The operational efficiency of a novel AnMBR treating antibiotic solvent wastewater in start-up stage

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ABSTRACT

The performance of a novel anaerobic membrane bioreactor (AnMBR) for treating antibiotic solvent wastewater was investigated in the start-up stage. The removal efficiencies of the four tested antibiotics were over 90%, mainly attributed to the biological process. Volatile fatty acid increased along with anaerobic sludge acclimation. pH (mean value 7.5) and a (mean value 0.12) remained stable. Mixed liquid suspended solids and mixed liquor volatile suspended solids increased along with the sludge acclimation as well. The protein and polysaccharide in suspended sludge decreased, while the protein/polysaccharide in exopolysaccharides increased. Microbial community analysis showed the abundance of *Methanosarcina* spp. fluctuated over time and was finally stable at 17%. The abundance of *Methanosaeta* spp. increased significantly. There are two kinds of hydrogen producing methane producing microorganisms (*Methanobacteriales* and *Methanomicrobiales*) in AnMBR. *Methanobacteriales* was the dominant methanogenus. These results indicate that an AnMBR can effectively treat antibiotic solvent wastewater in the start-up period.

Key words | AnMBR, antibiotic solvent wastewater, isopropanol (IPA), M-cresol (MC), N,N-Dimethylformamide (DMF), tetrahydrofuran (THF)

INTRODUCTION

Generally, drugs are manufactured through the fermentation and chemical synthesis techniques. Fermentation and chemical synthesis-based pharmaceutical wastewater usually has high chemical oxygen demand (COD) and contains a variety of toxic pollutants (Ramos et al. 2014; Pretel et al. 2015), making it difficult to treat. Among the toxic compounds, the antibiotic solvents are the most representative pollutants, as they have high biological toxicity and an inhibitory effect on microorganisms. Therefore, the disposal of pharmaceutical wastewater, especially antibiotic solvent wastewater, has attracted increasing public and research attention (Ramos et al. 2014; Pretel et al. 2015).

Currently, the traditional anaerobic-aerobic processes such as up-flow anaerobic sludge bed/blanket (UASB) and sequencing batch reactor activated sludge process are used widely to treat antibiotic solvent wastewater. Among these, the membrane bioreactor (MBR) is an effective method for treating high concentration antibiotic solvent organic wastewater. Moreover, most of the research on MBRs focuses on the aerobic treatment process. According to the literature (Ren et al. 2005; Kim et al. 2007; Snyder et al. 2007), the MBR process was found to be efficient for the treatment of antibiotic solvent wastewater and the removal of some pharmaceuticals, such as acetaminophen, ibuprofen and caffeine. However, the effluent COD of the MBR process does not meet the new emission standards (GB 21904-2008, COD < 120 mg/L) due to the higher COD concentration and poor biodegradability of antibiotic solvent wastewater with the
promulgation and implementation of new and more stringent standards for the discharge of water pollutants in China’s pharmaceutical industry (GB 21904-2008, COD <120 mg/L).

According to the literature (Chelliapan et al. 2006; Ramos et al. 2014; Pretel et al. 2015), the anaerobic membrane bioreactor (AnMBR) technology can improve the biodegradability of refractory wastewater, achieving the discharge standards of high concentration refractory wastewater, with less energy consumption. Therefore, AnMBR technology has become one of the key researches in foreign associations such as Water Environmental Research Foundation. To the authors’ knowledge, few studies have been carried out using an AnMBR for treating antibiotic solvent wastewater. Additionally, the shorter hydraulic retention time (SHRT) and the higher COD volumetric loading rate (HOLR) are two very important directly controllable operating parameters in an AnMBR. This is because SHRT-HOLR not only relates to the AnMBR operating efficiency and reactor size, but also relates to the microbial community characteristics and membrane fouling control in an AnMBR (Ramos et al. 2014; Pretel et al. 2015). Research on the operational efficiency of AnMBRs treating antibiotic solvent wastewater also is scarce under SHRT-HOLR conditions.

Therefore, the purposes of this work are: (a) to explore the feasibility of an AnMBR for treatment of antibiotic solvent wastewater containing M-cresol (MC), isopropanol (IPA), tetrahydrofuran (THF), and N,N-Dimethylformamide (DMF); and (b) to understand the removal efficiency for organic matter, the removal efficiency for characteristic pollution, the membrane efficiency and the structure change of the microbial community in the start-up stage of an AnMBR system.

**MATERIALS AND METHODS**

**Test apparatus**

A new split type AnMBR was designed as shown in Figure 1. The main body anaerobic fermentation tank in the AnMBR adopted continuous stirring and complete mixing. A three-phase separator was set for separating biological gas, wastewater and anaerobic sludge. The biogas was collected from the top of the reactor. The anaerobic sludge automatically slid down and settled to the bottom of the reactor. Wastewater flowed out from the clear zone. This not only...
reduced the loss of anaerobic sludge, increasing the anaerobic sludge concentration in the reactor, but also reduced the sludge content and sludge viscosity, thus effectively reducing membrane fouling.

**Synthetic wastewater**

In order to explore the optimized experimental conditions in the AnMBR, the experimental antibiotic solvent wastewater was artificially prepared. The main organic pollutants in the wastewater were MC, IPA, THF, and DMF. The specific water quality indicators are shown in Table 1.

The content of ammonia nitrogen in the simulated wastewater was low. The nitrogen, which is used by microorganisms, was insufficient. It was necessary to add the appropriate amount of ammonium chloride and potassium dihydrogen phosphate into the influent to supplement the nitrogen and phosphorus (C:N:P = 250:5:1) in this work. Glucose was added to the wastewater simultaneously as the co-substrate (1.032 g glucose COD is 1.0 g), forming the glucose and antibiotic solvent (MC, IPA, THF, DMF) mixed wastewater. The glucose concentration in the wastewater was gradually reduced, while the concentration of antibiotic solvent was increased in the experiment. Thus, the anaerobic sludge was domesticated, making it suitable for the degradation of antibiotic solvent. The influent eventually became a pure solvent of antibiotic wastewater. The dosages of glucose, buffer, nutrients and trace elements in the synthetic wastewater are shown in Table 2.

**Source of anaerobic sludge**

Anaerobic sludge was collected from four sources, namely the UASBAF (two-stage combined UASB and anaerobic biofilter) reactor at Harbin 2nd Traditional Chinese medicine factory, the UASB reactor at North China Pharmaceutical General Factory, the internal circulation reactor at Harbin Brewery Factory and the anaerobic pond at Harbin Wenchang Wastewater Treatment Plant, then mixed at the volume ratio of 1:1:1:1 and used to fill the AnMBR.

| Characteristic pollutant species          | MC          | IPA         | THF         | DMF         |
|------------------------------------------|-------------|-------------|-------------|-------------|
| COD (mg/L)                               | 1,000–5,000 | 1,000–5,000 | 15,000–25,000 | 8,000–25,000 |
| Antibiotic solvent COD equivalent (gCOD/g)| 2.52        | 2.4         | 2.44        | 1.86        |
| Biochemical oxygen demand (BOD) (mg/L)   | 200–1,000   | 400–2,000   | 2,000–5,000 | 2,000–6,500 |
| BOD/COD                                  | 0.2         | 0.4         | 0.2         | 0.25        |
| MC (mg/L)                                | 0–1,500     | –           | –           | –           |
| IPA (mg/L)                               | –           | 0–200       | –           | –           |
| THF (mg/L)                               | –           | –           | 200–4,500   | –           |
| DMF (mg/L)                               | –           | –           | –           | 50–2,000    |
| pH                                       | 6.0         | 6.2         | 8.5         | 6.5         |

| Characteristic pollutant species          |             |             |             |             |
|------------------------------------------|-------------|-------------|-------------|-------------|
| 1,3,5-Tribromobenzene (mg/L)             |             |             | 2,200–2,800 |             |
| 1-Br-3-nitrobenzene (mg/L)               |             | 2,500–2,900 |             |             |
| 1-Bromide (mg/L)                         |             |             | 2,800–3,200 |             |
| 1-Bromine propane (mg/L)                 |             |             | 2,600–3,000 |             |
| 3-Amino phenol (mg/L)                    |             |             | 2,500–2,900 |             |
| N-Acetylsulfanilyl chloride (mg/L)        |             |             | 450–550     |             |
| Para ester (mg/L)                        |             | 550–650     |             |             |
| SO$_4^{2-}$ (mg/L)                       |             | 1,000–5,000 |             |             |
| Influent temperature (°C)                |             |             | 20–25       |             |
| SS (mg/L)                                |             |             | 30–50       |             |
AnMBR start-up

There are two strategies to start up an AnMBR: (1) using a constant HRT and COD of concentration to start-up; (2) using a constant HRT and gradually increasing the COD concentration to start-up. In accordance with the antibiotic solvent wastewater characteristics, this work used the second strategy, and the specific operating conditions are listed in Table 3.

Analysis

Influent COD, supernatant COD, membrane effluent COD, mixed liquid suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) were measured by the national environmental protection department and the American Public Health Association standard method.

MC concentration was determined by high performance liquid chromatography (Chen et al. 2016); the concentration of IPA (Lenka & Sarkar 2015), THF (Shinde et al. 2018) and DMF (Józwiak et al. 2015) were determined by gas chromatography.

The total volatile fatty acid (VFA) concentration in the reactor (Scoma et al. 2016) and the alkalinity of hydrogen carbonate (Lafay et al. 2014) were measured by the titration technique. The compositions of VFAs were determined by a SP-502 gas chromatograph equipped with a hydrogen flame ion detector (Scoma et al. 2016). The microbial community structure of anaerobic sludge was analyzed by denaturing gradient gel electrophoresis (Aydin et al. 2015).

Removal rate calculation

The COD, MC, IPA, THF, DMF biological removal rate = (influent COD, MC, IPA, THF, DMF – supernatant COD, MC, IPA, THF, DMF)/influent COD, MC, IPA, THF, DMF.

Table 2 | Dosage of buffer, nutrient and trace element

| Item                     | Solution concentration (g/L) | Dosage (mg/gCOD) | Item                     | Solution concentration (g/L) | Dosage (mg/gCOD) |
|--------------------------|------------------------------|------------------|--------------------------|------------------------------|------------------|
| Glucose                  | Solid                        | Adjust according to the actual situation | Sodium bicarbonate         | Solid                        | Adjust according to the actual situation |
| Nickel sulfate           | 440                          | 5–12             | Calcium chloride         | 260                          | 10–25            |
| Ammonium chloride        | Solid                        | 220–320          | Ferric chloride          | 250                          | 3–11             |
| Two potassium hydrogen phosphate | 188                        | 22–57            | Manganese chloride       | 440                          | 0.8–5.2          |
| Potassium dihydrogen phosphate | 75                          | 8–25             | Zinc chloride            | 260                          | 0.5–2.1          |
| Magnesium sulfate        | 320                          | 32–84            | Cobalt chloride          | 220                          | 0.3–1.8          |
| Sodium citrate           | 170                          | 18–86            | Sodium metaborate        | 80                           | 0.1–0.9          |
| Copper chloride          | 130                          | 0.1–0.8          | Ammonium molybdate       | 160                          | 0.2–1.2          |

Table 3 | Operating conditions for AnMBR start-up

| Item                      | Start-up         |
|---------------------------|------------------|
| Running days (d)          | 1–23             |
| THF concentration of influent (mg/L) | 0–100       |
| DMF concentration of influent (mg/L) | 0–300          |
| MC concentration of influent (mg/L) | 0–200         |
| IPA concentration of influent (mg/L) | 0–20          |
| Influent COD (mg/L)       | 1,000–3,000      |
| HRT (h)                   | 48               |
| COD volume load (kg COD/m³·d) | 0.5–1.5          |
| THF volume load (kg THF/m³·d) | 0–0.05          |
| DMF volume load (kg DMF/m³·d) | 0–0.25          |
| MC volume load (kg MC/m³·d) | 0–0.05           |
| IPA volume load (kg IPA/m³·d) | 0–0.01          |
| Temperature (°C)          | 35 ± 1           |
| pH                        | 6.0–8.5          |
| Sludge age (d)            | No sludge        |
| Alkalinity (mg/L)         | >1,000           |
| Cross flow rate CFV (m/s) | 0.2 ± 0.1        |
| Transmembrane pressure TMP (kPa) | 10–30          |
| Membrane effluent discharge (L/h) | 2.0              |
The COD, MC, IPA, THF, DMF total removal rate = (influent COD, MC, IPA, THF, DMF – membrane effluent COD, MC, IPA, THF, DMF)/influent COD, MC, IPA, THF, DMF.

The COD, MC, IPA, THF, DMF physical removal rate = (supernatant COD, MC, IPA, THF, DMF – membrane effluent COD, MC, IPA, THF, DMF)/supernatant COD, MC, IPA, THF, DMF or = COD, MC, IPA, THF, DMF total removal rate – COD, MC, IPA, THF, DMF biological removal rate.

RESULTS AND DISCUSSION

COD removal efficiency

In order to investigate the operational efficiency of the AnMBR in treating the antibiotic solvent wastewater in the start-up stage, the influent COD, supernatant COD, membrane effluent COD, COD removal rate and the change of volume loading rate over time were measured in the start-up phase (0–23 days) (Figure 2).

It can be seen from Figure 2 that COD gradually increased from 1,000 mg/L to 3,000 mg/L. The starting load rate (OLR) was 0.5 kg COD/(m³·d) in AnMBR treatment for antibiotic solvent wastewater. The start-up duration was 23 days (the start-up duration was 30 days in UASB treating THF wastewater). The HRT was set at 48 h and the temperature was controlled at 35 ± 1 °C. The cross flow rate (CFV) was 0.2 ± 0.1 m/s, and the trans-membrane pressure was 10–30 kPa.

At the beginning of the experiment (1–15 days), the membrane effluent COD increased. After a few days, the membrane effluent COD decreased. After inoculation, the reactor did not produce gas and the total removal rate of COD was lower than 55%, indicating that microorganisms did not adapt well to the wastewater. When the reactor was run to day 16, OLR rose to 1.2 kg COD/(m³·d), microorganisms gradually adapted to the wastewater, the total removal efficiency of COD increased to 85%, and the membrane effluent COD was stable at 400–500 mg/L.

The supernatant and membrane effluent COD values were very similar throughout the beginning of the experiment. The supernatant COD was stable at 600–700 mg/L. COD removal efficiency through the biological role was around 77%. Physical COD removal efficiency was calculated by subtracting biological COD removal efficiency from total COD removal efficiency, which showed that the pollutants were removed by the physical interception of the membrane and the two dynamic biological membranes of the membrane surface. The physical removal efficiency of COD increased from 2.1% to 20%, during the start-up stage, indicating the formation of the two dynamic membranes (DMs). In addition, TMP was 10–30 kPa at the initial stage, due to the low-voltage start-up, similarly to the study by Yoo et al. (2012), where an anaerobic fluidized bed membrane bioreactor (SAF-MBR) was used to treat municipal wastewater, TMP was 25 kPa within 3 days of the start-up stage and the membrane flux was 11 L/(m²·h).

The application of DM technology is a new concept in AnMBRs (Ersahin et al. 2014). The suspended particles in the effluent, such as microbial cells, aggregates and inert substances, can accumulate on the membrane surface and form a dynamic filter cake layer. Pollutants can be trapped by the DM, which greatly reduces the pollution of the membrane components. In order to provide a stable operation for AnMBRs, the DM thickness should be controlled by the shearing stress on the membrane surface. The current research has mainly focused on the long-term operation of the dynamic film in the external AnMBR high concentration wastewater treatment processes, the investigation for the biogas injection rate and the effect of OLR on removal efficiency and filtration characteristics. The formation of an effective DM in AnMBRs could improve the removal efficiency of pollutants. The DM would be formed soon after the system start-up (Park et al. 2004; Hu et al. 2016), and the improved pollutant removal performance could be observed in a few days. The DM formation is dependent on the type of substrate, the concentration of sludge and the structure of membrane modules. As an example, Ersahin et al. (2014) showed that the DM formation was obtained in 15–20 d, and a high removal efficiency of COD was achieved.

Removal of characteristic pollutants

In order to investigate the operational efficiency of the AnMBR treating antibiotic solvent wastewater in the start-up stage, the influent (THF, DMF, MC, IPA), supernatant
(THF, DMF, MC, IPA), membrane effluent (THF, DMF, MC, IPA), (THF, DMF, MC, IPA) removal rate and the change of volume loading with time were measured in the start-up phase (Figure 3).

As shown in Figure 3, in the start-up period, THF was not detected in the influent until the 5th day. When the influent COD increased from 1,000 to 3,000 mg/L, the influent THF gradually increased from 20 to 100 mg/L, and the THF start loading rate was 0.01 kg THF/(m$^3$·d), the effluent THF was less than 5 mg/L, the total removal rate of THF was 95%.

The THF concentration in supernatant fluctuated from 13 to 24 mg/L, slightly higher than that in membrane effluent. The biological removal efficiency of THF was stable at about 80% throughout the start of the experiment, while the physical removal efficiency of THF was 11%.

As shown in Figure 3, the influent DMF increased from 0 to 300 mg/L, the corresponding DMF volumetric loading rate increased from 0 to 0.15 kg DMF/(m$^3$·d), the membrane effluent DMF and supernatant DMF were similar to each other with an average concentration of 26 mg/L and 39 mg/L, respectively. During the start of the experiment, the total removal efficiency of DMF increased from 49% to 92%, the DMF biological removal rate increased from 43% to 75%, and the average physical removal rate of DMF was 7%, which proves that the physical removal rate of DMF is also basically achieved by the physical entrapment of the membrane at start-up initial stage because the membrane surface did not form the two DMs.
Figure 3 | Comparison of various characteristic pollutant removal rates.
As shown in Figure 3, the influent MC increased from 0 to 200 mg/L, the corresponding MC volumetric loading rate increased from 0 to 0.099 kg MC/(m³·d), the membrane effluent MC and supernatant MC were similar to each other. When the reactor ran to day 15, the supernatant MC was higher than the effluent MC. During the whole experiment, the total removal efficiency of MC increased from 48% to 91%, the MC biological removal rate increased from 41% to 80%, and the average physical removal rate of MC was 8.7%.

As shown in Figure 3, influent IPA concentration was much lower than other characteristic pollutants. The influent IPA increased from 0 to 25 mg/L, the corresponding IPA volumetric loading rate increased from 0 to 0.099 kg IPA/(m³·d). The membrane effluent IPA and supernatant IPA were similar to each other, the average values were 11 mg/L and 2.2 mg/L respectively. During the whole experiment, the total removal rate of IPA increased from 53% to 96%, and the average physical removal rate of IPA was 7.3%.

As shown in Figure 3, the characteristic pollutant concentrations of influent during the start-up period were DMF > MC > THF > IPA. A similar trend was observed in the supernatant and membrane effluent. At start-up, IPA had the highest biological removal efficiency, while THF had the lowest. The physical removal efficiency of THF was the highest, and the physical removal efficiency of DMF was the lowest. The highest total removal efficiency of the characteristic pollutant was IPA, the lowest was MC. The same pattern also existed in the characteristic pollutant volume load and sludge load, namely, DMF > MC > THF > IPA.

Analysis of VFA and terminal fermentation products

In order to investigate the operational efficiency of the AnMBR treating antibiotic solvent wastewater in the start-up stage, the change of VFA and terminal fermentation product over time were measured in the start-up phase (Figure 4).

As shown in Figure 4, VFA increased from 58 mg/L (acetic acid) to 320 mg/L along with the anaerobic sludge acclimation, but the growth rate was slow, indicating that microorganisms in the reactor were adapting to wastewater, a few syntrophic acetogenic bacteria began to appear and VFA began to accumulate. However, other volatile acid concentrations did not significantly change.

Throughout the start-up period, the reactor performance was good, which could also be reflected in the pH (the average value was 7.5) and \( a \) value (the average value was 0.12). Poggi-Varaldo et al. (1997) showed that the critical \( a \) value corresponding to anaerobic reactor stability was 1.0; Khoufi et al. (2015) found the anaerobic reactor could be operated successfully when the \( a \) value was less than 0.3.

MLSS, MLVSS and sludge loading rate of COD

In order to investigate the operational efficiency of the AnMBR treating antibiotic solvent wastewater in the start-up stage, the change of MLSS, MLVSS and COD sludge loading over time were measured in the start-up phase (Figure 5).

As shown in Figure 5, the inoculation sludge MLSS concentration was 17 g/L, the concentration of MLVSS was 12 g/L, MLVSS/MLSS was 0.75, COD sludge load was 0.04 kg COD/(kg MLVSS·d). With the extension of running time, the MLSS and MLVSS values increased along with sludge acclimation. This was because the microorganisms adapted to the wastewater, resulting in the improvement of microbial activity. MLSS increased to 25 mg/L. MLVSS increased to 20 mg/L. MLVSS/MLSS also increased to 0.79 mg/L when the reactor was run to day 17.

AnMBR operating efficiency showed that the inoculation sludge, which was formed by sludge from a variety of anaerobic environments, provided sufficient sludge population to anaerobically digest and degrade organic matter. Moreover, these populations showed a high level of activity. Furthermore, in order to ensure the effective operation, the COD sludge loading rate was at least 0.04 kg COD/(m³·d). During this period, MLSS and MLVSS of sludge attaching on the membrane surface were not determined.

Analysis of EPS content in suspended sludge

In order to investigate the operational efficiency of the AnMBR treating antibiotic solvent wastewater in the start-up
stage, the exopolysaccharides (EPS) content in the suspended sludge was measured in the start-up phase (Figure 6). Protein and polysaccharide were the main components of EPS. The determination of protein and polysaccharide enabled the determination of the content of EPS in suspended sludge. The contents of protein and carbohydrate in inoculation sludge were 125 g/g sludge and 68 g/g sludge, respectively. During start-up, the sample was taken every two days for the determination of EPS content.

As shown in Figure 6, the concentration of protein and polysaccharide decreased over time in suspended sludge, while the protein/polysaccharide increased. The contents of protein and polysaccharide were 125, 116, 88 and 94 mg/g VSS and 68, 84, 38 and 54 mg/g VSS respectively at the 1st, 7th, 16th, and 23rd days. The protein/polysaccharide values were 1.8, 1.4, 2.3 and 1.7 respectively. In this study, the contents of protein and polysaccharide in sludge samples were slightly higher than those of other researchers.

**Microbial community dynamics in AnMBR**

In order to investigate the operational efficiency of the AnMBR treating antibiotic solvent wastewater in the start-up stage, the dynamic changes of the microbial community were investigated in the start-up stage (Figure 7).

As shown in Figure 7, there were four kinds of methanogens in the AnMBR, including \textit{Methanosetaeaceae},
Methanosarcinaceae, Methanobacteriales and Methanomicrobiales. Methanosarcinaceae can use acetic acid as the substrate and grow in a low concentration of acetic acid conditions. Methanosarcinaceae can use acetic acid, hydrogen and organic compounds as substrates and grow in high concentrations of acetic acid. Methanobacteriales and Methanomicrobiales just use hydrogen as the substrate.

Methanosaeta spp. was the predominant archaea (the relative abundance was 22%) in the start-up stage. In 6–9 d, the abundance of Methanosarcina spp. had been reduced (the relative abundance was 16%), however its relative abundance increased (mean value is 30%) in days 10–12, and then decreased in day 13 and remained at around 17%.

Methanosaeta spp. was able to grow in a low concentration of acetic acid (Lü et al. 2016) and had higher affinity for low acetic acid. Therefore, it enriched in the start-up stage. Because Methanosaeta spp. existed in the inoculation sludge, one third of the inoculated sludge was anaerobic granular sludge. It has been demonstrated that Methanosaeta spp. were the key methanogens in the granular sludge community in the UASB reactor (Montalvo et al. 2016).
and the anaerobic moving bed reactor (Borghei & Hosseini 2004).

In this work, the granular sludge inoculated in the AnMBR was crushed in the inoculation process. This was because AnMBR conditions could not stimulate the formation of granular sludge. Therefore, the increase of methane bacteria of bristle concentration was probably due to lower acetate concentration at the beginning of the operation.

There were two kinds of hydrogen utilization methanogens in the AnMBR (Methanobacteriales and Methanomicrobiales). In the initial 23 days of reactor operation, Methanobacteriales dominated the archaea community. The relative average abundance was 16%. During the start-up stage, Methanomicrobiales maintained a low abundance with an average value of 5.5%.

**CONCLUSIONS**

It was feasible for the novel AnMBR technology to effectively treat antibiotic solvent wastewater. The total removal rate of COD, THF, DMF, MC and IPA were 11%, 11%, 7%, 8.7% and 7.3%, respectively. VFA increased along with the anaerobic sludge acclimation. MLSS and MLVSS increased along with the sludge acclimation as well. The protein and polysaccharide in suspended sludge concentration decreased, but the protein/polysaccharide in EPS increased. The abundance of Methanosarcina spp. fluctuated with a mean value of 17%. The number of Methanosaeta spp. increased significantly. There were two kinds of hydrogen producing methane producing bacteria (Methanobacteriales and Methanomicrobiales) in the AnMBR. Methanobacteriales was the dominant archaea.

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