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Aerobic treatment of oilfield wastewater with a bio-contact oxidation reactor

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ABSTRACT

This paper investigated the use of Bacillus coagulans W-15 immobilized on carriers in a bio-contact oxidation reactor to treat oilfield produced water. It researched the effect of hydraulic retention time (HRT) on the removal efficiencies of chemical oxidation demand (COD) and total organic carbon (TOC). The results showed that, when the HRT was 24 and 32 h, the COD of effluent water were less than or equal to 352 mg/L, the removal efficiencies of COD and TOC were greater than or equal to 75% and 68%, respectively. The degradation efficiencies in the bio-contact oxidation reactor immobilized with W-5 were estimated to be 73% for total oil, and 86% for the gas chromatography resolved compounds. The quality of the effluent water met the professional emission standard of petrochemical industry of China.

Keywords: Produced water; Bio-contact oxidation; Immobilization; Squalane

1. Introduction

Oilfield wastewater, called 'produced water' in the scientific literature, accounts for the majority of the waste derived from the production of crude oil [1]. This wastewater is the main source of the oily water in China, because many Chinese oilfields are in their mid-or final-stage of development and the produced oil contains up to 90% (v/v) water [2]. However, until now, most of the oilfield produced water in China was directly re-injected into the underground formation without treatment, which may damage the stratum [3]. Moreover, the untreated produced water discharges from oil and gas operations may be toxic to the environment due to organic and inorganic matter contained in the wastewater, mainly salts and oil hydrocarbons [4]. Hydrocarbons in produced water are also known to impact species diversity in benthic communities close to shallow water discharges [5]. Neff et al. [6] found elevated total petroleum hydrocarbons (TPH) concentrations < 300 m from a shallow water discharge and < 100 m from an offshore discharge in coastal waters. Neff concluded that benthic communities within 20 m of both discharges were influenced by sediment contamination derived from produced water discharges.

It is of significance that produced water should be reused for enhancing oil recovery or discharged into the environment after effective treatment. Various technologies and methods exist for treatment of oilfield produced water. Ebrahimi et al. [1,4] applied different ceramic micro-, ultra-, and nanofiltration membranes for efficient treatment of oilfield produced water. Reverse osmosis has

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also been used for desalination and removal of oilfield produced water [7,8]. However, due to the presence of dissolved oil in untreated oilfield wastewater, the membrane equipment may become fouled and thus destabilize the treatment process, increasing operating costs [9,10].

The biological treatment has been widely used for removal of organic compounds from petrochemical wastewater [11,12]. Compared with physical and chemical processes, biological treatment is a cost-effective and environmentally friendly technique, and more compatible with existing plant facilities and operation. Hydrocarbon-degrading microbes such as bacteria, yeast and fungi, which can grow using petroleum hydrocarbons as a source of carbon and energy, have been widely used to treat oil-contaminated wastewater [11–15]. Biodegradation of crude oil by these microorganisms is one of the primary mechanisms by which petroleum and other hydrocarbons are eliminated from the environment.

Currently, because of its low cost and reasonable efficiency, conventional activated sludge (CAS) process is commonly used to decompose organic compounds in municipal wastewater. However, it may be difficult to meet the discharge criterion using CAS to treat oilfield wastewater, for oilfield produced water has a large flow rate, high suspended solid content, and contains complex components. Moreover, CAS process cannot operate efficiently over the long term as bulking and foaming tend to occur [16].

The application of immobilized microorganism technology has drawn considerable concerns in recent years. In addition to their use in pharmaceutical and food biotransformations, immobilized cell processes have been used in the degradation of toxic and recalcitrant compounds during wastewater treatment [17,18]. The immobilization process is ideal for reclamation and reuse of wastewater. It features excellent removal of organic matter and solid suspensions by maintaining high hydraulic loading rates and retaining a high biomass concentration to reduce environmental shock, resulting in less sludge formation, and promoting microorganisms grow. Moreover, it features easy maintenance and achieves energy-saving and space-conservation [17]. It has been demonstrated that the combination of bioreactor and microorganism carriers is able to remove about 99% biochemical oxygen demand (BOD), 92% chemical oxygen demand (COD), 74% of suspended solids, and 92% of total nitrogen [19].

In this study, a pure strain identified as *Bacillus coagulans* W-5 was isolated from petroleum-contaminated soil through enrichment using crude oil as sole source of carbon and energy. On a laboratory scale, oilfield produced water was treated with strain W-5 immobilized onto a carrier using a bio-contact oxidation (BCO) reactor. The characteristics of petroleum hydrocarbon biodegradation by the immobilized microbial cells were investigated.

2. Materials and methods

2.1. Microorganism screen and cultivation

Oil-degrading bacterium was isolated by enrichment culture technique from soil samples contaminated with crude oil procured from Dagang oil field in northern China. The minimal medium (SM) for isolating oil degrading bacteria contained (g/L): crude oil 2, NaCl 0.5, $(NH_4)_2SO_4$ 0.1, $NaNO_3$ 0.2, $MgSO_4{\cdot}7H_2O$ 0.025, NaH_2PO_4 0.5, pH 7.2. The isolation was carried out as follows: the contaminated soil was inoculated to three replicate 250-ml Erlenmeyer flasks containing 100 mL SM. The flasks were incubated on a rotary shaker at 45°C and 150 rpm. After one week of incubation, 0.5 mL of the supernatant was inoculated into fresh medium, and incubation was continued. After five cycles of enrichment, 1 mL of culture was diluted 104-fold into 0.1 M potassium phosphate buffer (pH 7.0), and 100 μL of the mixture was plated on SM agar plates. The plates were incubated at 45°C for one week and morphologically distinct colonies were reisolated by transfer to fresh SM agar plates until pure cultures of each kind of colonies were obtained. All purified cultures were maintained on SM agar slants at 4°C and transferred every four months.

The liquid medium used for cell cultivation was as follows (g/L): yeast extract 0.1, glucose 2.0, NaCl 0.5, $(NH_4)_2SO_4$ 0.1, $MgSO_4{\cdot}7H_2O$ 0.025, KH_2PO_4 0.2, pH 7.2. The bacteria were grown in 250-mL shake flasks containing 100 mL liquid medium at 45°C, 150 rpm for 24 h. Cells were harvested at the late of the exponential growth phase by centrifugation at 8000 rpm for 5 min at 4°C, and washed with sterile distilled water to eliminate medium components. The pellet was resuspended in the same volume of sterile distilled water as the cell suspensions (OD600, 0.8) and ready for use.

2.2. Cloning and sequencing of the 16S rRNA gene

High molecular weight DNA was extracted with a commercially available kit (Dingguo, China). The 16S rRNA genes were amplified by PCR in a Techgene thermocycler (FTGENE 5D, 112757-4, Techne Combridge Ltd. DUXFORD Cambridge UK), using the forward primer 5′-GAGAGTTTGATCCTGGCTCAG-3′ and the reverse primer 5′-CTGAGCCAGGATCAAACTCTC-3′. Reactions were done in 50 μL volumes with final concentrations of reactants as follows: 2.5 mM deoxynucleoside triphosphates, 5 μM of each primer, 0.1–0.5 μg of DNA template and 2.5 U of the Taq DNA polymerase (Sangon, Shanghai, China).

The cycling conditions were: initial denaturation at 95°C for 3.5 min; 5 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 4 min; 5 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 4 min; 20 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 4 min; and final extension for 10 min at 72°C. PCR products were resolved by electrophoresis

in 1% agarose gels at 120 V for 40 min in TAE buffer (20 mM acetic acid, 2 mM EDTA-Na_2, 40 mM Tris base, pH adjusted to 8.0 with NaOH), and then visualized by a UV transilluminator after staining with ethidium bromide (Nippon Gene, Tokyo, Japan). The sequence was determined on an ABI Prism 310 genetic analyzer (Vilber Lourmat, France).

2.3. BCO reactor system

A horizontal flow BCO reactor system was used in this study (Fig. 1). The rectangular reactor made of polymethyl methacrylate has a volume of 15 L, a length of 50 cm, a width of 20 cm, and is 15 cm high. The reactor was equipped with inlet and outlet ports for feeding and for effluent discharge, respectively. The inlet port was located on one end at the bottom of the rectangular reactor, while the outlet port was set on another end at the top of the reactor. The water flow was driven by gravity, from the bottom of the reactor to the top, by means of gravitational potential energy, namely the wastewater was fed into the bottom of the reactor from a sufficient height that the hydraulic head was sufficient to drive the water through the system.

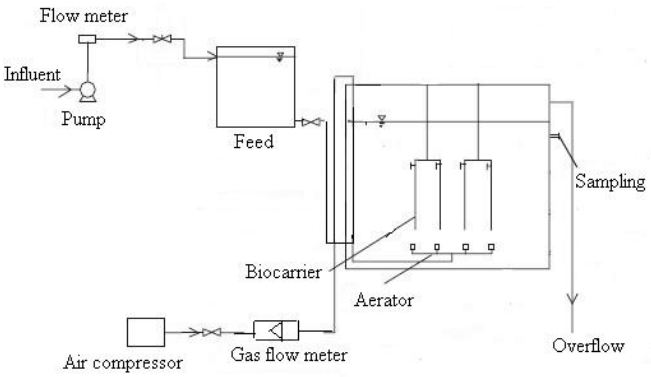

Fig. 1. Scheme of the bio-contact oxidation reactor.

One kind of combined semi-soft medium, constructed by plastic ring and synthetic fiber string, was chosen as the support for microorganisms (shown in Fig. 2). The packing had a theoretical specific surface area of 1520 m^2/m^3. The packing was hung from 2 cm below the top of the reactor to the bottom of the reactor. The level of liquid in the reactor was maintained at approximately 1 cm above the top of the support media, therefore the working volume of reactor was 14 L. Total volume of the packing material was 6.2 L.

When in operation, air was supplied to the reactor through air diffusers, located at the bottom of the reactor, to supply sufficient oxygen to the biomass and to stir the liquid as well as to exfoliate the aged biofilm.

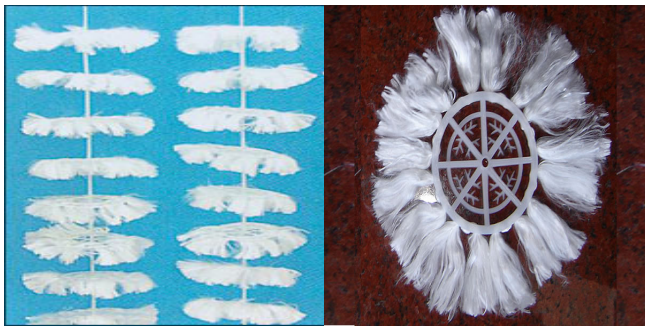

Fig. 2. Combined plastic packing material.

2.4. Produced water

The oilfield produced water was abstracted from the water injection pipe of Henan Oilfield, Central China. The wastewater was allowed to settle for 24 h, and the floating crude oil was discarded. The wastewater was stored at 4°C until required. The COD in the influent flow varied in the range of 315–352 mg/L. The characteristics of influent samples are listed in Table 1.

Table 1
Characteristics of oilfield produced water

pH	6.2–6.7
COD, mg/L	315–352
BOD_5, mg/L	95–113
TOC, mg/L	82–86
TPH, mg/L	23–26
Total dissolved solids, mg/L	4650–5325

2.5. Adaptation and immobilization of microorganisms

Microorganisms were first acclimated to produced wastewater operating with a 24-h cycle. The first four cycles marked the startup phase during which the reactor was operated with 22 h of aeration, and 2 h of quiescent condition, to allow the biomass to settle out of solution. At the start of each cycle, liquid paraffin and compound fertilizer NPK 15:15:15 salt were added to the reactor. During the acclimation stage, exhausted wastewater and excess sludge were removed daily, and then fresh wastewater was fed into the reactor. The reactor was cultivated over a 24-h fill-and-draw cycle to stimulate the formation of biofilm on the packing. The culturing was continued until the steady-state biomass loading on the plastic packing materials. After 10 days, a stable biofilm was formed on the plastic packing materials. The pH varied in the range 6.0–7.0 and the temperature was maintained at 45 ± 2°C.

2.6. Treatment of produced water in the BCO reactor

The wastewater in the reactor was withdrawn after immobilization. The oilfield wastewater was stored in a feed tank prior to being pumped to the BCO reactor. The wastewater was enriched with the inorganic nutrients by adding NH_4Cl as nitrogen source and K_2HPO_4 as phosphorus source based on a COD:N:P ratio of 100:5:1. The reactor was filled with wastewater and the air blowers turned on, with the influent and effluent stream valves opened. The dissolved oxygen (DO) concentration was maintained at 2–4 mg/L by adjusting the air flow. Reactor performance was studied at 16, 24 and 32 h of hydraulic retention time (HRT). While in operation the temperature was maintained at 45 ± 2°C.

In addition, a control reactor containing the packing material without microorganisms was prepared to enable the calculation of any non-biological effect on treatment efficacy. Each experiment was carried out in triplicate. Data collection was started after the steady state conditions were obtained. Operational parameters such as gas flow rate and liquid rate were adjusted if necessary, depending on the experimental needs.

2.7. Water quality detection

In order to ascertain the content of organics, the wastewater samples were acidified to pH < 2 using a solution consisting of 1:1 (v/v) H_2SO_4. Then, the samples were stored at 4°C, and analyzed within 24 h. The wastewater quality parameters were monitored according to standard methods [20]. COD was determined by filtering with a 0.45 μm filter and then oxidation with potassium dichromate under strongly acidic conditions and at an elevated temperature for 2 h. The oil content in the wastewater was determined by using an infrared spectrophotometry oil-measuring instrument (H3-OCMA-350, Japan). BOD_5 was determined by measuring the amount of oxygen absorbed by a sample of wastewater in the presence of microorganisms within five days at 20°C. Total organic carbon (TOC) was measured using a TOC analyzer (Shimadzu TOC-5000A). Additionally, temperature, DO and pH were routinely monitored with probes during the experimental period.

2.8. Biology observation

Morphological characteristics of microorganisms in the wastewater were monitored by a fluorescence microscopy (OlympusBX 60-FLA with software Image Pro-Plus).

2.9. Gas chromatography-mass spectrometric (GC-MS) analysis

A 50 mL sample of wastewater was extracted by 5 mL dichloromethane three times for pH 2, 12, and 7. The three extract layers were combined and dried using nitrogen, and the residue was dissolved in a 1 mL solution of n-hexane. Samples were analyzed by gas chromatography, using a Thermo-Finnigan SSQ710 GC-MS with HP-5MS elastic silica capillary columns (60 m × 0.25 mm × 0.25 μm). The injection volume was 1 μL. The carrier gas was helium at 37 kPa. Flow velocity was 1 mL/min. The analytical conditions were: initial temperature of 50°C, with isothermal operation for 1 min; heating to 120°C at a constant rate of 20°C/min; and heating to a final temperature of 310°C at a constant rate of 4°C/min, with a 30 min isothermal. Mass spectrometer conditions were: ionization mode: El, electron energy 70 eV; filament current 100 μA ; multiplier voltage, 1200 V; full scan.

3. Results and discussion

3.1. Isolation and screening of crude oil-degrading strains

In order to obtain the most efficient strain, we investigated the capacity of each isolate to degrade crude oil (data not shown). In the end, eight morphologically distinct bacterial colonies which could grow using crude oil as the sole carbon source were obtained from the petroleum-contaminated soil. The isolated bacterium with the highest degradation efficiency, marked as W-5, was used in the following work for further experimental study.

Cells of strain W-5 were rod-like, and had a dimension of about 2.0–2.5 μm in length and about 0.5 μm in width. Colonies were white, smooth, shining, convex, mucous and about 1.5 mm in diameter after they grew on nutrient broth agar at 45°C for 48 h.

The sequencing result was submitted to GenBank database and also done similarity search of the nucleotides by BLAST. After alignment with other 16S rRNA sequences in GenBank, strain W-5 was identified as *Bacillus coagulans*, and had a high degree of similarity (99%) to other members of genus *Firmicutes*.

3.2. Effect of HRT on COD removal efficiency

The COD was selected as the principal parameter for analysis in the present study, because the time required for the determination of the organic content in a sample is only 3–4 h, whereas the time required for the determination of other parameters like BOD_5 is a full 5 days. As such, the results of COD removal are particularly useful in determining the performance of the BCO system under shock loading environments so as to enable the operator to adjust the system appropriately in response to any adverse conditions encountered.

The COD removal efficiency of the BCO system is presented in Fig. 3. The results showed that at 32 and 24 h of HRT, COD removal efficiency from the wastewater was consistently higher than 75% except for a HRT of 16 h that gave a 62% removal. This indicated that the biomass can adapt to the wastewater characteristics. At HRT of 32 and 24 h, the COD of effluent could satisfy the professional emission standard (grade1) (CODCr <

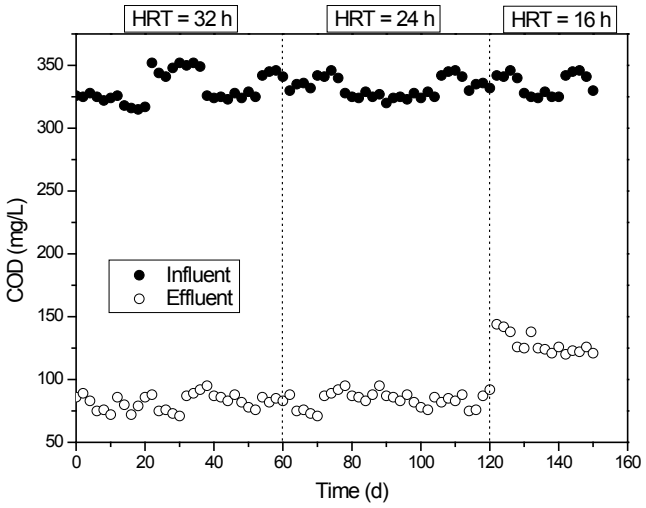

Fig. 3. Effect of HRT on COD removal during the operation of the BCO reactor.

100 mg/L) of petrochemical industry of China (GB4287-92). The results obtained in the present study suggest that a BCO process can achieve high removal efficiency and has strong adaptability to shock loading. This can be attributed to the higher biomass concentration that can be achieved in BCO system compared to CAS system which also matches the case of Wang and Chen et al. [21].

3.3. Effect of HRT on TOC removal efficiency

Fig. 4 represents the profile of TOC concentration. TOC removals were in the range of 60–72% for all the three HRT values studied. At lower loading rates (< 80 mg TOC/L·d, HRT of 24 and 32 h), the effect of HRT on TOC removal efficiency was not pronounced. In this range, TOC removal efficiency varied between 68–72% which was not significant. However, at HRT of 16 h, the effect of HRT became pronounced.

3.4. Effect of HRT on oil removal efficiency

Fig. 5 shows the profile of oil content during treatment. Oil removals were in the range of 70–85% for all the three HRT values studied. Although COD and TOC removal efficiencies were reduced at high loading rate (16 h of HRT), the effect of HRT on oil removal efficiency was not pronounced.

3.5. Biology observation

Normally, the operational status of reactor or the state of microorganisms would be revealed by biological constituents, which can be examined by a microscope with a magnification of 400. Higher microorganism can also be observed. In this study, the color of biomass in supporting media was brown throughout the sampling period. Higher microbial forms were observed in the reactor after 18 days of operation (Fig. 6). When a wastewater treatment plant operates with a good performance, the population of higher microorganisms would increase along with the bacterial proliferation to the disappearance of the bacteria. The grazing phenomenon from higher microorganisms contributes substantially to the reduction of suspended solids in effluents, in particular the dispersed bacteria, so this filtering activity of protozoa improves the quality of the effluent by the removal of suspend bacteria. Higher microorganisms did not act to decrease the purified water quality. This was likely because the biofilm was tightly packed to form large specifically organized matrix, protecting the bacteria within. Debeer et al. [22] reported that the pore diameter of the biofilm cell cluster matrix

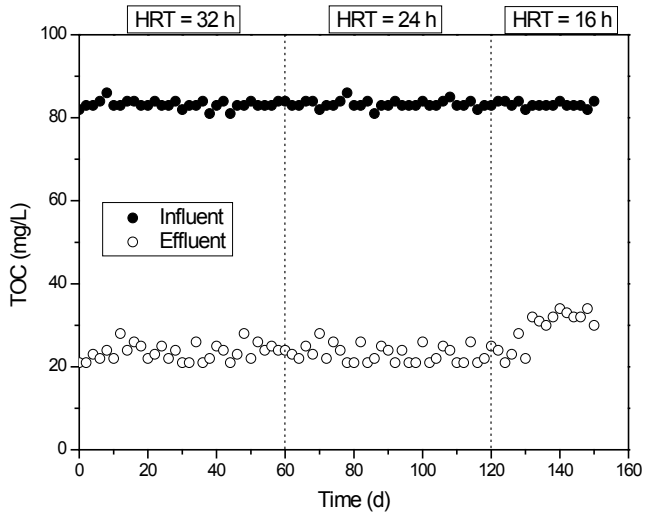

Fig. 4. Effect of HRT on TOC removal during the operation of the BCO reactor.

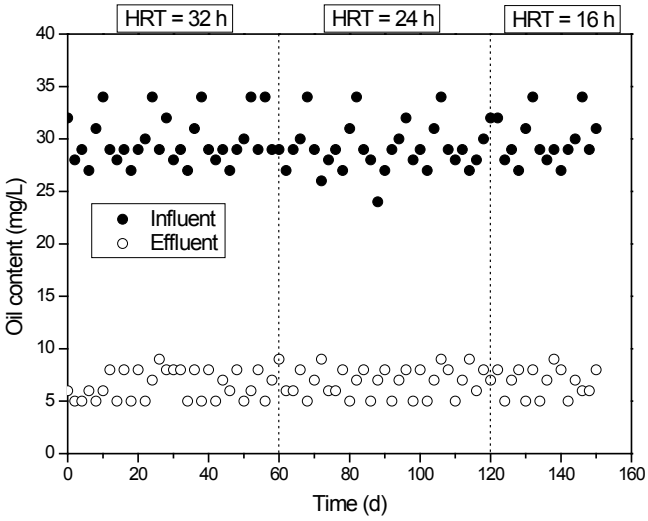

Fig. 5. Effect of HRT on oil removal during the operation of the BCO reactor.

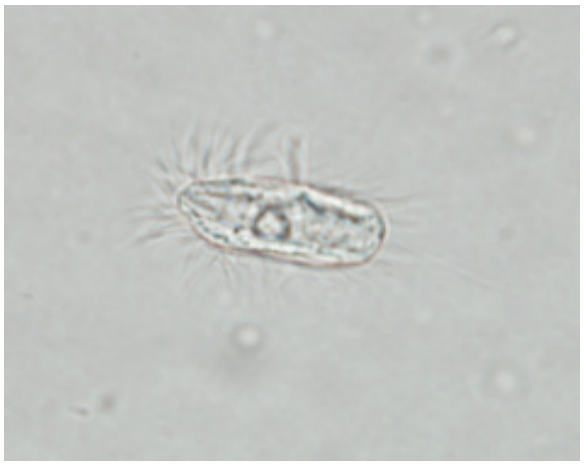

Fig. 6. Higher microorganisms (ciliates) existed in day 18.

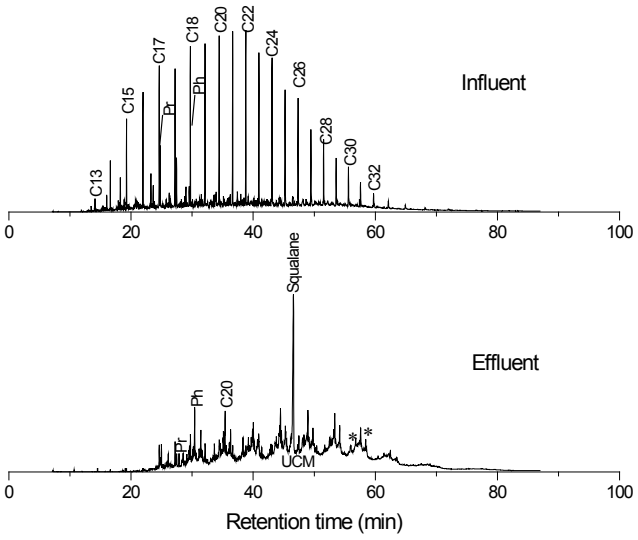

Fig. 7. Chromatographic profiles (GC-MS) of TPH extracts from 58 days. Pr, pristane; Ph, phytane;* saturated biomarkers of the hopane series.

was between 10 and 300 nm. This spacing was not large enough for the heavy grazing organisms to penetrate and consume the bacteria. Thus the ciliates had not influenced the organization of the biofilms and in turn had not affected the purification process conducted by the biofilms.

From day 90, cyanobacteria were observed in the upper biofilms and flourished gradually. Some publications suggest that cyanobacteria may produce substances toxic to bacteria [23,24]. Meanwhile, DO in the effluent was relatively high compared to that of other periods (data not shown), originating from photosynthetic oxygen evolution of the cyanobacteria. In order to block cyanobacteria photosynthesis and therefore their growth, the reactor was covered with a piece of black tarpaulin from day 95.

3.6. Biodegradation of hydrocarbons

Fig. 7 shows the results of biodegradation of crude oil in the produced water during the BCO process at HRT of 58 h. The initial crude oil dissolved in the wastewater contained an important amount of *n*-alkanes in the range n-C18 to n-C24. The ratio of pristane/phytane was 1.16, which characterized the maturity of this crude oil. The oil in the wastewater was extensively biodegraded after treatment (Fig. 7). Almost all of GC-resolved compounds were removed, even the pristane. After degradation, squalane was the most dominant compound, indicating that strain W-5 cannot effectively degrade squalane. The remaining compounds were biomarkers of the hopane series, as well as resistant polycyclic alkanes and aromatics contained in the unresolved complex mixture (UCM). Bacteria degrade normal alkanes readily, whereas the isoprenoidal alkanes are relatively resistant to microbial degradation, resulting in an increase in the ratio of isoprenoidal alkanes with increasing levels of degradation, and therefore the formation of UCM.

Squalane is a multiply branched saturated hydrocarbon and much less susceptible to microbial oxidation. Many investigators have used squalane as the stable internal standard to assess the degree of oil degradation, both natural and following bioremediation [25,26]. In this study, using squalane as the stable internal standard, the biodegradation percentage of the oil was estimated to be 73% for total oil, and 86% for the GC-resolved compounds. This confirmed the high biodegradative capacity of strain W-5 in the oilfield produced water environment.

4. Conclusions

Laboratory experiments have been carried out to investigate the performance of BCO reactor using immobilized microorganisms on plastic packing carriers. It is found that strain W-5 is effective at treating oilfield wastewater. The reactor containing immobilized W-5 was well operated during the entire 150-day period. The BCO reactor achieved a mean COD removal efficiency of 76%, a mean TOC degradation efficiency of 70%, when the HRT was 24 h, and the volume loading was 80 mg TOC/L·d. Higher microorganisms such as ciliates developed in the reactor, which would improve the quality of the effluent by the removal of suspend bacteria, and did not act to decrease the purified water quality. The GC-MS data showed that almost all of GC-resolved compounds in the influent oilfield wastewater, including pristine, were degraded.

Abbreviations

BOD — Biochemical oxygen demand
CAS — Conventional activated sludge
COD — Chemical oxidation demand

DO — Dissolved oxygen
HRT — Hydraulic retention time
SM — Minimal medium
TOC — Total organic carbon
TPH — Total petroleum hydrocarbons
UCM — Unresolved complex mixture

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