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Comparison of the treatment for isopropyl alcohol wastewater from silicon solar cell industry using SBR and SBBR

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Abstract In the present study, isopropyl alcohol containing wastewater generated from silicon solar cell manufacture was sequentially treated with sequencing batch biofilm reactor and sequencing batch reactor. Sequencing batch biofilm reactor could remove 90 % of isopropyl alcohol from wastewater efficiently as the chemical oxygen demand lower than 1,200 mg L^{-1}. However, 1,600 mg L^{-1} of chemical oxygen demand damaged the biofilm. The operation mode was changed to sequencing batch reactor on day 30, and sequencing batch reactor showed a greater ability to remove isopropyl alcohol. When the influent chemical oxygen demand was 1,600 mg L^{-1}, the reactors achieved stable removal efficiencies of >95 % for chemical oxygen demand, and the effluent chemical oxygen demand was lower than 100 mg L^{-1}. Denaturing gradient gel electrophoresis analysis showed an increase in bacteria diversity as the operation mode was switched from sequencing batch biofilm reactor to sequencing batch reactor, which might increase the stability of flocs in sequencing batch reactor. Though 13 bands were sequenced from the denaturing gradient gel electrophoresis and a phylogenetic analysis was conducted based on these sequences, it is difficult to analyze the function of these predominant strains in the reactors. Two models were constructed for interpreting the structure of biofilm in sequencing batch biofilm reactor and flocs in sequencing batch reactor, respectively. Higher efficient transfer rate of dissolved oxygen in flocs was proposed as the main reason for the higher isopropyl alcohol removal ability in sequencing batch reactor.

Introduction

The volume of isopropyl alcohol (IPA) wastewater that is yielded from the wafer cleaning process in the silicon solar cell (SSC) manufacturing industry has continuously increased due to the increase demand on solar cell to obtain clean electric power. IPA and its metabolite, acetone, act as central nervous system depressants (Burkhart and Kulig 1990). Besides, the IPA wastewater contains many other refractory and complex organic compounds, e.g., fluoride and suspended solids, which not only introduce direct or indirect contamination to environment but also are harmful to human health (Lin and Kiang 2003). However, few studies have been conducted aiming to solve this problem in the industry, and the wastewater has consequently brought the industry in China a bigger and bigger trouble since Chinese factories produced more than one-half of solar cells in the world since the year of 2010. Though methanol and ethanol are usually used as carbon source in wastewater treatment and bio-treatment of wastewater has been successfully applied as a low-cost treatment method in many other industrial applications, the use of bio-treatment for IPA wastewater has not been reported as successful as that in other industry.

Sequencing batch reactor (SBR) technology, a periodic discontinuous process with activated sludge, has been

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2382

Int. J. Environ. Sci. Technol. (2015) 12:2381–2388

successfully applied for treating various types of wastewater (domestic wastewater, medium- and low-strength landfill leachate, specific organic pollutants, and various types of industrial wastewater) using diverse types of reactor configurations (Kaballo et al. 1995; Mohan et al. 2007; Lotito et al. 2013; Cui et al. 2013). The process can be considered for IPA wastewater treatment since periodic operation imposes suitable oxygen gradients on the microorganisms that overwhelm natural variations in waste strength and composition (Woolard 1997). Furthermore, the operation can maintain effective cultures within the reactor. The resulting environment therefore offers robust microbial community capable to persist and metabolize at extremely adverse and diverse conditions.

Besides, it is evident that the biofilm-configured sequencing batch systems, i.e., sequencing batch biofilm reactor (SBBR), are well suited for treating wastewater containing poorly degradable compounds (Makinen et al. 1993). In SBBR, microorganisms are immobilized on fillers as biofilm, which results in high biomass hold up and enables the process to be operated at high liquid throughputs and organic loading rates. The attached biofilm acts as buffer to reduce the concentration of toxic chemicals during process operation and thereby benefits the treatment of low-biodegradable industrial wastewater that contains recalcitrant compounds (Bishop 1997). Compared with sludge-based systems, SBBR is generally less energy intensive and more resistant to shock loads to which wastewater treatment systems are frequently subjected (Chaudhry and Beg 1998). Besides, SBBR is particularly useful where high hydraulic loading variations occur and where slowly growing microbes with special metabolic capacities can be protected from washout (Woolard 1997). However, there are few experimental studies reported so far on the treatment of IPA wastewater by SBR or SBBR.

Since pilot experiments have showed that anaerobic treatment was unsuitable for treating IPA wastewater from SSC manufacturing industry, the object of this study was to evaluate the feasibility of using SBR or SBBR as a treatment technique for IPA wastewater. In addition, to investigate the microbial mechanism, molecular techniques, such as denaturing gradient gel electrophoresis (DGGE) and phylogenetic analysis, were employed to explore the bacterial community in the systems. The study was conducted in Changsha between November in 2009 and July in 2010.

Materials and methods

Reactors operation

Two parallel laboratory-scale reactors A and B with an effective volume of about 3 L (diameter: 12 cm; height:

30 cm) were used to perform the experiment. The reactors were inoculated with 500 ml of activated sludge (suspended solids: about 2.0 g L^{-1}; volatile suspended solids: about 0.8 g L^{-1}) from an oxidation ditch municipal wastewater treatment plant in Changsha, China. The biofilm was acclimated with IPA wastewater by continuous aeration to maintain a dissolved oxygen (DO) of 2.5–4.0 mg L^{-1} for 10 days. During the acclimation, the COD in IPA wastewater was about 300 mg L^{-1}. The IPA wastewater was taken from a SSC factory in Changsha, China, and the chemical oxygen demand (COD) in IPA wastewater was 1,500–1,700 mg L^{-1}.

Since some researchers have reported that the SBBR is evidently well suited for treating wastewater containing poorly degradable compounds (Makinen et al. 1993), we attempted to use SBBR to treat the IPA wastewater. Therefore, we firstly operated the reactors in SBBR mode after the 10-day acclimation. The COD in influent was increased from about 500–900, 1,200, 1,600 mg L^{-1} step by step. Soft polypropylene fiber was used as filler. In SBR mode, soft polypropylene fiber was removed, and the biofilm was washed off from the fillers as seeding sludge. Therefore, the main difference between SBBR and SBR mode was that the sludge was in biofilm form in SBBR and in suspended flocs form in SBR. The COD in influent was increased from about 800 to 1,200, 1,600 mg L^{-1} step by step. The reactors were operated in SBR mode for 50 days.

In this study, 0.14 g L^{-1} KH$_2$PO$_4$ and 0.05 g L^{-1} NH$_4$Cl were added to the influent as the P and N source during the acclimation and operation periods, respectively. One liter of effluent was replaced with 1 L of influent every 8 h, and the wastewater replacement was finished in 10 min. Therefore, the hydraulic retention time of the reactors was 24 h. The sludge retention time of the SBR was about 15 days. During the whole experiment, the two reactors were operated as the following repeats of a 3-h aeration phase and then a 1-h settling incubation. During the aeration, the DO in bulk solution was maintained at 2.5–4.0 mg L^{-1}. The mixed liquor suspended solids and mixed liquor volatile suspended solids (excluding the biofilm) in SBBRs were about 1.0 and 0.6 g L^{-1}, respectively. The mixed liquor suspended solids and mixed liquor volatile suspended solids in the SBRs were about 3.2 and 2.2 g L^{-1}, respectively. The pH was maintained at 7, and the temperature was 20–25 °C.

Analytical methods

Water quality parameters of COD, pH, and DO were measured every day. The pH was determined by a pH meter, DO was determined by a DO analyzer, and the measurements of COD were performed following standard

methods (Eaton and Franson 2005). Removal efficiency of COD (Re) was calculated as the following equation:

$$Re = (1 - Ci/Ce) \cdot 100\%,$$

where Ci was the concentration of COD in the added 1,000-ml influent, and Ce was the concentration of COD in the treated effluent. All tests have three repeats.

Sampling

Nine sludge or biofilm samples were sampled. One sample was sampled from the inoculate sludge and designated as sample 0. Samples A10, A25, A50, and A75 were sampled from reactor A in the 10th, 25th, 50th, and 75th day, respectively. Samples B10, B25, B50, and B75 were sampled from reactor B in the 10th, 25th, 50th, and 75th day, respectively. The samples were immediately stored at −20 °C for following analysis.

DNA extraction and PCR amplification

Genomic DNA was extracted from each 1 g of centrifuged samples (wet weight) with protease K and CTAB according to our previous report (Yang et al. 2007). Purified DNA was dissolved in 200 μL sterilized Milli-Q water (Millipore, USA), and the concentrations of purified DNA ranged from 200 to 300 ng μL^{-1} by applying 5 μL of DNA for agarose gel electrophoresis with a comparison with *Hind*III digested λDNA (TianGen, Beijing, China).

Primers were selected to amplify a part of 16S rDNA, positioning 341–926 in *Escherichia coli* position. The nucleotide sequence of the forward primer, which is universal for eubacteria, contains at its 5′ end a 40-base GC clamp (5′-CGCCCGCCGCGCCCCGCGCCCGGCCCGC CGCCCCGCCCG -3′) (GC341F; 5′-(GC clamp)-CCTACGGGAGGCAGCAG-3′) (Muyzer et al. 1998; Xiao et al. 2009). A universal consensus sequence was used as the reverse primer (517R: 5′-ATTACCGCGGCTGCTGG-3′) (Muyzer et al. 1993; Murray et al. 1996). Each 50 μL PCR mixture contained about 150 ng of genomic DNA, 5 pmol each primer (Sangon, Shanghai, China), 25 μL of 2 × PCR mix (BioTek, Beijing, China), 10 μg bovine serum albumin (BSA) V (Sangon), and about 22 μL of sterilized Milli-Q water. PCRs were performed using a MyCycler (Bio-Rad, Hercules, USA). PCR mixtures were pre-incubated at 95 °C for 4 min. Denaturing, annealing, and extension were carried out at 94, 55, and 72 °C, respectively, and the duration was 30 s in all the steps. This cycle was repeated 30 times and then incubated at 72 °C for 7 min for the final elongation.

Denaturing gradient gel electrophoresis

The DGGE was carried out by using a DCode^{TM} Universal Detection System instrument and gradient former model 475 (Bio-Rad) according to the manufacturer's instructions. The denaturant solution was prepared as Muyzer et al. (1998) reported, and in this study, the optimal acrylamide concentration in the gel was 8 % and the optimal denaturing gradient was 35–55 %. Gels were run in 1 × TAE buffer at 60 °C for 8 h at 120 V. Gels were stained with 1 × SYBR^{TM} Green I and visualized in UV light with the Gel Doc XR System (Bio-Rad). Bands were recognized both by the program QuantityOne V4.63 (Bio-Rad) and manual identify; therefore, some weak bands on the DGGE pattern also were sequenced.

16S rDNA cloning, sequencing, and phylogenetic analysis

Each manually recognized band was excised from DGGE gel under UV light, and the DNA was extracted from the gel by bathing the gels in water at 4 °C. The extracted DNA was amplified with primer pair GC341F/517R. The PCR products were cloned, re-DGGE affirmed, and sequenced as our previous study reported (Xiao et al. 2011a, b).

Phylogenetic identity was determined by comparing the partial 16S rDNA sequences of the clones with sequences which were found in GenBank using the BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and a phylogenetic tree was constructed using the program MEGA5 as that in our previous study (Tamura et al. 2011).

Nucleotide sequence accession numbers

The retrieved thirteen sequences of the 16S rRNA gene clones have been deposited orderly in the GenBank database under Accession No. JX872406-JX872418.

Results and discussion

Reactors performance

Figure 1 shows the average COD concentration (plus standard deviation) in influent and effluent in reactors A and B. The COD removal efficiencies in both reactors were also shown in this figure. Figure 2 shows the daily removed COD of each reactor.
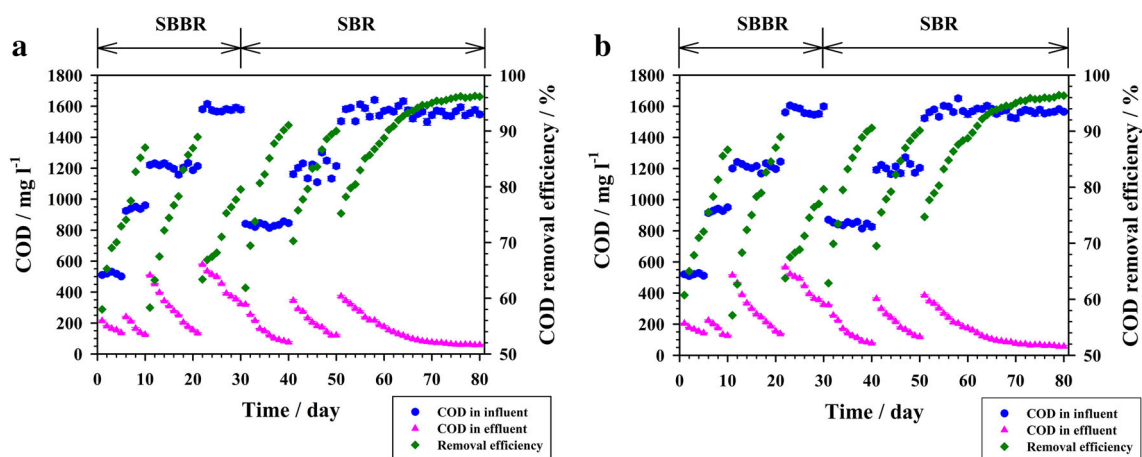
2384

Int. J. Environ. Sci. Technol. (2015) 12:2381–2388

Fig. 1 COD concentrations in influent and effluent and COD removal efficiencies in reactors A (**a**) and B (**b**). The COD concentrations were presented as average value with standard deviation (*n* = 3). *Circle*: COD in influent; *triangle*: COD in effluent; *diamond*: COD removal efficiency
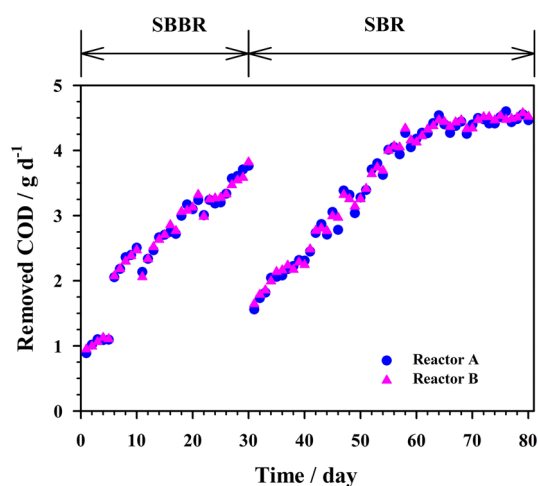

Fig. 2 Daily removed COD from reactors A and B. *Circle* reactor A; *triangle* reactor B

Fig. 3 DGGE analysis of the nine flocs/biofilm samples from inoculant sludge and reactors A and B

At the beginning of SBBR operation (day 1), COD in influent was increased from 300 mg L^{-1} to about 500 mg L^{-1}. While the effluent COD on the 5th day reduced to <150 mg L^{-1}, the emission concentration standard level 2 of Integrated Wastewater Discharge Standard in China, the influent COD was increased to about 900 mg L^{-1} on day 6 and then to about 1,200 mg L^{-1} on day 11. When the influent COD was increased to 1,600 mg L^{-1} on day 25, the biofilm began to fall off from the filler (mixed liquor suspended solids higher than 2,000 mg L^{-1}).

On the 26th day, one-half of raw IPA wastewater was reduced in influent, and 800 mg L^{-1} of glucose was added as we suspected the IPA wastewater was harmful to microbes. The biofilm continued to fall off in the following
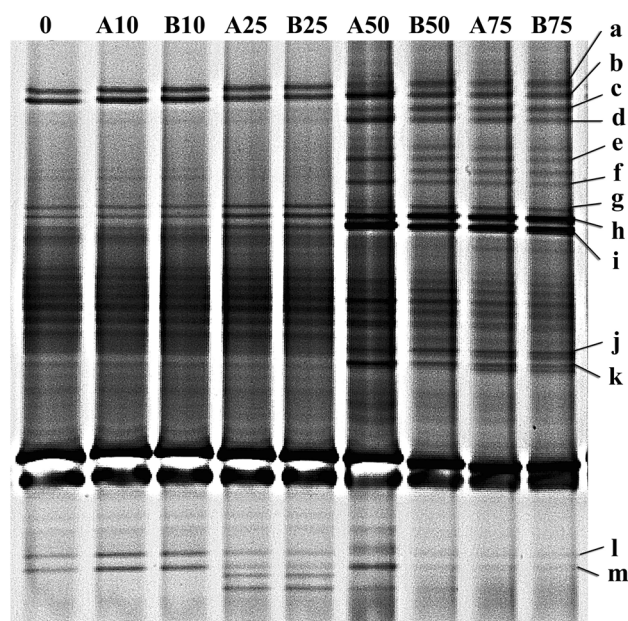
5 days although the COD removal efficiencies were still growing. We suspected that high concentration of IPA might have negatively impacted the formation of some extracellular substances which was very important for the attachment of microbes on filler. Based on the inference above, the operation mode was switched from SBBR to SBR in which the flocs did not attach on fillers. Since the DGGE analysis showed that the biofilm sampled at day 25 had very similar profile with that of the activated sludge from municipal wastewater treatment plant (Fig. 3), the sludge eluted from the filler was used to start the SBR.

At the beginning of SBR operation, no glucose was added and the influent COD was about 800 mg L^{-1}. Though the daily removed COD decreased to about 1.5 g day^{-1} on day 31 (Fig. 2), the COD removal efficiency quickly increased to 80 % on day 34 and then to 90 % on the 39th day (Fig. 1).

On day 41–50, the COD in influent was increased to 1,200 mg L^{-1} and the COD removal efficiencies increased stably from 70 % to about 90 %. While the influent COD was increased from 900 to 1,200 mg L^{-1} on the 11th day (SBBR mode), both the COD removal efficiencies and the daily removed COD were lower than that on day 10 (Fig. 2). But in SBR mode, when the influent COD was increased from 800 to 1,200 mg L^{-1} on day 41, the daily removed COD was higher than that on day 11 and day 40 (Fig. 2). This result meant that flocs in SBR had a stronger shockproof ability to high IPA, compared with biofilm in SBBR.

Then, the influent COD was increased to 1,600 mg L^{-1} on day 51, and the COD removal efficiencies in both reactors increased from about 74 to 90 % in the following 10 days. The daily removed COD stabilized at about 4.5 g day^{-1} in the last 20 days, and in the last 15 days, the effluent COD was lower than 100 mg L^{-1}, the emission concentration standard level 1 Integrated Wastewater Discharge Standard in China. The results showed that the COD removal ability of flocs in SBR was obviously higher than the biofilm in SBBR. Some researchers have reported that the SBBR is evidently well suited for the treatment of wastewater containing low-biodegradable compounds including composite chemicals (Mohan et al. 2007) or chlorophenols (Makinen et al. 1993) due to the greater biomass concentration to be maintained in the reactor (Singh and Srivastava 2011). However, this study indicates that SBR is more suitable for treating high concentration IPA wastewater rather than SBBR.

DGGE analysis of the bacterial community in biofilm/flocs

Figure 3 shows the DGGE profiles of nine samples collected from inoculum sludge and reactors A and B. Sample 0 was the inoculant sludge sampled from a municipal wastewater treatment plant. After being sequentially acclimated and operated in SBBR mode for 20 days, samples A10 and B10 showed almost the same DGGE profiles as sample 0, which indicated that the change of carbon source and operation mode had little effect on the community of predominant bacteria. The result surprised us as a previous study showed an obvious succession of dominant bacteria in a SBBR reactor even in 4 h (Xiao et al. 2009). Increased IPA in influent seemed not to have much impact on the community of predominant bacteria

either as the profiles of A25 and B25 were similar to that of 0, A10, and B10.

Switching the operation mode from SBBR to SBR dramatically increased the diversity of bacteria community, and no band in lanes of SBBR disappeared from the lanes SBR (Fig. 3). Previous report demonstrates that a more diverse system possesses more resistance to perturbation than a less diverse system (Girvan et al. 2005). Therefore, the flocs in SBR might be able to tolerate higher concentration of IPA than the biofilm in SBBR. After being operated for a continued 25 days with 1,600 mg L^{-1} COD in influent, the bacteria community in SBR had almost no change. The results meant that, comparing to SBBR, SBR can maintain a more diverse bacteria community and perform more stable function for removing high concentration IPA.

Thirteen bands, i.e., a to m in Fig. 3, were excised from DGGE gel and were successfully sequenced after being cloned into T-vector. Based on the sequences of these bands, a neighbor-joining tree (Fig. 4) was constructed by the program MEGA5 (Tamura et al. 2011). As showed in the phylogenetic tree, almost all the bands, except band g, showed high similarity to beta- or gamma-Proteobacteria bacteria.

Band a was 96 % similar to sequences from *Xenophilus* sp. and *Variovorax* sp., and band b showed highest similarity (97 %) to sequence from *Pelomonas* sp. Though none of these species have been reported as an IPA degrader, both bands might be very important for IPA degradation as they showed high intensity in all DGGE lanes (Fig. 3). Band c showed low intensity in lanes from SBBR, but had high intensity in samples from SBR, which meant that SBR operation could facilitate the growth of this strain. The band showed 98 % similarity to sequence from *Methylibium* sp. Some strains in genus *Methylibium* have been reported as alcohols degrader (Schäfer et al. 2011). Therefore, band c was expected to perform as a key player in degrading IPA in SBR. Band f showed highest similarity (95 %) to a strain of *Cupriavidus basilensis*, which was reported as a degrader for many recalcitrant contaminants (Fischer et al. 2010; Koopman et al. 2010; Wierckx et al. 2010). The band was only detected in samples from SBR, indicating that SBR might facilitate its ability to degrading IPA. Bands h and i, showing 100 % similarity to *E. coli* and 99 % similarity to *Azonexus* sp., respectively, both were detected in all samples and had higher intensity in samples from SBR than that from SBBR, which meant that SBR was more suitable for their growth in IPA wastewater. *E. coli* is a widespread bacterium in natural environment, but the result surprised us since there was no report on the degradation with the bacterium.

Several bands of d, e, g, j, k, l, and m could not be assigned to specific species. Thus, it is difficult to discuss
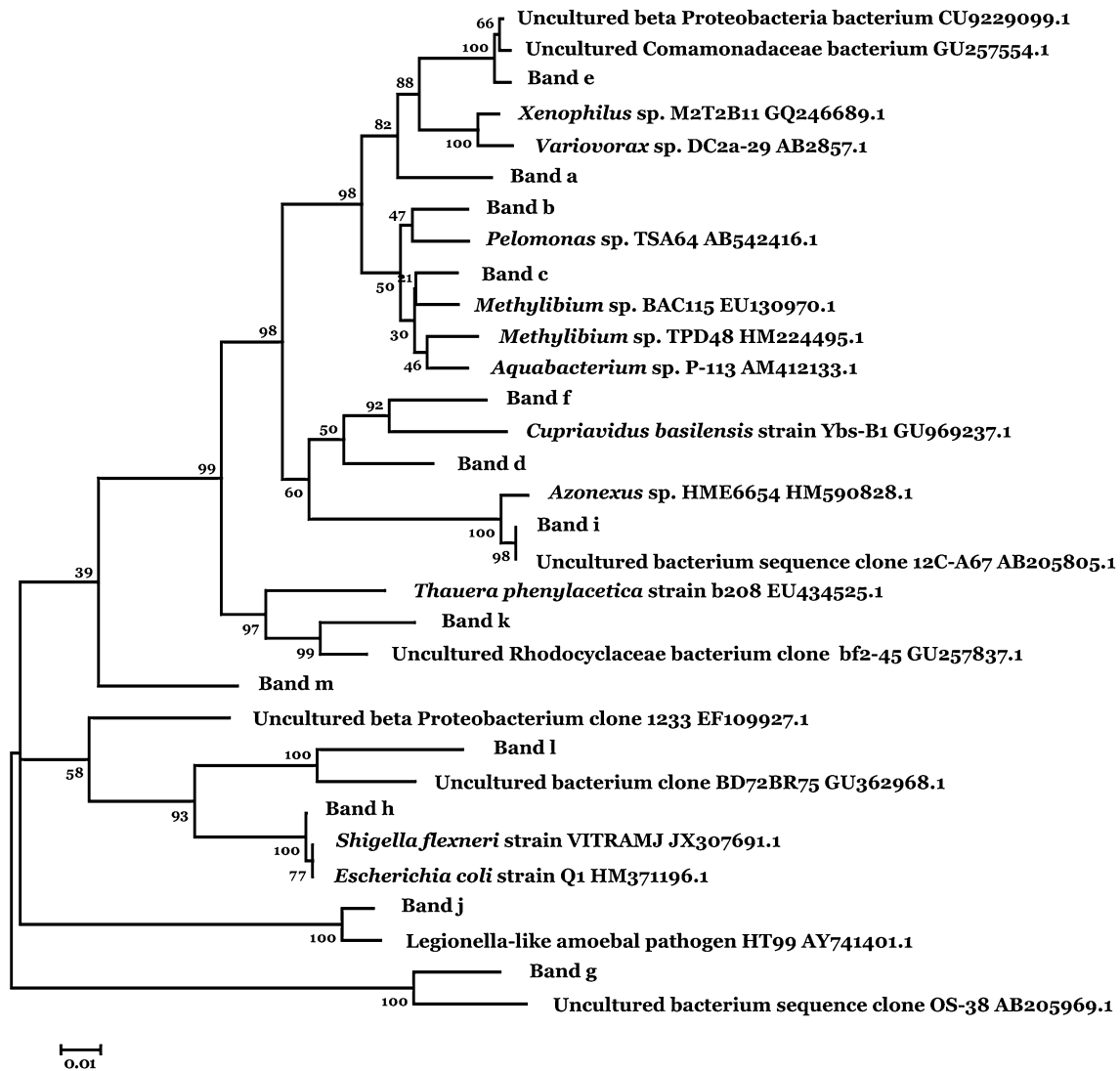
Fig. 4 Neighbor-joining tree showing the phylogenetic relationship of clones sourced from DGGE bands aligned with reference sequences from the domain *Bacteria* based on 16S rRNA gene sequences. Numbers at nodes indicate percentages of 1,000 bootstrap replications. Clones studied herein are presented as Band a to m

how they performed the function of degrading IPA in SBBR or SBR. Though, we could expect bands d, e, j, and k to play important roles in degrading IPA in SBR since they were detected with high intensity in samples from SBR.

## Potential effects of SBBR and SBR on biofilm/flocs

The operation results obviously showed that flocs in SBR could tolerate and remove higher concentration of IPA better than the biofilm in SBBR. The DGGE analysis showed an increase of bacterial diversity in SBR. Phylogenetic analysis was also used to explore the potential functions of the predominant bacteria in degrading IPA. Though, it is still uncertain how the bacterial community

was affected by the operation mode change from SBBR to SBR. In aerobic condition, IPA might be degraded to CO_2 by microorganisms. Therefore, DO and substrates transportation can influence the activity of biofilm/flocs, and we proposed two modes for substrates transportation and DO within biofilm in SBBR and flocs in SBR, respectively (Fig. 5).

The main difference between SBBR and SBR was the sludge structure which was in biofilm form in SBBR and suspended form in SBR, respectively. Different structure significantly affects the DO and substrates transfer rates inside the sludge aggregation. The thickness and compactness will also have impact on the DO/substrates transfer in biofilm/flocs. However, the thickness of biofilm (about 2 mm) is similar to the semi-diameter of flocs, and

Fig. 5 Schematic of DO and mass transfer inside biofilm in sequencing batch biofilm reactor (**a**) and flocs in sequencing batch reactor (**b**). The *bold arrows* present the direction of DO and mass transfer
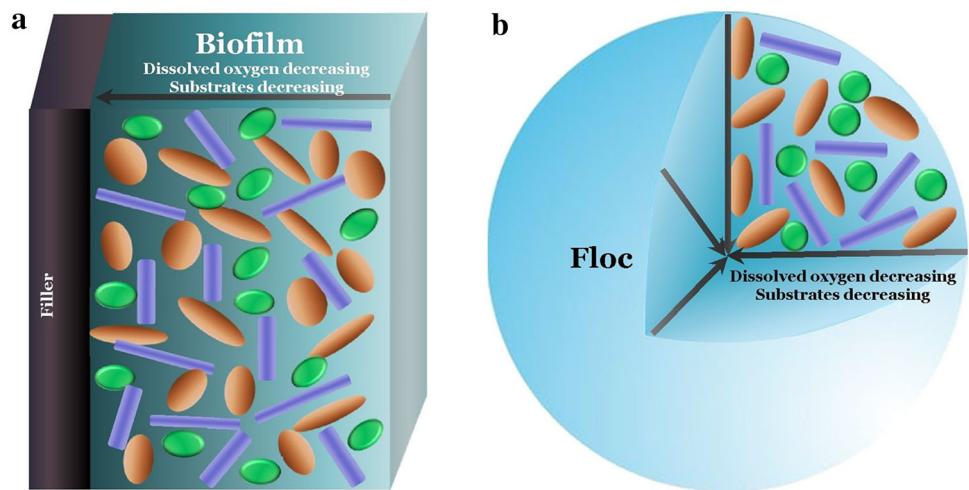
both the biofilm and flocs showed very similar compactness under a microscopy. Therefore, the sludge structure was the main factor influencing the DO and substrates transfer rates inside the sludge aggregation. The thickness of biofilm is much less than the length of fillers. So, the DO and substrates, affecting the growth of innermost microorganisms in biofilm, were mainly from the same plane with the bacteria (Fig. 5a). The flocs can be supposed to a spherical-like structure, and bacteria, even in the core, can acquire DO and substrates from any direction (Fig. 5b).

In wastewater biological treatment systems, DO is usually the major factor affecting the performance of microbial community. Studies have reported that DO will dramatically decrease as the depth of aggregation increases (Bishop et al. 1995; de la Rosa and Yu 2005). When the IPA concentration was low, such as 1,200 ml L^{-1} in this study, the DO might be available for bacteria in the innermost biofilm. As the COD was continuously increasing, DO consumed by the bacteria in surface biofilm increased and bacteria in the innermost biofilm could hardly gain enough DO. Thus, many aerobic bacteria in inner biofilm were going to die, and the biofilm became easily detached which might decrease the quality of effluent. Moreover, we had to increase the aeration rate to maintain a reasonable DO in the reactors, which further damaged the structure of biofilm. In flocs, DO could be efficiently transferred to the inner of flocs and bacteria would not die for lacking DO and substrates. The increase of COD would not induce disintegration of flocs. Moreover, IPA wastewater from SSC industry usually contain a mass of Na$_2$SiO$_3$, which will combine with extracellular polymers and improve the flocs settling. All in all, flocs in SBR was more conducive to the DO and substrates transfer than biofilm in SBBR, and therefore, SBR was more suitable for high IPA wastewater treatment.

Conclusion

IPA wastewater from SSC industry was sequentially treated with SBBR and SBR. SBR showed greater ability to remove IPA. When the influent contained 1,600 mg L^{-1} of COD, SBR achieved stable COD removal efficiencies of >95 %. Switching the operation mode from SBBR to SBR could increase the stability of flocs in SBR. Two structure models were constructed for biofilm in SBBR and flocs in SBR, respectively. High efficient DO transfer in flocs was proposed as the main reason for its stronger tolerance on high concentration of IPA and higher IPA removal efficiencies.

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2388

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