Analysis on the Spatial Difference of Bacterial Community Structure in Micro-pressure Air-lift Loop Reactor

L G Wan¹,2,3, Q Lin²,3, D J Bian²,3, Q K Ren²,3, Y B Xiao²,3 and W X Lu¹

¹ College of Environment and Resources, Jilin University, Changchun Jilin 130021, China
² School of Water Conservancy & Environment Engineering, Changchun Institute of Technology, Changchun Jilin 130012, China
³ Jilin Provincial Key Laboratory of Municipal Wastewater Treatment (Changchun Institute of Technology University), Changchun Jilin 130012, China

Corresponding author: D J Bian: biandj@163.com, W X Lu: luwenxi@163.com

Abstract. In order to reveal the spatial difference of the bacterial community structure in the Micro-pressure Air-lift Loop Reactor, the activated sludge bacterial at five different representative sites in the reactor were studied by denaturing gradient gel electrophoresis (DGGE). The results of DGGE showed that the difference of environmental conditions (such as substrate concentration, dissolved oxygen and PH, etc.) resulted in different diversity and similarity of microbial flora in different spatial locations. The Shannon-Wiener diversity index of the total bacterial samples from five sludge samples varied from 0.92 to 1.28, the biodiversity index was the smallest at point 5, and the biodiversity index was the highest at point 2. The similarity of the flora between the point 2, 3 and 4 was 80% or more, respectively. The similarity of the flora between the point 5 and the other samples was below 70%, and the similarity of point 2 was only 59.2%. Due to the different contribution of different strains to the removal of pollutants, it can give full play to the synergistic effect of bacterial degradation of pollutants, and further improve the efficiency of sewage treatment.

1. Introduction

The Micro-pressure Air-lift Loop Reactor is a new type of sewage treatment setup designed on the basis of the fully mixed activated sludge process. It utilizes the micro-pressure structure to subtly extend the stroke of the aeration bubbles in the reactor and make full use of the energy provided by the bubbles to cause the mixture to form a looping flow inside the reactor, thereby, the flow rate, dissolved oxygen, sludge concentration, substrate concentration inside the reactor to form a spatial distribution of differences, to achieve a different area within the reactor unique biochemical environment. Compared with the traditional sewage treatment process, it has the advantages of simple structure, low running cost and strong degradation of pollutants [1]. At present, the research on the Micro-pressure Air-lift Loop Reactor is mainly focused on the treatment efficiency of the reactor, the fluid flow characteristics inside the reactor and the sludge distribution characteristics [2-3], the community structure of bacterial in the reactor has not been studied. Actually, the bacterial population structure and bacterial diversity distribution in the reactor have an important role in revealing the relationship between the bacterial and the pollutants removal effect in each functional area, it is important to guide the reactor to be efficient and stable [4]. In this study, PCR-DGGE technology was used to study the
activated sludge bacterial in the Micro-pressure Air-lift Loop Reactor. The differences in bacterial diversity and similarity in the internal space of the reactor and the evolution of bacterial community was investigated, it aims to create a bridge between the operation of the process and the dynamic changes in microbes and their functions. The process parameters were adjusted according to the bacterial community structure and the characteristics of the dominant flora in different spatial locations within the reactor, so as to enhance the special effect of the functional microbes in each region and further improve the effluent treatment efficiency of the reactor.

2. Materials and Methods

2.1. Experimental Setup and Sampling Conditions

The experimental setup is made of organic glass, composed of micro-pressurized gas recirculation flow reactor and sedimentation tank, as shown in Figure 1. The structure of the upper part is 80 mm × 100 mm × 600 mm, the main role is to raise the water level, so that the next part of the reaction zone to withstand micro-pressure; the next part of the main reaction zone, the structure size is: 800 mm × 100 mm × 600 mm. Compressed air from the bottom of the single side by the aperture of 1.2 mm perforated aeration pipe into the gas flow meter to adjust the amount of aeration.

The activated sludge was inoculated in a municipal sewage treatment plant in Changchun, and the initial sludge concentration in the reactor was controlled at about 2500 mg·L⁻¹. Reactor water from the sewage plant grit chamber effluent, the influent rate is 198.5 L·d⁻¹, water quality is in Table 1. After 20 days of culture and domestication, the experimental Setup was placed in a constant temperature room of 20 ± 1°C for 110 days. During operation, the sludge reflux ratio was 100% and the aeration rate was about 0.15 m³·h⁻¹. In order to study the spatial distribution of the bacterial community structure in the reactor, a sampling point as shown in Figure 2 was designed. On the 55th day of the stable operation of the reactor, five sampling points were designed and analyzed. Samples were taken three times and the sludge mixture was immersed for 30 min. The precipitated fraction was used for analysis.

Table 1. Quality of experimental water

| Item     | pH  | COD mg·L⁻¹ | NH₄⁺-N mg·L⁻¹ | TN mg·L⁻¹ | TP mg·L⁻¹ | SS mg·L⁻¹ |
|----------|-----|------------|---------------|-----------|-----------|-----------|
| Range    | 7.1~7.8 | 150~556   | 18.2~36.3    | 38.3~55.1 | 5.8~18.7  | 78~260    |

2.2. Total DNA extraction, polymerase chain reaction (PCR) amplification
Take the sediment sludge, DNA was extracted using Power Soil DNA Isolation Kit, MO BIO Laboratories (Carlsbad, CA, US) according to the manufacturer instruction. DNA concentration and quality were measured using a NanoDrop spectrophotometer. 16S rRNA gene fragments were amplified from the extracted total DNA from the samples by using the bacterial primer sets PRBA357F-GC (5' - CGC CCG CCG CGC GCG GCGGGC GGG GCG GGG GCA CGG GGG GCC TACGGG AGG CAACAG-3') and PRUN 518R (5'-GTAT-TACCGCGGCTGCTGG-3' ) [5]. The conditions used for the PCR were according to the literature [6].

2.3. DGGE analysis
DGGE of PCR products, using the DCode System (Bio-Rad Laboratories Inc, USA), was carried out on 1× TAE buffer, 8% polyacrylamide gels with linear gradient ranging from 30% to 60%. The denaturant concentration increases from the top to the bottom of the gel. DGGE gels were stained for 30 min with SYBR Green I (Invitrogen, Life Technology, USA). The electrophoresis was performed at 85 V and 60°C for 16 h. Then, the electrophoretic results were observed and photographed using a BIO-RAD GelDoc 2000 gel imaging system.

2.4. Analysis of Bacterial Diversity and Similarity
The diversity of microbial communities is expressed by the Shannon-Wiener (H) index. The formula is:

\[ H = -\sum p_i \log p_i \]  
\[ p_i = \frac{n_i}{N} \]

where \( n_i \) is the peak area and \( N \) is the all peak area.

The calculation of \( H \) is based on the location of the DGGE tape and the strength of the strip, and the strength of the strip is represented by the peak area obtained by the quantity one software analysis. By comparing the similarity coefficient (Cs) to reflect the similarity of bacterial in different space points in the reactor, Cs as in equation(3):

\[ Cs = \frac{2j}{a+b} \times 100 \]

where \( a, b \) are the number of bands corresponding to the lane in the DGGE pattern of the samples taken at different spatial points, \( j \) is a, b in a total number of bands.

3. Results and discussion

3.1. DGGE Map Analysis
The denaturing gradient gel electrophoresis results of the PCR products are shown in Figure 3. From the 5 sample lanes, it can be seen that there is a significant difference in the richness and diversity of bacterial in the mixed sludge at different spatial points in the reactor. The internal mixture of the reactor flows vertically under the action of unilateral aeration, 5 different space points of the sludge samples, representing the sewage in the reactor within the five different treatment stages, the corresponding processing time corresponding to each stage. From the DGGE map, each stage of the sample lane both has always maintained a stable number of dominant species and has some processing time with the space to change the environment to cultivate the dominant species. There are also large numbers of strains in different treatment stages. For example, the strains represented by bands a, b, e, f, h, k, and l were always present in five different treatment stages in the reactor internal space, and the number changed little and the strength remained stable, indicating that the bacterial are well adapted to the internal environment of the reactor, the removal of pollutants is prominent; belongs to the dominant species [7]. The strains represented by bands i, j and n were always been there, but in different stages of treatment of the bacterial and the larger changes in the band brightness is weak, indicating that the bacterial are indispensable strains, but not the dominant species [8]. The strains
represented by bands o and p were found in the lanes of sample 1 and sample 5, but it is obvious in the lanes of sample 2, 3 and 4, indicating that the survival of the flora was affected by the influent impact, but with the adaptation to the external environment gradually showed a dominant position, and then with the lack of carbon source, endogenous respiration intensified, the bacterial are gradually being eliminated.

![DGGE profile of total bacteria in activated sludge samples](image)

**Figure 3.** DGGE profile of total bacteria in activated sludge samples

3.2. Analysis of Bacterial Community Diversity

It can be seen from Table 2 that there is a difference in the total bacterial Shannon-wiener biodiversity index of the five sludge samples at different spatial locations within the reactor, mainly due to the difference in the distribution of organic substrate and nitrogen and phosphorus nutrient concentration, dissolved oxygen concentration, sludge concentration and pH value in different areas the vertical circulating flow of the internal mixture of the reactor. The bacterial diversity index of the lane 5 was the smallest, only 0.92, which was mainly located near the point 5, and the concentration of contaminants in the effluent entering the reactor was higher. The microbes near the point 5 have a certain impact, making some of the sewage does not meet the quality of bacterial growth at a disadvantage, the biodiversity index is small. Sewage into the reactor after the ring to do the clockwise circulation, the point 3 of the bacteria gradually adapt to the sewage, bacterial species and the number increased [9], the biodiversity index rose to 1.25. Point 4 near the side of the aeration tube, the mixture dissolved oxygen concentration is higher, while the amount of organic matter decreased with the flow of sewage flow, microbial nutrition decreased, the biodiversity index decreased to 1.20. The point 1 is at the end of the flow-through circulation, a mount of organic mass in the sewage significantly reduced, its growth was severely inhibited and the biodiversity index was reduced to 1.09. Point 2 is located in the middle of the reactor, the flow rate of the mixed solution is small, the activated sludge is easy to flocculate into the group, the type and quantity of bacteria are larger, and the concentration of dissolved oxygen and organic mass in this region is different from that in the outer loop, the formation of a more special bacterial ecological environment, the biodiversity index of up to 1.28.
Table 2. Shannon-Wiener index of biodiversity on various bacteria

| Lane | 1     | 2     | 3     | 4     | 5     |
|------|-------|-------|-------|-------|-------|
| H    | 1.09  | 1.28  | 1.25  | 1.20  | 0.92  |

3.3. Similar analysis of bacterial community

In order to analyze and compare the similarity between the samples, the similarity coefficient (Cs) of each sample was calculated according to the DGGE pattern as shown in Table 3. Overall, the similarity between sample 2 and sample 4 was higher than 80%, and the similarity of sample 3 and sample 4 was the highest, which was 87.4%. The similarity between sample 5 and other samples lower than 70%, of which sample 5 and sample 2 the lowest similarity, only 59.2%. The reason for this phenomenon may be due to the fact that point 3 and point 4 are in the middle of the mixed fluid recirculation flow at which the external environment of bacterial growth does not change significantly so that their bacterial community is mostly of the same; and the point 5 is located near the influent point, at the beginning of the circulating flow, affected by the impact of water quality impact, where the growth of bacterial and other points of the larger differences, resulting in sample 5 and other similarity of the sample is low. The above analysis shows that the microbial population structure undergoes complex succession changes during the operation of the reactor, and there is a large difference in space.

Table 3. Similarity matrix of DGGE

| Lane | 1     | 2     | 3     | 4     | 5     |
|------|-------|-------|-------|-------|-------|
| 1    | 100.0 | 73.8  | 66.2  | 73.5  | 67.7  |
| 2    | 73.8  | 100.0 | 83.0  | 81.8  | 59.2  |
| 3    | 66.2  | 83.0  | 100.0 | 87.4  | 59.6  |
| 4    | 73.5  | 81.8  | 87.4  | 100.0 | 67.2  |
| 5    | 67.7  | 59.2  | 59.6  | 67.2  | 100.0 |

4. Conclusions

There are some conclusions as follows:

1. The spatial difference of the bacterial community structure in the Micro-pressure Air-lift Loop Reactor is related to the flow characteristics of the mixed sludge in the reactor. The vertical circulation of the mixed solution causes the concentration of the substrate, the dissolved oxygen concentration, the sludge concentration and pH distribution difference, thereby, resulting in different spatial location of activated sludge bacterial flora diversity and similarity there is a big difference. Near the point of influent point 5 sample of the smallest biodiversity index, and as the sewage recirculates the biodiversity index increased first and then decreased. The active sludge in the middle of the reactor is easy to flocculate, and the species and quantity of the bacteria are larger, and the biodiversity index of point 2 is the highest. The similarity of the flora between the point 2, 3 and 4 was 80% or more, respectively. The similarity of the flora between the point 5 and the other samples was below 70%, and the similarity of point 2 was only 59.2%.

2. The different spatial positions of the Micro-pressure Air-lift Loop Reactor represent the different treatment stages of the sewage. The bacterial bands of each stage are significantly different. Both have always maintained a stable number of dominant species (such as bands h, k, l, etc.) and with the internal environment of space to cultivate the advantages of bacterial (such as bands o, p). Due to the different contribution of different strains to the removal of pollutants, it can give full play to the synergistic effect of bacterial degradation of pollutants, and further improve the efficiency of sewage treatment.

Acknowledgements

This work was supported by Chinese Major Science and Technology Program for Water Pollution Control and Management (No. 2014ZX07201-011-004, 2012ZX07202-009-01), Jilin Provincial Key Laboratory of Municipal Wastewater Treatment, Changchun Institute of Technology (No.
20160622013JC), Science and Technology Development Program of Jilin Province, China (20140312001ZG), Science and Technology Research Project of the Education Department of Jilin Province, China (No. 2014329) and Youth Fund of Changchun Institute of Technology (No. 320130031).

References

[1] Tian X, Wan L G and Bian D J 2010 Environ. Sci. Technol. 33 374-375
[2] Wan L G, Bian D J, Ai S S, Ren Q K, Tian X and Zuo Y 2012 Environ. Pollut. Preventi. 34 18-22
[3] Bian D J, Ren Q K, Wan L G, Ai S S, Tian X and Qu H 2011 Adv. Mater. Res. 255-260 2740-44
[4] Yin J, Chen Y X, Liu H and Wang Y P 2004 Environ. Sci. 25 11-15
[5] Muyzer G, De Waal E C and Uitterlinden A G 1993 Appl. Environ. Microbiol. 59 695-700
[6] Miura Y, Hiraiwa M N, Ito T, Itonanga T, Watanabe Y and Okabe S 2007 Water Res. 41 627-637
[7] Amann R I, Ludwig W and Schlefier K-H 1995 Microbiol. Rev. 59 143-169
[8] Buzzini A P, Sakamoto I K, Varesche M B and Pires E C 2006 Process Biochem. 41 168-176
[9] Gao D W, Li X X, An R, Fu Y and Ren N Q 2010 China Environ. Sci. 30 209-215