Study on the membrane bioreactor for treating surfactant wastewater
Zhilin Ran, Jia Zhu, Ke Li, Li Zhou, Pei Xiao and Bing Wang

ABSTRACT
The main component of surfactant is linear alkylbenzene sulfonate (LAS), which is toxic to the ecological environment and can cause serious harm. In this study, some activated sludge was taken from the aerobic and anaerobic tank of a sewage treatment plant in Shenzhen, then cultivated and domesticated in a membrane bioreactor with artificial surfactant wastewater. The start-up phase of the reactor adapted the constant-flux filtration, and the HRT was 12 h. The pH was below 5.5, which needed the addition of NaHCO₃ after 6 days to adjust to the more optimal level (pH 6.5–7.5). After operation for 20 days, the start-up of the system was considered successful. At the early stage, the removal rates of chemical oxygen demand (COD) and LAS were relatively stable, reaching as high as 85.49%–93.31% and 80%, respectively. When the LAS concentration reached over 175 mg/L and the COD declined to about 83%, the removal rate of LAS also significantly decreased. LAS removal rate further decreased to about 60% when the dosage reached 200 mg/L, indicating that the resistance of microorganisms against LAS toxicity was also limited. LAS degradation could have been mainly driven by Dechloromonas, Gemmata, Pseudomonas and Zoogloea in the system.

INTRODUCTION
Surfactant is common in chemical, machinery, petroleum, metallurgy and other fields, and whose main component is linear alkylbenzene sulfonate (LAS) (Duarte et al. 2010a). LAS is toxic to the ecological environment and can cause serious harm to humans. Biodegradation is an important method to remove LAS. However, at present, most studies have focused on the cultivation and domestication of the degrading bacteria whose sole carbon source is LAS, and there are few reports on the degradation of LAS by co-metabolism (Terzić et al. 1992; Brenner et al. 2005; Carosía et al. 2014).

In order to investigate the effect of co-metabolism of LAS, some activated sludge was taken from the aerobic and the anaerobic tank of a sewage treatment plant in Shenzhen, then cultivated and domesticated in a membrane bioreactor (MBR) with artificial surfactant wastewater. First is the start-up phase, in which the influent contains glucose as the sole carbon source but no LAS. When the removal rate of chemical oxygen demand (COD) remains stable and efficient, the start-up can be considered finished. The start-up phase lasts 20 days. Next is the domestication phase. LAS is started to be added into the influent and the concentration increases gradually. In the early phase of domestication, the removal rates of COD and LAS were respectively 85.49%–93.31% and about 80%, at relatively stable levels. This showed that LAS could be degraded by co-metabolism in the presence of growth medium. When the concentration of LAS reaches 175 mg/L, the removal rate of COD (about 83%) and LAS (from 78.31% to 69.89%) decreased obviously, which showed that there was a limit to the adaptation of microorganisms to LAS toxicity.
METHODS

Experimental device

We used the integrated MBR to treat LAS, and the process is schematically shown in Figure 1. The bioreactor was made from polymethyl methacrylate, with dimensions of 500 × 300 × 500 mm (L × W × H) and the effective volume was 60 L. During the operation, the liquid level was maintained at 400 mm with an elevation of 100 mm. The film modules, composed of inorganic ceramic plate films, were commercially manufactured by Meidensha Corp., major parameters of which are shown in Table 1. For this study, two films were used with a total effective film area of 0.25 m².

The raw wastewater was suctioned using a peristaltic pump and transferred to the reactor, which was fully mixed with the microbial flocs using a stirring rake. Then, using the suction of the outlet pump, the water was introduced to both sides of the film, which was collected by catch pipes and then pumped out by the outlet pump. Both of the backwash and outlet pumps were alternately and periodically operated to increase and promote film pollution. The aeration system was set up under the film modules. The gauges for the liquid level, dissolved oxygen, pressure, flow, peristaltic pump and valves were all connected with the programmable logic controller in the electric control cabinet, to allow self-control and real-time monitoring.

Source of sludge

The sludge used in this trial was a mixture of the sludge from the aerobic and anaerobic wastewater tanks of a treatment plant in Shenzhen city.

Water quality of raw water

We used an artificial surfactant wastewater, whose characteristics are summarized in Table 2.

Detection method

Concentration of LAS was measured using fluorescence spectrophotometry (Chakraborty et al. 2005). Briefly, a 1.5 mL water sample was centrifuged for 10 min at 8,000 rpm, from which 1 mL supernatant was taken and added into a 25 mL colorimetric tube and mixed with 5 mL NH₃-NH₄Cl buffer solution (pH = 10.6). Deionized water was further added until it reached 25 mL. The reagent blank was used as the reference, and measured at excitation wavelength of λex = 230 nm and emission wavelength of λem = 290 nm. Specifically, we calculated for fluorescence

Figure 1 | Process flow chart of the trial.
intensity \( F \), and the LAS concentration was determined from a standard curve and the \( F \) value.

On the other hand, COD concentration was determined following the confined catalysis digestion method. The HACH CRB200 digester was specifically used to digest samples at a temperature of 150 \(^\circ\)C. After cooling to normal temperature, reading of data was carried out in a HACH CR3900 desktop visible spectrophotometer.

### High-throughput sequencing

The microbial population within the system was determined using high throughput sequencing approach. Sludge samples were taken at different phases, and HTS of the amplicons was carried out in Shenzhen BGI using the Roche 454 GS FLX Titanium platform. The sequencing included five samples, which were labeled as S1–S5. S1 denotes the original sludge, S2 those samples during the last phase of startup, S3 were the samples of the middle phase of domestication, S4 were from the last phase of domestication, and S5 were the samples from the stable phase.

### RESULTS AND DISCUSSION

#### Start-up of the system

The inoculated sludge was mixed at a volume ratio of 1:1 before being added into the reactor. The volume of the mixed sludge accounted for 77\% of the reactor’s effective volume. The initial sludge concentration within the reactor was 1,576 mg/L. The raw water used for the trial was artificial wastewater, with glucose as the carbon source, while nitrogen, phosphorus and potassium were provided in the form of \( \text{NH}_4\text{Cl}, \text{K}_2\text{HPO}_4 \) and \( \text{KCl} \), respectively. Small amounts of trace metals such as \( \text{Fe} \) and \( \text{Mg} \) were also added. The initially prepared COD concentration was 200–300 mg/L, and the C/N ratio was 4/1–5/1. The reactor startup stage adopted constant-flux filtration, with an effluent flow rate of 5 L/h, film flux of 20 L/(m\(^2\)h) and HRT of 12 h. The operation conditions included outlet for 8 min, backwash for 2 min and backwash flux of 30 L/(m\(^2\)h).

#### Change in pH condition

At the early stage of the startup phase, the pH of the reactor was lower than 5.5, which mainly resulted from mass propagation of acid-producing bacteria. Since the pH range suitable for the growth of methanogenic bacteria was 6.5–7.8, the resulting environment was not favourable for methanogens, indicating that excessive acidification would result in system collapse. Hence, to adjust the pH value of the system, on the 6th day of the startup, \( \text{NaHCO}_3 \) was added into the influent. As shown in Figure 2, the pH within the system gradually increased, and after the 10th day it was maintained within 6.5–7.5.

#### Change of COD

As shown in Figure 3, during the startup phase, COD concentration of the influent significantly changed from 200 to 300 mg/L. In contrast, the water quality of the effluent was relatively stable and the COD concentration remained at a relatively lower level, with removal rate maintained above 90\%. With the prolonged startup time, the COD concentration of effluent gradually declined, until it reached and
was maintained at 25 mg/L after 9 days. The start-up reaction was considered successful when it lasted 20 days of operation.

Domestication of the system

After the successful start-up, the low concentration of LAS was added into the influent first, with the dosage gradually increased at an increment, domesticating the microbial system in the reactor. During this period, the other parameters were kept the same as those during the start-up phase. To make the microbial system gradually acclimatize to LAS toxicity, the same dosage was maintained at each increment for a certain period before being increased.

Effects of the removal of LAS

During the domestication phase, the LAS concentration in the influent gradually increased from 25 mg/L to 200 mg/L, with a total of eight concentration gradients and with each concentration being maintained for 10 days before increasing. As shown in Figure 4, the microbial system had relatively good removal efficiency for LAS, and its removal rate was not affected by the increase in the dosage, and was consistently maintained above 78.31%. When LAS concentration reached 150 mg/L, the LAS concentration in the effluent was below 30 mg/L. This indicates that the mixed sludge had a microbial assemblage that can readily adapt to changes in water quality and LAS toxicity. At the same time, the novel film separation technology in the bioreactor’s film lengthened the retention time of refractory organic matter and LAS in the bioreactor, guaranteeing the water quality of the effluent. Further, when LAS was increased to above 175 mg/L, its removal rate also significantly declined. However, when the dosage reached 200 mg/L, the removal rate of LAS was only about 60%, indicating that the removal efficiency of LAS out of the system was limited.

The change of removal rate of COD

The removal rate of COD was relatively stable during the whole domestication phase (Figure 5). With the increase of LAS dosage, the COD concentration of the influent ranged between 273.0 and 408.3 mg/L with relatively high
fluctuation. However, the COD concentration of the effluent was below 50 mg/L, and the removal rate of COD remained within the range of 85.49%–93.31%, indicating relatively good removal effect. Nevertheless, when domestication time lasted for 6 days, LAS concentration in the effluent reached over 175 mg/L, with the removal rate for COD declining by about 83%. This implies that the microbial activity was influenced by the LAS toxicity.

Changes in microbial colonies in the sludge samples during the start-up and domestication phases of the reactor

Sludge samples from different phases were collected for high-throughput sequencing of the microbial communities to determine their populations and diversity. Results showed that samples S1 and S5 had 1,168 OTUs in total (Figure 6), but only 195 OTUs were found in all samples. When the original sludge was incubated until it reached stability, a total of 759 OTUs disappeared and evolved into 214 new microorganisms. Putting the five samples together for analysis, showed that during each phase of the trial, the sludge samples were accompanied by the disappearance of old microbes and the production of new microbes.

Figure 7 demonstrates the distribution of microbial populations in the sludge based on higher taxonomic ranking (i.e., Class). Specifically, the Proteobacteria class accounted for the largest proportion of the community, which was mainly composed of Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria and Gammaproteobacteria, accounting for 55.39%, 68.43%, 64.76%, 57.93% and 55.66% in S1, S2, S3, S4 and S5, respectively. At the level of Order (Figure 8), the microbial community of the sludge was dominated by Rhizobiales, Burkholderiales, Saprospirales and Xanthomonadales. Meanwhile, Comamonadaceae, Methylocystaceae and Xanthomonadaeae accounted for the larger proportion compared with the taxa at the level of Families (Figure 9). However, at this time, it was difficult to find the dominant families with larger proportion. This indicates that during the whole trial process, the degradation of organic pollutants did not mainly depend on few bacteria. Instead, degradation occurred due to the synergetic efforts of the...
entire microbial community, as seen in its high diversity and abundance, allowing the bioreactor to reach equilibrium.

Figure 10 shows the distribution of the microbial classification in the sludge samples at the level of Genus. Among the detected groups included are *Dechloromonas* (Chakraborty & Coates 2005; Chakraborty et al. 2005; Duarte et al. 2010b), *Gemmata* (Krieg et al. 2010), *Pseudomonas* (Lode & Coon 1971; Jimenez et al. 1991; Cook et al. 1998; Stover et al. 2000; Almendariz et al. 2001; Lara-Martin et al. 2007; Oliveira et al. 2010) and *Zoogloea* (Brenner et al. 2005; Oliveira et al. 2010), which were all related to the entire microbial community, as seen in its high diversity and abundance, allowing the bioreactor to reach equilibrium.

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Degradation of LAS. *Dechloromonas* was a facultative anaerobe, while *Gemmata*, *Pseudomonas* and *Zoogloeae* were all aerobic bacteria. All of the four bacteria potentially participate in the degradation of aromatic compounds. In addition, *Pseudomonas* is also involved in β- and ω-oxidation reactions. The relevant genera and their features related to LAS mentioned in some of the literature are listed in Table 3.

**CONCLUSIONS**

The following conclusions were reached:

1. After operation for 20 days, the start-up of the system was considered successful, the pH basically remained between 6.5 and 7.5, the removal rate was steady above 90% and the COD concentration of the effluent remained at around 25 mg/L.
2. During the domestication phase, the added LAS concentration was gradually increased from 25 mg/L to 200 mg/L. During this phase, DO basically remained low, within the range of 0–1.5 mg/L. At the early stage, the removal rates of COD and LAS were relatively stable, reaching as high as 85.49%–93.31% and 80%, respectively. When the LAS concentration reached over 175 mg/L and the COD declined to about 83%, the removal rate of LAS also significantly decreased. LAS removal rate further decreased to about 60% when the dosage reached 200 mg/L, indicating that the resistance of microorganisms against LAS toxicity was also limited.

3. During the whole trial process, the degradation of organic pollutants did not depend on only a few bacteria. Instead, it was a result of the synergetic activity of the entire microbial community, as seen with its high diversity and abundance, allowing the bioreactor to reach equilibrium. LAS degradation could have been mainly driven by Dechloromonas, Gemmata, Pseudomonas and Zoogloea in the system.

<table>
<thead>
<tr>
<th>Genera</th>
<th>Type</th>
<th>Fracture of benzene ring</th>
<th>Degrade sulphonates</th>
<th>β/ω-oxidation</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dechloromonas</td>
<td>Facultative anaerobe</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>This paper (Chakraborty &amp; Coates 2005; Chakraborty et al. 2005; Duarte et al. 2010b)</td>
</tr>
<tr>
<td>Gemmata</td>
<td>Aerobic</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>This paper (Krieg et al. 2010)</td>
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<tr>
<td>Pseudomonas</td>
<td>Aerobic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>This paper (Duarte et al. 2010b)</td>
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<tr>
<td>Zoogloea</td>
<td>Aerobic</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>This paper (Brenner et al. 2005; Oliveira et al. 2010)</td>
</tr>
<tr>
<td>Aeromonas</td>
<td>Facultative anaerobe</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Brenner et al. (2005) and Oliveira et al. (2010)</td>
</tr>
<tr>
<td>Comamonas</td>
<td>Aerobic</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Jimenez et al. (1991), Brenner et al. (2005) and Schleheck et al. (2010)</td>
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<tr>
<td>Desulfoomonile</td>
<td>Aerobic</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>Chakraborty et al. (2005)</td>
</tr>
<tr>
<td>Desulphonema</td>
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<td>+</td>
<td>–</td>
<td>–</td>
<td>Chakraborty et al. (2005)</td>
</tr>
<tr>
<td>Desulfoacetrix</td>
<td>Aerobic</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Chakraborty et al. (2005)</td>
</tr>
<tr>
<td>Geobacter</td>
<td>Anaerobic</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Brenner et al. (2005), Lara-Martin et al. (2007) and Delforno et al. (2012)</td>
</tr>
<tr>
<td>Rhizobium</td>
<td>Aerobic</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>Brenner et al. (2005)</td>
</tr>
<tr>
<td>Synergistes</td>
<td>Anaerobic</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Allison et al. (1992) and Kumar et al. (2010)</td>
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</table>

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**REFERENCES**


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