Enhanced biodegradation of 4-aminobenzenesulfonate in membrane bioreactor by *Pannonibacter sp.* W1

Jinsong Zhang, Yanqing Wang, Ji Ti Zhou, Rong Wang, Shuwen Goh and Anthony G. Fane

ABSTRACT

4-Aminobenzenesulfonate (4-ABS), an aromatic amine and recalcitrant toxic pollutant, is widely used in the dye and pharmaceutical industry. *Pannonibacter sp.* W1 is a specialized microbial strain which can efficiently degrade 4-ABS. This study shows the feasibility of using the specialized strain in an MBR system to treat synthetic wastewater containing large amount of 4-ABS. Due to membrane retention, the biomass concentration is able to reach 5 g/L within two months of continuous operation. *Pannonibacter sp.* W1 is able to adapt to the high loading rate of 1000 mg 4-ABS/L and achieve a remarkable 4-ABS removal efficiency of 99% within 6 h. Strain W1 grows well under the MBR continuous operation and remains as the dominant bacterium at the end of 60 days continuous operation. Minor membrane fouling has been detected within 40 days of operating at 15 LMH. At a flux of 25 LMH, the system experiences the 'TMP jump'. The high organic removal rate and low membrane fouling results illustrate the excellent performance of the bioaugmented MBR system in 4-ABS wastewater treatment.

Key words | 4-aminobenzenesulfonate (4-ABS), membrane bioreactor, industry wastewater, membrane fouling

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INTRODUCTION

Sulfonated aromatic amines such as 4-aminobenzenesulfonate (4-ABS) are key intermediates in the production of sulfonated azo dyes, dye mordants, spices, food pigments, pharmaceuticals and pesticides. The reduction of sulfonated azo dyes under anaerobic conditions is another key industrial process which results in sulfonated aromatic amines generation. Sulfonated aromatic amines are more toxic and carcinogenic than their azo dyes predecessors (Chung & Cerniglia 1992; Oh *et al.* 1997; O'Neill *et al.* 1999). The sulfonate group on the aromatic ring confers a xenobiotic and polar nature and hence, resistance to biodegradation by non-acclimatized activated sludge (Rozgaj & Glancer 1992; Feigel & Knackmuss 1993; Alexander & Lustigham 1996). 4-ABS is a representative compound of sulfonated aromatic amines, and is also intermediate of some sulfonated azo dyes (Figure 1). It is synthesized in large quantities and easily discarded to the surface waters due to its high water-solubility (Alexander & Lustigham 1996). The generation of large quantity of industrial wastewater containing the recalcitrant toxic 4-ABS warrants significant interest in the development of methods for the removal of 4-ABS.

Biological treatment is the most economical method to treat 4-ABS contaminated wastewater. However, there were few reports on the use of pure and mixed culture in the biodegradation of the substance in the last two decades. A defined co-culture consisting of *Hydrogenophaga palleronii* S1 and *Agrobacterium radiobacter* S2 could utilize 4-ABS as the sole carbon and energy source (Feigal & Knackmuss 1993). Strain S1 was reported to convert 4-ABS to catechol-4-sulfonate, which was further degraded by strain S2. Further studies by Dangmann *et al.* (1996) showed that strain S1 was able to mineralize 4-ABS directly with the supplementation of specific vitamins while strain S2 was unable to do so. It was reported that some pure bacterial strains such as *Pseudomonas paucimobilis* (Perei *et al.* 2001) and *Agrobacterium sp.* PNS-1 (Singh *et al.* 2004, 2006) have the ability to completely degrade 4-ABS through mineralization.

Due to the toxic and recalcitrant property of 4-ABS, mixed culture used in previous studies exhibit synergetic

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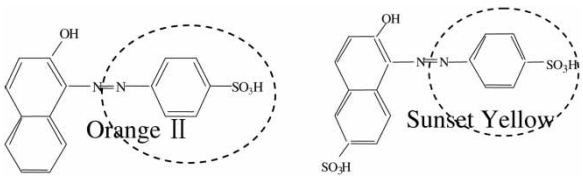

Figure 1 | Structures of some azo dyes containing 4-ABS as intermediates.

degradation while single strain was also reported to be inhibited by high concentration of 4-ABS. This resulted in low 4-ABS degradation efficiency. Wang *et al.* (2009) reported a strain of genus *Pannonibacter* which utilize 4-ABS as the sole carbon and energy source. However, in the conventional-activated sludge (CAS) operations, this *Pannonibacter sp.* W1 will acclimate very slowly due to feed limitation and bacteria losses during sludge wasting.

The biomass can be immobilized in the bioreactor in two ways, namely: (i) immobilized within the calcium alginate gel bead in a fluidized bed bioreactor (Singh *et al.* 2006) or (ii) as a suspension in the bioreactor. The immobilized microorganism can withstand higher substrate inhibition but the medium diffusion resistance would also be higher. The free suspended culture can degrade the 4-ABS more efficiently but faces stronger substrate inhibition at high feed to microorganism (F/M).

The membrane bioreactor (MBR) technology combines the conventional biological treatment with membrane separation to retain the biomass in the reactor, thus allowing long sludge retention time, high mixed liquor suspended solids (MLSS) and low organic sludge loading. As such, the MBR is favored over CAS in the treatment of stable, recalcitrant pollutants (Stephenson *et al.* 2000). Bioaugmentation of special strains in membrane processes are fast becoming a trend in treatment of recalcitrant pollutants due to its high rate of biodegradation and efficiency (Barrios-Martinez *et al.* 2006).

To date, there is no publication on bioaugmented 4-ABS degradation with MBR technology. The dual objectives of this study are: (i) to acclimatize the *Pannonibacter sp.* W1 in a continuous operation using 4-ABS as the sole carbon and nitrogen source, (ii) to investigate the feasibility and performance of 4-ABS removal in MBR bioaugmented with the novel 4-ABS degrading strain *Pannonibacter sp.* W1.

METHODS

Synthetic wastewater and microorganism

The synthetic 4-ABS wastewater used in the study contained Analytical grade 4-ABS (Tianjin Guangfu Fine Chemical Institute, Tianjin, China) and mineral medium. The compositions are shown in Table 1. The maximum absorbance wavelength of 4-ABS is 249 nm and its molecular mass is 173. Strain W1 was isolated from activated sludge in our previous study and has been identified as *Pannonibacter sp.* according to the 16S rDNA sequence analysis (Wang *et al.* 2009).

Experimental set-up and operating conditions

The MBR system (Figure 2) comprised of a bioreactor (2 L aerated tank) with submerged flat sheet MF module (0.012 m^2, Millipore, PES, pore size 0.22 μm). The tank was partitioned into three compartments by two baffles to create an airlift loop for circulation and membrane filtration. Air diffusers were installed beneath the flat sheet membrane modules. The synthetic wastewater was continuously

Table 1 | Synthetic wastewater composition

Chemicals	Concentration/g/L
4-ABS	1
$Na_2HPO_4{\cdot}2H_20$	2
KH_2PO_4	1
NH_4Cl	0.5
$MgSO_4{\cdot}7H_2O$	0.25
$FeSO_4{\cdot}7H_2O$	0.002
K_2SO_4	0.06
$CaCl_2{\cdot}H_2O$	0.035

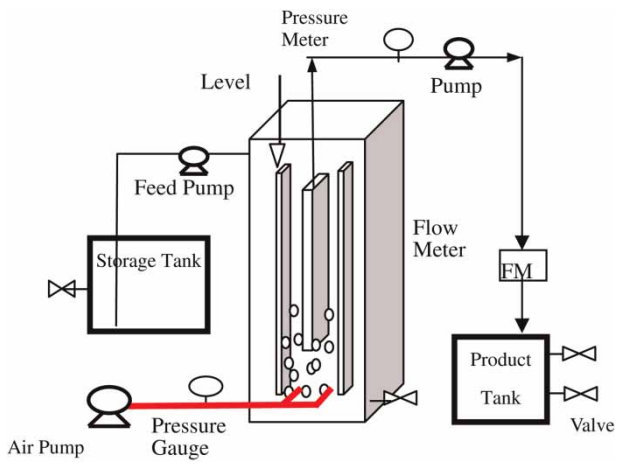

Figure 2 | Schematic diagram of MBR.

pumped into the reactor, which was controlled by a level sensor to maintain a constant volume in the reactor. LABVIEW data logging system (National Instruments TM, PCI 6014, software: Labview 7) were applied for system control and data acquisition of parameters such as transmembrane pressure (TMP) and permeate flowrate.

Analytical methods

MLSS of activated sludge, the specific oxygen uptake rare (SOUR) and the chemical oxygen demand (COD) were determined in accordance to Standard methods (APHA 1998). 4-ABS concentration was analyzed by either UV-Spectrophotometer at 249 nm wavelength or HPLC (HP1100, Hewlett-Packard, USA). The HPLC was equipped with a 4.6 × 250 mm C18 column (Waters, USA) and a UV-visible detector. A mixture of methanol and deionized water with 1 g/L tetraethylammonium bromide was used as the solvent and flow rate was maintained at 0.8 mL/min. Linear gradient elution was carried out for 40 min using the solvent with methanol/deionized water (10:90, v/v) to 100% methanol, followed by 10 min of washing with 100% methanol. The emerging peaks were detected at 249 and 254 nm using the UV-visible detector. For total organic carbon (TOC) measurement, the samples were centrifuged at 12,000 rpm for 10 min, filtered with a 0.22 μm membrane and the TOC content of the filtrates were determined with a TOC analyzer (TOC-VCPH, SHIMADZU, Japan).

RESULTS AND DISCUSSION

Microorganism acclimation and MLSS accumulation in MBR

In order to acclimatize the strain *Pannonibacter sp.* W1 to the MBR mode of operation, the 4-ABS loading to bacteria was incrementally increased by slowly decreasing the hydraulic retention time (HRT) from 14 to 10 h and eventually, 6 h. As shown in Figure 3, at a HRT of 14 h, MLSS concentration increased from 0.5 to 3.5 g/L within 8 days. The MLSS increased slowly and managed to reach 5 g/L with HRT as short as 6 h. The MLSS was stabilized at around 5 g/L as equilibrium was reached between cell proliferation and endogenous respiration. With the high MLSS concentration, the system was able to endure the impact of increased loading and continuously achieve high 4-ABS removal rate.

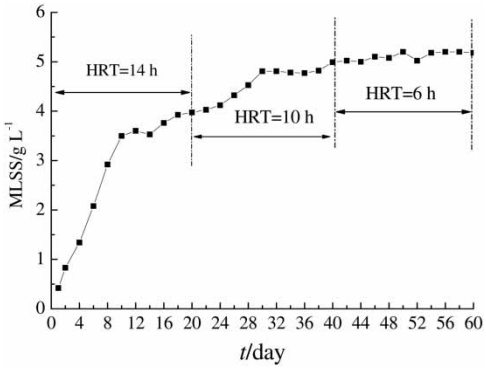

Figure 3 | MLSS accumulation in MBR.

4-ABS degradation in MBR

The feasibility of the degradation of 4-ABS was studied over two months of continuous MBR operation. The 4-ABS concentration in the influent and effluent, the 4-ABS removal rate were shown in Figure 4. The influent 4-ABS concentration was kept constant at about 1,000 mg/L throughout the experiment. The 4-ABS removal rate remained at 99.4% with 60 days operation, indicating good system performance in the degradation of 4-ABS.

During the initial biomass acclimation stage (HRT = 14 h), *Pannonibacter sp.* W1 exhibited slight inhibition to the high concentration 4-ABS, however, the system managed to overcome this quickly to achieve higher treatment efficiency. This shows that *Pannonibacter sp.* W1 is able to effectively degrade 4-ABS in continuous culture and the acclimatization of microorganism plays a significant role in abating the initial inhibition effects of toxic compounds during the biodegradation of toxic and recalcitrant compound.

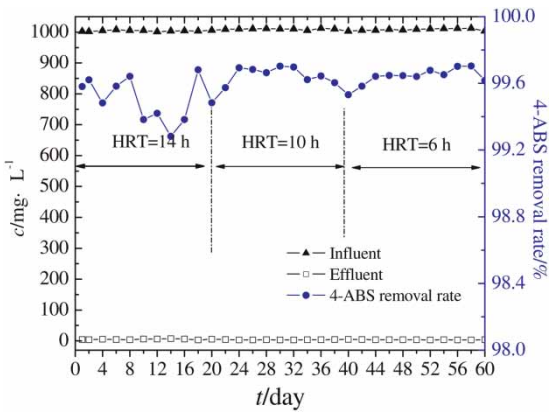

Figure 4 | Removal of 4-ABS by strain W1 in MBR.

Removal of TOC in MBR

Pannonibacter sp. W1 is capable of utilizing 4-ABS as its sole carbon, energy, nitrogen and sulfur source (Wang *et al.* 2009). The TOC and 4-ABS analysis indicates that 4-ABS was mineralized to a large extent. Figure 5 shows that the influent TOC remained constant at around 400 mg/L, while effluent TOC remained consistently below 20 mg/L during 60 days operation. The maximum TOC removal rate was 95%, which was lower than 4-ABS removal rate of 99.4%. During the 60 days operation, the *Pannonibacter sp.* strain W1 managed to enhance biodegradability of the product, resulting in a slightly lowered effluent TOC which stabilize with time. HPLC analysis showed that no other aromatic intermediate products accumulated during 4-ABS degradation (Wang *et al.* 2009).

Effect of Influent loading on 4-ABS removal

In Figure 6, it was shown that, at a fixed influent COD concentration, shortening the HRT increased volume loading from 1.7 kgCOD/ (m^3 day) to 4.2 kgCOD/ (m^3 day). In spite of the maximum loading, the 4-ABS degradation performance remained consistently good.

In Figure 7, the sludge loading (F/M) increase from 0.5 to 0.8 kg COD/ (kgMLSS day). This is not proportional to the volume loading increase due to the increased biomass concentration. The COD removal rate remained of 95%, indicating strain *Pannonibacter sp.* W1 is adaptability to increased 4-ABS loading.

Effect of dissolved oxygen on 4-ABS removal

Aeration will affect both the dissolved oxygen (DO) concentration in the MBR, as well as membrane fouling. The effect of DO concentration on 4-ABS degradation rate was shown in Figure 8. In the startup period, the aeration rate increase from 0.03 to 0.13 m/s (airflow rate 2–8 L/min) as the MLSS concentration increased from 1.08 to 1.18 mg/L in the first 8 days. The 50% COD removal rate was achieved at the DO below 4.5 mg/L at corresponding aeration rate of

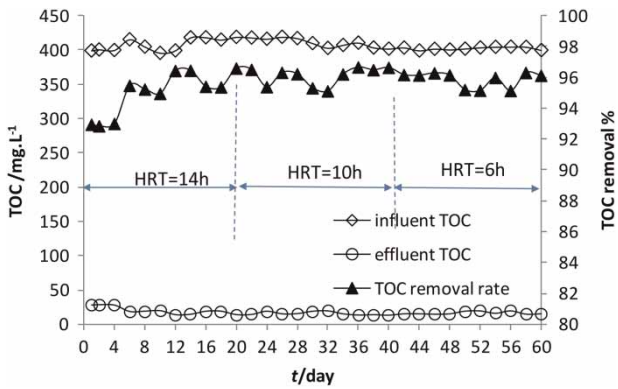

Figure 5 | TOC removal in MBR during operation.

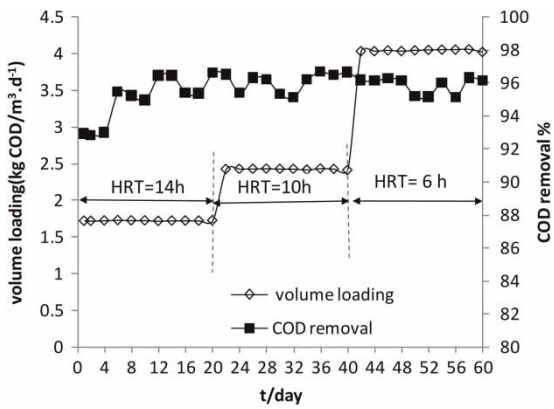

Figure 6 | Effect of influent volume loading on 4-ABS removal.

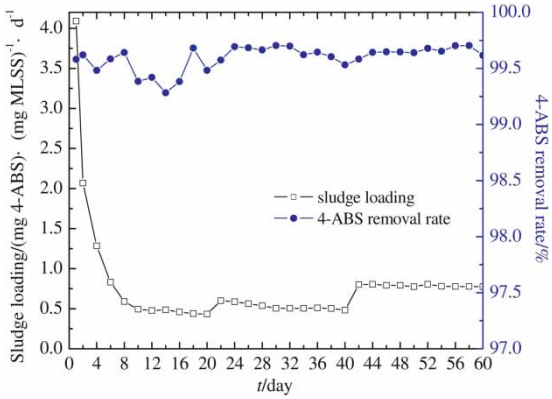

Figure 7 | Effect of sludge loading on 4-ABS removal.

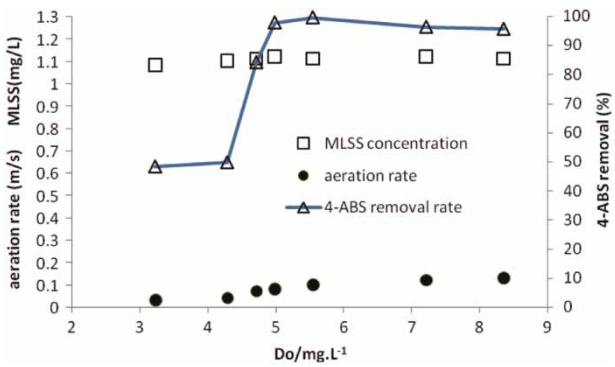

Figure 8 | Effect of DO on 4-ABS degradation in MBR.

0.07 m/s (airflow rate 4 L/min). As the aeration rate increased to 0.08 m/s (airflow rate 5 L/min), the DO reached 5 mg/L and there was a corresponding increase in degradation rate (more than 95%), indicating the significant effect of DO on biodegradation rate. Thus, the aeration rate was fixed at the 0.08 m/s to achieve a DO concentration of at least 5 mg/L.

Change of SOUR during the MBR running

SOUR is the oxygen consumption rate per unit mass of sludge, and it is a key parameter in characterizing biological activity from the perspective of microbial respiration rate and metabolic activity of the activated sludge.

In Figure 9, SOUR was stable and remained at high level during the operation of MBR, indicating that the *Pannonibacter sp.* strain W1 was kept at high activity in the MBR culture mode and hence, were able to endure the increasing 4-ABS loading impact. The SOUR of seed bacteria increased after the startup period of MBR. After 40 days of operation, SOUR decreased slightly due to increased sludge concentration and aging sludge.

pH tolerance comparison in shaking flask and MBR

A comparison of the pH tolerance range for a shaking flask mode and continuous MBR operation mode shows that the continuous MBR operation mode can tolerate a wider pH range than the shaking flask mode (Figure 10). 4-ABS degradation can continue under acidic and alkaline conditions in continuous MBR operation; however, similar performance could only be achieved at neutral and metaacid condition in the shaking flask.

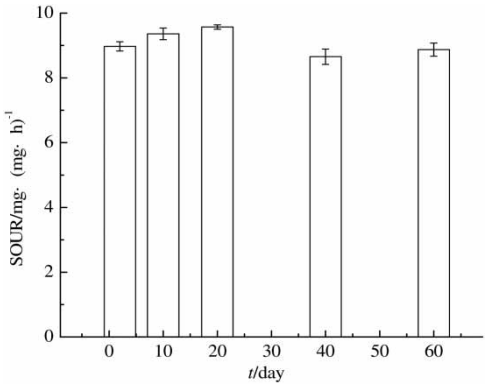

Figure 9 | Time course of SOUR in MBR.

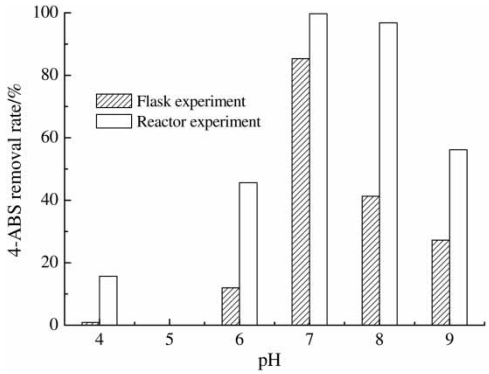

Figure 10 | Comparison of pH tolerance between shaking flask and continuous MBR culture.

Stability of bacteria consortium in MBR

After two months of MBR operation, 1 mL of sludge sample was taken from the reactor for bacteria plate culture to determine the strain purity. The morphology of bacteria colony was investigated 24 h after plate culture. The result is shown in Table 2. Similar bacteria colonies containing *Pannonibacter sp.* W1 make up 70% of the total colonies. Hence, it could be inferred that strain W1 remained the dominant species during the final stage of MBR operation. This result is just a rough indication and further microbiology work is required to justify this.

Supernatant and effluent TOC

SMP, sludge supernatant or rejected organics in the supernatant of mixed liquor are recognized as significant membrane foulant. The supernatant TOC and effluent TOC was measured during the experiment. The result was shown in Figure 11. The TOC in the supernatant was high at beginning, then stabilized after 5 days between 20 and 30 mg/L. the effluent TOC concentration follow the same trend, with the concentration stabilizing at 15–20 mg/L

Table 2 | Distribution of different colonies in MBR

Kinds of consortium	Number of consortium on the plate	Cells in unit volume (cells/mL)	Proportion (%)
Similar to strain W1	32	3.2×10^{10}	69.6
Bacteria consortium	9	9×10^{9}	19.5
Fungi consortium	5	5×10^{9}	10.9

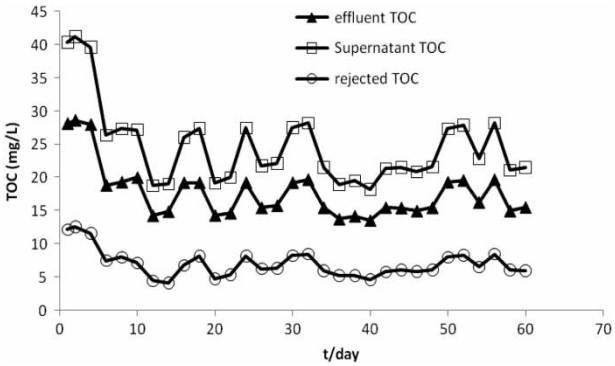

Figure 11 | TOC in supernatant and effluent of MBR.

after initial 5 days. The retentive TOC, an important membrane foulant, was calculated by supernatant TOC minus effluent TOC. The rejected TOC concentration was very low at around 7.03 ± 2.02 mg/L.

Membrane fouling

The long term TMP profiles in Figure 12 were obtained during the entire operation period of 60 days. During the first 20 days start up period (HRT = 14 h), a constant flux of 10 LMH was maintained. The flux was increased to 15 LMH to achieve 10 h HRT in the next 20 days operation. During the last 20 days of operation, the flux was set at 25 LMH to achieve a HRT of 6 h. At a flux of 10 LMH, the TMP increased quickly from 1.5 to 2 kPa within the first 8 h, followed by gradual increase at a rate of approximately 0.007 kPa/day. At 15 LMH flux, the TMP increased slowly at a rate of 0.008 kPa/day. At 25 LMH flux, the TMP increased gradually at a rate of approximately 0.01 kPa/day for the initial 10 days before experiencing a rapid increase known as 'TMP jump'. This phenomenon has been described in the author's previous work (Zhang *et al.* 2006).

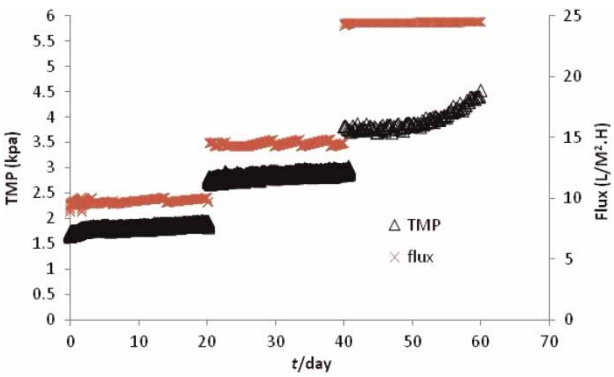

Figure 12 | TMP profile at flux of 10, 15 and 25 LMH.

DISCUSSION

From these series of controlled experiments, it is evident that the combination of MBR with specialized bacteria strain is very effective. In the shaking flask culture, strain *Pannonibacter sp.* W1 could fully degrade 1,000 mg/L 4-ABS within 24 h (Wang *et al.* 2009). However, in the MBR, this could be achieved in just 6 h. A possible reason for this significantly higher degradation rate is the high concentration of biomass retained in MBR. The wider tolerant pH range and resilience to increased 4-ABS impact loading in the MBR operations also indicate better performance than that observed in previous shake flask experiments. This could be due to the high concentration biomass and also, the formation of bigger aggregate bioflocs in the MBR which allows the strain *Pannonibacter sp.* W1 to better withstand higher loading and greater pH fluctuation.

Membrane fouling is less significant at lower flux of 10 and 15 LMH. This could be due to the low concentration of rejected TOC within the MBR system as well as the low imposed membrane flux. The rejected TOC was in the low end of the range reported in MBR papers (Meng *et al.* 2009). The slight increase in fouling tendency observed at flux 25 LMH could be explained by the higher imposed flux. Since the reported critical flux of a commercial MBR is in the range of 15–20 LMH, the flux 25 LMH was likely to be higher than the critical flux and this resulted in rapid fouling and 'TMP jump' (Zhang *et al.* 2006). Future work will investigate the EPS, polysaccharide and protein content of the mixed liquor, as well as the analysis of the shift in the biocommunity using DGGE.

CONCLUSIONS

The study demonstrated the feasibility of combining the MBR system with the strain W1 for the treatment of 4-ABS synthetic industrial wastewater. The results showed good permeate quality with 4-ABS removal rate up to 99% and TOC lower than 20 mg/L. Active MLSS was able to accumulate to high concentration. The optimum DO for the operation was determined as 5–6 mg/L, and the pH tolerance range in MBR operations was observed to be wider than that observed in shake flask experiments. A simple test using the dilution plate method showed that strain W1 remained the dominant bacterium at the end of 60 days continuous operation, indicating that the species can continue to survive under MBR operation. Membrane fouling was not significant during the 40 days operation at

flux 10, 15 LMH, while operation at the higher flux of 25 LMH would result in TMP jump in 20 days operation.

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