Korean J. Chem. Eng., 32(5), 874-881 (2015)
DOI: 10.1007/s11814-014-0307-9

pISSN: 0256-1115
eISSN: 1975-7220

Biodegradation potential of hydrocarbons in petroleum refinery effluents using a continuous anaerobic-aerobic hybrid system

Niloofar Nasirpour, Seyyed Mohammad Mousavi†, and Seyed Abbas Shojaosadati

Biotechnology Group, Chemical Engineering Department, Tarbiat Modares University, P. O. Box 14115-143, Tehran, Iran
(*Received 8 January 2014 • accepted 9 October 2014*)

Abstract−We investigated a novel wastewater treatment method for the remediation of crude oil refinery effluents with large number of recalcitrant organic compounds. The treatment system consists of an up-flow anaerobic sludge blanket (UASB) reactor and an aerobic packed-bed biofilm reactor (PBBR) in combinatory pattern to increase the efficiency of treatment and to remove polycyclic aromatic hydrocarbons (PAHs) of the wastewater. The mean chemical oxygen demand (COD) removal efficiency in the UASB reactor and PBBR over 118 days of sampling was 68.48% and 38.28%, respectively. The total COD removal efficiency of the system was 81.07%. The GC-MS abundance and area values for each of the substances in the effluent decreased greatly from the corresponding value in the influent. Specifically, the PAHs were totally removed during the treatment process. This study presents a feasible technology for the treatment of refinery effluents.

Keywords: Biodegradation, Chemical Oxygen Demand, Polycyclic Aromatic Hydrocarbons (PAHs), Petroleum Refinery Effluent, Up-flow Anaerobic Sludge Blanket (UASB)

INTRODUCTION

The petroleum industry generates series of liquid effluents containing large amounts of priority pollutants during the petroleum-refining process. The major pollutants found in these industries are petroleum hydrocarbons, specifically aliphatic hydrocarbons of 1-40 carbon atoms, along with cycloalkanes and aromatic compounds including monoaromatic and polycyclic aromatic hydrocarbons [1,2]. Since PAHs exhibit toxic, mutagenic and carcinogenic properties [3], there is a serious concern about their environmental presence [4]. Treatment of petroleum refinery wastewater is commonly performed through a series of on-site treatment technologies, such as American Petroleum Institute (API) separators, tilted plate interceptor (TPI) separators and dissolved air floatation (DAF) units. These operations are followed by biological treatment in a suspended growth process. But, suspended growth processes commonly have numerous operational problems, which include poor ability of sludge to settle due to low food-to-microorganism ratio; production of extra-cellular polymers consisting of lipids, proteins and carbohydrates that adversely affect sludge settling; biological inhibition due to toxic compounds, which necessitates very long sludge retention time; and long period of acclimation or start-up and production of large amount of biological sludge [5,6]. The mentioned problems have created an attempt searching for an alternative to suspended growth process.

Sequential anaerobic-aerobic processes exploit the advantages of the two systems in the most cost-effective set-up. In comparison with conventional aerobic technologies, the combined anaerobic-aerobic system consumes distinctly less energy, produces less excess sludge, and is less complex in operation [7-9].

The anaerobic treatment is performed in different reactor types; the most widely used are the high-rate up-flow anaerobic sludge blanket (UASB) bioreactors [10]. The UASB reactor has been well accepted for treatment of various sewage and industrial wastewaters due to good removal efficiency of organic substances [11-13]. It has been widely used as for treating printing and dyeing wastewater [14], and landfill leachate [15]. Packed bed biofilm reactors (PBBR) exhibit high surface area available for microbial growth. Also, these systems present a higher potential for use than suspended growth biomass reactors since the former can retain a higher concentration of biomass with higher metabolic activity when operated with continuous processes. Furthermore, the attached biomass is sometimes claimed to be more resistant to toxicity; it has been reported that biofilm cells can be up to 500 times more resistant to antibacterial agents than freely suspended ones [16-19].

As for treating refinery wastewater, some individual aerobic and anaerobic processes as an alternative for activated sludge process were investigated, including ultrafiltration membrane bioreactor [20], fixed film bioreactor [21], fluidized bed bioreactor [22], air-lift loop bioreactor [23], and up flow anaerobic sludge blanket bioreactor [24]; but hybrid systems have not been assessed for the treatment of petroleum refinery wastewaters, and specifically, the combination of UASB and aerobic PBBR was not utilized for the treatment of refinery effluents.

The anaerobic process is mediated by a sequential series of fermentation reactions carried out by diverse groups of microorganisms in which hydrocarbons are converted to simple molecules and ultimately to CO_2 and CH_4. Metabolic interactions between the various groups of organisms are essential to successful and complete mineralization of the organic molecules [25,26]. An increas-

†To whom correspondence should be addressed.
E-mail: mousavi_m@modares.ac.ir

ing number of novel microorganisms have been shown to utilize aliphatic and aromatic hydrocarbons as growth substrates under strictly anoxic conditions. These microorganisms use nitrate, ferric iron or sulfate as electron acceptors for anaerobic respiration, grow in syntrophic cocultures with other anaerobes or grow by anoxygenic photosynthesis [27,28]. The anaerobic degradation of alkanes with sulfate or nitrate was first demonstrated by the quantitative measurement of substrate consumption with novel isolates that differed from any known species [25-31]. Furthermore, alkane degradation by methanogenic communities was also shown [28,30,32].

Aerobic bacteria growing on hydrocarbons, O_2 is not only the terminal electron acceptor for respiratory energy conservation, but also an indispensable reactant in the activation mechanism [32, 33]. By the action of monooxygenases (on aliphatic and certain aromatic hydrocarbons) or dioxygenases (on aromatic hydrocarbons), one or two oxygen atoms, respectively, are directly incorporated from O_2 leading to hydroxylated products [25]. The principal mechanism for aerobic metabolism of PAHs by bacteria is oxidation of the aromatic ring, followed by ring fission and subsequent metabolism of the resulting organic acids, ultimately to carbon dioxide. One of the benzene rings within the PAHs is initially oxidized by the action of dioxygenase enzymes to form *cis*-dihydrodiols. These are then dehydrogenated to form dihydroxylated intermediates, which are further metabolized via catechols to carbon dioxide and water [34].

The main objective of this research was to develop an efficient hybrid system for treating petroleum refinery wastewater, and make use of advantages of both up-flow anaerobic sludge blanket and packed bed biofilm reactors to degrade the highly toxic compounds of refinery wastewater such as PAHs and other refractory compounds. In this study, the performance of a UASB reactor and PBBR hybrid treatment system was evaluated for the remediation of complex refinery effluents. Real wastewater of the petroleum refinery was utilized as the feed to experimental set-up, which demonstrates the exact and actual performance of the system. The efficiency of the system in COD and TPH removal during the steady-state operation was measured. The GC-MS analysis of the influent and effluent demonstrated that the system performed well in the removal of almost organic compounds. Specifically, biodegradation of the PAH compounds was investigated in a thirty-day continuous experiment.

MATERIALS AND METHOD

1. Wastewater Source and its Characteristics

The wastewater was collected from the effluent of the API separator of the Tehran refinery effluent treatment unit. The characteristics of this wastewater are shown in Table 1. All experiments were conducted according to the standard methods for the examination of water and wastewater [40].

2. Biomass Culturing Medium

The growing medium for the PBBR was a mixture of mineral salts containing water and the following chemicals ($g \cdot l^{-1}$): K_2HPO_4 (0.8), KH_2PO_4 (0.2), KNO_3 (1), $MgSO_4 \cdot 7H_2O$ (0.2), $CaCl_2 \cdot 2H_2O$ (0.1), NaCl (0.1), $FeCl_3 \cdot 6H_2O$ (0.01) and yeast extract (0.1). All of the chemicals were obtained from Sigma-Aldrich, Sigma and Merck and were of pure analytical grade.

Table 1. Characteristics of the original wastewater provided from a petroleum refinery treatment unit

Parameter	Average value
COD ($mg \cdot l^{-1}$)	435
BOD_5 ($mg \cdot l^{-1}$)	110
TPH ($mg \cdot l^{-1}$)	1520
$N\text{-}NH_4^+$ ($mg \cdot l^{-1}$)	16
$P\text{-}PO_4^{3-}$ ($mg \cdot l^{-1}$)	0.5
H_2S ($mg \cdot l^{-1}$)	3
Total PAH ($mg \cdot l^{-1}$)	10.33
TSS ($mg \cdot l^{-1}$)	160
pH	8

3. Experimental Setup

The UASB reactor was made of glass with an internal diameter of 10 cm and a height of 80 cm, equipped with a jacket. The wastewater was fed into the lower part of the reactor. The substrate passed upward through an anaerobic sludge bed that expanded with the flow rate of the influent, gas production and effluent recycling. A gas-solid-liquid separator was positioned at the top of the reactor to enable the sludge that washed out of the reactor to settle and automatically return to the reactor. The reactor temperature was maintained at 35±1 °C, which is nearly the optimum temperature of mesophiles in UASB reactors. At present most of the UASB systems are operated under mesophilic condition because the thermophiles consume too much energy, while psychrophiles meet many obstacles, so operating the reactor by mesophiles is the optimum condition for UASB [35]. Temperature control was by heated water that circulated through the jacket of the reactor.

The aerobic up-flow PBBR was made of glass with an effective volume of 2.2 *l*, a diameter of 10 cm, and a height of 50 cm. Pumps drive the direct up flow of wastewater through the reactors, with the influent entering at the bottom and the treated effluent leaving near the top. Mineral natural supports were used to immobilize the microorganisms. The mineral packing used in the PBBR as a support for biomass attachment and growth was analyzed by X-ray diffraction (XRD) to ensure that it did not contain any toxic substances, which could inhibit microbial growth or increase wastewater pollution. Fig. 1 shows the schematic diagram of the system, respectively.

4. Sludge Preparation for the UASB Reactor

Although the best seed material for a UASB reactor is the granular sludge from another UASB reactor treating similar wastewater, it is difficult and often impossible to obtain. In general, a mixture of several kinds of sludge is considered as adequate seed material because it has a diverse microbial population that is suitable for the treatment of many kinds of wastewater [10].

Because the anaerobic granular sludge from a reactor treating similar wastewater was unavailable, the seed sludge for the experiments was prepared by combining the waste activated sludge of the treatment plant of the Tehran refinery unit with the granular sludge of a UASB reactor that was treating the wastewater of a dairy unit. The waste activated sludge was stored in 25 °C in sealed containers for approximately two weeks to gain anaerobic inocula [24]. The mixture of this sludge with the granular sludge was the inocu-

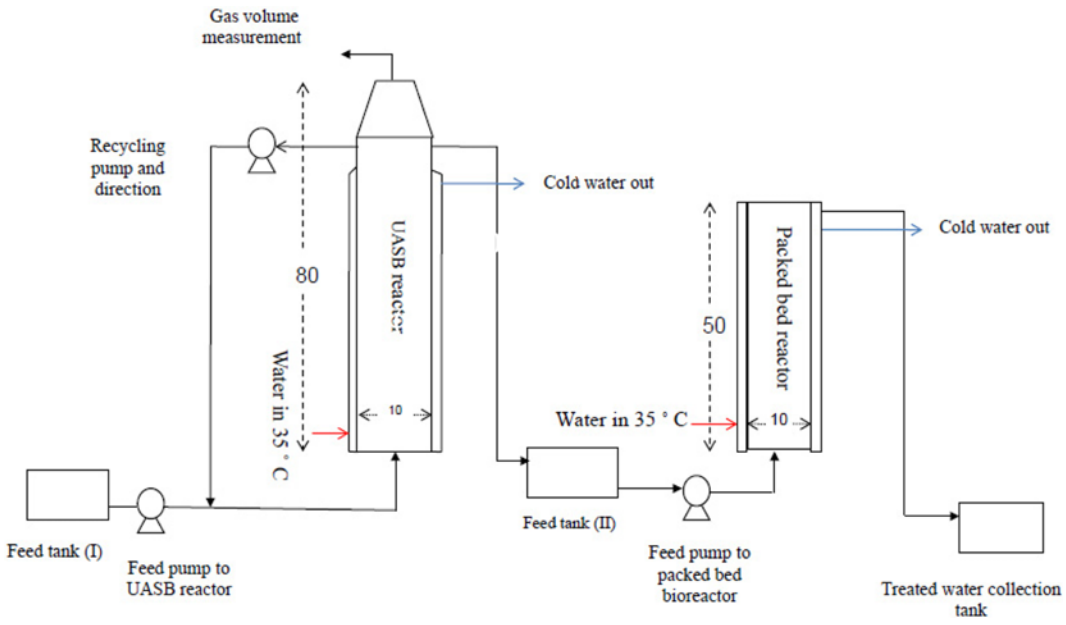

Fig. 1. Schematic diagram of the experimental setup comprising the UASB reactor and the PBBR.

lum of the UASB reactor. The MLSS and MLVSS of the sludge was 17.1 and 15.1 g·l^{-1}, respectively.

5. UASB Reactor Startup

The reactor startup is a very important process step because the stability of the operation and satisfactory performance of the reactor depend on it. The duration of the startup depends on numerous biological, chemical and physical parameters. The startup duration is influenced by the composition, strength, activity and adaptability of the inoculum. Environmental parameters, such as the temperature, pH, and presence of nutrients and trace elements, and operation parameters, such as the loading rate, retention time, reactor configuration, and liquid mixing, also influence the startup duration [10]. The volume of sludge used to inoculate the reactor was 15% of total reactor volume.

For the first step, a batch feed was used to maintain the reactor influent COD at 1,000 mg·l^{-1} for two days. After the reactor had been filled, the transition to a continuous operation with a hydraulic retention time of 72 h was started. The startup was followed by increasing the organic loading rate, step by step, to produce the most rapid biomass acclimation and development. Organic loadings were enhanced by decreasing the hydraulic retention time influent upon the attainment of a pseudo-steady state. Pseudo-steady state was defined based on the stable COD removal efficiency. During the startup step, the ratio of C : N : P was maintained at 300 : 5 : 1; this ratio changed to 600 : 5 : 1 in the steady-state operation phase [36].

To increase the linear up-flow velocity in the reactor, the recycling pump periodically extracted liquid from the top and reintroduced it to the bottom part of the reactor.

For biological treatments, BOD-to-COD ratio is an important index for determining biodegradability of the wastewater. Generally, when the ratio is greater than 0.5, the wastewater is easily biodegraded. However, it is refractory when the value is below 0.3 [11]. In the present study, the BOD to COD ratio was 0.25, which means that wastewater was recalcitrant for biological treatment, so molasses was used as a co-substrate. Co-substrates are known to enhance biodegradation of many recalcitrant or slow biodegradable organic compounds [37]. The addition of molasses caused an increase in the COD of the raw wastewater from 435 mg·l^{-1} to 876, 517 and 480 mg·l^{-1} in the three steps. To make use of the real wastewater as feed for system, molasses addition was decreased stepwise; thus the total COD of influent hybrid system decreased.

6. Biomass Culturing and Immobilization in the PBBR

Biomass culturing was performed according to following procedure: the mineral support media that served as the carriers were acid washed, then carefully placed, without compression, into the reactor under dry conditions. The growing medium was also introduced into the bioreactor [22]. To start the growth of the microorganisms on the support, a fed-batch culture was initiated by introducing 20% of inoculum into the reactor. The carbon source for the bacteria growth was refinery effluent. As the COD of the media was decreased to below 200 mg·l^{-1} wastewater was added to the bioreactor to bring back the COD approximately 500 mg·l^{-1}. The inoculum was a mixture of the effluent and the influent of the DAF system in the treatment plant of Tehran refinery unit. After 2 months, the support media was completely covered with biofilm.

Common wastewater bacteria can function in a pH range of 6.0 to 8.5. The optimal pH for the bioreactor system is between 6.5 and 7.5. In this system, the pH was fixed at 7.3±0.1. For aerobic treatment of the wastewater, oxygen must be supplied for biomass growth; operation of the system under anaerobic conditions is not recommended, Therefore, at least 2-3 mg·l^{-1} of DO was maintained in PBBR. The reactor temperature was maintained at 35±1 °C.

7. Measurements and Observations

TSS and daily COD measurements were performed according to the standard methods for the examination of water and wastewater. To control the system, the pH of the effluent in the UASB reactor and the PBBR was measured daily with a pH meter (Metrohm model 827).

Table 2. GC-MS operating conditions and parameters

Item	Condition
Apparatus	GC Agilent 7890A with MSD 5975C
Column	HP-5MS (30 m×250 μm×0.25 μm)
Carrier gas	Hydrogen
Column pressure	7.65 Psi
Mode	Split
Split flow	5 ml·min^{-1}
Split ratio	5 : 1
Injector temperature	280 °C
Oven temperature	Initial 50 °C
	50 °C→300 °C, 8° min^{-1}
Electron source	Electron impact

The water displacement method was used to measure the biogas volume produced in the UASB reactor.

For observations with the scanning electron microscope, the supports from different parts of the reactor were collected. The samples were treated with glutaraldehyde (2.5% solution buffered with sodium phosphate) to fix the cells and then dehydrated serially in ethanol, freeze dried, and sputter-coated with gold. Scanning electron micrographs were taken by a scanning electron microscope.

The GC-MS data were obtained when the HRT was 24 h. A 5 ml sample of wastewater was extracted with 5 ml of dichloromethane three times at pH 2, 12, and 7. The three extract layers were combined and dissolved in a 1 ml solution of n-hexane; the details of the method are described in Table 2.

The concentrations of naphthalene (Nap), phenanthrene (Phe) and pyrene (Pyr) were determined for all of the samples by GC, during 30 days of operation.

RESULTS AND DISCUSSION

1. Variation of Optical Density and pH During Biomass Culturing and Immobilization in PBBR

The concentration of free cell suspension was measured as an optical density (OD) at 620 nm with a spectrophotometer (Cary win 100, Varian Company). OD and pH of suspended media were measured over 80 days of PBBR batch start-up. It is useful to mention that biofilm covered the packing of PBBR completely in 60 days after start-up. Fig. 2 shows the OD variation in PBBR, the OD of media has increased from zero to maximum value of 2.4 in 28th day of sampling and then decreased to nearly zero. Increment of OD until day 28th is because of biomass growth; thus the OD has an increasing trend, but after that the biomass settled on packing, which caused OD to decrease.

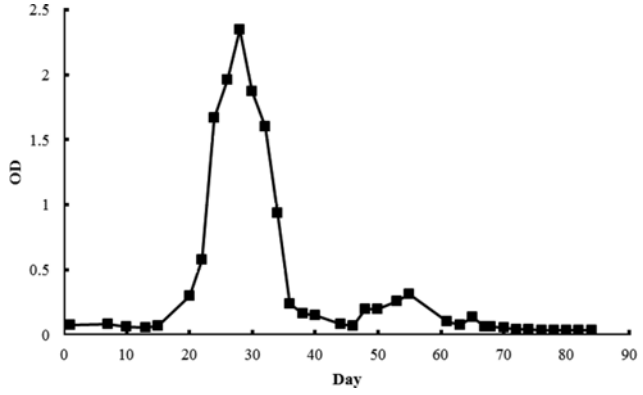

Fig. 2. Daily variation of optical density in PBBR during fed-batch culture.

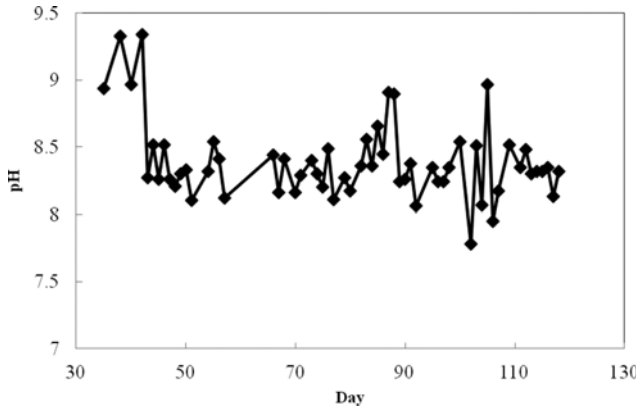

Fig. 3. Daily variation of pH in PBBR during fed-batch culture.

Fig. 3 shows pH variation in PBBR, during start-up process. Mean pH value during 84 days of sampling was 7.7. The maximum pH value of PBBR media was 8.3. Variation of pH is an indicator of the microorganism growth and activity. Acidic and basic metabolites resulting from biodegradation of pollutants cause this variation. By addition of wastewater to batch reactor as carbon source, the pH of samples decreased and then increased to a constant value.

At the end of the study, samples of biofilm packing from various bed locations were taken to measure the biomass weight. The amount of dry biomass formed on supports of PBBR was 47±6 mg per gram of dried packing.

2. Treatment Efficiency of the System

The influent and effluent COD to UASB and PBBR, organic loading rate (OLR) and removal efficiency of the COD in the UASB and PBBR systems during the period of operation is shown in Fig. 4(a) and (b), respectively. OLR increased stepwise by decreasing the retention time in system. During the initial startup period in UASB reactor, the COD concentration in the influent was approximately 1,000 mg·l^{-1}, and the organic loading rate (OLR) was 0.3 kg·m^{-3} d^{-1}. The COD removal efficiency for UASB, PBBR and hybrid system was calculated according to Eqs. (1) to (3).

COD removal efficiency of UASB

$$= \frac{COD_{influent\ to\ UASB} - COD_{effluent\ from\ UASB}}{COD_{influent\ to\ UASB}} \times 100 \quad (1)$$

COD removal efficiency of PBBR

$$= \frac{COD_{influent\ to\ PBBR} - COD_{effluent\ from\ PBBR}}{COD_{influent\ to\ PBBR}} \times 100 \quad (2)$$

Total COD removal efficiency

$$= \frac{COD_{influent\ to\ UASB} - COD_{effluent\ from\ PBBR}}{COD_{influent\ to\ UASB}} \times 100 \quad (3)$$

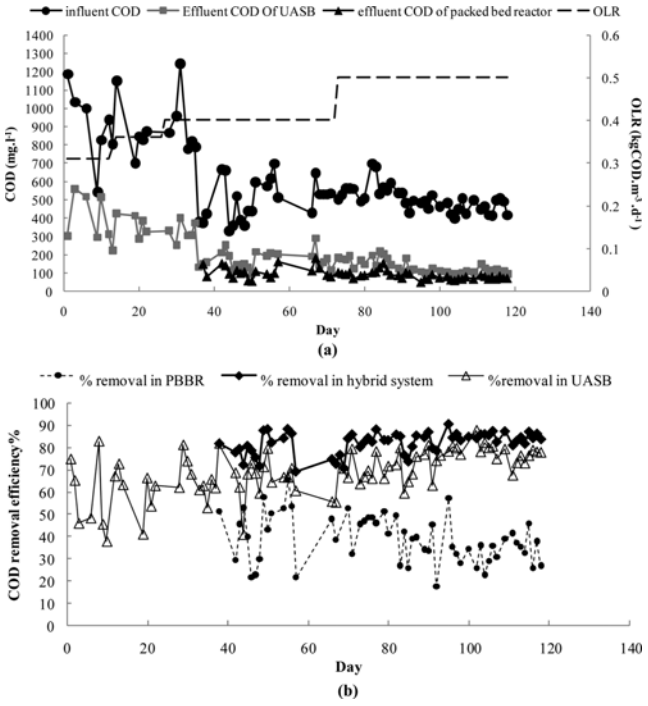

Fig. 4. (a) Influent and effluent COD of UASB; PBBR and hybrid system and organic loading rate into the hybrid system; (b) COD removal efficiencies of UASB, PBBR and hybrid system.

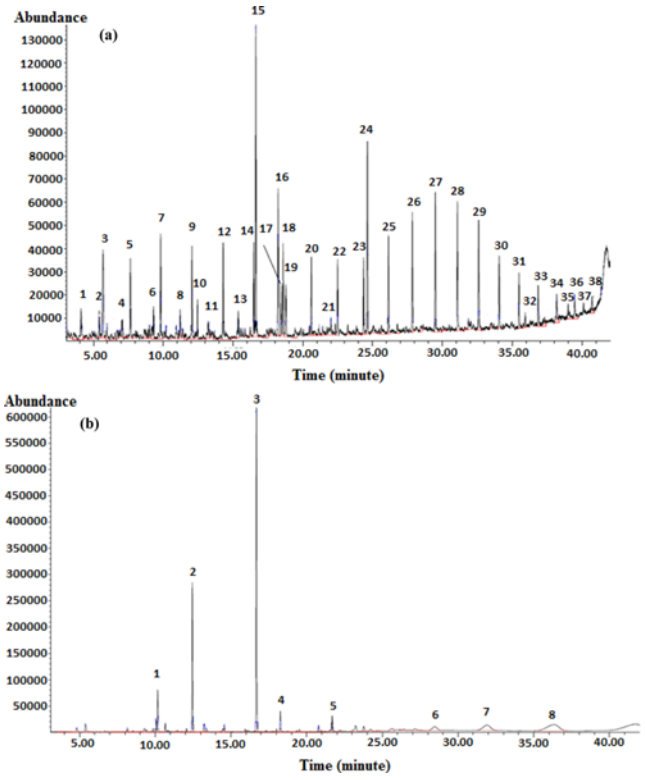

Fig. 5. Spectra of the organic components in the influent and effluent of the hybrid system; (a) Influent organic components: (1) undecane (2) cyclopropane, 1,1-dimethyl-2-(1-methyl-2-propenyl) (3) naphthalene (4) methoxyacetic acid (5) tridecane (6) dodecane, 2,6,10-trimethyl- (7) tetradecane (8) heptadecane, 2,6,10,14-tetramethyl (9) pentadecane (10) butylatedhydroxytoluene (11) hexadecanol (12) hexadecane (13) pentadecane, 2,6,10-trimethyl (14) dodecane, 1-chloro (15) cyclopantane carboxylic acid, 4-hexadecyl ester (16) phenanthrene (17) octadecane (18) hexadecane 2,6,10,14-tetramethyl (19) oxalic acid, cyclobutylpentadecyl ester (20) nonadecane (21) 9,10-anthracenedione (22) eicosane (23) heneicosane (24) pyrene (25) docosane (26) heptadecane (27) tetracosane (28) heneicosane, 11-(1-ethylpropyl)- (29) tetratriacontane (30) tetratetracontane (31) sulfurous acid, butyl tridecyl ester (32) 1,5,9-decatriene, 2,3,5,8-tetramethyl (33) sulfurous acid, 2-propyl tridecylester (34) tritetracontane (35) 8-hydroxy-1-(2-hydroxyethyl)-1,2,5,5-tetramethyl-cis-decalin (36) 2,2-dimethylpropanic acid, 4-hexadecyl ester (37) nonahexacontanoic acid (b) organic components in the effluent of the system: (1) isoindole-1,3-dione, perhydro-2-butyl-spiro-5,2-1,3-dioxolane (2) butylatedhydroxytoluene (3) allyl dimethyl silane (4) 2H-indazol-3-amine, 2-methyl-(5) sulfurous acid, cyclohexylmethyl isobutyl ester (6) docosane (7) heneicosane (8) tetracosane.

The results obtained for the UASB reactor are as follows: the COD removal efficiency ranged from 34.1% to 87.6% and the mean COD removal efficiency and mean outflow COD were 68.48±10.54% and 191.4±107.09 mg·l^{-1}, respectively. After the initial startup of the UASB reactor, there were some fluctuations in the COD removal efficiency, which stabilized after 30 days, and the amplitudes of the variations in efficiency decreased. Sludge adaptation to wastewater during the startup step caused a nearly constant removal efficiency in the next days of operation. In the study of Ghavipanjeh and Shayegan [24] maximum COD removal efficiency for UASB reactor, treating the identical wastewater was 70%. Compared to their study the UASB in hybrid system had better performance in COD removal efficiency. UASB startup method in the present study was different from the previous, as the inocula of UASB reactor were different, and also molasses was used as co-substrate in the present study; and this may have caused the lower COD removal efficiency in the latter. As examined in this investigation, without addition of molasses, the UASB reactor worked improperly and was unable to remove TPHs.

The corresponding results for PBBR are as follows: the COD removal efficiency ranged from 17.4% to 65.24% and the mean COD removal efficiency and mean outflow COD were 38.3% and 88.8 mg·l^{-1}, respectively. The total COD removal efficiency in the hybrid system and the mean outflow COD during the 118 days of sampling were 82% and 88.8 mg·l^{-1}, respectively.

A comparison of the COD removal efficiencies in the UASB reactor and PBBR showed that the major organic compounds were degraded in UASB reactor, and the organic residues in the effluent, which are difficult to remove, remained to be biodegraded in the PBBR. The PBBR performance was good in removing recalcitrant substances. In this case, the COD of outflow from the PBBR meets the criteria specified by discharge standards for water pollutants from oil industry. However, the effluent COD from the UASB reactor was variable, and the final effluent COD from the hybrid system was found to remain constant, so the PBBR acts as a compensator to the treatment system, which ensures the quality of outflow from treatment.

The results of COD removal efficiency for refinery effluent by ultrafiltration membrane were 30%, which is much lower than the efficiency of the current hybrid system [20], but the result of fluidized bed bioreactor for COD removal was better; COD removal efficiency for the three-phase fluidized bed was 90% [22]. Comparing the results of this hybrid system with the results of traditional activated sludge shows the better performance of the hybrid system in treating the refinery effluents, as the mean COD removal efficiency in traditional activated sludge process is between 50-60% [21].

3. Biodegradability of Organic Substances

Fig. 5 represents the abundance of different organic substances, whose identities were obtained using GC-MS, in the influent and effluent streams of the hybrid system when the HRT was 24 hours. The influent wastewater stream contains 75 different organic substances; the highest peak response occurs at 16.608 min, corresponding to cyclopentanecarboxylic acid, 4-hexadecyl ester. The influent contains six types of branched-chain alkanes and 15 types of straight-chain alkanes that range from undecane ($C_{11}H_{24}$) to tetratetracontane ($C_{44}H_{90}$). The influent stream also contains five aromatic compounds, including naphthalene, phenanthrene, pyrene, 9,10-anthracenedione and butylated hydroxytoluene; naphthalene, phenanthrene and pyrene are polycyclic aromatic hydrocarbons. All of the aromatic compounds in the influent stream, except butylatedhydroxytoluene, were completely degraded; butylatedhydroxytoluen is known as fuel additive in the petroleum industry and also finds uses as turbine and gear oils. Butylatedhydroxytoluene has a benzene ring surrounded by methyl groups and one hydroxyl group, which can cause structural restriction to biodegradation. However, biodegradation of this compound has not been examined thoroughly in literature and the exact reason of being unchanged during treatment process is unknown. The effluent contains 62 different types of organic substances; the three highest peaks occur at 10.165, 12.454 and 16.688 min correspond to isoindole-1,3-dione-perhydro-2-butyl-spiro-5,2′-1′,3′-dioxolane, butylatedhydroxytoluene and allyl dimethyl silane. Docosane, heneicosane and tetracosane are straight-chain alkanes which were not completely degraded; however, the peak area values corresponding to these chemicals are considerably reduced. A comparison of Fig. 4(a) and (b) shows that the GC-MS abundance and area values for each of the substances in the effluent decreased greatly from the corresponding value in the influent, confirming the capability of hybrid system to decompose organic substances.

4. Biodegradation of TPH and PAH

A GC-MS analysis was used to measure TPH amounts. Samples from effluent of the hybrid system were taken for TPH measurements on the 82th and 86th day of operation, when the HRT was 24 hours. The threshold for measurement was 1 mg·l^{-1}. The TPH compounds were completely degraded in the combined system. Total petroleum hydrocarbons (TPH) describe a large family of several hundred chemical compounds that originate from crude oil. Therefore, the amount of TPH measured is a suitable parameter to reliably evaluate the pollution levels in effluents from the petroleum industry. The hybrid system can treat TPH-containing effluent at steady state conditions, suggesting that the combination of the UASB and PBBR systems may perform well in the treatment of effluent from the petroleum industry.

The influent stream contained naphthalene, phenanthrene and pyrene, three PAH compounds for which the corresponding response values occurred at 5.690, 18.210 and 24.647 min. GC analyses were performed over 30 days of operation to measure PAH concentrations. The total concentration of PAHs was 10.33 mg·l^{-1} in the influent stream. PAH compounds in the influent were degraded to non-detectable levels by the combined system. The aerobic and anaerobic parts of wastewater treatment contain different consortia of bacteria; assemblages of different bacterial species with broad enzymatic capabilities have a high capacity to degrade complex PAH mixtures [38,39]. The effluent stream was devoid of PAH compounds, suggesting that these components were completely removed but not necessarily biodegraded. Because the PAH compounds have a low water solubility and hydrophobic properties, they may be removed from the wastewater partly by biodegradation and partly by sorption into the sludge [29]. Sorption causes PAH retention in the treatment system where it may be ultimately biodegraded [39]. The sludge of industrial wastewater treatment systems, which may contain hazardous compounds such as PAHs, requires appropriate biological treatment for safe disposal into the environment.

6. Biogas Production in the UASB Reactor

The variation in the daily biogas production of the UASB reactor throughout the 62 day operation is illustrated in Fig. 6. The mean daily biogas production is 1904±417 cm^3. By comparing the COD of added molasses and the refinery effluent it is revealed that the main portion of biogas production resulted from the degradation of refinery effluent. During the first 56 days of operation, although the COD was degraded, the biogas volume was not measurable and water displacement did not occur. We concluded that the degraded COD transformed into insoluble organic compounds, possibly caused by the delay in the adaptation of the methanogens to the refinery effluent.

Biogas composition was analyzed by gas chromatography, and the methane-to-carbon dioxide ratio was 12 to 1. Also, trace amounts of H_2S and H_2O were present in the produced biogas. Higher amount of methane compared with carbon dioxide demonstrates the presence of H_2-utilizing and CO_2-utilizing bacteria growth condition in UASB. As the influent COD was low, bacteria consumed the produced CO_2 in biochemical reaction pathways. Similar results were

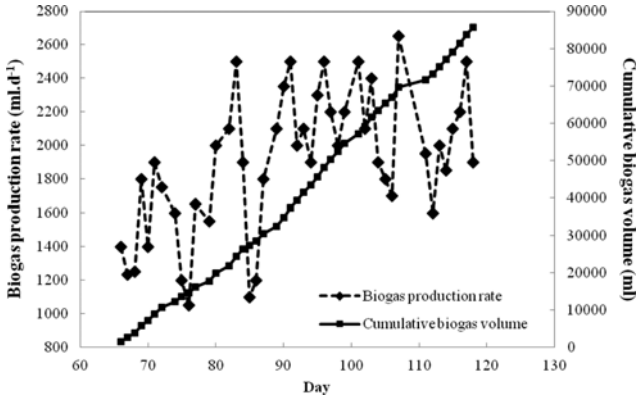

Fig. 6. Biogas production rate in the UASB reactor.

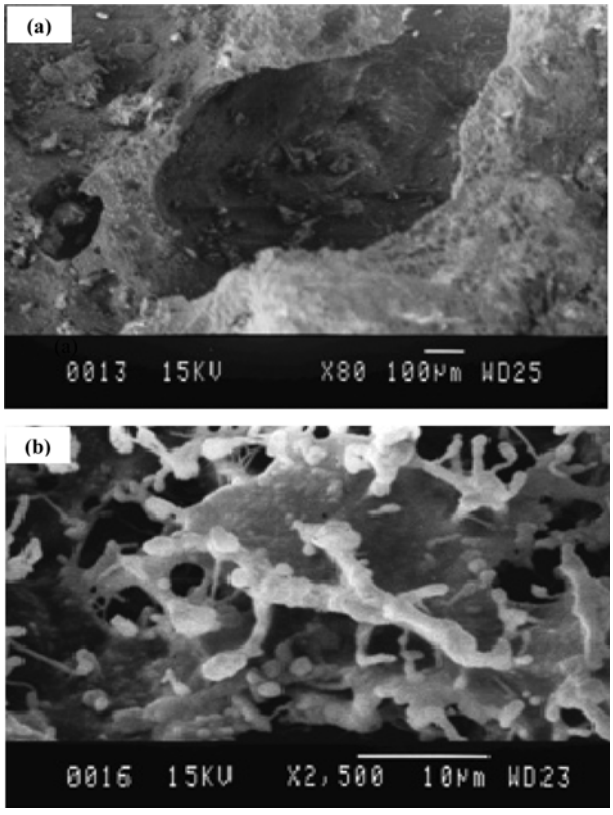

Fig. 7. SEM images of carriers in the PBBR. (a) Carrier without microorganisms, showing the space and capacity for the immobilization of microorganisms on the carrier (×80); (b) carrier covered by microorganisms, and a clump of cells (×2500).

reported in the study carried out at low influent CODs by Maat and Habbets [41].

7. Surface Morphology of Biofilms in PBBR

After biomass immobilization, samples of the support media from various bed locations of the packed reactor were collected for SEM observations. Fig. 7(a) shows the carrier without microorganisms. The surface of the natural rock does not have a uniform pattern and the structure is completely chaotic. The shallow cavities on the surfaces of the carriers are appropriate locations for the growth and attachment of microorganisms, but because of the non-uniformity of the surface, cells are distributed differently on various parts of support. In a biofilm, the developing biofilm on the exterior prevents the transfer of oxygen and nutrients to the interior locations of the biofilm. In practice, the carrier protects the microorganisms that are immobilized on it from being shocked by any sudden change in COD load or increase in toxicity. Fig. 7(b) shows a carrier that is covered by microorganisms; the presence of extracellular polymeric substances (EPS) and the bacteria clumps can be clearly observed in the images. Note that there is different cell morphology in SEM of the carrier, which is the result of the mixed culture inocula, as the influent and effluent of DAF was used as inocula to PBBR. Also the addition of wastewater during the fed-batch culture has enriched the microbial consortium. This suggests that various kinds of microorganism with significant population were responsible of different organic compounds degradation.

EPS production by bacteria plays an important role in floc formation, because of EPS's adherent property. Formation and maintenance of microcolonies within the floc is considered to be related with the EPS production. Microcolonies influence the spatial distribution of different microorganisms and also they become the hotspots for microbial-aided organic transformation and elemental cycling. The formation of aggregates also helps in the sequestering of nutrients, trace metals and other essential elements, thereby increasing their accessibility to the microorganism. Because the COD levels in the influent stream to the PBBR are low and highly toxic compounds are present, microorganisms that have grown on the carriers have the ability to tolerate these conditions and metabolize the toxic compounds.

CONCLUSION

We have demonstrated the treatment of refinery effluent with a hybrid UASB-PBBR system. The system operated efficiently up to an OLR of 0.5 kg COD m^{-3} d^{-1} for a period of 118 days. The UASB operation was performed without any sludge flotation and biogas production observed after 56 days of operation, and a complete layer of biofilm was formed on the carriers after 60 days. During this period, the mean COD removal efficiencies in the UASB reactor and the PBBR were 68.5% and 38.3%, respectively, resulting in an overall COD removal efficiency of 82%; the outflow COD was 88.8 mg·l^{-1} from the hybrid system. The GC-MS data showed that the organic substances in the refinery effluent included alkanes ranging from $C_{11}H_{24}$ to $C_{44}H_{90}$ and three polycyclic aromatic hydrocarbons, including naphthalene, phenanthrene and pyrene. The hybrid system performed well in the degradation of alkanes; also, the degradation of PAHs was complete in 30 days of sampling. The TPH removal efficiency in the hybrid system was 100%.

ACKNOWLEDGEMENTS

This study was financially supported by National Iranian Oil Refining and Distribution Company (NIORDC) under Grant number 88-1078. The authors thank Tehran refinery for their kind provision of wastewater.

REFERENCES

1. B. Gargouri, F. Karray, N. Mhiri, F. Aloui, S. Sayadi *J. Hazard. Mater.*, **189**, 427 (2011).
2. H. Baheri and P. Meysami, *J. Hazard. Mater. B*, **89**, 279 (2001).
3. C. E. Cerniglia, *Biodegradation*, **3**, 351 (1992).
4. United States Environmental Protection Agency, *Remediation Technologies Screening Matrix and Reference Guide*, EPA 542-B-93-005 (1993).
5. A. Chavan and S. Mukherji, *J. Hazard. Mater.*, **154**, 63 (2008).
6. R. D. Tyagi, F. T. Tran and A. K. M. M. Chowdhury, *Environ. Pollut.*, **76**, 61 (1992).
7. G. Kassab, M. Halalsheh, A. Klapwijk, M. Fayyad and J. B. van Lier, *Bioresour. Technol.*, **101**, 3299 (2010).
8. A. vanHaandel and G. Lettinga, *Anaerobic Sewage Treatment*, Wiley (1994).

9. M. von Sperling and C. A. L. Chernicharo, *Biological Wastewater Treatment in Warm Climate Regions*, IWA Publishing (2005).
10. J. Berrueta, A. Gutikrrez and G. Fueyo, *J. Chem. Technol. Biotechnol.*, **67**, 302 (1996).
11. G. Liua, Zh. Yea, K. Tong and Y. Zhang, *Biochem. Eng. J.*, **72**, 48 (2013).
12. A. Schellinkhout and E. Osorio, *Long-term experience with the UASB technology for sewage treatment on large scale*, in: *7th Int. Symp. on Anaerobic Digestion*, The Netherlands, 251 (1994).
13. P. Bhunia and M. M. Ghangrekar, *Bioresour. Technol.*, **99**, 2132 (2008).
14. J. Liu, J. E. Zuo, Y. Yang, S. Q. Zhu, S. L. Kuang and K. J. Wang, *J. Environ. Sci.*, **22**, 777 (2010).
15. H. W. Sun, Q. Yang, Y. Z. Peng, X. N. Shi, S. Y. Wang and S. J. Zhang, *J. Environ. Sci.*, **22**, 481 (2010).
16. M. Farhadian, D. Duchez, C. Vachelard and C. Larroche, *Water Res.*, **42**, 1325 (2008).
17. A. R. Pedersen and E. Arvin, *Biodegradation*, **6**, 109 (1995).
18. N. K. Sahoo, K. Pakshirajan and P. K. Ghosh, *J. Hazard. Mater.*, **190**, 729 (2011).
19. J. Paca, M. Halecky, J. Barta and R. Bajpai, *J. Hazard. Mater.*, **163**, 848 (2009).
20. C. C. Teodosiu, M. D. Kennedy, H. A. Van Straten and J. C. Schippers, *Water Res.*, **33**, 2172 (1999).
21. G. C. Jou and G. C. Huang, *Adv. Environ. Res.*, **7**, 463 (1999).
22. W. Sokol, *Biochem. Eng. J.*, **15**, 1 (2003).
23. L. Xianling, W. Jianping, Y. Qing and Zh. Xueming, *Biochem. Eng. J.*, **27**, 40 (2005).
24. F. Ghavipanjeh and J. Shayegan, *IJCHE*, **1**, 3 (2004).
25. O. B. D. Thabet, H. Bouallagui, J. L. Cayol, B. Ollivier, M. L. Fardeau and M. Hamdi *J. Hazard. Mater.*, **167**, 1133 (2009).
26. F. Widdel and R. Rabus, *Curr. Opin. Biotechnol.*, **12**, 259 (2001).
27. F. Aeckersberg, F. Bak and F. Widdel, *Arch. Microbiol.*, **156**, 5 (1991).
28. P. Ehrenreich, A. Behrends, J. Harder and F. Widdel, *Arch. Microbiol.*, **173**, 58 (2000).
29. P. Rueter, R. Rabus, H. Wilkes, F. Aeckersberg, F. A. Rainey, H. W. Jannasch and F. Widdel, *Nature*, **372**, 455 (1994).
30. R. T. Anderson and D. R. Lovley, *Nature*, **404**, 722 (2000).
31. K. Zengler, H. H. Richnow, R. Rosselló-Mora, W. Michaelis and F. Widdel, *Nature*, **401**, 266 (1999).
32. S. Harayama, H. Kishira, Y. Kasai and K. Shutsubo, *J. Mol. Microbiol. Biotechnol.*, **1**, 63 (1999).
33. D. T. Gibson, *Microbial degradation of organic compounds*, New York, Marcel Dekker (1984).
34. S. M. Bamforth and I. Singleton, *J. Chem. Technol. Biotechnol.*, **80**, 723 (2005).
35. Ch. Zhao, *Effect of Temperature on Biogas Production in Anaerobic Treatment of Domestic Wastewater UASB System in Hammarby Sjöstadsverk*, Masters Level Degree Project (2011).
36. Metcalf, Eddy, *Wastewater Engineering: Treatment and Reuse*, Chapter 10, 4th Ed., McGraw-Hill, New York (2004).
37. H. Shim, E. Shin and S. T. Yang, *Adv. Environ. Res.*, **7**, 203 (2002).
38. D. Trzesicka-Mlynarz and O. P. Ward, *Can. J. Microbiol.*, **41**, 470 (1995).
39. W. Stringfellow, M. Alvarez Cohen and L. Alvarez Cohen, *Water Res.*, **33**, 2535 (1999).
40. APHA, AWWA & WPCF, *Standard Methods for the examination of water and wastewater*, 18th Ed. American Public Health Association, Washington, D.C. (1992).
41. D. Z. Maat and L. H. A. Habbets, *Pulp and Paper Canada*, **88**, T410-T414 (1987).