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Performance and microbial diversity of a full-scale oilfield wastewater treatment plant

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ABSTRACT

In this study, the performance and microbial diversity of an oilfield wastewater treatment plant with an integrated anaerobic baffled reactor (ABR) and sequencing batch reactor (SBR) system were investigated. The analysis showed that the range of influent chemical oxygen demand and oil concentration was 215–731 and 9–52 mg/L, while the effluent concentration decreased to 30–87 and 2–8 mg/L after treatment, giving mean removal efficiencies of 88.5% and 85.0%, respectively. The removal efficiency of other indices in the wastewater (S^{2-}, total suspended solids, and NH_3–N) reached 99.0%, 94.0%, and 80.1%, respectively. The microbial community analysis showed that the dominant bacterial and fungal species identified in the systems are halotolerant, suggesting that the process performances stated in this study were made possible by the adaptation of halotolerant microorganisms. Moreover, the results indicated that bacterial genera including *Marinobacterium*, *Marinobacter*, *Thiomicrospira*, *Methylophaga*, and *Pseudomonas* were likely to be actively involved in the decomposition processes of oil pollutants in the system. Furthermore, fungal communities were evenly distributed, with *Ascomycota*, *Basidiomycota*, and *Zygomycota* as the main phyla. Among them, *Alternaria, Meyerozyma, Cryptococcus, Aspergillus, Candida, Stachybotrys, Fusarium, Blastobotrys, Mortierella, Rasamsonia,* and *Geminibasidium* were abundant in the ABR and SBR systems, and may involve in the degradation process.

Keywords: Oilfield wastewater; Integrated ABR–SBR system; Degradation performance; Illumina MiSeq; Bacterial diversity; Fungal diversity

1. Introduction

Oilfield wastewater, which is also known as produced water, is generated in association with the production of crude oil and accounts for the largest ratio of wastewater generated during crude oil production. The quality of oilfield wastewater varies considerably, but it is usually hypersaline and contains high concentrations of refractory organic pollutants, oilfield chemicals, suspended solids, and heavy metals; accordingly, it can cause considerable environmental impacts if discharged without effective treatment.

Many technologies have been developed for the treatment of oilfield wastewater, including membrane filtration, reverse osmosis, electrochemical oxidation, land disposal, and biological treatment [1,2]. Among these, biological

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treatment has been shown to be a cost-effective and environmentally friendly method that is compatible with existing plant facilities [3,4]. Additionally, oilfield wastewater contains high concentrations of salts; therefore, the utilization of halophilic microorganisms seems to be a reasonable approach for its treatment [5]. Halophilic organisms may be used in activated sludge for higher effectiveness [6], and sequencing batch reactors (SBRs) are usually applied for the treatment of hypersaline wastewater [5,7]. However, since most oilfield wastewater contains recalcitrant compounds such as halohydrocarbons, surfactants, and phenols, as well as high salt concentrations and low nutrients, it is difficult to treat such wastewater effectively using a single aerobic biological technology. In addition, some refractory organic compounds would remain unaffected during aerobic treatment, but can undergo reductive transformation under anaerobic conditions [8]. Therefore, to improve SBR effluent quality, an anaerobic baffled reactor (ABR) can be coupled to the SBR for pretreatment of oilfield wastewater.

Weizhou terminal treatment plant (Zhanjiang Branch, China National Offshore Oil Corporation), covers over 300,000 m^2, making it China's largest offshore oil and gas processing terminal. Electrolysis was initially employed for the treatment of oilfield wastewater generated by this site. However, as the oil production platforms increased, the amount of wastewater generated exceeded the capacity of this method. In addition, the electrolysis method has the disadvantages of high operating cost, high energy consumption, and toxic gas generation. Therefore, our research group conducted a pilot-scale study, after which a biological treatment plant with an integrated ABR and SBR system was established for the treatment of oilfield wastewater. Nevertheless, information describing the operation performance and microbial community structure in the system has not yet been presented. In addition, although there were many reports on the bacterial community structure in the full-scale municipal wastewater treatment plant, the study of microbial community diversity, especially both the bacterial and fungal diversity in the oilfield wastewater treatment plant was rare.

Therefore, this study was conducted to (1) evaluate the operation performance of the integrated ABR and SBR system during the treatment of oilfield wastewater; (2) investigate the microbial community diversity (bacterial/fungal) in the system and to understand the functional microbial (bacterial/fungal) populations in the ABR and SBR. The results of the present study will improve understanding of the integrated ABR and SBR system for the treatment of oilfield wastewater.

2. Materials and methods

2.1. Full-scale plant description

A biological wastewater treatment plant combined with an ABR–SBR system was established in 2006 to treat oilfield wastewater from the Weizhou Island Oil Production Plant (Zhanjiang Branch, China National Offshore Oil Corporation). A schematic diagram of the wastewater treatment system is presented in Fig. 1. The plant is located in southeastern Beihai, Guangxi Zhuang Autonomous Region, China. The oilfield wastewater was pretreated by an oil and grease trap (a horizontally oriented tank designed to trap oils and grease at source and prevent discharge to the subsequent treatment system), after which it was fed to an equalization tank with a capacity of 800 m^3. Next, the stabilized supernatant slurry was pumped into the ABR reactor (21.0 m × 16.6 m × 6.5 m) with retention time of 36 h for microbial anaerobic treatment to remove some organics and further enhance the biodegradability of the wastewater. The ABR reactor adopts multi-compartments structure and no special gas–solid–liquid separation system is employed. The effluent from the ABR was then passed into the SBR reactors (20.0 m × 12.0 m × 5.8 m, three reactors) for aerobic treatment. The effective volumes of the ABR and three SRBs were 1,500, 360, 360, and 360 m^3, respectively. The ABR works in continuous flow while the three SBRs work in sequence. There is small variation in the volume of the wastewater pumped into the system and the ABR usually operates in half load. All of the SBRs were operated according to the following strategies: filling (1.0 h), reaction (aeration, 8.0 h), settling (2.0 h), extraction (1.0 h), and idle, with total retention time of 12 h.

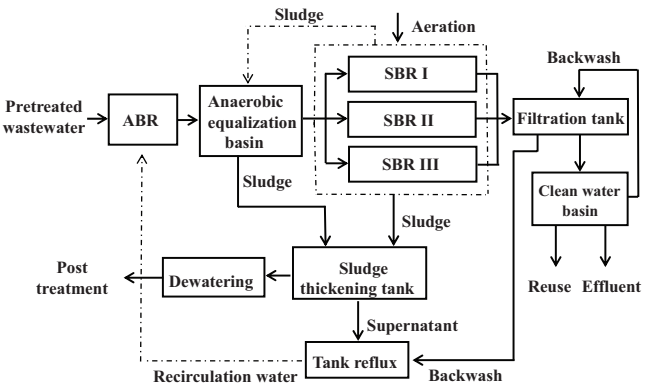

Fig. 1. Schematic diagram of the Weizhou oilfield wastewater treatment plant.

2.2. Characteristics of the oilfield wastewater and chemical analysis

Weizhou Island Oil Production Plant (China National Offshore Oil Corporation) is China's largest offshore oil and gas processing terminal, producing over 4,000 m^3 of oilfield wastewater daily. After primary treatment, around 1,000 m^3 of wastewater were distributed to the system for further treatment. The wastewater is grayish brown in color, and has a strong smell. The characteristics and analysis methods used to evaluate the oilfield wastewater are shown in Table 1. The temperature and pH of the influent wastewater were analyzed daily using a temperature meter (ME-200, China) and a pH meter (HACH model 53, USA). The concentrations of chemical oxygen demand (COD), biochemical oxygen demand within 5 d (BOD_5), ammonium nitrogen (NH_3–N), S^{2-}, Cl^-, total suspended solids (TSS), and total salinity were analyzed using the Chinese standard methods [9]. The oil content was measured by infrared spectrophotometry. The water samples (50 mL) were acidified first to pH < 2.0 using hydrochloric acid, and the organic substances were extracted from the aqueous phase using tetrachloromethane (10 mL) with the presence of 1.0 g NaCl.

A GC/MS analyzer (Agilent 7890A/5975C) equipped with a HP-5ms capillary column (30 m × 0.25 mm × 0.25 μm) was

Table 1
Characteristics of the oilfield wastewater and the analysis methods

Parameter (unit)	Value	Analysis method
pH	7.8–8.2	pH meter
Temperature (°C)	43–50	Temperature meter
Total salinity (g/L)	27.4–31.8	Weight method
CODcr (mg/L)	215–731	The rapid digestion spectrophotometry
Oil content (mg/L)	9–52	Infrared spectrophotometry
NH_3–N (mg/L)	11–13	Nessler's reagent colorimetry
TSS (mg/L)	540–710	Weight method
Cl^- (mg/L)	14,000–15,000	Silver nitrate titration
S^{2-} (mg/L)	11.8–20.1	Iodometric method

used to qualitatively analyze the composition of the organic constituents. The water samples (200 mL) were extracted twice using dichloromethane (50 mL). The extracts were evaporated to 1 mL before analysis. The sample loop volume was 1.0 μL. Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The oven temperature program was as follows: starting at 60°C for 2 min, increasing the temperature to 300°C at a rate of 5°C/min, and holding 300°C for 3 min. The temperature of GC injection and MS ion source was 280°C and 250°C, respectively. The MS was performed in scan mode with the electronic impact ionization energy of 70 eV, and scanning ranged from 50 to 400 *m*/*z*.

2.3. DNA extraction and pyrosequencing

Water samples from influent and effluent of the system, as well as sludge samples from the ABR and SBR reactors were taken in a sterile bottle and stored at 4°C for microbial experiments. Genomic DNA in these samples was extracted using an E.Z.N.A.® Water DNA Kit (Omega Bio-Tek, Norcross, USA) according to the manufacturer's instructions. The extracted nucleic acids were kept at –80°C until use.

Barcodes that allow sample multiplexing during pyrosequencing were incorporated into primers 515F (GTGCCAGCMGCCGCGG) and 907R (CCGTCAATTCMTTTRAGTTT) for bacterial 16S rRNA gene amplification, and ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGCGTTCTTCATCGATGC) for fungal ITS gene amplification. PCR amplification was performed using a GeneAmp PCR System® 9700 (Applied Biosystems, Foster City, CA, USA) with a total volume of 20 μL containing 4 μL of 5× FastPfu buffer, 2 μL of 2.5 mmol/L dNTPs, 0.8 μL each of 5 μmol/L primer, 0.4 μL of FastPfu polymerase (TransGen Biotech, China), and 10 ng of DNA template. Thermal cycling conditions for bacterial 16S rRNA gene sequences were initial denaturation at 95°C for 3 min, followed by 27 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, with a final extension at 72°C for 10 min. Thermal cycling conditions for fungal ITS gene sequences were initial denaturation at 95°C for 3 min, followed by 35 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, with a final extension at 72°C for 10 min. Following amplification, PCR products of the same sample were purified from agarose gels using an Axy Prep DNA Gel Purification Kit (Axygen Biotechnology, Taizhou, China), then quantified using the QuantiFluor™ system (Promega, Milano, Italy). Pyrosequencing was performed on an Illumina MiSeq platform by Majorbio BioPharm Technology Co., Ltd. (Shanghai, China).

2.4. Phylogenetic and statistical analyses

All sequence reads obtained from pyrosequencing were quality checked using the trimmomatic software [10]. Sequence adapters and any poor quality reads were removed. Good quality sequences were clustered into operational taxonomic units (OTUs) based on 0.97 (i.e., species level) sequence similarity thresholds with the Uclust algorithm [11]. Representative OTUs were selected based on the most abundant sequences in the samples and the Ribosomal Database Project classifier was used for taxonomic assignments [12]. Additionally, the shared OTUs were used to estimate similarity between communities based on membership and structure. The mothur software [13] was employed to generate rarefaction curves, construct distance matrices, and calculate richness estimators and diversity indexes, including the abundance-based coverage estimator (ACE), Chao1 richness estimator, and Shannon diversity index. Pyrosequencing data were deposited in the NCBI Sequence Read Archive under accession number SRP071730.

3. Results and discussion

3.1. Characteristics of influent and effluent of the biological wastewater treatment

Generally, the COD of the influent fluctuate between 150 and 730 mg/L, with total salinity of 27.4–31.8 g/L. The temperature was 50°C ± 3°C in the ABR and 46°C ± 2°C in the SBR, with a pH of 7.4 ± 0.2 in the ABR and 7.8 ± 0.2 in the SBR. The BOD_5/COD ratio of the influent was 0.29 ± 0.05, which was considered as slowly biodegradable. However, the single aerobic biochemical treatment was unable to effectively degrade COD as concluded from the 1 year operation using a single aerobic tank. Therefore, an ABR was constructed, and the integrated ABR–SBR system was used for the treatment of oilfield wastewater.

The organic constituents in oilfield wastewater, ABR effluent, and SBR effluent were analyzed by GC–MS. The organic pollutants belonged to three main categories: petroleum-based normal alkanes (C6–C34), aromatic hydrocarbons (toluene, xylene, styrene, benzaldehyde, phenol, and phthalates), polycyclic hydrocarbons (indene, azulene, and naphthalene), and others (nitriles, esters, alcohols, amines, heterocyclic matters, and others). The abundance of each category is shown in Table 2. The dominant pollutants in influent were *n*-alkanes, the content of which increased by ABR process. It is worthwhile to note that the number and abundance of some persistent organic pollutants such as aromatics and polycyclics decreased, while the content of *n*-alkanes increased by ABR process. This proved that ABR reactor could decompose the refractory organics in the oilfield

Table 2
Abundance of different hydrocarbon categories in the samples[a]

Sample	*n*-Alkanes (%)	Aromatics (%)	Polycyclics (%)	Others[b] (%)
Influent	63.3	15.8	13.4	7.5
ABR effluent	84.1	3.6	7.0	5.3
SBR effluent	–	21.3	13.5	65.2

[a]The presented percentages are calculated from peak area of each component in GC chromatograms.
[b]Other compounds include nitriles, esters, alcohols, amines, heterocyclic matters, and others.

wastewater at some extent. Most of the contaminants were degraded following the subsequent SBR process.

3.2. COD and oil pollutants removal in ABR

The oilfield wastewater treatment plant was run over a total period of 270 d under the operating conditions. The corresponding degradation efficiency of ABR for COD and oil pollutants is shown in Fig. 2. As shown in Fig. 2(A), the influent COD concentration ranged from 215 to 731 mg/L, while the influent oil content ranged from 9 to 52 mg/L throughout the year. Following anaerobic microbial degradation in the ABR reactor, the effluent COD concentration decreased to the range of 170–590 mg/L, with the lowest removal rate being 12.4% and the highest removal rate 35.9%. Meanwhile, the effluent oil content decreased to 5–27 mg/L, with the lowest removal rate being 37.0% and the highest removal rate 54.6%.

As shown in Fig. 2(A), the COD removal rate was basically positively correlated with influent COD load. Nevertheless, there was no obvious correlation between the oil removal rate and the oil load of the influent, with the average oil removal stabilized at 45.8% (Fig. 2(B)). The factors influencing COD removal in the ABR might be attributable to the influent hydraulic loading and temperature, as high hydraulic loading in the ABR could cause short flow phenomenon, resulting in loss of granular sludge and consequently influencing the degradation efficiency. Moreover, the high influent temperature would inhibit the enzyme activity of the anaerobic microorganisms and slow down the metabolic activity.

Overall, the major role of the ABR reactor was to increase the biodegradability of oilfield wastewater, facilitating the following aerobic treatment. The BOD_5/COD ratio increased from 0.29 ± 0.04 in influent to 0.43 ± 0.06 in ABR effluent, indicating that the biodegradability of the petrochemical wastewater was improved by ABR treatment. This suggested that the anaerobic microorganisms in the ABR were responsible for the preliminary, but crucial, treatment of refractory substances in the wastewater, such as the conversion of macromolecular substances into small molecules, which made it easier for further aerobic degradation in SBRs. Other studies also reported that anaerobic biological treatment facilitated the hydrolysis of complex organic matter, and some refractory organic compounds could only be transformed under anaerobic conditions [14–16].

3.3. COD and oil pollutants removal in SBR

The effluent from the ABR was evenly divided into three separate SBR reactors for further aerobic degradation, and

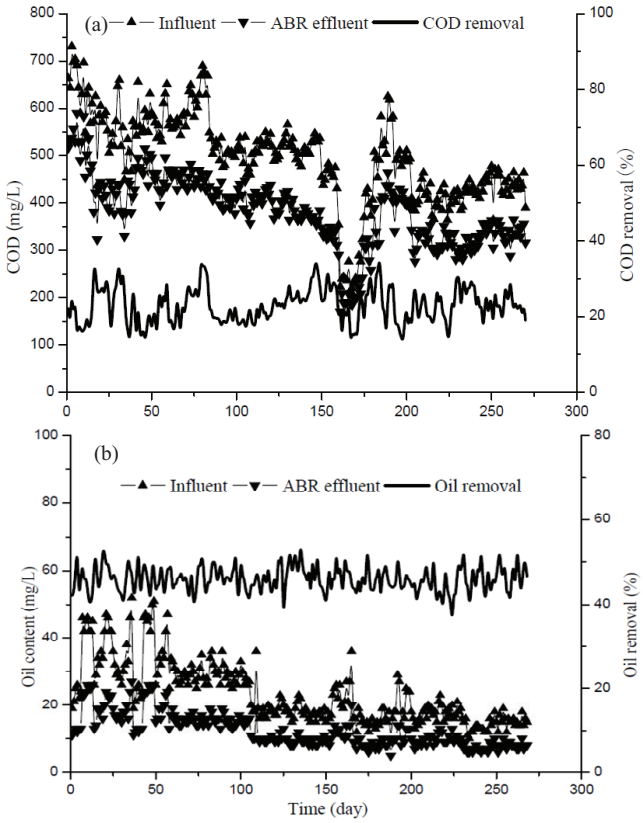

Fig. 2. Evolution of COD (A) and oil pollutants (B) in the influent (▲), effluent (▼), and corresponding removal (—) as a function of time during anaerobic wastewater treatment in the ABR.

the corresponding degradation efficiency of SBR for COD and oil pollutants are shown in Fig. 3. After aerobic treatment in the SBR, the effluent COD concentration was decreased to 30–87 mg/L, while the effluent oil content decreased to 2–8 mg/L throughout the year (Fig. 3(A)). Accordingly, the degradation efficiency reached as high as 80%–90% for COD and 60%–80% for oil content (Fig. 3(B)).

It was considered that the refractory pollutants in the wastewater were subjected to hydrolysis acidification by the anaerobic granular sludge in the ABR, which greatly improved the biodegradability of wastewater, thereby facilitating the subsequent aerobic degradation in the SBR. Overall, the integrated ABR–SBR system could effectively enhance the degradation efficiency for COD and oil pollutants, reaching a maximum removal rate of approximately 94% for COD, and 92% for oil pollutants.

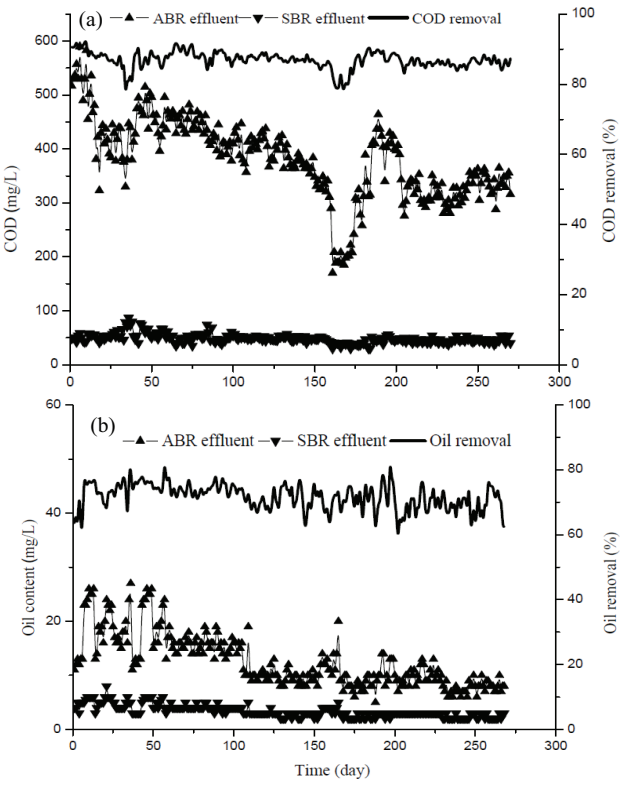

Fig. 3. Evolution of COD (A) and oil pollutants (B) in the influent (▲), effluent (▼), and corresponding removal (—) as a function of time during aerobic wastewater treatment in the SBR.

3.4. Removal of other pollutants by the integrated ABR–SBR system

Other pollutants including NH_3–N, S^{2-}, and TSS in the influent and effluent during the integrated ABR–SBR treatment were also monitored daily. The concentration of NH_3–N, S^{2-}, and TSS in the influent fluctuates in the range of 11–13, 11.8–20.1, and 540–710 mg/L, respectively. As shown in Fig. 4, the concentration of NH_3–N, S^{2-}, and TSS in the effluent decreased after treatment with the integrated ABR–SBR system.

The results showed that NH_3–N concentration in the SBR effluent was in the range of 041–4.32 mg/L, with an average concentration of 2.38 mg/L (Fig. 4). The total NH_3–N removal in the ABR–SBR system was around 80.1%, with an average influent concentration of 12.0 mg/L in the influent. For S^{2-}, the concentration in the SBR effluent was in the range of 0.11–0.46 mg/L, with an average concentration of 0.22 mg/L. The S^{2-} concentration was higher in the ABR effluent than in the influent, whereas it decreased to around 0.22 mg/L after SBR treatment (data not shown). These findings suggest that anaerobic microorganisms in the ABR converted organic sulfur compounds and SO_4^{2-} in the wastewater into S^{2-} as an intermediate product, which explains the relatively high concentration of S^{2-} in the ABR effluent. The generated S^{2-} was then oxidized in the subsequent SBR treatment. The TSS concentration in the effluent was 25–39 mg/L, and the maximum removal rate reached as high as 94% relative to the influent concentration in the range of 540–710 mg/L. Overall, these pollutants could be effectively removed by the ABR–SBR system.

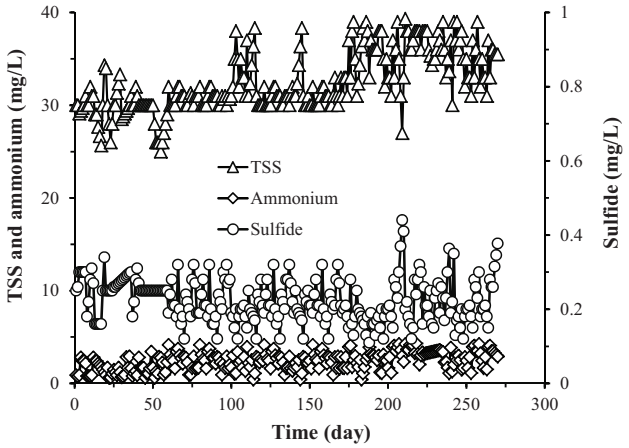

Fig. 4. Evolution of TSS (△), ammonium (◊), and sulfide (○) in the SBR effluent as a function of time.

3.5. Sequence diversity analysis

Pyrosequencing yielded a total of 183,129 high-quality sequences of the 16S rRNA gene and 192,541 high-quality sequences of the ITS gene for all samples (Table 3). Based on the clustering of these sequences at 97% gene similarity, 387 bacterial OTUs were found in the influent, while 259, 258, and 231 bacterial OTUs were found in the granular sludge of ABR, activated sludge of SBR, and the effluent, respectively (Table 3). Meanwhile, 211 fungal OTUs were found in the influent, while 268, 266, and 286 fungal OTUs were found in the granular sludge of the ABR, activated sludge of the SBR, and the effluent, respectively (Table 3). The bacterial OTUs richness in the influent was higher than that in the effluent, while fungal OTUs richness showed the opposite trend, as expressed by ACE and Chao1 indices. In addition, Shannon and Simpson analyses indicated that influent had the highest bacterial diversity and effluent had the lowest, while fungal diversity showed the opposite results.

3.6. Bacterial community and composition

The structure of the bacterial community at different taxonomic levels was provided for all samples. At the class level, clear differences in the bacterial community structure between influent and the other samples were observed (Table 4). The dominant bacteria in the influent include *Alphaproteobacteria* (30.17%), *Gammaproteobacteria* (25.27%), *Phycisphaerae* (13.43%), *Betaproteobacteria* (8.52%), and *Cytophagia* (4.31%), with other minor classes belonging to *Flavobacteria* (2.63%), *Actinobacteria* (2.48%), *Planctomycetacia* (2.42%), and *Sphingobacteriia* (1.65%). During treatment with the ABR–SBR system, most representative classes shifted to *Gammaproteobacteria* (54.74%–60.17%), *Sphingobacteriia* (17.98%–20.84%), *Alphaproteobacteria* (2.74%–6.19%), *Deltaproteobacteria* (5.11%–5.73%), and *Clostridia* (1.69%–5.73%), with other classes including *Betaproteobacteria* (0.78%–2.57%), *Synergistia* (1.40%–2.39%), *Thermotogae* (1.07%–2.47%), and *Spirochaetes* (0.51%–1.38%).

Table 3
Observed OTUs and α-diversity indexes of the microbial phylotypes in the treatment systems

Sample		Reads[a]	0.97					
			OTU[b]	ACE[c]	Chao1[d]	Coverage	Shannon	Simpson
Bacterial	Influent	45,583	387	392	390	0.999	3.65	0.0695
	ABR	38,118	259	301	293	0.998	2.91	0.108
	SBR	46,623	258	301	297	0.999	2.86	0.112
	Effluent	51,805	231	284	288	0.999	2.78	0.1224
Fungi	Influent	53,637	211	217	215	0.999	4.15	0.0315
	ABR	41,147	268	275	275	0.999	4.37	0.0258
	SBR	44,176	266	276	279	0.999	4.21	0.033
	Effluent	53,581	286	292	294	0.999	4.39	0.0261

[a]Trimmed reads that passed quality control.
[b]The operational taxonomic units (OTUs) were determined with a 3% width.
[c]ACE richness estimates.
[d]Chao1 richness estimates.

Table 4
Identified bacterial and fungal classes in the ABR–SBR system

Bacterial community composition (relative abundance)					Fungal community composition (relative abundance)				
Taxon	Influent (%)	ABR (%)	SBR (%)	Effluent (%)	Taxon	Influent (%)	ABR (%)	SBR (%)	Effluent (%)
Acidobacteria	0.79	0.00	0.01	0.02	*Agaricomycetes*	1.20	2.62	2.00	1.65
Actinobacteria	2.48	0.15	0.21	0.59	*Agaricostilbomycetes*	0.00	0.00	0.00	0.04
Alphaproteobacteria	30.17	5.88	6.19	2.74	*Archaeorhizomycetes*	0.00	0.10	0.00	0.00
Anaerolineae	0.93	0.03	0.03	0.03	*Ascomycota*	9.48	5.75	9.68	6.34
Bacteroidia	0.09	0.30	0.32	0.03	*Basidiomycota*	1.71	1.64	1.64	1.71
Betaproteobacteria	8.52	1.13	0.78	2.57	*Chytridiomycetes*	0.11	0.00	0.00	0.00
Chlorobia	1.11	0.02	0.06	0.00	*Cystobasidiomycetes*	0.00	0.01	0.04	0.20
Clostridia	0.29	2.11	1.69	5.73	*Dothideomycetes*	8.87	11.75	9.66	21.74
Cytophagia	4.31	0.00	0.00	0.01	*Eurotiomycetes*	11.49	8.61	14.13	10.48
Deferribacteres	0.00	0.45	0.45	2.06	*Exobasidiomycetes*	0.00	0.01	0.10	0.07
Deltaproteobacteria	0.58	5.11	5.63	5.73	*Leotiomycetes*	0.45	0.21	0.28	0.24
Flavobacteria	2.63	0.28	0.27	0.01	*Microbotryomycetes*	0.17	0.23	0.25	0.19
Gammaproteobacteria	25.27	60.17	56.91	54.74	*Orbiliomycetes*	0.00	0.14	0.00	0.00
Lentisphaeria	0.62	0.13	0.13	0.02	*Pezizomycetes*	6.15	6.16	1.70	5.35
Phycisphaerae	13.43	0.13	0.17	0.09	*Saccharomycetes*	12.72	12.01	19.07	10.25
Planctomycetacia	2.42	0.01	0.00	0.00	*Sordariomycetes*	18.60	20.42	22.69	20.67
Sphingobacteriia	1.65	17.98	20.84	20.14	*Tremellomycetes*	10.88	7.96	8.29	6.95
Spirochaetes	0.11	1.30	1.38	0.51	*Ustilaginomycetes*	0.00	0.08	0.02	0.00
Synergistia	0.16	2.39	2.00	1.40	*Wallemiomycetes*	3.42	4.87	2.29	3.31
Thermotogae	0.00	1.93	2.47	1.07	*Zygomycota*	2.61	5.82	1.34	3.66
Bacterial_others	4.44	0.50	0.46	2.51	Fungi_unclassified	12.12	11.60	6.82	7.16

At the genus level (Fig. 5), distinct differences in the bacterial community composition in the influent were observed relative to other samples. Over 60 minor genera were found exclusively in the influent, and were grouped together under the "Others" category. Other representative genera included unclassified *Rhodobacteraceae* (15.89%), *Pseudidiomarina* (13.30%), *Nitrosomonas* (7.63%), uncultured *Rhodothermaceae* (4.20%), *Marinobacter* (3.56%), *Mycobacterium* (2.17%), *Parvibaculum* (1.88%), *Owenweeksia* (1.86%), *Methylophaga* (1.81%), *Legionella* (1.59%), and *Planctomyces* (1.46%), most of which were only found in the influent. The ABR and SBR were dominated by *Marinobacterium* (24.99%, 19.76%), *Marinobacter* (16.36%, 15.60%), *Thiomicrospira* (7.59%, 12.71%), *Methylophaga* (4.52%, 3.62%), and *Pseudomonas* (4.03%, 3.27%), with other genera including *Desulfuromonas* (2.26%, 2.31%), *Roseovarius* (2.07%, 1.95%), *Desulfotignum* (1.36%, 1.69%), and *Spirochaeta* (1.27%, 1.36%). The effluent was mainly composed of *Halothiobacillus* (23.65%), *Marinobacterium*

(14.78%), *Desulfuromonas* (5.02%), *Pseudomonas* (4.78%), *Proteiniclasticum* (4.46%), *Marinobacter* (3.91%), *Thiomicrospira* (2.75%), *Pusillimonas* (2.43%), *Roseovarius* (2.26%), and *Methylophaga* (2.25%).

The integrated ABR–SBR system was constructed to treat the oilfield wastewater with low biodegradability. However, the anaerobic biological process is known to be inhibited by high salinity mainly due to the presence of cations [17]. In spite of this, a certain number of processes have been operated successfully for the anaerobic treatment of saline wastewater, some of which used a halophilic inoculum [18,19], whereas others required the adaptation of a non-halophilic inoculum to salt environment [20,21]. In this study, sewage sludge was used as inoculum, and the results in this study suggested that the adaptation of this non-saline sludge to high salinity was performed successfully. Further molecular analysis showed that the dominant microbial species identified in the system. For example, *Marinobacterium*, *Marinobacter*, *Thiomicrospira*, etc., are halotolerant, suggesting that the process performances stated in this study were made possible by the adaptation of halotolerant microorganisms.

The dominant bacterial communities in the ABR and SBR, *Marinobacterium*, *Marinobacter*, *Thiomicrospira*, *Methylophaga*, and *Pseudomonas* were also frequently detected in the oil reservoirs or in oil plume waters [22]. Among them, *Marinobacterium* and *Marinobacter* were first discovered in marine environment and could grow at the saline environment with NaCl concentration up to 75 mg/L. Together with *Pseudomonas*, they were characterized relative to their aliphatic and (polycyclic) aromatic hydrocarbon metabolizing ability [23,24]. Moreover, it has been reported that *Marinobacterium* functioned as nitrogen-fixing bacteria [25] and utilized dimethyl sulfide as the sulfur source [26], while *Marinobacter* consumed low carbon number *n*-alkanes and were associated with denitrification [27]. Moreover, *Thiomicrospira* were commonly classified as sulfide-oxidizing and denitrifying chemolithoautotrophs [28], while the methylotrophic *Methylophaga* was involved in the dissimilatory reduction of nitrate [29], suggesting that they were attributable to the sulfide and nitrate removal from wastewater in the system. Recent findings have also shown that *Methylophaga* were involved in the degradation of hydrocarbons [30]. In view of the above findings, it is believed that *Marinobacterium*, *Marinobacter*, *Thiomicrospira*, *Methylophaga*, and *Pseudomonas* are actively involved in the decomposition processes of the oilfield wastewater in the system.

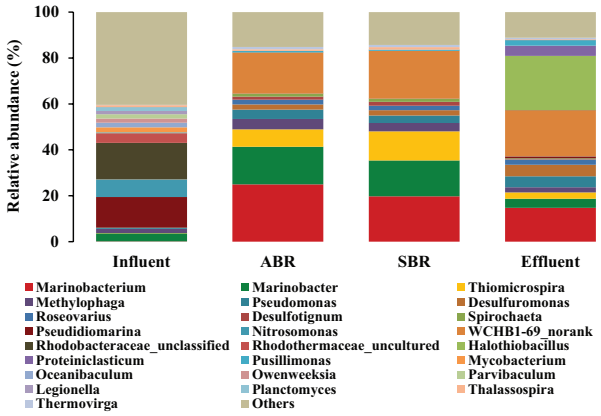

Fig. 5. Relative abundance of bacterial OTUs represented by their taxonomic group at the genus level for the samples. The 25 most abundant OTUs for all samples combined are listed, whereas all other OTUs were combined and shown as "Others".

3.7. Fungal community and composition

The structure of the fungal community at different taxonomic levels was provided for all samples. At the class level, the representative groups were consistent among all the samples (Table 4). Generally, all samples were dominated by *Sordariomycetes* (18.60%–22.69%), *Dothideomycetes* (8.87%–21.74%), *Saccharomycetes* (10.25%–19.07%), *Eurotiomycetes* (8.61%–14.13%), *Tremellomycetes* (6.95%–10.88%), *Ascomycota* (5.75%–9.48%), and *Pezizomycetes* (1.70%–6.16%), with other minor classes including *Wallemiomycetes* (2.29%–4.87%), *Zygomycota* (1.34%–5.82%), *Agaricomycetes* (1.20%–2.62%), and *Basidiomycota* (1.64%–1.71%).

At the genus level (Fig. 6), the majority of fungal groups were evenly distributed among samples. Generally, the samples were abundant with *Stachybotrys* (2.18%–7.61%), *Alternaria* (3.86%–8.97%), *Cryptococcus* (4.28%–7.50%), *Aspergillus* (2.67%–7.49%), *Candida* (4.23%–7.12%), *Fusarium* (3.47%–5.20%), unclassified *Ascomycota* (5.74%–9.45%), unclassified *Sordariomycetes* (3.85%–6.60%), and other genera such as *Rasamsonia* (1.84%–3.35%), *Mortierella* (1.34%–5.82%), *Meyerozyma* (1.24%–10.27%), *Blastobotrys* (1.02%–3.41%), and *Geminibasidium* (1.74%–4.42%) were also detected.

In general, fungi have stronger hydrocarbon-degrading abilities than bacteria [31]. In this study, the fungal genera dominant in the ABR and SBR system included *Stachybotrys*, *Alternaria*, *Cryptococcus*, *Aspergillus*, *Candida*, *Fusarium*, *Blastobotrys*, *Meyerozyma*, *Mortierella*, *Rasamsonia*, and *Geminibasidium*. Among them, *Blastobotrys*, *Meyerozyma*,

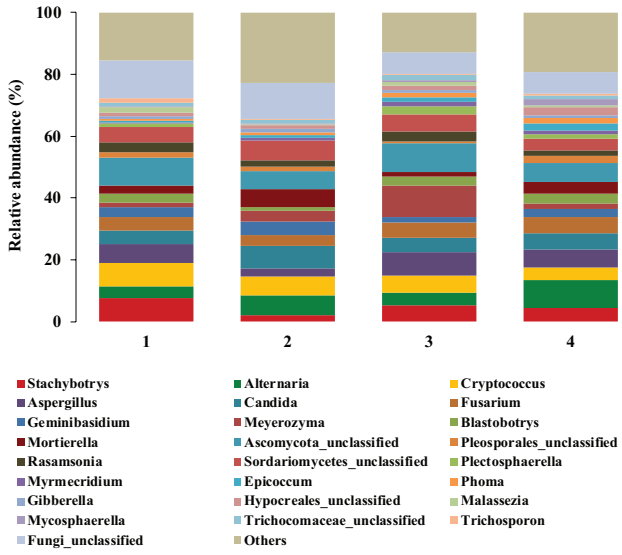

Fig. 6. Relative abundance of fungal OTUs represented by their taxonomic group at the genus level for the samples. The 25 most abundant OTUs for all samples combined are listed, whereas all other OTUs were combined and shown as "Others".

and *Cryptococcus* belong to the yeast phylum. Yeast have been shown to assimilate a variety of carbon compounds, including adenine, aliphatic amines, diamines and hydroxyamines, phenolics and other benzene compounds, and polysaccharides [32,33]. Moreover, degradation of crude oil by *Aspergillus* has been reported, especially the metabolism of polycyclic aromatic hydrocarbons [34]. It has been suggested that the extracellular enzymes from *Aspergillus* spp. were efficient at degrading crude oil, especially the cytochrome P-450 monooxygenase enzyme systems. In addition, *Fusarium* has been reported to degrade the aliphatic fraction of all crude oils at high concentrations [35], while *Mortierella* were shown to be involved in the degradation of herbicides [36] and *Alternaria* were involved in the degradation of ether-type polyurethane [37]. However, there have been few investigations of the degradation activity of hydrocarbons or crude oil by *Rasamsonia* or *Geminibasidium*.

4. Conclusions

The performance and microbial community structure of an oilfield wastewater treatment plant with integrated ABR–SBR treatment processes were investigated. The results indicated that the combined biological process could tolerate the high temperature and saline condition of the oilfield wastewater, and exhibits effective and stable degradation performance. In addition, the microbial community composition in the system was investigated. Bacterial genera including *Marinobacterium*, *Marinobacter*, *Thiomicrospira*, *Methylophaga*, as well as *Pseudomonas* were likely to be actively involved in the decomposition processes of the oilfield wastewater in the system, while fungal genera including *Blastobotrys*, *Meyerozyma*, *Cryptococcus*, *Aspergillus*, *Alternaria*, *Fusarium*, and *Mortierella* functioned as potential responsible degraders. Lastly, it is worthy of note that the dominant bacterial and fungal species identified in the ABR and SBR systems, for example, *Marinobacterium*, *Marinobacter*, *Thiomicrospira*, *Alternaria*, and *Aspergillus*, are halotolerant, suggesting that the process performances stated in this study were made possible by the adaptation of halotolerant microorganisms.

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