Two-Stage UASB Process to Treat PTA Wastewater: Process Performance and Microbial Community Analysis

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\textbf{Abstract}

Purified terephthalic acid (PTA) manufacturing industry generates a high-strength organic wastewater containing acetate (HAc), terephthalate (TA), benzoate (BA) and p-toluate (pTOL) as major pollutants. This PTA wastewater is usually treated by an anaerobic process, which is achieved by a syntrophic culture system where diverse acidogenic and methanogenic microbes are involved. This syntrophic culture system is known to have a distinct feature that the activities of the acidogenic microbes responsible for the degradations of BA, TA and pTOL are severely inhibited by HAC which is also redundant in the PTA wastewater. In this study, we operated a two-stage UASB process using a synthetic PTA wastewater. Contrary to the previous reports, we focused on the pTOL degradation which was revealed as the most hardly-biodegradable pollutant in the PTA wastewater. It was found that all the major pollutants except pTOL were completely degraded in the first reactor and the remaining pTOL was removed in the second reactor at the HRT 48hr. In the ways of reaching this state, it was also confirmed that the biodegradability of the major pollutants was in the order of BA, TA and pTOL. Also, difference of microbial population of granule samples obtained from each stage was also investigated by using fluorescence in situ hybridization (FISH) technique combined with confocal laser scanning microscopy (CLSM). In situ hybridization with bacterial- and archaeal-domain probes within granule sections clearly showed that both first reactor and second reactor granules had different layered structures. In the results of microbial community analysis, the granule of first reactor was mostly dominated by the methanogenic archaea cells whereas the granule of the second reactor was dominated by the acidogenic bacteria cells. Tough not elucidated in this study, it is expected that the acidogenic bacteria dominant in the second reactor would be different from those of the first reactor, being accountable for the pTOL degradation. These results revealed the spatial organizations of methanoges and bacteria and their in situ morphologies and metabolic functions in both first reactor and second reactor granule sludges.

1. Introduction

Purified terephthalic acid (PTA) manufacturing industry generates a high-strength organic wastewater containing acetate (HAc), terephthalate (TA), benzoate (BA) and p-toluate (pTOL) as major pollutants (Kleerebezem et al., 1997; Joung et al, 2009). PTA wastewater was treated by a aerobic biological treatment process. The aerobic biological process was a several disadvantages as followeds: excess sludge production, highly energy requirement, and etc. Since the last in 1980, however, the anaerobic treatment process, which is achieved by a syntrophic culture system where
diverse acidogenic and methanogenic microbes are involved, have been applied to PTA wastewater treatment process because of several advantages such as less surplus sludge production, lower nutrients requirement, and energy recovery (Kleerebezem et al., 1997; Joung et al., 2009). This syntrophic culture system is known to have a distinct feature that the activities of the acidogenic microbes responsible for the degradations of BA, TA and pTOL are severely inhibited by HAc which is also redundant in the PTA wastewater (Macarie and Guyot, 1992; Fajardo et al., 1997; Kleerebezem et al., 1997).

The UASB allows the build-up of a high biomass concentrations as granule sludge, resulting in good process efficiency and stability (McHugh et al., 2003; Najafpour et al., 2006). Granular sludge consists of different physiological types of anaerobic microorganisms. One feature of the granules is the spatial organization of the microorganisms. Usually, the inner layer consists mostly of aceticlastic methanogens and the outer layer is comprised of fermentative bacteria (Guyot et al., 1992; Sekiguchi et al., 1999). Various studies have provided the microbial community and structure of different types of granule sludges in UASB reactors using molecular biological techniques, such as oligonucleotide hybridization, denaturant gradient gel electrophoresis (DGGE), fluorescence in situ hybridization (FISH), and gene cloning, based on 16s rRNA gene (Diaz et al., 2006; Sekiguchi et al., 1999; Zheng et al., 2006). As a possible solution to minimize this inhibition effect, two-stage bioreactor configuration has been often considered (Young et al., 2000; Kleerebezem et al., 2005).

In this study, we operated a two-stage UASB process using a synthetic PTA wastewater. Contrary to the previous reports, we focused on the pTOL degradation which was revealed as the most hardly-biodegradable pollutant in the PTA wastewater. Furthermore, microbial community analysis of anaerobic granule sludge cultivated in both stages were carried out using molecular biological method such as fluorescence in situ hybridization technique combined with confocal laser scanning microscopy (CLSM) to visualize the locations of several microorganisms of particular interest in both granule sludges in first stage and second stage reactor.

2. Experimental methods

The lab-scale UASB process consists of two identical UASB-type bioreactors each of which has the total working volume of 5 L (diameter 100 mm x height 600 mm). The influent and recycle flow rates were controlled by synthetic wastewater was supplied from the bottom by a peristaltic pump, and effluent liquids were discharged from the top of the reactor. A temperature was keeping each reactor at 38 °C using a circulated water bath. The total hydraulic retention time (HRT) of the reactor was 48 h, initially. The synthetic wastewater contained HAc (1,000 mg/L), BA (500 mg/L), TA (500 mg/L), pTOL (500 mg/L), and other minor minerals of which composition was adjusted following Kleerebezem et al. (1999) as shown Table 1.

The collected samples were centrifuged at 3500 rpm for 3 minutes (MF550, HANIL), and then the supernatants were used for the following analyses after filtered using 0.2μm syringe filter. All aromatic compounds presented in PTA wastewaters were analyzed by HPLC (P680, DIONEX) with UV-detector (UVD170U, DIONEX) at the wavelength of 230nm. Acetate was analyzed by GC (GC 6890N, Agilent) with a FID detector. In the FISH experiment, we only compared the relative dominance of acidogenic and methanogenic microbes in each reactor by using the bacteria- and archaea-specific oligonucleotides (Stahl and Amann, 1991; Amann et al., 1995).
Table 1. Composition of Synthetic wastewater in two stage lab-scale UASB reactor

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Synthetic wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.5</td>
</tr>
<tr>
<td>TOC (mg/L)</td>
<td>Initial conc. 2000, final conc. 3500</td>
</tr>
<tr>
<td>HAc (mg/L)</td>
<td>Initial conc. 500, final conc. 1200</td>
</tr>
<tr>
<td>BA (mg/L)</td>
<td>Initial conc. 500, final conc. 600</td>
</tr>
<tr>
<td>TA (mg/L)</td>
<td>Initial conc. 500, final conc. 600</td>
</tr>
<tr>
<td>pTOL (mg/L)</td>
<td>Initial conc. 500, final conc. 800</td>
</tr>
<tr>
<td>Nutrients (mg/L)</td>
<td>Kleerebezem et al., 1999</td>
</tr>
</tbody>
</table>

3. Results and Discussion

Figure 1 represents the overall process performances along with the changes of the applied HRT. During the experiment we tried to find out an HRT condition under which none other than pTOL was introduced into the second UASB reactor, because this strategy make the pTOL-degrading microbes enriched in the second reactor. At the HRT of 48 hr, all the major pollutants except pTOL were completely degraded in the first reactor and the remaining pTOL was removed in the second reactor. In the ways of reaching this state, it was also confirmed that the biodegradability of the major pollutants was in the order of BA, TA and pTOL.

![Figure 1. Influent and effluent concentrations of BA, TA and pTOL during the operation.](image)

After 105 days of operation, granule samples were obtained from each reactor and their microbial populations were analyzed. As shown in figure 2, the granule of the first reactor was mostly dominated by the methanogenic archaea cells whereas the granule of the second reactor was dominated by the acidogenic bacteria cells. Tough not elucidated in this study, it is expected that the acidogenic bacteria dominant in the second reactor would be different from those of the first reactor, being accountable for the pTOL degradation.
4. Conclusions

This study demonstrated that pTOL degradation which was revealed as the most hardly-biodegradable pollutant in the PTA wastewater using two stage UASB reactor. Also, difference of microbial community in anaerobic granule cultivated in both stages elucidated using molecular biological method such as fluorescence in situ hybridization technique combined with confocal laser scanning microscopy (CLSM). In conclusions, fluorescence in situ hybridization with bacterial- and archaeal-domain probes within granule sections clearly showed that both first reactor and second reactor granules had different layered structures. In the results of microbial community analysis, the granule of first reactor was mostly dominated by the methanogenic archaea cells whereas the granule of the second reactor was dominated by the acidogenic bacteria cells.

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