Dynamic changes of key enzymes activity in a bromate and perchlorate-reducing hydrogen-based membrane biofilm reactor

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Abstract. In this study, the microorganisms in different stages of MBfR were cultured, by using kits to detect the dynamic changes of key enzymes activity, and exploring the effects of biofilm microorganisms on key enzymes activity of MBfR. The results indicated that function or role played by microorganism lived on the outer surface of membrane directly caused the removal of NO3--N, BrO3- and ClO4- from water in the MBfR. The key enzymes activity is related to NO3--N and degraded substances. When the concentration of NO3--N is 5mg/L, the activities of denitrification enzyme were obviously inhibited.

1 Introduction

Hydrogen-based membrane biofilm reactor (MBfR) is a novel and special membrane bioreactor using H2 as an electron donor to reduce kind of oxidated pollutants from water and wastewater[1-4]. As a clean energy, H2 has the advantages of no pollution and low preparation cost, causing extensive concern[5-7]. Although being successful in dealing with water pollution, MBfR also adversely affects the behavior of hydrogen autotrophic microorganisms. Hyun[8] reported that MBfR can enrich the hydrogen-otrophic methanogens, and inhibit the growth of methanogens in acid operations. The research arouse our interests in the study about of biofilm microorganisms on key enzymes activity of MBfR. However, the effects were still unknown and only little attention was paid to the effects on biofilm microorganisms.

In MBfR, hydrogenotrophic denitrification bacte-ria were generally accounted for the most part of the biofilm microorganisms. Hydrogenotrophic denitrif-ication bacteria can successfully promote MBfR re-oval of oxidized contaminants, such as arsenate[9], chromate[10], nitrate[6], perchlorate[11], selenite[12], trichlorathene[13] and N-nitrosodimethylamine[14]. Therefore, studying the effects of biofilm micro-organisms on MBfR is important, which can benefit to the application of biofilm microorganisms. However, the effect among microbial community and key enzymes activity of MBfR has not been reported. This study is based on MBfR degradation of NO3--N, BrO3- and ClO4-, the effects of biofilm microorganisms on key enzymes activities of MBfR were investigated.

2 Materials and methods

2.1. Experimental setup

The fiber inner and outer diameters are 1.0mm, 1.5mm, respectively. The working-volume was 1.8L. The MBfR contained a bundle of 60 hollow-fiber membranes (Watercode Company, Guangzhou, China) with the pore size of 0.02μm. The reactor was 64cm in height and the fiber surface area was 0.28m². The Figure 1 shows the schematic of the MBfR used in this study.

![Fig. 1. Schematic of the hydrogen-based membrane biofilm reactor (MBfR)](image)

2.2. Experimental design

We set up the nitrate reducing and perchlorate reducing MBfR in different conditions through stages 1-8, tap water blank forming biofilm sludge (KM), domesticated sludge in MBfR (XN), forming biofilm sludge in MBfR with a
single BrO₃⁻ at 1mg/L (BM) and forming biofilm sludge in MBfR at a concentration of 1mg/L at a single ClO₄⁻ (GM), setting the concentration of BrO₃⁻ and ClO₄⁻ to 1mg/L, changing the concentration of NO₃⁻N in the influent (1.5, 2.5, 5, 10mg/L), and taking the biofilm sample attached to the hollow fiber membrane (BGM-1.5, BGM-2.5, BGM-5, BGM-10), respectively. The operating conditions of each stage are listed in Table 1.

### Table 1. Conditions for culturing microorganisms.

| Sample number | Operation time (d) | Influent concentration of NO₃⁻N (mg/L) | Influent concentration of BrO₃⁻ (mg/L) | Influent concentration of ClO₄⁻ (mg/L) |
|---------------|--------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| KM            | 52(0-52)           | 1.5                                    | —                                      | —                                      |
| XN            | 35(53-87)          | 10.0                                   | —                                      | —                                      |
| BM            | 31(88-118)         | 5.0                                    | 1.0                                    | —                                      |
| GM            | 32(119-150)        | 5.0                                    | —                                      | 1.0                                    |
| BGM-1.5       | 33(151-183)        | 1.5                                    | 1.0                                    | 1.0                                    |
| BGM-2.5       | 33(184-216)        | 2.5                                    | 1.0                                    | 1.0                                    |
| BGM-5         | 32(217-248)        | 5.0                                    | 1.0                                    | 1.0                                    |
| BGM-10        | 35(249-283)        | 10                                     | 1.0                                    | 1.0                                    |

Notes: Other experimental conditions are hydrogen pressure is 0.04MPa, HRT is 15h, and initial pH is 7.0.

The original sludge (YN), and the biofilm samples attached to the hollow fiber membrane during the stabilization period of each stage (KM, XM, BM, GM, BGM-1.5, BGM-2.5, BGM-5, BGM-10), each taken 1 part, using the kit to measure enzymes activity and high-throughput metagenomic microbial sequencing method to detect it, analyzing the microbial enzyme activity, and selecting the OTU clustering results in the overall abundance of the sequence for analysis to investigate the changes of the microbial population structure in the biofilm under the influence of NO₃⁻N before and after acclimation.

### 3 Results and Discussion

The biofilm on the outer surface of the hollow fiber membrane was sampled 8 times in total. Table 2 shows the specific sampling time and the operational conditions as well as the water quality characteristics of the reactor at the time of sampling.

### Table 2. Water quality parameters and operational conditions at biofilm sampling time

| Sampling time (d) | KM | XN | BM | GM | BGM-1.5 | BGM-2.5 | BGM-5 | BGM-10 |
|-------------------|----|----|----|----|---------|---------|-------|--------|
| Electron acceptor |    |    |    |    |---------|---------|-------|--------|
| NO₃⁻N (mg/L)      | 1.5| 10 | 5  | 5  | 1.5     | 2.5     | 5     | 10     |
| NO₂⁻N (mg/L)      |    |    |    |    |---------|---------|-------|--------|
| BrO₃⁻ (mg/L)      |    |    |    |    | 1       | 1       | 1     | 1      |
| ClO₄⁻ (mg/L)      |    |    |    |    | 1       | 1       | 1     | 1      |
| Influent concentration of NO₃⁻N (mg/L) | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |
| Hydrogen pressure (MPa) | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |
| HRT (h)           | 15 | 15 | 15 | 15 | 15      | 15      | 15    | 15     |
| Effluent concentration of NO₃⁻N (mg/L) | ND  | 0.31 | ND | ND | ND      | 0.23    | 0.35  |
| Effluent concentration of NO₂⁻N (mg/L) | —   | —   | ND | —  | 0.01    | ND      | 0.57  |
| Effluent concentration of BrO₃⁻ (mg/L) | —   | —   | ND | —  | 0.02    | 0.01    | 0.55  |
| Effluent concentration of ClO₄⁻ (mg/L) | —   | —   | —  | ND | 0.02    | 0.01    | 0.55  |

Notes: ND means not detected.

As can be seen from the above table, the sampling time is the last day of the reactor operation to each stable state, and the difference in working conditions is the time of electron acceptor (pollutant) addition. According to the water quality characteristics in the table, the removal effect of the reactor on various pollutants is obviously different under different working conditions.

#### 3.1. The effect of key enzymes activity in hydrogen autotrophic microorganisms

Compared with the original sludge (YN), the tap water blank forming biofilm sludge (KM) of the tap water has a significantly reduced denitrifying enzyme activity caused by the presence of heterotrophic denitrifying bacteria in the original sludge (YN), and the tap water blank forming biofilm sludge (KM) is an autotrophic type of bacteria. With the reducing of the biomass, the enzyme activity is being lowered Figure 2. On this basis, 10mg/L domesticated sludge (XN) was continuously added, the biomass of autotrophic denitrifying bacteria increased, and the activity of denitrifying enzyme upwarded, but a reduction was found in the activity of nitrate reductase, which indicates that the activity of nitrite reductase was significantly enhanced. The denitrifying enzyme and nitrate reductase activities of domesticated sludge (XN) at...
a concentration of 10 mg/L of NO$_3$-N were 226.87 U/L and 70.98 U/L, the two enzyme activities for simultaneous degradation of BrO$_3^-$ and ClO$_4^-$ (BGM-10) at the same NO$_3$-N concentration were 343.47 U/L and 76.67 U/L, denitrifying enzyme activity was significantly improved, and nitrate reductase activity had no essential change, which indicated that the addition of BrO$_3^-$ and ClO$_4^-$ had a little effect on nitrate reductase activity, but had a strong effect on denitrification enzyme activity. At the same time, the denitrifying enzyme activities of single degradation of BrO$_3^-$ (BM) and ClO$_4^-$ (GM) under the condition of NO$_3$-N concentration of 5 mg/L were 165.11 U/L and 158.02 U/L, and the denitrifying enzyme activity of BrO$_3^-$ and ClO$_4^-$ (BGM-5) was 317.58 U/L, which was significantly lower than the same concentration of NO$_3$-N, which proved that a part of denitrifying enzyme acts to reduce BrO$_3^-$ and ClO$_4^-$. Past studies have shown that most of the ClO$_4^-$ reducing bacteria belong to denitrifying bacteria[12,13] and BrO$_3^-$ can be cometabolized by denitrifying enzyme or ClO$_4^-$ reductase to produce nontoxic Br$^-$ under denitrification or ClO$_4^-$ reduction conditions[14,15], similar to this research. The sludge enzyme activities of simultaneous degradation of BrO$_3^-$ and ClO$_4^-$ under different NO$_3$-N concentrations showed that the activities of denitrifying enzyme and nitrate reductase increased linearly with the increase of NO$_3$-N concentration.

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