Investigation of Microbial Diversity in Sludge Treatment Reed Bed

Junwen Ma[1,2][a], Yubo Cui[1,3,*][b], Chengdong Ma[4][c], Wanjun Zhang[3][d], Zhongwei Zhang[3][e] and Ke Zhao[5][f]

[1]*Key Laboratory of Biotechnology and Bioresources Utilization, Ministry of Education, Dalian Minzu University, Dalian 116600, China*

[2]*School of Environment Science & Technology, Dalian University of Technology, Dalian, 116024, China*

[3]*College of Environment and Resources, Dalian Minzu University, Dalian, 116600, China*

[4]*Department of Marine Ecological Environment Information, National Marine Environmental Monitoring Center, Dalian, 116023, China*

[5]*Key Laboratory of Songliao Aquatic Environment, Ministry of Education, Jilin Jianzhu University, Changchun, 130118, China*

Keywords: Sludge Treatment Reed Beds, Microbial Diversity.

Abstract: This paper fully characterized the diversity of the bacterial flora in sludge treatment reed beds (STRBs) by sampling samples from the STRBs in stages and overall studying the changes in the bacterial flora in the natural stabilization period, with sludge samples as the main focus, divided into bottom and surface sludge discussions, and combined with reed samples and natural substrate samples to give auxiliary analysis. We found that species changes of the dominant bacterial community in sludge mainly changed with different periods of ecological stabilization, and the diversity as well as the homogeneity of the bacterial community was increasing after the stabilization period, indicating that the root activity in plant growth can influence the activity of nearby bacterial communities.

1 INTRODUCTION

At present, China's sewage treatment process is becoming more and more mature, but the amount of residual sludge generated by the sewage treatment process is huge, so the disposal of residual sludge should not be underestimated (Wu, et al., 2000). Specifically, the amount of residual sludge produced today is up to more than 35 million tons, and its water content is all around 80%. The main treatment measures for residual sludge are composting, incineration, landfill, drying and digestion, but still 80% of the sludge cannot be properly treated. Therefore, the secondary pollution problem is still prominent. Secondary pollution problems mainly exist in terms of high sludge treatment costs, poor product marketing and the existence of pollution transfer (Cui, et al., 2018). From a technical and economic point of view, incineration and composting for agricultural use are reliable technologies for the treatment and disposal of huge amounts of sludge, but the high investment and operating costs hinder the widespread application of incineration technology, and the high cost of composting technology also requires consideration of agricultural safety issues, which are the challenges that must be faced in promoting the technology. Therefore, sludge treatment or stabilization requires consideration not only of internal factors of operating technology, but also of external factors such as economic costs and environmental operation. The sludge treatment reed bed (STRB) is a combination of traditional sludge drying beds and artificial wetland technology, which not only effectively dewater the remaining sludge, but also additionally produce a mineralized substance that can be used as a land improvement and agricultural fertilizer (Uggetti, et al., 2010, Nielsen, et al., 2016, Hardej, et al., 2002). The

[a] https://orcid.org/0000-0001-5716-9446
[b] https://orcid.org/0000-0001-8950-5889
[c] https://orcid.org/0000-0002-2933-1220
[d] https://orcid.org/0000-0002-3342-3734
[e] https://orcid.org/0000-0001-5413-554X
[f] https://orcid.org/0000-0002-7481-0503

Ma, J., Cui, Y., Ma, C., Zhang, W., Zhang, Z. and Zhao, K.
Investigation of Microbial Diversity in Sludge Treatment Reed Bed.
DOI: 10.5220/0011180300003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 59-63
ISBN: 978-989-758-595-1

diversity of sludge flora in STRBs is important for evaluating the effectiveness of STRBs in treating residual sludge. Previously, there is still a gap in the research on the diversity of bacterial flora in STRBs during the stabilization period. With the continuous advancement of technological innovations in molecular biology, the use of denaturing gradient gel electrophoresis (DGGE) to study microbial changes in microecological environments has been widely used, but a complete cycle study of microbial flora diversity during the ecological stabilization of residual sludge has not been carried out.

In conclusion, it is necessary and urgent to investigate the changes of microbial flora in STRBs during the stabilization period using DGGE.

2 MATERIALS AND METHODS

2.1 Sampling

The STRB system is divided equally into three units, all of which are 3.0 m x 1.0 m x 1.3 m. Unit 1 is a conventional STRB with an aeration structure (two aeration risers); Unit 2 is a STRB with an aeration structure (two aeration risers) and reeds are grown; Unit 3 is a normal STRB with only reeds but no aeration structure. The time period selected for the study was eight months, from April to November in the resting period, according to seasonal variations.

The reed bed is mainly composed of packing layer and mud storage layer. The packing layer is filled with 20 cm of slag, 20 cm of gravel, and 25 cm of British sand filter material in sequence from bottom to top, of which 5 cm of coarse sand and the rest are fine sand; the mud storage layer is set to 65 cm, In order to provide enough space for sludge accumulation in the later stage. Aeration risers are installed along 1/3 and 2/3 of the length of the drain pipe and extend to the space above the mud storage.

2.2 Methods

In this experiment, DGGE was performed using Bio-Rad's denaturing gradient gel system, and the basic operating conditions for this experiment were determined after repeated adjustments and tests. The concentration of polyacrylamide gel denaturant used in this study was 8%, and the gradient of denaturing gel was 30-60% in 1× TAE buffer, and electrophoresis was carried out at 18 mA for 18-20 h.

3 RESULTS AND DISCUSSION

3.1 Cluster Analysis and Diversity Index of Bacteria

3.1.1 Analysis of May and November Samples from Strbs

According to the results of cluster analysis, Figure 1 shows that lane 4 (5-2S) and lane 5 (5-3B) have the lowest similarity with the rest of the samples, indicating that the dominant bacteria in the bottom sludge of unit 2, which is planted with reeds and aerated, and the surface sludge of unit 3, which is planted with reeds and not aerated, have similarity and belong to two groups with the rest of the samples during the budding stage of reed growth in the natural stabilization period. In the natural stabilization period of STRBs, the surface sludge and bottom sludge of each unit did not belong to the same taxon in the same sampling period. This can also be seen by the greater differentiation between 11# (11-3B) and 12# (11-3S). In one unit, the similarity between 1# (5-1B), 2# (5-1S), 7# (11-1B), and 8# (11-1S) was not high. According to the related study from the germination stage of reed, soil respiration rate increased with the increase of temperature, and soil temperature and near surface temperature reached the highest value in July-August, and soil respiration rate peaked correspondingly in the vigorous growth period; after entering the wilting stage, soil respiration in both reed wetlands gradually decreased with the decrease of soil temperature and near surface temperature, due to the The lowest value of soil respiration rate was reached in the overwintering period due to the limitation of low temperature. Unit I, as a STRB, the role of flora was mainly related to soil respiration rate, indicating that the dominant flora of each sample in Unit I were not in the same range. In unit II, the dominant bacterial groups between 3# (5-1B) and 10# (11-1S) belonged to the same taxon, indicating that the sludge potential bacterial groups in unit II planted with reeds did not change much and had some stability.

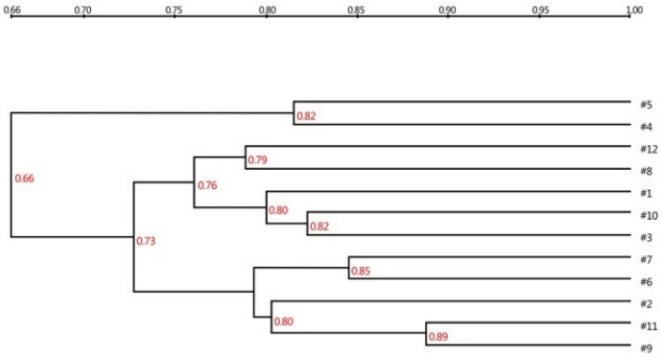

Figure 1: The dendrogram of the DGGE fingerprint of the V4-V5 gene of 16S rDNA of the bacteria from twelve sample. 1-6: The samples in May; 7-12: The samples in November

The diversity values of the same sludge sample in May and November were compared longitudinally, and it was found that the difference between the two units in the bottom sludge sample was the smallest, and the H value increased from 1.44 (3#5-2D) to 1.61 (9#11 -2D); bed number 3 rose from 1.27 (5#5-3D) to 1.86 (11#11-3D), with the largest fluctuation; while unit 1 dropped significantly from 1.93 (1#5-1D) to 1.41 (7#11-1D), in the process of research and application, the diversity index represents the degree of species diversity of each sample bacteria, and is a comprehensive index of richness and uniformity. Mud was closest to the degree of species diversity at the beginning and end of the study cycle. The surface sludge was analyzed, and it was found that the fluctuation between the first unit and the third unit was not large, and the second unit had a certain fluctuation relatively. The H value increased from 1.40 (4#5-2B) to 1.72 (10#11-2D), The results showed that in the comparison of the surface sludge diversity index, the two units had the largest difference in species diversity at the beginning and end of the study period. It can be seen from Figure 3.6 that the fluctuation of the evenness index EH value also characterizes the above trend. There is little difference in the richness of the sample colonies, the highest is 13 (11#11-3D), and the lowest is 9 (5#5-3D).

On the whole, the diversity index in May and November did not fluctuate much, and the sludge had a certain degree of stability.

3.1.2 Analysis of July and September samples from STRBs

According to the results of cluster analysis, Figure 2 shows that the reed samples (8#, 9#, 10#, 18#, 19#, 20#) and the sludge samples (from 1# to 7# and from 11# to 17#) are clearly divided into two major clusters with a similarity of 0.37. Comparing the reed samples, it can be seen that the similarity between the 9# (7-L2) and 10# (7-L3) samples is as high as 0.94, indicating that these two samples of reeds showed great consistency in July because they were both growing in the sludge. In contrast, the comparison of 19# (9-L2) and 20# (9-L3) revealed that the reeds began to show some variability in growth in September. the similarity between the reed samples in July (8#, 9#, 10#) and September (18#, 19#, 20#) was arranged apart, indicating that the reed endophytes' changed in July and September.

There are some obvious fluctuation patterns in the sludge samples, within one unit, the samples 1# (7-1B) and 2# (7-1S) in July do not belong to one taxon and the similarity value between the two taxa is 0.75, and the corresponding samples 11# (9-1B) and 12# (7-1S) in September also do not belong to one taxon and the similarity value becomes smaller to 0.67, indicating that in the sludge with only contemporaneous structure The difference in the sludge surface and bottom layers of the sludge in the dryer bed and the fluctuation of the dominant bacterial group. A cross-sectional comparison of the samples from the three units shows that the sludge samples 5# (7-3B) and 6# (7-3S) from July were not matched to the same taxon, but the samples 15# (9-3B) and 16# (9-3S) from September had high similarity values (0.85) and belonged to the same taxon; a longitudinal comparison revealed that 5# (7-3B) and 15# (9-3S) belonged to two taxa (0.71) and 6# (7-3B) and 16# (9-3S), indicating that the three units (reedbeds without aeration structures) changed significantly after the growth period. Among the three units, the lanes of 3# (7-2B), 4# (7-2S) and 13# (9-2B) were sequentially adjacent to each other in the analysis chart with high similarity; 14# (9-2S) represented a sludge sample at the peak of reed

growth with some variability from the above samples (similarity value 0.71). The natural substrate samples 7# and 17# belong to one taxon, but the similarity is low (0.59), indicating that the natural substrate samples also changed in July and September.

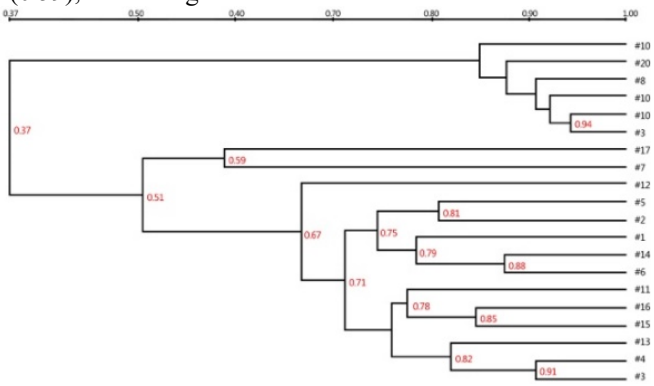

Figure 2: The dendrogram of the DGGE fingerprint of the V4-V5 gene of 16S rDNA of the bacteria from twenty sample. 1-10: The samples in July; 11-20: The samples in September.

Compared with the diversity index in July and September, it was found that the fluctuation of the diversity index of the sludge sample was significantly larger than that of the reed sample, and the diversity index of the natural sediment sample was closer to the reed sample, which was relatively stable. The diversity index of the reed samples was similar in two months. Compared with the uniformity index, it was found that the reed samples in the second unit had the most stable changes before and after (9#, 19#).

In the control of sludge samples, the one-unit bottom sludge diversity index without reed plants fluctuated the least, and the H value changed from 1.40 (1#7-1D) to 1.44 (11#9-1D). The bottom sludge diversity index of the three units planted with reeds fluctuated the most, and the H value changed from 2.03 (5#7-3D) to 1.20 (15#9-3D). In the comparison of the surface sludge, the diversity index of pool 1 dropped significantly, and the diversity index of pool 2 fluctuated less. In general, the diversity index of samples in September was lower than that of samples in July.

The trend graph of the evenness index EH value also characterizes the above trend. There is a certain difference in the abundance of sludge sample colonies, the highest is 14 (5#7-3D), the lowest is 6 (16#9-3B), and the richness of natural sediment is 5 (7#7-T and 17#9-T); the richness of the reed samples differed little, both in July and in September.

3.2 Bacterial Species and Affinity Analysis

3.2.1 Species and Affinity Analysis of May and November Samples

The research showed that it can be concluded that each band represents Bacteria (bacteria), bands 2, 5 and 6 do not specify the specific dominant bacterial genus, and after comparison, it is known to belong to the environmental sample species.

Band 8 belongs to Bacteroidetes (Bacteroidetes), which is widely found in nature, including soil, sediment and seawater, as well as animal skin and viscera, and according to the results, this species is the dominant species in the samples of each unit in May, while the dominant characteristics in the samples of November are concentrated in the bottom sludge.

The rest of the bands in the map represent the dominant genus of bacteria belong to Proteobacteria (Phylum Amoebae), bands 3, 4, 7, 11, 12, 13 and 14 are from Gammaproteobacteria (γ-Amoebacteria), bands 4, 13 and 14 are bacteria Rhodanobacter, band 3 is bacteria Dyella, bands 8 and 12 are only compared to Xanthomonadaceae (Order Xanthomonadaceae).

Bands 9 and 10 both belong to Betaproteobacteria (β-Amastigotes), band 9 represents Burkholderiales (Burkholderiales) - Comamonadaceae (Trichomonadaceae) - Simplicispira; band 10 represents Hydrogenophilales (Hydrogenophilales) - Hydrogenophilaceae (Hydrogenophilaceae) - Thiobacillus (Thiobacillus

spp.), a dominant bacterium that grows mainly in environments with pH values between 3 and 4 and produces sulfuric acid to improve fertilization efficiency.

3.2.2 Species and Affinity Analysis of July and September Samples

The comparison results showed that band 4 was Uncultured Acidobacteriales, which belongs to Acidobacteria, a newly discovered bacterium and less studied.

Band 6 was Uncultured Sphingobacteria, i.e. Sphingobacteria (Phylum Sphingobacteria), which are more frequent in the dominant group analysis and have been shown to remove ammonia nitrogen from water. This band only appeared in the sludge samples of the second unit in July and in the bottom sludge of the first unit in the same period, indicating that the sludge of the second unit in July had a certain bacterial richness.

Band 7 is Uncultured Bacteroidetes, which is Bacteroidetes (phylum Bacteroidetes), a specialized anaerobic bacterium that generally exists widely in manure wastewater and thickened sludge. The presence of this band in lanes 2# (7-1S) and 5# (7-3B) indicates that the sludge in units 1 and 3 showed an anaerobic environment in July, and presumably the sludge in unit 2 had a higher oxygen content.

Band 10 was Gloeobacter, which showed predominance in all samples. It is a Cyanobacteria (phylum Cyanobacteria), a group of bacteria that produces oxygen. It belongs to Gloeobacter (genus Gloeobacter) in the Gloeobacteraceae (family Mucoraceae).

Band 16 is Uncultured Alcanivorax, also belonging to Gammaproteobacteria (γ-Amastigotes) in Proteobacteria (Phylum Proteobacteria), but Alcanivorax (Alcanivorax spp.) in Oceanospirillales (Order Oceanospirillales). The profile showed the occurrence of this class of bacteria in the second unit of sludge as well as in natural sludge in July.

4 CONCLUSIONS

In this paper, we found significant differences in the DGGE profiles of bacterial populations in different periods and sampling locations by sampling samples in STRBs. The changes in the dominant bacterial species in sludge mainly changed with different periods of ecological stabilization, and the diversity as well as the homogeneity of the dominant bacterial flora generally increased after the stabilization period of action, indicating that the root activity in plant growth can stimulate the activity of nearby bacterial flora to some extent.

Sequence comparison revealed that during the stabilization period of STRBs, the dominant bacteria were Bacteroidetes and Proteobacteria at lower temperatures, and Thiobacillus (Thiobacillus spp.), which can produce sulfuric acid to improve fertilization efficiency, was always present. The dominant species were widely distributed at higher temperatures, including Proteobacteria (Phylum Anamorphobacteria), Acidobacteria (Phylum Acidobacteria), Sphingobacteria (Phylum Sphingobacteria), Bacteroidetes (Phylum Anamorphobacteria), and Cyanobacteria (Phylum Cyanobacteria). Among them, Acidobacteriales (Acidobacteria), which is abundant in soil, appeared in all samples of sludge and natural substrate. Gloeobacter which can produce oxygen was shown in all samples at high temperature. The specialized anaerobic bacteria, Bacteroidetes (phylum Sphingobacter), was not shown in the rest of the samples, except in the samples of 1S and 3B in May and July.

ACKNOWLEDGEMENTS

The research was financed by the Natural Science Foundation of Liaoning, China (2020-MZLH-02) and Science and Technology Innovation Foundation of Dalian, China (2018J12SN080).

REFERENCES

Cui, Y. B., Wu, X. H., Liu, Z. S., Liu, J.Z., Lin, Y.Z. (2018). Ecological stabilization of thickened wastewater sludge from CAST process. Water Sci. Technol. 58, 1911-1916.

Hardej, M & Ozimek, T. (2002). The effect of sewage sludge flooding on growth and morphometric parameters of Phragmites australis (Cav.). Trin. Ex Steudel. Ecol. Eng. 18, 343–350.

Nielsen, S & Larsen, J. (2016). Operational strategy, economic and environmental performance of sludge treatment reed bed systems - based on 28 years of experience. Water Sci. Technol. 74 (8), 1793-1799.

Uggetti, E., Ferrer, I., Llorens, E., García, J. (2010). Sludge treatment wetlands: a review on the state of the art. Bioresour. Technol. 101, 2905-2912.

Wu, L., Ma, L. Q., Maetinez, G. A. (2000). Comparison of methods for evaluating stability and maturity of biosolids compost. J. Environ. Qual. 29, 424-429.