitrifies and others, can further consume organic materials, NO₂⁻ and NO₃⁻, and mainly generate CH₄ and N₂, respectively.

With the increasing shortage of freshwater resources in the world, many countries take the advantage to widely utilize seawater, accompanied by a large amount of sewage containing high salinity. At present, some scholars are focusing on the treatment of wastewater with high salinity in constructed wetlands[6-7]. Fu studied the influences of salinity fluctuations on the denitrifying microbial community structure.
in constructed wetlands[8], the results show that a variety of aerobic denitrifying bacteria are enriched in constructed wetlands under different salinities. There are about 10-13 aerobic denitrifying bacteria enriched in the upper layer of the constructed wetland, with a relative abundance of 19.1±7.9%, the enriched microbial community improves the nitrogen removal performance of constructed wetlands, while enhancing the adaptability to salinity stress. Little research aimed to the effects of gradually increased salinity on microbial community structure in different zone of constructed wetland has been explored. In view of this, the novel patent intermittent aeration vertical flow constructed wetland (IA-VFCW) was employed, the structure and transition of microbial community in different zone of constructed wetlands under different salinity and influent NH$_4^+$-N concentration was investigated. The research object is to provide a theoretical basis for the treatment of different salinity-containing wastewater using vertical flow constructed wetlands.

2. Materials and methods

2.1. Experimental setup and operation

The intermittent aeration vertical flow constructed wetland was shown in Fig.1, which is cylindrical and made of plexiglass with a diameter of 30cm and a height of 80cm. The device was filled with coarse gravel, gravel, cinder and sand sequentially from bottom to top. The upper part of the device is provided with concentric circles, the aeration zone, the soil zone and the water outlet zone were separately installed from inside to outside. The *prundo phragmites* was planted around circular soil zone, also defined as aquatic plant area.

The intermittent aeration vertical flow constructed wetland was operated under constant HRT of 48h and influent COD concentration of 200mg/L. The salinity of the influent was set to 0 ‰, 5 ‰, 10 ‰, and 20 ‰, termed CW1, CW2, CW3 and CW4, respectively. The samples taken from the aquatic plant area(aeration zone) and matrix in the lower section of the IA-VFCW(anaerobic zone) were referred to as P1 and P2, respectively. The NH$_4^+$-N concentration of the influent is respectively set to 10mg/L, 25mg/L, 40mg/L under individual salinity. The sampling was performed regularly for microbial community analysis.

2.2. Microbiological sample analysis

Use Illumina HiSeq high-throughput sequencing technology to perform sequencing in HiSeq 2500 system. To simplify analyses, reads within a 16S rRNA gene dataset are typically collapsed into operational taxonomic units (OTUs). This is carried out based on sequence similarity between reads. Convention is typically to group reads that share at least 97% identity, which is considered “species” level. Count the composition of each sample at the genus level to study the effect of salinity on the microbial community structure and abundance.
3. Result and discussion

Fig. 2 is a Venn diagram and petal diagram based on OTU. In the R1 group, CW1P1, CW2P1, CW3P1, CW4P1 have a total of 276 OUTs, and the unique OTUs are 218, 101, 79, and 119 respectively. In the R2 group, CW1P2, CW2P2, CW3P2, and CW4P2 have a total of 307 OUTs, and the unique OTUs are 122, 121, 112, and 89, respectively. The CW1P2 to CW4P2 have a total of 198 OUTs, and the unique OTUs are 92, 17, 16, 40, 11, 36, 28, 31, respectively. The R1 group and R2 group have a total of 1482 OUT numbers, and the unique OUT numbers are 214 and 215, respectively.

Fig. 2 Venn diagram and petal diagram

Fig. 3 is a joint graph of clustering trees of different salinity samples and relative abundance of species based on gate level. It can be used to judge the similarity of species. In the R1 group, the samples of CW3P1 and CW4P1 are similar. It shows that the diversity of microbial species in the soil layer of constructed wetlands at 10‰ and 20‰ salinity is closer. In the R2 group, the diversity of microbial species in the soil layer of constructed wetlands at 5‰ and 20‰ salinity is closer.

Fig. 3 unweighted unifrac tree analysis

As shown in Fig. 4, the microorganisms in the constructed wetland are classified and compared at the genus level. The dominant bacteria in each stage of this experiment are *Ferritrophicum*, *Thiothrix*, *Halomonas*, *Thiobacillus* and *Psychrobacter*, etc. *Ferritrophicum* participates in the iron oxidation process and is of great significance to the biogeochemical cycle of iron and carbon, nitrogen, phosphorus and other elements[9-10]. The abundances of *Ferritrophicum* in CW1P1, CW2P1, CW3P1, CW4P1, CW1P2, CW2P2, CW3P2 and CW4P2 are 1%, 9.8%, 2.4%, 8.7%, 6%, 11.6%, 3.9% and 14.8%, respectively. *Thiothrix* is often used as a filamentous fungus carrier. The abundances are 5.9%, 7.9%, 71%, 39.7%, 55.0%, 28.2%, 41.0%, 14.7%, respectively. The abundances of *Halomonas* are higher in CW1P1 and CW4P1 samples. They are 28% and 2.3%, respectively. The abundances of *Thiobacillus* are 2.8% to 12.4%. *Psychrobacter* only exists in CW3P1, CW4P1, CW1P2, CW2P2, CW3P2, CW4P2, the abundances of it are 1.2%, 1.3%, 1.4%, 1.4%, 8.1%, 9.8%, respectively. This shows that
Psycrobacter is a new dominant strain emerging under salt stress. Cyanobacteria was only detected in CW1P1 with an abundance of 9.9%. This shows that Cyanobacteria is the dominant strain of CW1P1. In addition, we found 10 kinds of microorganisms associated with the removal of nitrogen: Halomonas, Thiobacillus, Cyanobacteria, Denitratisoma, Arcobacter, Acinetobacter, Sulfurimonas, Nitrosomonas, Dechloromonas and Hydrogenophaga[11-14].

Fig.4 Taxonomic classification of microbial community at genus

4. Conclusions
The experimental results showed that at the genus level, because of the salt concentration increases from 0‰ to 20‰, bacterial communities have changed. Ferritrophicum and Psychrobacter show an increasing tendency and the maximum abundance was 14.8% and 9.8%, respectively. As the salt concentration increases, Cyanobacteria gradually disappears. At total of 8 samples were carried out by microbiology, we found 10 kinds of microorganisms associated with the removal of nitrogen: Halomonas, Thiobacillus, Cyanobacteria, Denitratisoma, Arcobacter, Acinetobacter, Sulfurimonas, Nitrosomonas, Dechloromonas and Hydrogenophaga.

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