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Microbial community dynamics in hybrid biological reactor treating petrochemical wastewater

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ABSTRACT

Hybrid biological reactor (HBR) was developed by adding carriers to the aeration tanks, and could be applied to improve the poor nitrification ability of overloaded petrochemical wastewater treatment plants. Using the normal activated sludge process (ASP) as a control, the performance of ASP combined with HBR for the treatment of petrochemical wastewater was studied. When the influent load was 0.97 kg COD_{Cr}/m^3 d (COD_{Cr}, Chemical Oxygen Demand with the oxidant of $K_2Cr_2O_4$), effluent of ASP contained 138.2 mg/L COD_{Cr} and 8.2 mg/L NH_4^+-N, and the COD_{Cr} and NH_4^+-N concentration in effluent of HBR was 120.8 and 1.6 mg/L, respectively, indicating that both processes had good removal capacities for petrochemical wastewater. When the load was increased to 1.94 kg COD_{Cr}/m^3 d, there was no apparent change in effluent COD_{Cr} values of HBR and ASP. However, a great difference in the removal efficiency of NH_4^+-N between HBR and ASP was observed. NH_4^+-N concentration in effluents of ASP and HBR was 16 and 1.7 mg/L, respectively, suggesting that HBR had a much better treatment capacity for NH_4^+-N than ASP. Using ribosomal intergenic spacer analysis fingerprinting and 16S rDNA sequence analysis, a big difference in microbial community structure was found between the two processes. And there existed simultaneous nitrification and denitrification in HBR system. Compared with ASP, HBR had more advantages, such as high utilization of oxygen, less sludge generation, and lower power consumption.

Keywords: Petrochemical wastewater; Hybrid biological reactor; Organic loading rate; Nitrification; RISA

1. Introduction

At present, petrochemical wastewater is difficult to treat because of the complex components containing oil, sulfur, phenol, cyanide, polycyclic aromatic hydrocarbons, alkalis, and low ratio of BOD_5/COD_{Cr} (COD_{Cr}, Chemical Oxygen Demand with the oxidant of $K_2Cr_2O_4$). It has long been seen as recalcitrant industrial sewage. Ever since 1960s, traditional or improved sequential processes of oil separation–air floatation-activated sludge treatment have been used by Chinese petrochemical plants to dispose petrochemical wastewater. The main process, based on activated sludge process (ASP), has made great

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contribution to the removal of COD_{Cr} and BOD_5, but it had a low NH_4^+-N removal efficiency. The concentration of effluent ammonia nitrogen often exceeded the discharge limit. In recent years, the expanding scale of crude oil processing resulted in the increase of effluents amount of petrochemical wastewater and deterioration of water quality. On the other hand, the government has increased the wastewater discharge requirements, which could not be met by traditional ASP. Thus, the development of an effective process based on ASP to expand treatment capacity has become a key to solve the problem. The generally used methods include building hybrid biological reactor (HBR, carriers are fixed) [1,2], moving bed biological reactor (carriers are moving) [3–6], and membrane biological reactor [7].

HBR was developed by addition of carriers to the aeration tanks. This introduced a new phase of attached biomass into a regular suspended growth system (ASP) [8,9]. This process had native advantages of both ASP and biological membrane process, and improved the disposal capacity and operation stability of the system [10–13]. In addition, due to its little modification of the original ASP it had slight influence during operation and could be applied to reform the petrochemical wastewater treatment plants overloaded or with poor nitrification ability. HBR has been studied for a long time, which can be ascended to 1980s, but research dealing with the petrochemical wastewater denitrification performance was rare [14–16].

The treatment of petrochemical wastewater by traditional ASP and HBR was investigated in this study. The pollutant removal performance, shock load resistance, sludge properties, and utilization rate of dissolved oxygen (DO) of the two processes were compared. The results demonstrated the feasibility of using hybrid process to upgrade ASP process and improve the capacity of petrochemical wastewater treatment. Moreover, ribosomal intergenic spacer analysis (RISA) was used to analyze the changes of the sludge community structure in the reactor and explain the performance difference between the two processes.

2. Materials and methods

2.1. Description of the reactor

Bench-scale experiments were conducted to compare the performance of ASP and HBR for the degradation of organic and persistent compounds present in petrochemical wastewater. The anaerobic–aerobic HBR (Fig. 1) was made of Perspex sheets. The anaerobic and aerobic columns had a liquid volume of 4.5 L (Φ100 mm × 850 mm) and 17.8 L (Φ141 mm × 1,500 mm), respectively. Two such reactors were connected in series. Elastic fillers were placed in both reactors as fixed carrier material. The inlet to the anaerobic column was fixed at the bottom. The outlet of the anaerobic column was connected to the inlet of the aerobic column by a hose so that effluent from anaerobic column flowed as the influent to aerobic column. The effluent from aerobic column flowed to the settling tank where the mixed liquor suspended solids (MLSS) settled at the bottom and the clear supernatant was discharged. The settled sludge from the settling tank was recycled to anaerobic column by a peristaltic pump. As a comparison, systems without the addition of elastic filler were used for study of ASP.

2.2. Startup and operation of the reactor

After seeding with activated sludge from petrochemical wastewater plant at Dalian Petrochemical Company, HBR and ASP systems were started at hydraulic retention time (HRT) of 44.6 h. The HRT was reduced stepwise to 22.3 h while substrate concentration in the influent was held constant. Pseudo-steady state was assumed to be achieved when the variation in successive effluent COD_{Cr} values was observed to be insignificant.

The influent petrochemical wastewater had a COD_{Cr} of 860.7–951.4 mg/L, NH_4^+-N of 39.8–45.4 mg/L, NO_2^--N of 0.24–0.54 mg/L, NO_3^--N of 4.9–6.7 mg/L, TN of 48.9–53.2 mg/L, S^{2-} of 19.5–26.4 mg/L, oil of 43.8–48.6 mg/L, and pH of 7.8–8.4. DO inside the aerobic reactors was maintained in the range of 3–3.5 mg/L. The temperature inside the reactors was kept within the range of 25–30℃ throughout the study. In HBR and ASP, in order to keep the sludge concentration in the range of 8–10 g/L, measures like sludge recycling might be a good solution.

The experiment could be divided into two stages based on organic loading of the influent (0.97 or 1.94 kg COD_{Cr}/m^3 d).

2.3. Analytical methods

The samples were taken three times from ASP and HBR every day. They would mix together and sampled for the measurement of COD_{Cr}, NH_4^+-N, NO_2^--N, NO_3^--N, TN, oil, S^{2-}. All the parameters were analyzed according to the standard methods for the examination of water and wastewater [17].

The sludge concentration included MLSS and attached solids. MLSS were determined according to Chinese NEPA Standard Methods [18]. The attached solid was determined following the procedures described by Wang [10].

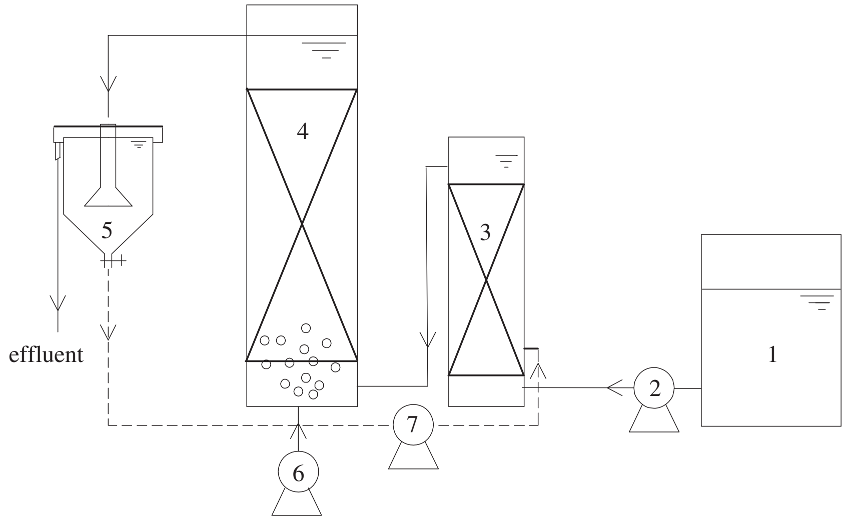

Fig. 1. Experimental set-up and flow chart: (1) storage tank; (2) metering pump for inlet; (3) anaerobic column; (4) aerobic column; (5) settled tank; (6) air pump; and (7) pump for return mixed liquor.

2.4. Genomic DNA extraction, PCR, and construction of clone libraries

The genomic DNA was extracted from the sludge samples by the method described previously [19].

The universal primers S926f (5´-CTYAAAKGAATTGACGC-3´) and L189r (5´-TCATGAGATGYTTMARTTC-3´) were used. PCR amplification was conducted as described previously with the exception of annealing temperature (48˚C) [20]. PCR amplified fragments were separated on native polyacrylamide (4.5%) gels, which were stained with ethidium bromide and photographed for RIS analysis.

Clone libraries of RIS-rDNA amplicons were constructed from the corresponding sample in RIS analysis of ASP (aerobic column, 50 d) and HBR (aerobic column, 50 d) using a TA Cloning Kit (TaKaRa, Dalian Co., Ltd.).

2.5. 16S rDNA sequencing and phylogenetic analysis

The recombinant plasmids with the rDNA inserts of different sizes from each library were selected randomly and the 16S rDNA regions were sequenced by TakaRa Biotechnology, Dalian Co., Ltd.

The determined partial 16S rDNA sequences (ca. 600 bp) were analyzed with the BLAST algorithm. And the sequence most similar to each clone was used as the reference affiliation. Phylogenetic trees were constructed using Mega 3.1 by the neighbor-joining method, and the evolutionary distances were calculated according to the model of Juke–Cantor [21]. Bootstrap analysis for 1,000 replicates was performed to estimate the confidence of tree topologies.

3. Results and discussion

3.1. Performance of HBR under shock loads

Fig. 2 show the influent COD_{Cr} of two processes under different loading conditions. When the influent organic loading reached 0.97 kg COD_{Cr}/m^3 d, the average COD_{Cr} values in anaerobic and aerobic effluents of HBR were 314.0 and 120.8 mg/L, respectively. Those in effluents of ASP were 395.3 and 138.2 mg/L, respectively. Therefore, both of the two processes had good COD_{Cr} removal capacities in petrochemical wastewater. HBR was a little more effective than ASP.

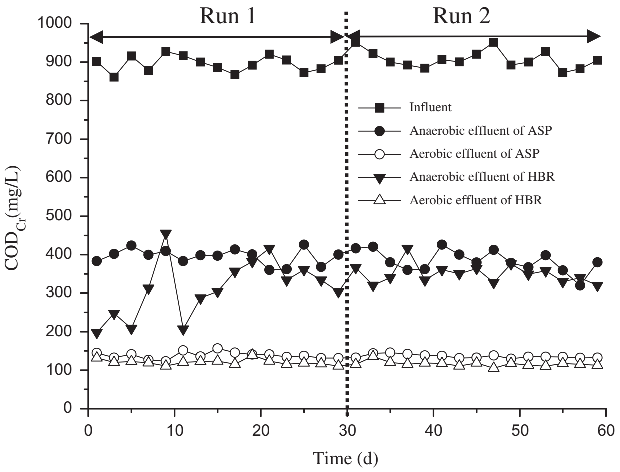

Fig. 2. The influent and effluent COD_{Cr} concentrations of the two processes during continuous operation. Run 1: the loads are 0.97 kg COD_{Cr}/m^3 d; Run 2: the loads are 1.94 kg COD_{Cr}/m^3 d.

When influent loading was increased to 1.94 kg COD_{Cr}/m^3 d, the COD_{Cr} removal capacity had no obvious changes in the two processes (350.3 and 116.2 mg/L for HBR and 384.1 and 136.0 mg/L for ASP). Thus, the two processes still had relatively good COD_{Cr} removal efficiencies when influent organic loading was increased.

Fig. 3 show changes of NH_4^+-N concentration in effluents of the two processes under different conditions. When the organic loading was 0.97 kg COD_{Cr}/m^3 d, the average NH_4^+-N concentrations in anaerobic and aerobic effluent of HBR were 21.8 and 1.6 mg/L, respectively. Those in effluents of ASP were 36.9 and 8.2 mg/L, respectively. Thus, the two processes had good removal capacities towards NH_4^+-N in petrochemical wastewater and HBR was more effective than ASP. When organic loading was increased to 1.94 kg COD_{Cr}/m^3 d, the NH_4^+-N removal capacity had no obvious change in the two processes (30.0 and 1.7 mg/L for HBR and 35.6 and 16.0 mg/L for ASP). In addition, the NO_2^--N, NO_3^--N, and TN concentrations in the effluents of two reactors with the influent loads of 1.94 kg COD_{Cr}/m^3 d were also determined. The results were undetectable, 2.4 and 20.4 mg/L in ASP and undetectable, 0.33 and 4.86 mg/L in HBR (detailed data not shown).

Therefore, when the influent organic loading was increased, HBR still had a relative good nitrogen removal efficiency, whereas ASP could not meet the treatment needs. The filler played a stranded role in the growth of nitrobacteria, which could prevent the nitrobacteria with low flocculability from flowing out along with water [22,23]. Thus, the HBR had a better nitrification ability.

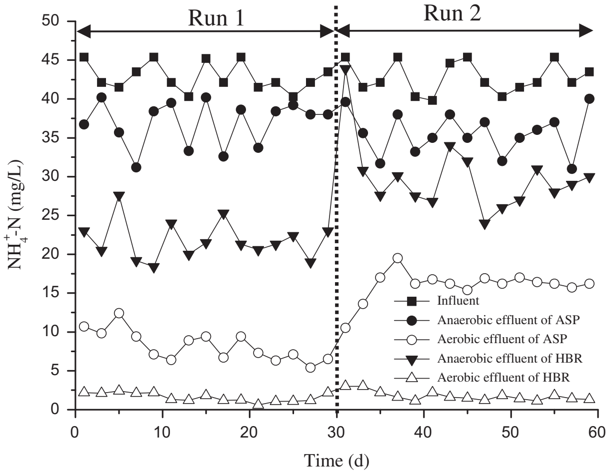

Fig. 3. The influent and effluent NH_4^+-N concentrations of the two processes during continuous operation. Run 1: the loads are 0.97 kg COD_{Cr}/m^3 d; Run 2: the loads are 1.94 kg COD_{Cr}/m^3 d.

HBR and ASP had similar sulfide and oil removal efficiencies under the two loading conditions. These two processes could obtain a high-removal efficiency of 99% towards sulfide. And the effluent sulfide concentration was lower than 0.1 mg/L. The average oil concentration in effluent of HBR and ASP was 12.1 and 14.4 mg/L, respectively (removal rate of 74.3 and 69.4%).

To sum up, compared with ASP, HBR showed a more superior removal efficiency and higher organic loading resistance for petrochemical wastewater treatment. Monitored Pollutant Parameters of the effluent have reached the requirements of the local discharge standard (COD <50 mg/L, NH_4^+-N <8 mg/L, TN <20 mg/L).

3.2. Oxygen utilization

At the reactor's continuous operation stage, the same aeration amount was applied in HBR and ASP. However, through the study of DO concentration of the two reactors, we found that DO concentration in HBR aerobic reactor was higher than that in ASP aerobic reactor (Fig. 4).

That was because the filler in HBR could collide with and cut the bubbles for many times, which increased the specific surface area. The filler could also adsorb these bubbles and extended the gas–liquid contact time. All these functions promoted the dissolving of oxygen, and improved the oxygen utilization rate. On the other hand, the high-sludge concentration in the ASP reactor increased the liquid viscosity, decreased the mass transfer rate, and thus, resulted in a lower DO concentration. Thus, when the same

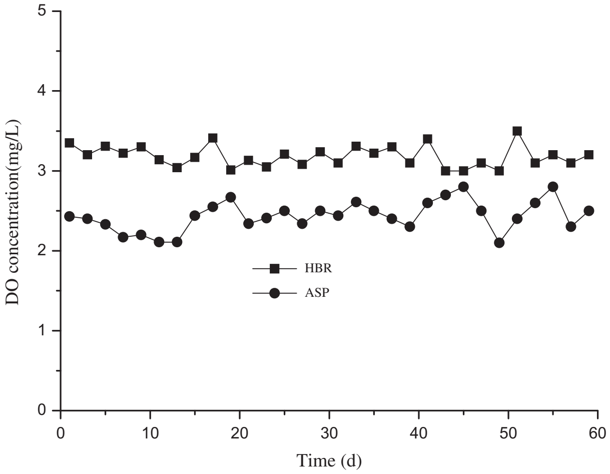

Fig. 4. Variation of DO concentration in aerobic reactor.

aeration amount was applied, HBR aerobic reactor possessed a higher DO concentration. The higher oxygen utilization rate could result in improved economical efficiency of HBR.

3.3. Micro-organism community analysis

Fig. 5 presents the polyacrylamide gel electrophoresis of RIS fingerprints. It had a good separation effect and could meet the needs of fingerprints polymorphism analysis [24,25].

Series of strips between 1 and 1.6 kb were observed for both processes, whereas the similarity of chromatographic fingerprint was not high (Fig. 5). Therefore, the micro-organism community in the two processes might belong to certain big species. However, there were great discrepancies between structures of their microbial community. The highest abundance strip of the ASP bacterial community appeared at around 1.4 kb, where strip abundance of HBR was very low. Instead, the highest strip abundance of HBR appeared between 1.2 and 1.3 kb. The great discrepancies observed in microbial community structures of the two processes corresponded to their differences in the pollutant removal and sludge properties.

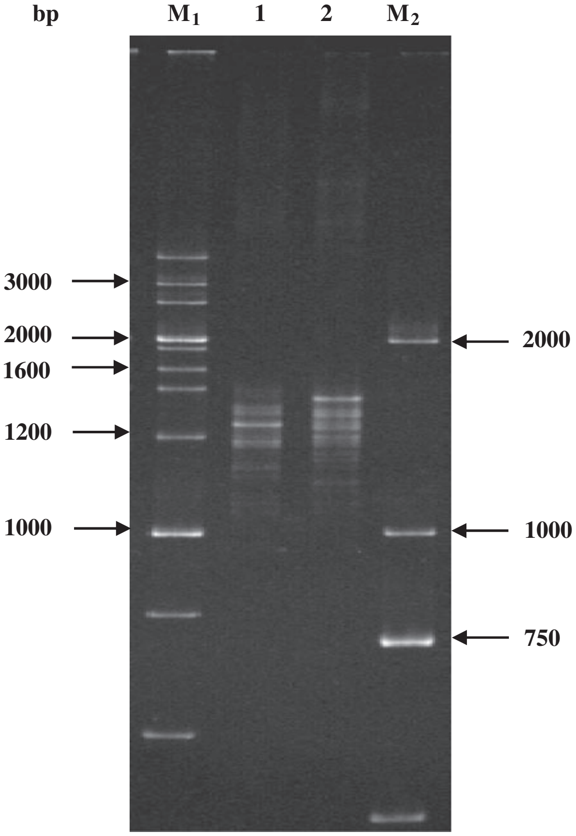

Fig. 5. RIS fingerprints of microbial communities of aerobic reactors of the two processes. Lane designations: M_1: 200 bp DNA Ladder Marker; M_2: DL2000 DNA Marker; 1: hybrid process community; and 2: activated sludge community.

Although RISA permits meaningful comparisons of community similarity, it does not estimate the accurate community structure [26]. After analyzing the bacterial community samples of HBR and ASP, two libraries (1# and 2#) were constructed, respectively, for evolution analysis of community species through sequencing. The rDNA-RIS fragment contained 600 bp 16S rDNA and could meet the needs of analyzing and identifying the uncultured clones to genus level. Monoclones with different insert fragments were selected randomly for sequencing (20 samples for each library). After sequencing, their homology with known 16S rDNA sequences from GenBank was compared for genus identification and phylogeny tree construction (Figs. 6(a) and 6(b)). The evolution analysis showed that the sequences from library 1# basically belonged to six different orders, while the sequences from library 2# basically belonged to five different orders. *Burkholderiales* was the only order detected in both libraries. The results further proved the great discrepancies between microbial communities of the two processes. However, most of the orders identified from library 1# and library 2# belonged to *Betaproteobacteria* and *Gammaproteobacteria*, and all of them belonged to *Proteobacteria*. Thus, it could be concluded that the general composition of microbial species of the two processes was contain the similarity. The bacteriode was the main bacterial community in the ASP.

In library 1#, 35% sequence belonged to *Oceanospirillales*, which had the highest abundance and might be the dominant bacterial community in hybrid process aerobic system. The *Alcanivorax* species attached to *Oceanospirillales* were efficient oil degrading bacteria, which could use straight chain and branched alkanes as sole carbon and energy sources [27–29]. Moreover, the *Brachymonas* attached to *Burkholderiales* could also degrade the hydrocarbon and aromatics in petroleum [30,31]. These might explain the stronger removal efficiency of HBR towards petroleum. In library 1#, there were also some clones with abilities of denitrification and desulfurization, such as *Thiobacillus denitrificans* and *Thioalkalivibrio*. *T. denitrificans* had the synchronous desulfurization and denitrification capacity. It could use sulfide as the electron donor to obtain energy, and simultaneously reduce nitrate to nitrogen to realize denitrifying effect [32]. Some studies showed

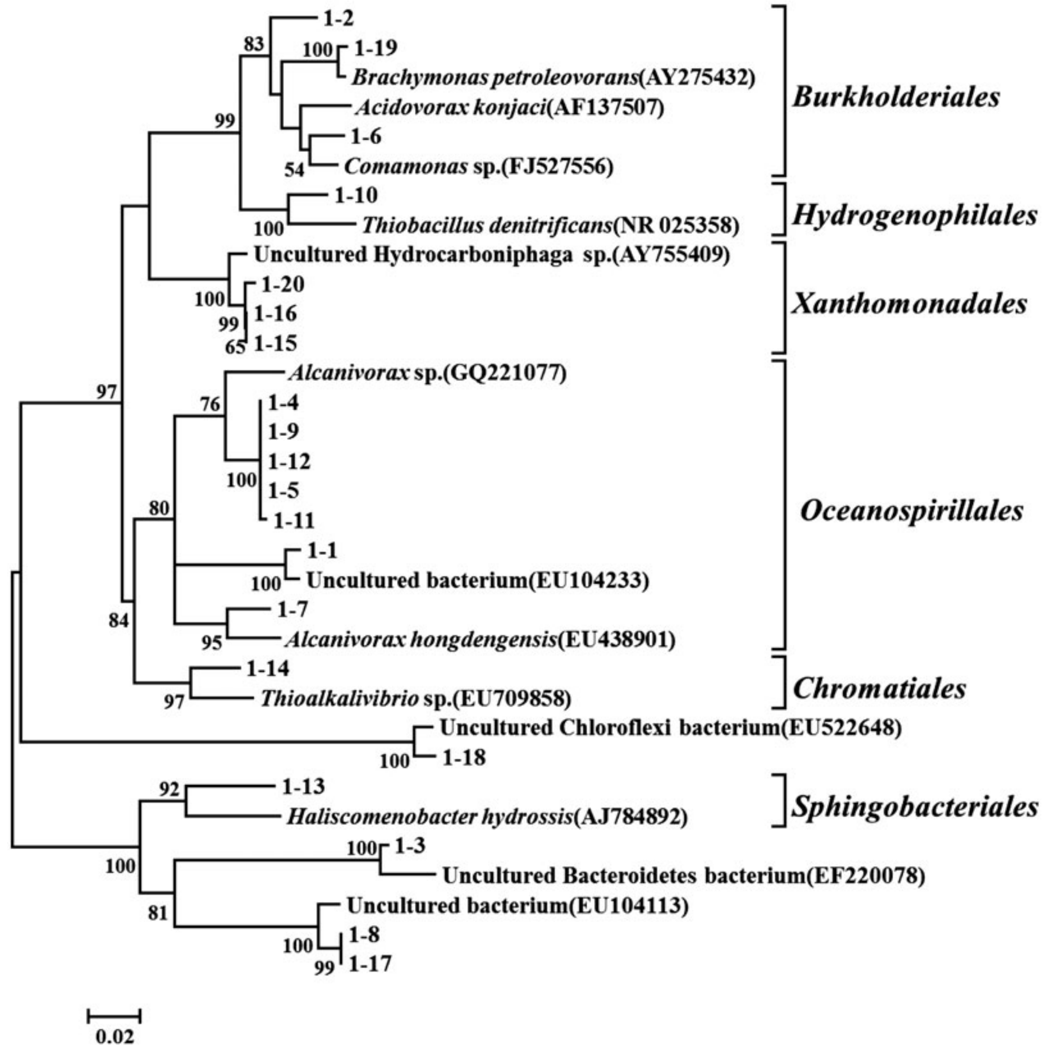

Fig. 6(a). Phylogenetic analysis of clone libraries 1#.

that *Thioalkalivibrio* had the capacity of sulfide oxidation, denitrification, thiocyanate degradation, and many other metabolic functions [33]. Thus, we speculated that there could be effects of simultaneous nitrification and denitrification in the HBR aerobic system.

In library 2#, 55% sequences belonged to *Burkholderiales*, which had the highest abundance and were all *Hydrogenophaga*. A study showed that *Hydrogenophaga* could degrade the pollutants like polyaromatic hydrocarbons and polychlorinated benzene compounds [34,35].Bacteria capable of degrading organic pollutant and sulfide were identified in library 2#, while no denitrification bacteria were found. These might explain poor denitrification performance of ASP.

According to the above RIS fingerprint analysis and the results of 16S rDNA sequencing and phylogenetic tree, although microbial communities of these two processes have great discrepancies, most of the bacterial community belonged to *β*, *γ-proteobacteria*. And the two systems also demonstrated certain similarities. In HBR, we found the bacteria with the ability of denitrification, which could explain the more efficient denitrification performance of HBR. Therefore, using RIS fingerprint analysis technology and 16S rDNA sequencing analysis technology could reveal the discrepancy of microbial community structures of these two processes and explain the macroscopic performance differences of them as well.

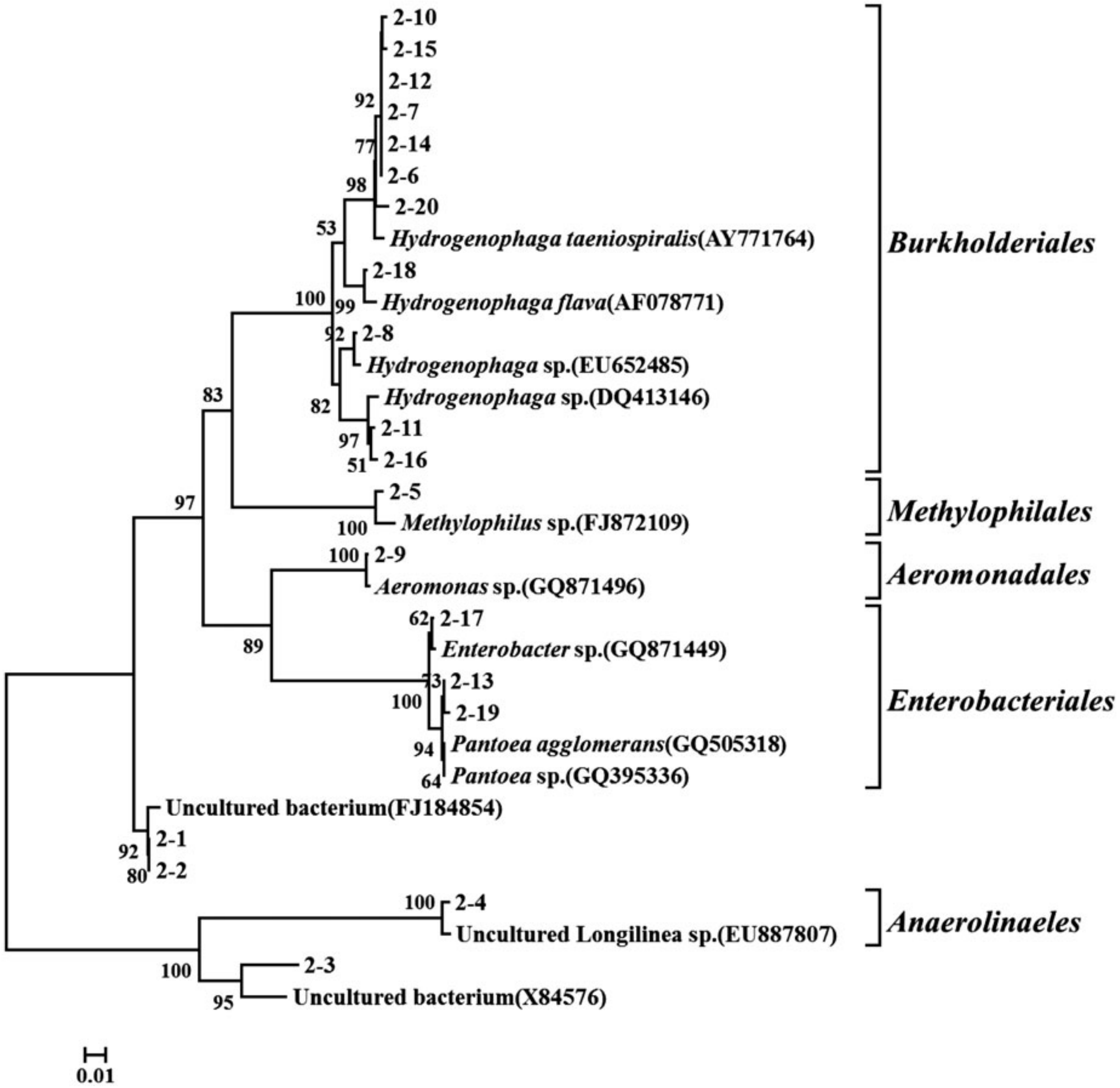

Fig. 6(b). Phylogenetic analysis of clone libraries 2#.

4. Conclusions

HBR could achieve an efficient removal of COD_{Cr}, NH_4^--N, and oil indicators in the petrochemical wastewater. It was shown that Monitored Pollutant Parameters of the effluent have reached the requirements of local discharge standard. Meanwhile, RIS fingerprint analysis also indicated that the microbial community structures of HBR were richer. The dominant bacteria of HBR were *Oceanospirillales* and *Burkholderiales*, which both had the removal efficiency towards petroleum. Besides, there were also some microbes with denitrification ability, which also explained the macroscopic performance differences of these two processes in microscopic. This research documents that if an ASP is converted into a HBR, the removal efficiency of the main regulated pollutants will increase and their concentration in the treated effluent will guarantee the respect of the legal requirements.

Acknowledgments

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