Optimization of the degradation conditions of swine wastewater and microbial community structure analysis in immobilized microbial technology

Ying Wang 1, Xuefei Sun 1, Yuge Chang 1, Yue Quan 2, Mingji Jin 2,*

1 Science College of Yanbian University, Yanji, China
2 Agricultural College of Yanbian University, Yanji, China

*Corresponding author e-mail: jinmingji@ybu.edu.cn

Abstract. In this paper, the treatment characteristics of immobilized microbial technology on swine wastewater under different operating conditions were investigated, so as to optimize the operating conditions of the system. At the same time, the microorganism community structure in the optimal condition was analyzed by high throughput sequencing technology. As a results, the optimal conditions for the degradation of TN in swine wastewater by immobilized microorganism were the filling rate of 25%, HRT of 27.5h and C/N of 5.48. In this case, the actual value of the TN removal rate was 74.81%. With the operation of the system, the abundance and diversity of the microbial community in the system are decreased, while the diversity and abundance of the microbial community in the denitrification tank are generally higher than that in the nitrification tank. Proteobacteria and Bacteroidetes are the dominant bacterial phylum. Hydrogenophaga are the dominant bacterial genus. The function of microbial community in the system is mainly "Metabolism".

1. Introduction
Swine wastewater is a kind of low carbon and high nitrogen wastewater [1], and traditional biological nitrogen removal process requires high carbon source [2]. Therefore, the treatment of swine wastewater has always been a fascinating area in the field of wastewater treatment [3, 4, 5]. Among the varies deep treatments of livestock and poultry wastewater, the immobilized microbial technology has many advantages [6, 7, 8], which make it become an excellent choice in the field of deep treatment of livestock and poultry wastewater at domestic and overseas [9, 10, 11]. In view of this, At the same time, the characteristics of microbial community structure in the system under optimal conditions were analyzed with high-throughput sequencing technology.

2. Materials and methods

2.1. Experimental setup
As shown in Figure 1., the Experiment Reactor is made of polyvinyl chloride barrel and is divided into two parts: a nitrification tank and a denitrification tank. An aeration device was installed in the nitrifying tank. A certain proportion of immobilized cubes were filled in the nitrifying tank and denitrifying tank...
respectively. 1cm³ (1cm×1cm×1cm) polyurethane foam (PUF) was used as the immobilized cube carrier.

![PUF Cube](image)

**Figure 1.** Experiment Reactor

2.2. **Wastewater**

The system operation conditions were optimized by using simulated wastewater with glucose as carbon source, NH₄Cl and KNO₃ as nitrogen source, and MgSO₄.7H₂O, MnSO₄.H₂O and CaCl₂ as microbial growth trace elements. The system treatment performance experiment under the optimal operation condition adopts the actual swine wastewater.

The original activated sludge was taken the municipal wastewater treatment plant and the nitrification / denitrifying bacteria were enriched and acclimate from the activated sludge respectively for the preparation of immobilized cubes.

2.3. **Methods of Analysis**

The water quality indicators measured by the experiment refer to the “Measures for Water and Wastewater Monitoring and Analysis” of the National Environmental Protection Agency of China [12]. In order to analyse the structure of the microbial community and the dynamic changes under optimal conditions, sludge samples from the experimental group were collected and Microbial samples subjected to high-throughput sequencing under the optimal conditions. Microbial samples were collected from The initial sludge, the nitrification and denitrification tanks at the initial stage (1st day) and the later stage (48th day), and the specific samples were labelled as IS (The initial sludge), Before_A and Before_B (Nitrification/denitrification tank sludge during initial operation), After_A and After_B (Nitrification/denitrification tank sludge during late operation) respectively.

2.4. **Experimental Design**

According to the results of single factor experiment, the HRT (A), carrier filling ratio (B) and C/N (C) (3 factors and 3 level response surface) were measured by taking the best experimental condition group as the center point [13], shown as Table 1. In this way, the operating conditions of the system are optimized.

| Code | Factor     | Level |
|------|------------|-------|
| A    | HRT        | -1    |
|      |            | 0     |
|      |            | 1     |
| B    | Filling ratio | 10%  |
|      |            | 20%   |
|      |            | 30%   |
| C    | C/N        | 2.5   |
|      |            | 5     |
|      |            | 7     |
3. Results and discussion

3.1. Response surface experimental results analysis

3.1.1. Model building. Multiple regression fitting of the experimental results was carried out with Design Expert 8.0.6 software [14]. The quadratic model Eq. (1) was formed considering the response quantity (TN degradation rate) and the factors (HRT, Filling ratio, C/N). A, B, and C in the formula indicate the encoded values.

\[
Y=78.27+2.11A+3.35B+2.48C+3.56AB-0.035AC+0.41BC-6.88A^2-4.08B^2-7.01C^2
\]  

(1)

The regression analysis of the model ANOVA and the significance test results are shown in Table 2. P < 0.01 for Model indicates that the model was highly significant [15], had a better fitting degree, and that its response surface could be used for subsequent optimization designs. Simultaneously, the Pr > F value > 0.05 in the Lack of Fit indicated that the model was not significant, the equation had a better fitting degree to the experiment [16]. In addition, \( R^2 \) is 0.978 (\( R^2_{adj} = 0.9497 \)), indicates that the predicted values are in good agreement with the measured values [17]. In the model, the effect of interaction between AC and BC on the removal rate of TN was not significant (P>0.05). The effects of other factors and their interactions on the removal rate of TN were extremely significant (P<0.01). In this experiment, the effect on the removal rate of TN was B > C > A.

Table 2. ANOVA of response surface regression equation

| Source    | Squares | df | Mean Square | F Value | Prob > F |
|-----------|---------|----|-------------|---------|----------|
| Model     | 754.61  | 9  | 83.85       | 34.59   | < 0.0001 |
| A         | 35.53   | 1  | 35.53       | 14.66   | 0.0065   |
| B         | 89.91   | 1  | 89.91       | 37.09   | 0.0005   |
| C         | 49.30   | 1  | 49.30       | 20.34   | 0.0028   |
| AB        | 50.55   | 1  | 50.55       | 20.85   | 0.0026   |
| AC        | 4.9 \times 10^{-3} | 1  | 4.9 \times 10^{-3} | 2.02 \times 10^{-3} | 0.9654 |
| BC        | 0.66    | 1  | 0.66        | 0.27    | 0.6189   |
| A^2       | 199.48  | 1  | 199.48      | 82.29   | < 0.0001 |
| B^2       | 70.19   | 1  | 70.19       | 28.96   | 0.0010   |
| C^2       | 206.79  | 1  | 206.79      | 85.30   | < 0.0001 |
| Residual  | 16.97   | 7  | 2.42        |         |          |
| Lack of Fit | 4.98  | 3  | 1.66        | 0.55    | 0.6724   |
| Pure Error | 11.99 | 4  | 3.00        |         |          |
| Cor Total | 771.58  | 16 |             |         |          |

\[ R^2=0.9780 \quad R^2_{adj}=0.9497 \quad R^2_{pred}=0.8724 \]

Adeq Precisior=15.506

Note: A-HRT, B-Filling Ratio, C-C/N, and Prob > F value less than 0.05 indicate that the model or factors have significant influence. Less than 0.01 means extremely significant.

According to the quadratic model Eq. (1), we determined that the best TN removal rate of the system were HRT of 27.5h, Filling ratio of 25%, and C/N of 5.48. The optimal TN removal rate of the system was predicted to be 79.73%. As a result, the TN removal rate of swine wastewater is 74.81%, with the maximum relative error of the predicted value and the measured value less than 5%. The optimized degradation conditions were accurate and reliable [18, the model can thus effectively predict experimental results.
3.2. High throughput sequencing analysis

High-throughput sequencing results are shown in Table 3, the evenness of the 5 samples is in order IS > Before_B > After_B > Before_A > After_A; the order of richness was Before_B > IS > After_B > Before_A > After_A. IS original sludge is taken from a sewage treatment plant and contains many microbial communities; with the enrichment and domestication of nitrification/denitrifying bacteria, some microbial communities were eliminated and the microbial community diversity was reduced; the microbial community diversity in the nitrification tank is generally lower than that in the denitrification tank, which is consistent with the results of Xiao Huang [19].

Table 3. Microbial community diversity index

| Sample   | Number of sequences | OTUs | Evenness | Coverage | Richness |
|----------|---------------------|------|----------|----------|----------|
|          |                     |      | Shannon  | Simpson  | ACE      | Chao1    |
| IS       | 52501               | 2569 | 6.18     | 6.8 × 10^-3 | 0.99 | 3170.08 | 3082.67 |
| Before_A | 73934               | 1967 | 5.36     | 0.02     | 0.99 | 2467.88 | 2386.38 |
| Before_B | 65512               | 2561 | 6.12     | 7.2 × 10^-3 | 0.99 | 3235.57 | 3144.72 |
| After_A  | 52668               | 1571 | 5.35     | 0.02     | 0.99 | 2155.60 | 2113.78 |
| After_B  | 62525               | 2079 | 5.72     | 9.5 × 10^-3 | 0.99 | 2714.56 | 2611.27 |

In order to explain the species and proportion of the samples with high abundance at different taxonomic levels, the microbial communities of the samples at phylum and genus level were analyzed.

Results are shown in Figure 2. the relative abundances of the top 8 dominant phylum in the five samples with high abundance in IS, Before_A, Before_B, After_A and After_B were 87.15%, 88.84%, 86.36%, 97.65% and 96.11%, respectively. Among them, Proteobacteria, Planctomycetes and Bacteroidetes are the dominant bacteria with relatively high abundance. With the long-term operation of the system, the abundance of Planctomycetes decreased. However, the abundance of Proteobacteria and Bacteroidetes increased significantly, and gradually dominated in the system, which was the dominant bacteria for the systematic degradation of swine wastewater.

As Figure 1, the relative abundances of the top 8 dominant genus in the five samples, the most abundant bacteria in IS, Before_A, Before_B, After_A and After_B were Parcubacteria (3.17%), Thermogutta (11.47%), Dokolonella (4.66%), Hydrogenophaga (10.11%) and Hydrogenophaga (5.92%). The relative abundance of dominant bacteria Pirellula, Thermogutta and Dokdonella decreased significantly with the long-term operation of the system. The relative abundance of Hydrogenophaga, Comamonas and Gemmabacter increased significantly. Especially, Hydrogenophaga had the highest abundance in the nitrifying and denitrifying tanks at the later stage of operation, and was the dominant genus in the system.

Figure 2. Microbial community structure and relative abundance of each sample at the phylum level
Figure 3. Microbial community structure and relative abundance of each sample at the genus level

The microbial community function of 5 samples was predicted, as shown in Figure 4. "Metabolism" was the most dominant category, of which "amino acid metabolism", "carbohydrate metabolism" and "energy metabolism" were the main pathways. There was no significant difference in microbial community function between the five samples, with the enrichment and domestication of nitrification/denitrifying bacteria and the operation of the system, the functions of microbial communities are still basically similar. It means that microbial communities can maintain their original functions through constant adaptation in different environments, this is similar to the results of Yang et al. [20]. In addition, the N transformation-related enzymes in 5 samples were further analyzed. The results showed that the N transformation-related enzymes in IS, Before A, Before B, After A and After B accounted for 4.74%, 4.30%, 4.74%, 5.24% and 5.12% of the total gene abundance, respectively. With the long-term operation of the system, the N transformation-related enzymes conversion increased, and the nitrogen conversion ability of microbial community in the system was strengthened.

Figure 4. Abundances of KEGG pathways in level-2 of the functional prediction by PICRUSt
4. Conclusion
In this study, the characteristics of immobilized microbial technology in the treatment of swine wastewater under different operating conditions were investigated, and the system operating conditions were optimized. At the same time, the microbial community structure was analyzed by high-throughput sequencing technology. The results are as follows:

1) According to the results of response surface optimization, the optimal conditions for TN degradation were HRT of 27.5 h, Filling ratio of 25%, and C/N of 5.48. At this time, the predicted value of TN removal rate is 79.73%, and the measured value of TN removal rate of swine wastewater under this condition is 74.81%. There is no significant difference between the predicted value and the measured value, and the model can effectively predict the TN removal rate.

2) High-throughput sequencing results showed that the microbial community diversity in the system decreased with the operation of the system, while the microbial community diversity in the denitrification tank was generally higher than that in the nitrification tank. Proteobacteria and Bacteroidetes, as well as Hydrogenophaga, are the dominant phylum and genus in the treatment of swine wastewater by immobilized microbial technology. The function of microbial community in the system is mainly "metabolism". With the operation of the system, the N transformation-related enzymes conversion increased, and the nitrogen transformation ability of the system microbial community was strengthened.

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