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Biological treatment of a saline and recalcitrant petrochemical wastewater by using a newly isolated halo-tolerant bacterial consortium in MBBR

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ABSTRACT

A halo-tolerant bacterial consortium comprised of *Pseudomonas pseudoalcaligenes strain R1*, *Bacillus subtilis* subsp. *inaquosorum R2* and *Shewanella chilikensis strain AM1* were isolated and used as inoculums in a moving bed bioreactor for treatment of a saline petrochemical wastewater. Observations demonstrated the halo-tolerant capability of isolated strains up to around 3.2%. The influence of varying organic loading rates and TDS concentrations were evaluated on bioreactor efficiency and biokinetic coefficients. A COD removal of 77% was observed for organic loading rate of less than 2.7 kg COD m^{-3} d^{-1} and TDS concentrations of 25,000 and 30,000 mg L^{-1}. Growth yield (Y) varied from 0.178 to 0.129 mg VSS mg COD^{-1} in different TDS concentrations. Results indicated that the biokinetic coefficients were in the range close to typical ranges reported for similar industrial wastewaters, except that of the half saturation constant (K_s).

Keywords: Biokinetic coefficient; Halo-tolerant bacteria; Moving bed bioreactor; Petrochemical wastewater treatment; Saline wastewater

1. Introduction

Petrochemical industries produce a complex wastewater containing high chemical oxygen demand (COD), total dissolved solids (TDS), heavy metals, phenolic compounds and so on which are highly toxic and biologically recalcitrant [1,2]. Among various treatment processes, biological technologies are known as the most extensively applied approaches for treatment of petrochemical wastewater, because of their cost-effectiveness, ease of operation, good performance and simplicity [3,4]. Biofilm-based processes have shown an appropriate performance for the removal of organics and the other toxic substances found in wastewater. In biofilm based reactors some of the limitations of activated sludge process including large reactor size, need to secondary settling tank and biomass recycling are avoided [5–7]. The moving bed biofilm reactor (MBBR) is an attached growth approach which has successfully been applied in different urban and industrial wastewater treatment plants [8–11]. In MBBR, the carriers with efficient surface area are continuously kept in

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the aeration tank and move freely in the reactor [5,12]. Freely moving carrier media have lots of advantages such as better oxygen transfer, application of higher organic loading rates (OLRs), a shorter hydraulic retention time (HRT), and resistance to hydraulic and toxic shocks [13–15]. Application of MBBR technology has been studied for treatment of mature landfill leachate [16], pharmaceutical and hospital wastewater [17,18], nutrients [19], dye-contained wastewater [20], aniline wastewater [8], biodegradation of natural and synthetic estrogens [21], municipal wastewater [22] and nitrification of industrial and domestic saline wastewater [23,24]. There are two problems in treatment of saline petrochemical wastewater: (i) salinity and (ii) the recalcitrant nature of organic matter present in wastewater. Direct biological treatment of the saline wastewater (usually considered as those having salt concentration of more than 20–30 g L^{-1}) is inefficient, due to the harmful influence of salinity on biomass [25,26]. High salinity disorders the microbial metabolism, poor settling of the activated sludge, cell plasmolysis, decreasing biomass respiration rates, etc. [23,27]. Moreover, salt tends to increase the suspended solids in effluents due to lysis of biomass [28]. Therefore, biodegradation of high-saline wastewater is limited, due to the requirement for high salinity tolerance. A possible approach for hyper-saline wastewater treatment is isolation of halo-tolerant bacterial strains with capability of degrading recalcitrant organics [29,30]. The main characteristic of these microorganisms is their ability to live in high salt concentrations, ranging between 3% and 6% NaCl [31]. In our previous study, a salt tolerant consortium was isolated and used as inoculums in a suspended growth activated sludge system for treatment of a saline wastewater [32]. For improving the obtained results, the application of a biofilm-based system comprised of halo-tolerant strains was studied in the present work as a first report. The main purpose of the current research was to evaluate the possibility of isolation of halo-tolerant bacterial strains to provide a bacterial consortium in an MBBR for treatment of a real saline petrochemical wastewater.

2. Materials and methods

2.1. Experimental setup

In this study, a bench-scale Plexiglas bioreactor with total operating volume of 7 L (16 cm in diameter and 35 cm in height) and filling grade of 50% with carriers (type: 2H-BCN 014 KLL, material: HDPE, surface area: 767 m^2 m^{-3} and mass of 1 m^3: 151 kg) was used for performing experiments. The contents of bioreactor were aerated and mixed by means of three aerator pumps with an injection rate of 4–6 L_{air} min^{-1}. A peristaltic pump with adjustable flow rate of 2–6 L h^{-1} was used for continuous injection of influent wastewater. Fig. 1 illustrates the schematic diagram of lab-scale MBBR.

2.2. Isolation of halo-tolerant bacteria

The isolation and enrichment of halo-tolerant bacteria were performed based on method described in our previous study [30]. High saline petrochemical wastewater was used as a source of halo-tolerant bacterial strains. The enrichment medium contained (g L^{-1}) K_2HPO_4, 6.3; KH_2PO_4, 1.8; NH_4Cl, 1; $MgSO_4{\cdot}7H_2O$, 0.1; $CaCl_2{\cdot}H_2O$, 0.1; $FeSO_4{\cdot}7H_2O$, 0.1; $MnSO_4{\cdot}H_2O$, 0.1 and 1 mL L^{-1} of trace elements solution. The trace elements solution contained (g L^{-1}) H_3BO_3, 0.03; $ZnSO_4{\cdot}7H_2O$, 0.01; $CoCl_2{\cdot}6H_2O$, 0.02; Na_2MoO_4, 0.006; $CuSO_4{\cdot}2H_2O$, 0.001. Determined amounts of NaCl were added to the enrichment medium for achieving the required salt concentrations and pH was also set at 7.0. All of the culture media were sterilized by autoclaving. The enrichment medium was supplemented with 5 mL saline wastewater as the carbon and energy source. Experimental flasks were incubated at 31°C in a shaker incubator (Model: IKM 4000, Germany) at 180 rpm during 1 week. Afterwards, 5 mL of enrichment culture was added into another 250 mL flask with 95 mL fresh saline wastewater + enrichment medium [33]. This procedure was repeated eight times. Serial dilution technique was adopted and the separated colonies (>10^{-4} dilution) were isolated in nutrient agar + solid NaCl (Merck, Germany)

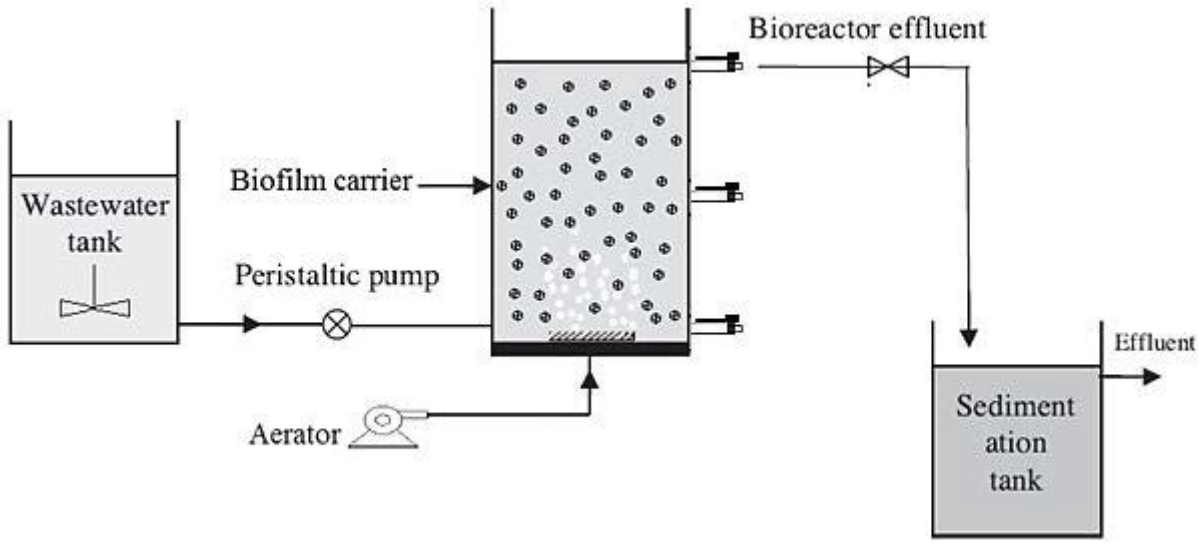

Fig. 1. Schematic of lab-scale MBBR.

plates (varying salt concentrations from 0.5 to 2.5 M NaCl, covering all moderate salt tolerant ranges) and incubated at 31°C for 24 h. Based on the salt tolerance limit and adaptation time, three potent moderate halo-tolerant bacterial strains were screened. Isolated halo-tolerant strains were maintained on nutrient agar slant contained 0.5 M NaCl at 4°C.

2.3. Identification of halo-tolerant strains

The extraction of the genomic bacterial DNA in order to identify the halo-tolerant bacteria was performed by boiling method [34]. Furthermore, the following universal primers were applied for amplification and sequencing the 16S rRNA gene: fD1 (5′-AGA GTT TGA TCC TGG CTC AG-3′) and rD1 (5′-AAG GAG GTG ATC CAG CC-3′) [25]. Each reaction was run with 50 μL mix using i-Taq Maxime PCR Premix (iNtRON Biotechnology, Korea). The thermal cycling protocol began with a denaturing step of 95°C for 300 s, then 35 cycles at 95°C 30 s, 52°C 30 s, 72°C 90 s, and finished with a final extension of 72°C for 900 s [32]. In addition, PCR products were sequenced by the Sanger dideoxynucleotide method using a 3730XL DNA analyzer instrument (Applied Biosystems, USA) under contract by Bioneer Inc., (South Korea). Sequence reads were edited and assembled a using DNA sequence assembler v4 (2013). The sequence data were analyzed by BLASTn from NCBI (http://www.ncbi.nlm.nih.gov) and classified by means of EzTaxon server (http://ezbiocloud.net/eztaxon) [35]. Evolutionary analysis was conducted in MEGA6 by maximum likelihood algorithm using Kimura 2-parameter distances [36] and 1,000-bootstrap replication.

2.4. MBBR start-up

Isolated halo-tolerant strains were transferred to 1-L flasks containing nutrient broth and incubated in a shaker incubator (Model: IKM 4000, Germany) at 180 rpm and 31°C to growth sufficiently ($OD_{600\ nm}$ = 2) as initial seed for MBBR. Thereafter, 90% of MBBR was filled with liquid culture and the remaining with raw saline wastewater. Nitrogen and phosphorus were provided by adding determined quantities of NH_4Cl and K_2HPO_4/KH_2PO_4, respectively, for obtaining the C:N:P ratio of 100:5:1. The reactor was first operated in batch mode for around 3 months until the biofilm coverage on carriers was completed. DO was adjusted to 3–6 mg L^{-1}. In startup period, the aerators were switched off, 2 L of supernatant was decanted and replaced with fresh raw saline wastewater after settlement of biomass, each day. The proportion of influent wastewater was increased gradually and reached to 5.5 L, during 3 months. Enhancement of COD removal was considered as a criterion for increasing the influent wastewater proportion [37,38]. The characteristics of raw wastewater are presented in Table 1.

Table 1
Raw saline petrochemical wastewater

Parameter	Range	Average
COD (mg L^{-1})	1,088–1,850	1,322 ± 289
TDS (mg L^{-1})	16,000–25,000	22,340 ± 2,170
BOD_5 (mg L^{-1})	87–185	110 ± 23
TOC (mg L^{-1})	598–1,164	680 ± 118
TSS (mg L^{-1})	300–500	385 ± 70
BOD_5/COD	0.08–0.1	
pH	8.5–9.5	

Microscopic observations using the scanning electron microscopy (SEM) (Fig. 2) were carried out for evaluation of the surfaces of the carriers either with or without biofilm. The small pieces of carrier media were collected from the reactor at the first day of bioreactor inoculation and at the end of start-up phase (after 90 d). Microscopic observations analysis revealed the formation and growth of a thick biofilm layer onto the surfaces of carrier media indicating a good acclimation procedure.

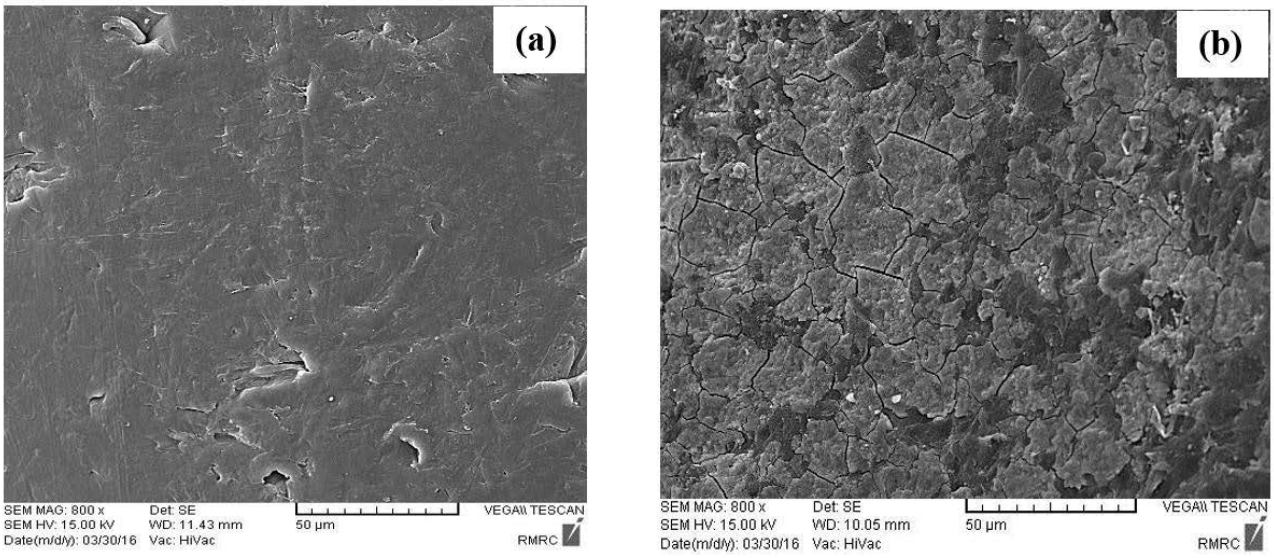

Fig. 2. Scanning electron microscopy (SEM) image of MBBR carrier before (a) and after (b) biofilm growth.

2.5. Experimental procedure

After obtaining the desired results in terms of biofilm growth in batch-mode operation, the process was divided into six sequencing operational runs. OLR was increased through depletion of HRT from 72 h in the first run to 4 h in the sixth run. The operational conditions are presented in Table 2. Upon obtaining steady-state conditions in the first step with naturally TDS concentration wastewater (25,000 mg L^{-1}), the effect higher salinities of 30,000 and 35,000 mg L^{-1}, based on TDS content were investigated. In the present study, the steady-state conditions were defined as no significant changes in the effluent characteristics, in terms of COD at 7–10 d of continuous operation.

2.6. Biokinetic coefficients determination

Kinetic parameters in biological treatment systems are used to carry out kinetic modelling, which is an important tool for prediction of system performance and design of the biological process. Monod model is accepted as a common and widely applied model to determine the biokinetic coefficients in activated sludge processes. The specific growth rate (μ) can be defined using Eq. (1) [39] as follows:

$$\mu = \mu_{\max}\left(\frac{S}{K_s - S}\right) \tag{1}$$

where μ_{max} is the maximum specific growth rate (d^{-1}), S is the substrate concentration (mg L^{-1}) and K_s is the half saturation constant (mg L^{-1}).

Critical kinetic coefficients half saturation constant (K_s), overall reaction rate (k), biomass yield (Y) and biomass decay coefficient (k_d) were calculated by integration of Monod equation and mass balance equations as follows in Eq. (2):

$$V\left(\frac{dX}{dt}\right) = \mu XV - k_d XV - Q_w X \tag{2}$$

When steady-state conditions are attained, Eq. (2) becomes:

$$\mu = k_d + \left(\frac{Q_w}{V}\right) \tag{3}$$

The solid retention time (SRT) is defined using Eq. (4) and then Eq. (5) can be attained by substituting Eq. (4) into Eq. (3):

$$\mathrm{SRT} = \frac{VX}{Q_w X} = \frac{V}{Q_w} \tag{4}$$

$$\mu = k_d + \left(\frac{1}{\mathrm{SRT}}\right) \tag{5}$$

Then Eq. (6) is obtained with substituting the value of μ from Eq. (5) into Eq. (1) as follows:

$$S = \frac{K_s\left(\frac{1}{\mathrm{SRT}} + k_d\right)}{\mu_m - \left(\frac{1}{\mathrm{SRT}} + k_d\right)} \tag{6}$$

The biokinetic coefficients, μ_m and K_s, were calculated by linear regression of SRT/[1 + (SRT k_d)] vs. 1/S, rearranging Eq. (6), yields Eq. (7):

$$\frac{\mathrm{SRT}}{1 + (\mathrm{SRT}\, k_d)} = \frac{K_s}{\mu_m}\left(\frac{1}{S}\right) + \frac{1}{\mu_m} \tag{7}$$

The substrate mass balance also can be written as Eq. (8):

$$V\frac{d_s}{d_t} = QS_0 - \mu\frac{XV}{Y} - S(Q - Q_w) - Q_w S \tag{8}$$

Under steady-state conditions, Eq. (8) becomes:

$$\frac{Q}{V}(S_0 - S) = \mu\frac{X}{Y} \tag{9}$$

The biomass concentration can be expressed as Eq. (10). Substituting Eq. (5) into Eq. (9) yields:

$$X = Y\frac{Q}{V}\frac{S_0 - S}{k_d + \frac{1}{\mathrm{SRT}}} \tag{10}$$

Table 2
Operational conditions of MBBR in continuous flow experiments

Flow rate (L d^{-1})	HRT (d)	TDS: 25,000 mg L^{-1}		TDS: 30,000 mg L^{-1}		TDS: 35,000 mg L^{-1}	
		OLR (kg COD m^{-3} d^{-1})	COD_{in} (mg L^{-1})	OLR (kg COD m^{-3} d^{-1})	COD_{in} (mg L^{-1})	OLR (kg COD m^{-3} d^{-1})	COD_{in} (mg L^{-1})
2.4	3	0.46	1,421 ± 198	0.48	1,450 ± 214	0.455	1,340 ± 148
3.6	2	0.74	1,421 ± 118	0.72	1,450 ± 56	0.688	1,340 ± 178
6.96	1	1.37	1,421 ± 90	1.392	1,450 ± 126	1.292	1,340 ± 94
13.92	0.5	3.05	1,421 ± 76	2.784	1,450 ± 252	2.585	1,340 ± 38
21.12	0.33	3.46	1,421 ± 142	4.224	1,450 ± 114	3.922	1,340 ± 135
42	0.16	8.57	1,421 ± 90	8.4	1,450 ± 82	7.8	1,340 ± 174

Then, k_d and Y were subsequently obtained by linear regression of Q (S_0–S)/VX vs. 1/SRT by rearranging Eq. (10) as follows to yield Eq. (11):

$$\frac{Q}{VX}\left(S_0 - S\right) = \frac{1}{Y}\frac{1}{\mathrm{SRT}} + \frac{k_d}{Y} \tag{11}$$

2.7. Analytical methods

COD, biochemical oxygen demand (BOD_5), TSS and mixed liquor volatile suspended solids were determined, based on the Standard Methods for Examination in Water and Wastewater [40]. Total organic carbon (TOC) analyser instrument (Shimadzu, TOC-VCSH, Japan) was applied for measuring TOC. DO was monitored by a portable DO meter (Hach Company, USA). Furthermore, pH values were measured frequently by a digital pH meter (Hach Company, USA) and were also adjusted with sodium bicarbonate solution, if necessary. TDS and electrical conductivity (EC) were also determined via a digital portable EC meter (Hach, USA). The organic matter compositions in the influent wastewater were detected by GC equipped with mass spectrometer (GC/MS) analyzer (Model: Agilent 7890, USA) with HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm film thickness, 5% phenyl- 95% methyl siloxane phase). Field-emission scanning electron microscopy (FESEM) by means of a TESCAN microscope (Mira 3, Czech Republic) was also employed for observing biofilm formation on carrier surfaces. The experimental results were reported as an average of three replicates. In addition, atomic force microscopy (AFM) (NanoWizard II, Germany) was applied to take some images of pure salt-tolerant bacteria. Ultimately, the removal efficiency (R%) was calculated based on COD concentration via Eq. (12):

$$R\left(\%\right) = \left(\frac{C_0 - C_t}{C_0}\right) \times 100 \tag{12}$$

where C_0 and C_t are the initial COD concentration (mg L^{-1}) and COD concentration (mg L^{-1}) at specified time periods, respectively. The biofilm mass was calculated via 100 biofilm carrier that was sampled randomly from MBBR. The carriers were separated from MBBR and dried until constant weight. The dried samples were weighted for determination of the constant weight in an oven at 103 total mass (M_{total}) composed of carrier element mass (M_{media}) and the attached biomass. The biomass was then washed off, the clean carriers were weighted, and the amount of biofilm attached to the 100 media elements was calculated using Eq. (13). The biomass quantity in the reactor was subsequently obtained as the overall carrier elements in the reactor with 50% filing grade was determined [38].

$$\mathrm{Biofilm}_{100} = M_{\mathrm{total}} - M_{\mathrm{media}} \tag{13}$$

3. Results and discussion

3.1. Raw wastewater characteristics

The real saline petrochemical wastewater characteristics are reported in Table 3. The low BOD_5/COD range value of 0.08–0.1 and TDS value of greater than 16,000 mg L^{-1} showed the non-biodegradable nature of studied wastewater and also confirmed that the conventional biological treatment processes are not suitable for this type of wastewater. The dominant organic substances of the studied wastewater, based on GC-MS analysis, are presented in Fig. 3.

3.2. Identification and characterization of halo-tolerant bacteria

As previously mentioned, according to salt tolerance limit and the adaptation time, three potent moderate halo-tolerant bacterial strains were screened. Based on the phylogenetic analysis of 16SrRNA gene sequence, these isolates were identified as *Pseudomonas pseudoalcaligenes strain R1*, *Bacillus subtilis subsp. inaquosorum R2*, and *Shewanella chilikensis strain AM1* (Fig. 4). The GenBank accession numbers for the sequences reported in this paper are KY629003-5. The summary characteristics of isolated halo-tolerant strains are reported in Table 3 and AFM images of pure cultures can be found in Fig. 5.

3.3. Effect of salinity on MBBR performance

The MBBR reactor was monitored during 84 d after 3 months of startup period in order to complete the biofilm growth on the carriers and also obtaining sufficient bacterial mass in suspended growth portion. Based on the variations of HRT, the desired values of OLRs were adjusted and COD removal efficiencies were monitored (Table 4). A COD removal of 16%–81% was observed for OLRs values in the range of 8.571–0.461 kg COD m^{-3} d^{-1}, TDS concentration of 25,000 mg L^{-1} and influent COD value of 1,421 mg L^{-1}. A high COD removal of around 80% was obtained for HRT of 12 h (OLR of 3.05 kg COD m^{-3} d^{-1}), indicating the extensive capability of enriched bacterial consortium. Also, the comparison of the bacterial mass in runs 1–4 showed that an enhancement in OLR along with high COD removal has led to increasing the biomass. Therefore, the overall pollutant mass removal has been increased due to higher biofilm

Table 3
Characteristics of isolated halo-tolerant bacterial strains

Bacterial strain	Shape	Gram staining	Mobility	Fluorescence	Spore forming	Salinity threshold (%)
Pseudomonas pseudoalcaligenes strain R1	Rod	Negative	Motile	No	No	3.4
Bacillus subtilis subsp. *inaquosorum R2*	Rod	Positive	Motile	No	Yes	3.2
Shewanella chilikensis strain AM1	Rod	Negative	Motile	No	No	3.5

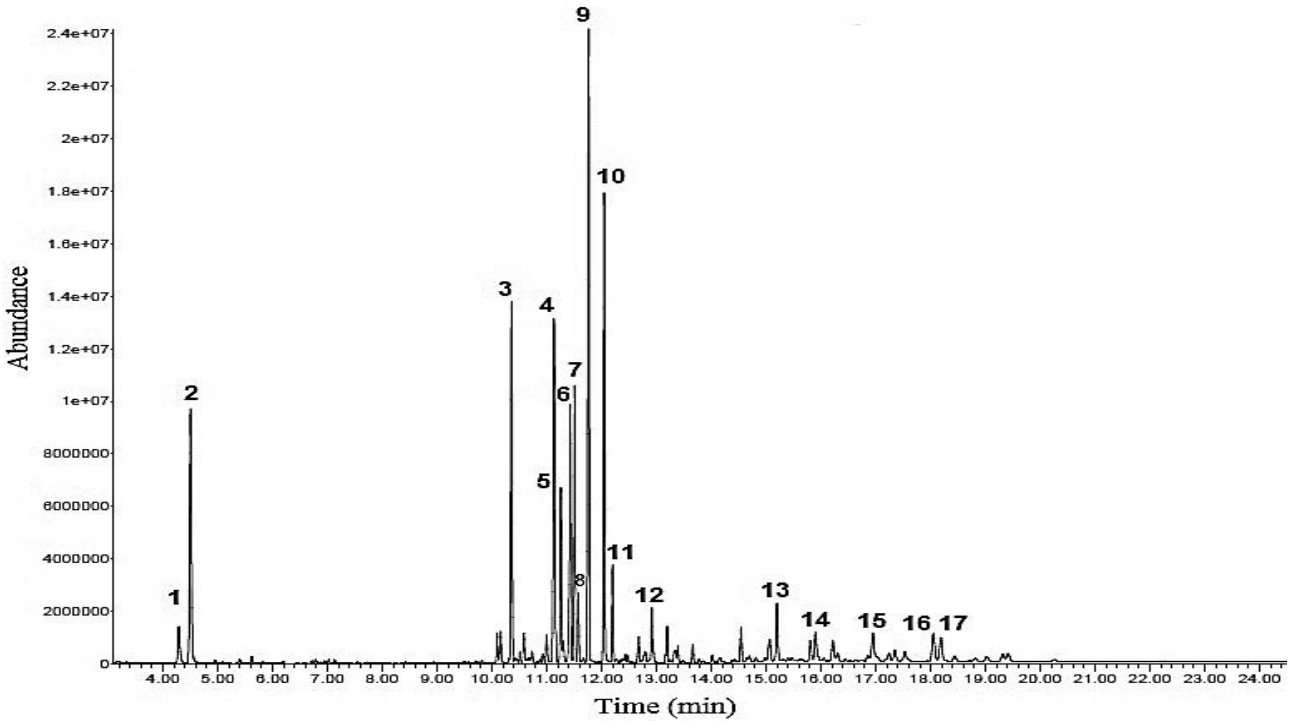

Fig. 3. GC-MS analysis of raw petrochemical wastewater. (1): 1-cyano-3-methyl-1,3-cyclohexadiene, (2): 2,5-Dimethyl-1,3-cyclopentadiene-5-carbonitrile, (3): o-Terphenyl, (4): p-Dicyclohexylbenzene, (5): 1-phenyl-3-phenylthio-butane, (6): 5-(4-Tolyl)dipyrromethane, (7): p-Dicyclohexylbenzene, (8): 9-(Methoxycarbonyl) phenanthrene, (9): 1,5-Bis(2,4-cyclopentadien-1-ylidene) cyclooctane, (10): 9,10-Anthracenedione, (11): p-Terphenyl, (12): Methylbis(phenylmethyl)benzene, (13): 1,1′:2′,1′′-Terphenyl, 4′-phenyl, (14): m-Terphenyl, 4′-phenyl, (15): 12,13-dimethoxypodocarpa-8,11,13-trien-19-oic acid, (16): Eicosanoic acid, (17): 1-(p Methoxyphenyl)-4-(2-imidazolinyl)-5-(phenyl)-1,2,3-triazole.

growth [37]. A similar trend was observed in the next step with TDS concentration of 30,000 mg L^{-1}. A COD removal of 18%–80% was observed for OLR values between 8.4 and 0.48 kg COD m^{-3} d^{-1}. The quantitative analysis of bacterial consortium and visual observations revealed favourable conditions till HRT of 12 h and thereafter a gradual decrease in VSS occurred which in turn led to decrease in COD removal efficiency. Increasing the salinity content over 30,000 mg L^{-1} adversely affected the MBBR performance and halo-tolerant biomass decreased significantly. Based on the obtained results in TDS concentration of 35,000 mg L^{-1}, COD removal values of 55.9%–8% were observed in the OLR range of 0.44–7.8 kg COD m^{-3} d^{-1}, along with the least biomass concentration of approximately 800 mg L^{-1} in the operational runs of 17 and 18. By decreasing the HRT to 8 and 4 h (OLR of 4.36 and 8.57 kg COD m^{-3} d^{-1} in runs 5 and 6, respectively), removal decreased significantly and reached 26% in run 6. This observation can probably be derived from the adverse effects of higher OLRs of greater than 3.05 kg COD m^{-3} d^{-1} on metabolic activity of microbial mass, insufficient contact time with biomass as a result of short HRT and increasing the wash out rate of biomass [38]. Results indicated the negative influences of higher salinity on MBBR performance. As the salinity increased, biomass was declined because of the harmful effects of high salinity on enzymatic function of the cells and occurrence of plasmolyzation [41]. This could be verified by appearance of the turbidity and TSS in the effluent which was attributed to the entrance of death cells into the effluent. On the other hands, increasing the OLR decreased the COD removal, due to high applied load. Salinity and low BOD_5/COD ratio were considered as the challenging issues of the studied wastewater and, isolated halo-tolerant strains demonstrated acceptable capabilities in overcoming such conditions [32,33]. These results are in a good agreement with literature. In a similar study by Lefebvre et al. [42] on treatment of tannery soak liquor containing 34 g NaCl L^{-1}, a COD removal of 95% was observed using halo-tolerant bacterial consortium in a sequencing batch reactor. Sharghi and Bonakdarpour [25] studied the treatment of hyper-saline water (NaCl content of 100–250 g L^{-1}) in an MBR inoculated with moderately halo-tolerant bacterial consortium and found the good organic and turbidity removal with no associated membrane fouling. Nakhli et al. [43] used a 10-L MBBR inoculated with a mixed culture of active biomass gradually acclimated to phenol and salt and concluded that MBBR could remove up to 99% of phenol and COD from the feed saline wastewater at salt contents of over 40 g L^{-1}. The comparison of the results of this study and the other studies suggest that various parameters such as the acclimation time period, type of wastewater, bacterial consortium, HRT and salinity content of the influent can affect the bioreactor efficiency.

3.4. Biokinetic coefficient

The biokinetic coefficients of isolated halo-tolerant bacteria for treatment of raw saline petrochemical wastewater were

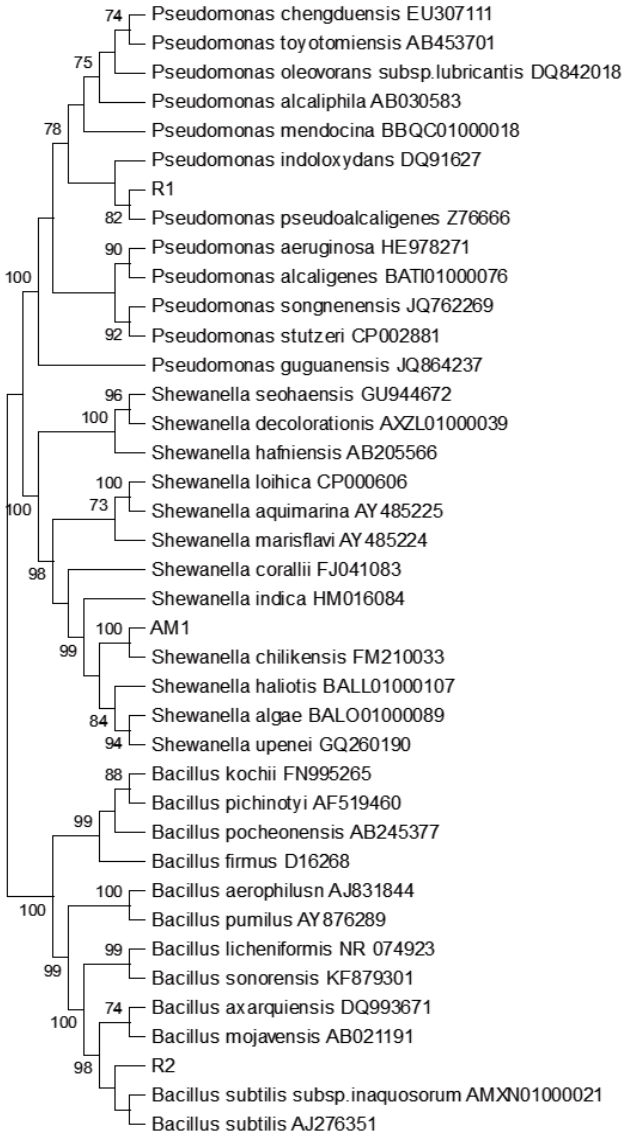

Fig. 4. Phylogenetic tree of 16s rRNA sequences. The evolutionary distances were computed using the Kimura 2-parameter method with 1,000 bootstrap replications. Phylogenetic analyses were conducted in MEGA6.

determined through collecting data at various steady-state operational conditions. Biokinetic coefficients, k_d (coefficient of endogenous decay [d^{-1}]) and Y (cell yield coefficient) expressed as mg cells formed per mg of removed substrate were calculated by plotting Eq. (11) in which k_d and Y were obtained from the slope and the intercept, respectively (Figs. 6a, c and e). Results are presented in Table 5. The Y values were calculated as 0.178, 0.168 and 0.129 g VSS $gCOD^{-1}$, respectively. Results indicated a decreasing trend along with TDS increase from 25,000 to 35,000 mg L^{-1}. In other words, Y was dependent to COD removal and since the removal has decreased in high TDS concentration, as a sequence, Y values declined at high salinities. Presence of the attached biomass enhanced the resistance of system against the qualitative and hydraulic shocks. The values for k_d are almost close to each other in different TDS concentration, but are generally much lower than that reported in literature [32,41,44]. This can be attributed to the compatibility of biomass to saline wastewater and presence of attached biomass. In order to calculate the biokinetic coefficients, μ_{max} (maximum specific growth rate, d^{-1}) and K_s (half velocity constant, mg L^{-1}), Eq. (7) was used in which μ_{max} and K_s were obtained from the slope and the intercept of drawing linear plot (Figs. 6b, d and f), respectively. These biokinetic coefficients (μ_{max} and K_s) are influenced by different factors such as temperature, type of carbon source as substrate, microorganism type and population and salinity content. In this case, K_s value that shows the affinity of the microbial mass to substrate is defined as the quantity of substrate when the specific growth rate is equal to one-half of the maximum growth rate [45]. The obtained K_s values were 662, 518 and 7,007 mg L^{-1} at TDS concentrations of 25,000, 30,000 and 35,000 mg L^{-1}, respectively. Based on COD removal efficiencies of MBBR, since the salinity enhanced, the effluent quality showed a decreasing trend. Since the COD removal was almost similar in TDS levels of 25,000 and 30,000 mg L^{-1}, the values of K_s were close. For TDS concentration of 35,000 mg L^{-1}, the MBBR performance decreased rapidly along with OLR enhancement. The shocks resulting from operational conditions decreased the affinity of biomass to substrate and a higher substrate concentration is required to reach one-half of the maximum growth rates [45,46]. The overall reaction rate (K, d^{-1}) was obtained in the range of 0.994–3.72 d^{-1} by dividing the μ_{max} by Y for studied range of TDS and the highest value of 3.72 d^{-1} belonged to TDS concentration of 35,000 mg L^{-1}. According to Table 4, the biomass concentration for TDS concentration of 35,000 mg L^{-1} reached its lowest values (run 13–18), due to the disturbances of enzymatic functions and bacterial plasmolyzation. The survived biomass demonstrated adaptive features for such salinity content through K balancing mechanism which in turn leads to higher K values. In addition, it seems that the attached biomass can play a key role in degradation of substrate in high saline conditions and keeping constant the biological reaction rate. According to Table 6, a comparison is presented between the calculated biokinetic coefficients in this work and those reported in the literatures and the typical values for conventional activated sludge processes. Generally, the obtained biokinetic coefficients showed values in the reported range in similar studies for the treatment of different wastewater [39,47]. The obtained biokinetic coefficients were in the range of values for conventional activated sludge processes, except that of K_s value that was much higher than the reported value [45]. Moreover, results showed that MBBR sludge yield represented the value lower than the values in the range of conventional activated sludge processes in municipal wastewater treatment plants. This can clearly attribute to the fact that the obtained biokinetic coefficient is affected by some factors such as bacterial consortium, growth medium, concentration and type of substrate, terminal electron acceptor, pH and temperature [32,46].

4. Conclusion

A saline petrochemical wastewater was studied for biological treatment using an enriched salt tolerant consortium. The activated sludge from a high TDS petrochemical

Table 4
Experimental data obtained under steady-state conditions for MBBR inoculated with isolated halo-tolerant strains for treatment of saline petrochemical wastewater

Run	Operation (d)	HRT (d)	SRT (d)	OLR (kg COD m^{-3} d^{-1})	Total bacterial mass (mg L^{-1})	Attached bacterial mass (mg L^{-1})	TDS (mg L^{-1})	COD_{in} (mg L^{-1})	COD_{out} (mg L^{-1})	Removal (%)
1	9	3	25	0.46	1,831	844	25,000	1,353 ± 19	247 ± 22	82 ± 3
2	7	2	21	0.74	2,332	1,198	25,000	1,474 ± 11	276 ± 45	81 ± 2
3	10	1	15	1.37	2,449	1,289	25,000	1,374 ± 9	277 ± 34	80 ± 1
4	13	0.5	12	3.05	4,060	1,580	25,000	1,526 ± 7	297 ± 36	81 ± 2
5	7	0.33	10	4.36	3,100	1,420	25,000	1,373 ± 142	588 ± 76	57 ± 2
6	11	0.16	9	8.57	2,512	1,244	25,000	1,429 ± 9	1,058 ± 1	26 ± 4
7	12	3	23	0.48	1,790	945	30,000	1,450 ± 214	288 ± 14	80.1
8	15	2	21	0.72	1,962	1,026	30,000	1,450 ± 56	280 ± 19	80.6
9	14	1	16	1.392	2,348	1,535	30,000	1,450 ± 126	297 ± 7	79.5
10	12	0.5	13	2.784	3,890	1,834	30,000	1,450 ± 252	330 ± 9	77.2
11	11	0.33	11	4.224	2,860	1,450	30,000	1,450 ± 114	906 ± 15	37.5
12	10	0.16	9	8.4	2,437	1,308	30,000	1,450 ± 82	1,188 ± 13	18.06
13	18	3	29	0.445	1,496	820	35,000	1,340 ± 148	590 ± 14	55.9
14	16	2	28	0.668	1,386	956	35,000	1,340 ± 178	648 ± 16	51.6
15	17	1	25	1.292	1,364	932	35,000	1,340 ± 94	890 ± 10	33.5
16	12	0.5	21	2.585	1,396	1,086	35,000	1,340 ± 38	956 ± 8	28.6
17	8	0.33	18	3.922	815	608	35,000	1,340 ± 135	1,174 ± 6	12.3
18	8	0.16	9	7.8	806	570	35,000	1,340 ± 174	1,227 ± 11	8.4

Table 5
Biokinetic coefficient of halo-tolerant consortium in MBBR for treatment of a real saline petrochemical wastewater

TDS (mg L^{-1})	Biokinetic coefficient				
	Y (mg VSS mg COD^{-1})	k_d (d^{-1})	K (d^{-1})	K_s mg L^{-1}	μ_{max} (d^{-1})
25,000	0.178	0.004	1.12	662	0.2
30,000	0.168	0.006	0.994	518	0.168
35,000	0.129	0.005	3.72	7,007	0.481

Table 6
Biokinetic coefficients for treatment of different wastewaters

Treatment system	wastewater type	Biokinetic coefficients					References
		Y (mg VSS mg COD^{-1})	k_d (d^{-1})	k (d^{-1})	K_s mg COD L^{-1}	μ_{max} (d^{-1})	
MBBR	Synthetic	0.54	0.08	0.7	62.9	0.37	[44]
Activated sludge bioreactor	Saline petrochemical	0.54	0.014	1.23	1,315.6	0.66	[32]
Powdered activated sludge/activated sludge (PACT)	Saline petrochemical	0.199	0.038	0.48	1,598	0.097	[48]
MBBR	Municipal wastewater	0.61	–	3.59	–	0.55	[22]
SB-MBR	Municipal wastewater	0.61	–	–	20	6	[49]
MBBR-MBR	Municipal wastewater	0.54	0.024	–	3.01	0.007	[19]
MBR	Municipal wastewater	0.46	0.03	–	16.47	0.019	[50]
MBR	Municipal wastewater	0.71	0.025	–	3.43	0.007	[19]
Hybrid MBBR-MBR	Municipal wastewater	0.55	0.036	–	8.88	0.026	[50]
Conventional activated sludge	Municipal wastewater	0.3–0.6	0.06–0.15	2–10	10–60	–	[45]
Current study	Saline petrochemical	0.129–0.178	0.004–0.006	0.994–3.72	518–7,007	0.2–0.481	–

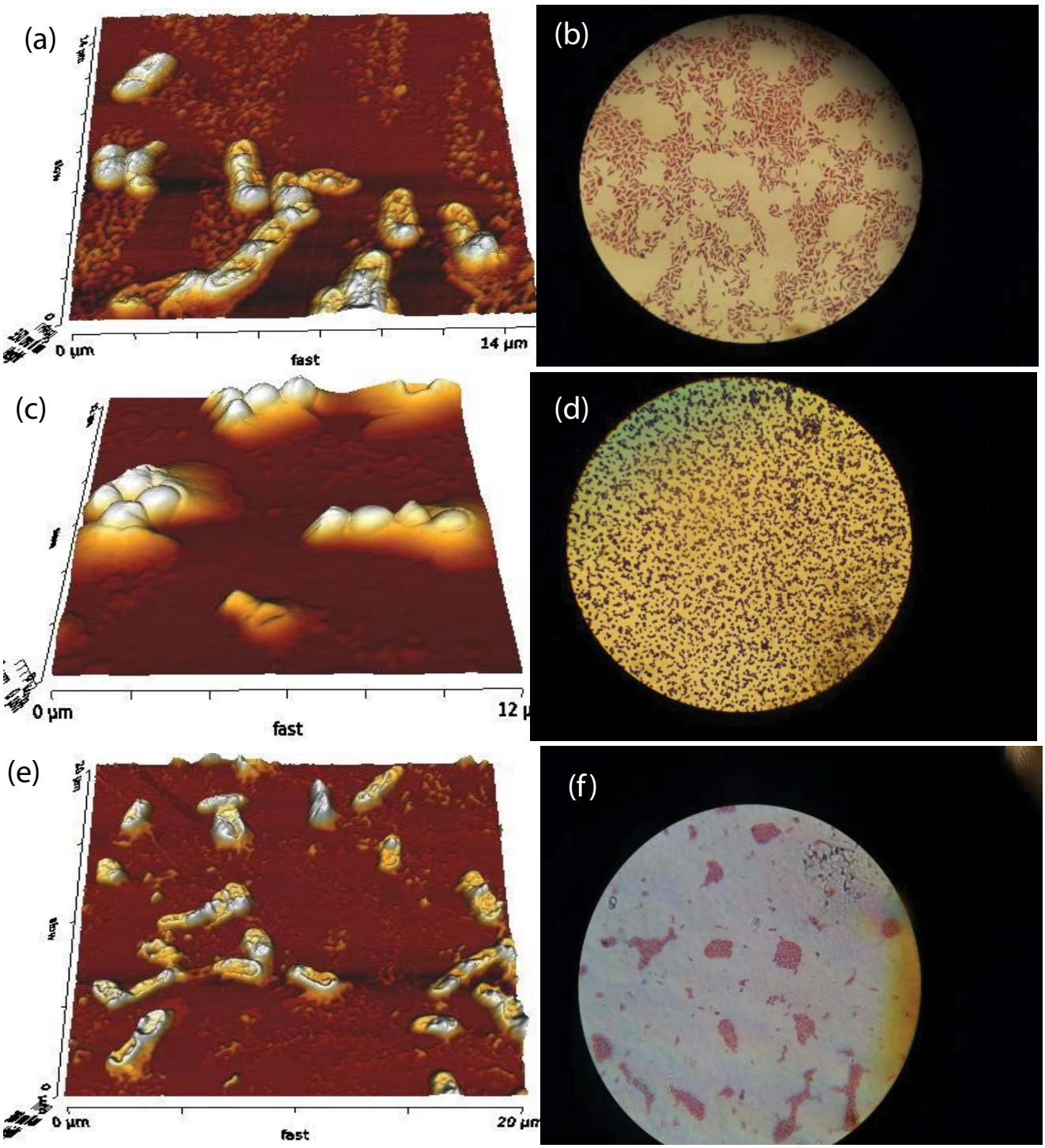

Fig. 5. AFM image (a) and gram staining (b) of *Shewanella chilikensis strain AM1,* AFM image (c) and gram staining (d) of *Bacillus subtilis* subsp. *inaquosorum R2* and AFM image (e) and gram staining (f) *Pseudomonas pseudoalcaligenes strain R1.*

wastewater was used as a halo-tolerant bacterial strains source. Following an enrichment procedure, three potent moderate halo-tolerant bacterial strains were screened and inoculated to startup a lab-scale MBBR. Based on the phylogenetic analysis of 16S rRNA gene sequence, these isolates were identified as *Pseudomonas pseudoalcaligenes strain R1*, *Bacillus subtilis* subsp. *inaquosorum R2*, and *Shewanella chilikensis strain*. A lab-scale setup was applied for determination of the biokinetic coefficients of an MBBR treating a real petrochemical wastewater with BOD_5/COD ratio of less than 0.1 and COD concentrations of 1,088–1,850 mg L^{-1}. The process performance under varying OLRs and COD

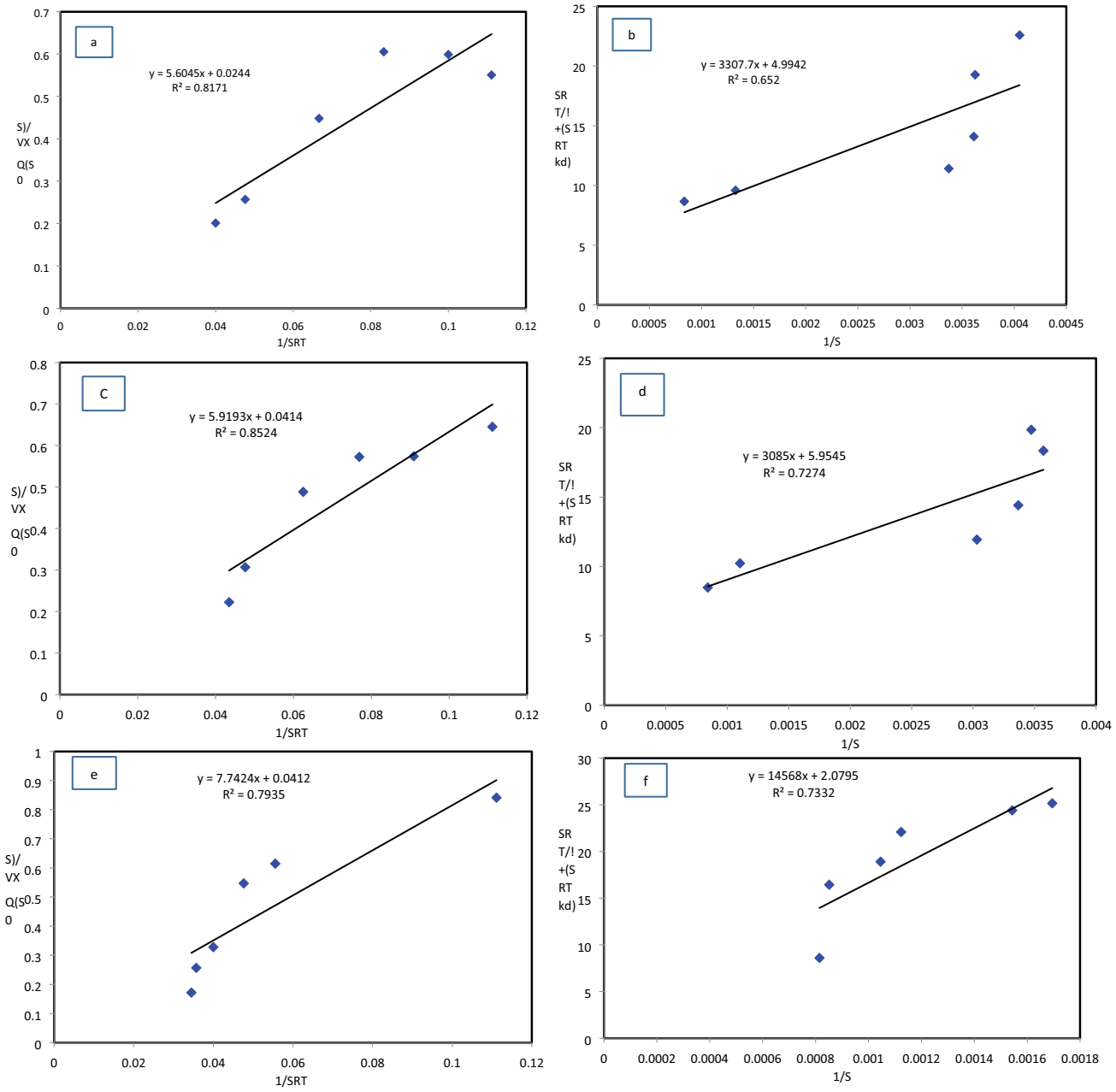

Fig. 6. Determination of biokinetic coefficients of halo-tolerant consortium in MBBR, (a) Y and k_d and (b) μ and K_s for TDS concentration 25,000 mg L^{-1}, (c) Y and k_d and (d) μ and K_s for TDS concentration 30,000 mg L^{-1}, (e) Y and k_d and (f) μ and K_s for TDS concentration 35,000 mg L^{-1}.

removal efficiency variations was studied. The findings of this study showed that MBBR had a great potential in saline industrial wastewater treatment and could remove over 80% of COD in optimum HRT and OLR of 12 h and 3.05 ± 0.15 kg COD m^{-3} d^{-1}, respectively. Regarding to the biokinetic coefficients of the MBBR process obtained using Monod model, most of the kinetic coefficients, except K_s, were at the range close to the typical range reported for conventional activated sludge processes. Also, the MBBR sludge yield showed the value less than the values in the range of conventional activated sludge processes in municipal wastewater treatment plant and emphasized that some factors such as bacterial consortium and the type of substrate impact on the kinetic coefficients.

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Declarations of interest

None

Symbols

V	—	Reactor volume, L
k	—	Overall reaction rate, d^{-1}
k_d	—	Biomass decay rate, d^{-1}
K_s	—	Half saturation constant, mg L^{-1}
S_0	—	Influent substrate concentration, mg L^{-1}
S	—	Effluent substrate concentration, mg L^{-1}
Y	—	Biomass yield coefficient, g VS produced/g substrate utilized
Q_w	—	Wastage flow rate, S^{-1}
HRT	—	Hydraulic retention time, h
μ_m	—	Maximum specific growth rate, d^{-1}
μ	—	Specific growth rate, d^{-1}
X	—	Biomass concentration in the reactor, mg VSS L^{-1}
SRT	—	Solid retention time, d
t	—	Time, s

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