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Post-treatment of anaerobically degraded azo dye Acid Red 18 using aerobic moving bed biofilm process: Enhanced removal of aromatic amines

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ABSTRACT

The application of aerobic moving bed biofilm process as post-treatment of anaerobically degraded azo dye Acid Red 18 was investigated in this study. The main objective of this work was to enhance removal of anaerobically formed the dye aromatic metabolites. Three separate sequential treatment systems were operated with different initial dye concentrations of 100, 500 and 1000 mg/L. Each treatment system consisted of an anaerobic sequencing batch reactor (An-SBR) followed by an aerobic moving bed sequencing batch biofilm reactor (MB-SBBR). Up to 98% of the dye decolorization and more than 80% of the COD removal occurred anaerobically. The obtained results suggested no significant difference in COD removal as well as the dye decolorization efficiency using three An-SBRs receiving different initial dye concentrations. Monitoring the dye metabolites through HPLC suggested that more than 80% of anaerobically formed 1-naphthylamine-4-sulfonate was completely removed in the aerobic biofilm reactors. Based on COD analysis results, at least 65–72% of the dye total metabolites were mineralized during the applied treatment systems. According to the measured biofilm mass and also based on respiration–inhibition test results, increasing the initial dye concentration inhibited the growth and final mass of the attached-growth biofilm in MB-SBBRs.

1. Introduction

Azo dyes are the largest class (60–70%) of synthetic dyes in the textile, food, rubber, plastic, paper, and cosmetic industries [1]. The release of colored wastewaters in the environment even at low concentrations of water soluble azo dyes (10–50 mg/L) not only is a matter for aesthetic point of view, but also leads to the reduction in sunlight penetration diminishing the photosynthesis and oxygen solubility [2]. Moreover, both mutagenic and carcinogenic effects of several azo dyes and their intermediates have been reported so far [3,4].

Various physicochemical processes including electrochemical [5], adsorption [6], chemical coagulation/flocculation [7], advanced oxidation [8] and photocatalysis [9] have been effectively used for treatment of azo dye-containing wastewaters. However, most physicochemical dye removal methods are quite expensive and energy consuming [10] and usually generate large amounts of sludge which require safe disposal [10,11]. These methods also interfere with other wastewater constituents [3] and in some cases generate toxic by-products which are difficult to dispose [2]. Therefore, biological azo dye removal methods as the environmentally friendly and cost-competitive alternatives to the physicochemical degradation processes have been considered in several researches [12–14].

Due to the large degree of complex aromatics present in azo dyes molecules and also strong electron-withdrawing property of the azo group (–N=N–), most azo dyes are recalcitrant to the conventional aerobic treatment [15]. Combined anaerobic–aerobic biological processes have been studied for treatment of wastewaters containing different azo dyes such as monoazo acid orange 7, 8 and 10; hydrolyzed and non-hydrolyzed reactive black 5 and diazo reactive red 141 [16–19]. In the case of azo dye Acid Red 18 (AR18), the previous researches are limited to the study accomplished by FitzGerald and Bishop. They utilized a two stage reactor system which consisted of an anaerobic fixed-film fluidized bed reactor followed by a conventional aerobic reactor for treatment of a wastewater containing low concentration (10 mg/L) of AR18 [20]. In two-stage anaerobic–aerobic processes, the reductive cleavage of the azo bond occurs in the anaerobic stage resulting in formation of the dye aromatic intermediates [4], while further mineralization of these intermediates is expected in the aerobic stage, [3,21]. Although the intermediate metabolites residue from anaerobic degradation of azo dyes can theoretically be mineralized aerobically, previous studies have shown that several aromatic amines

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such as aminobenzene sulfonates and naphthylamine sulfonates are resistant to degradation even under the aerobic conditions [10]. Aside from the mineralization difficulties, the autoxidation of azo dyes breakdown metabolites which leads to the formation of more aerobically recalcitrant products, has been mentioned by some researchers [17,22]. Therefore there is still a need to research on modified biological treatment techniques to achieve complete biodegradation and mineralization of azo dyes and their aromatic metabolites.

The attached-growth biofilm systems have shown to be more drastic than suspended-growth processes for the removal of compounds which are difficult to degrade [23]. It has also been reported that the biofilm cells are more resistant to toxicity than freely suspended ones [24]. Moving bed biofilm reactor (MBBR) as one of the attached-growth biofilm systems was introduced about 15 years ago in order to offer the advantages of former biofilm processes without their limitation including head loss, clogging and hydraulic instability [25]. MBBRs have been efficiently used for treatment of different municipal and industrial wastewaters during the last decade [25–27]. Recently, some reports have been published on successful application of the MBBR process (individually or in combination with other treatment methods) in biodegradation of some aromatic compounds such as aniline [28], phenol [29] and polycyclic aromatic hydrocarbons [30] and also for treatment of wastewaters containing aerobically recalcitrant compounds such as pesticides [31]. This indicates that MBBR can be a good option to be applied as post-treatment of anaerobically degraded azo dyes, which to our knowledge has not been considered so far.

The main objective of the present study was to investigate the performance of moving bed sequencing batch biofilm reactor (MB-SBBR) as post-treatment of anaerobically degraded azo dye Acid Red 18 (AR18) in order to enhance the removal breakdown aromatic amines. For this purpose, the anaerobic–aerobic degradation of the dye was studied and the concentration of 1-naphthylamine-4-sulfonate (1N-4S) as one of the main aromatic constituents of azo dye AR18 was monitored through the applied treatment systems. In addition, the change of attached-growth biofilm mass during the operation period as well as the biofilm morphology was investigated.

2. Materials and methods

2.1. Reactors configuration

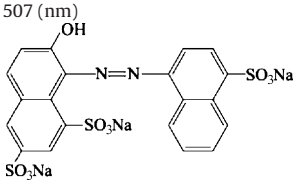
The study was carried out using three separate lab-scale sequential anaerobic–aerobic treatment systems (treatment system 1, 2 and 3). As shown in Fig. 1, each treatment system consisted of an anaerobic sequencing batch reactor (An-SBR) followed by an aerobic moving bed sequencing batch biofilm reactor (MB-SBBR). The reactors were made of plexiglas having an inner diameter of 14 cm and height of 50 cm.

A type of plastic biofilm carrier (2H-BCN017KL, Germany) was used for biomass immobilization in MB-SBBRs. About 50 percent of the effective volume of MB-SBBRs was filled with these carriers and a coarse bubble aeration system made them thoroughly immersed.

2.2. Composition of synthetic wastewater

The composition of synthetic wastewater (SWW) was as follows: glucose (1.5 g/L), lactose (1.5 g/L), urea (116.5 mg/L), KH_2PO_4 (23.3 mg/L), K_2HPO_4 (30 mg/L) and $NaHCO_3$ (1.5 g/L) supplied with different concentration of AR18 including 100, 500 and 1000 mg/L as the feed solution of An-SBR1, An-SBR2 and An-SBR3, respectively. The SWW was prepared in tap water and the chemicals were analytical grade (Merck, Germany). The COD/N/P ratio was adjusted to 100/2/0.3 in the feed solution of An-SBRs. In order to keep the COD/N/P ratio favorable for the aerobic biofilm process, additional nutrients (N and P) were added to the MB-SBBRs at the beginning of each aerobic reaction cycle. The azo dye C.I Acid Red 18 (AR18) was obtained from Alvan Sabet Company (Tehran, Iran) and used without further purification. The general characteristics of the dye AR18 are listed in Table 1.

Table 1
General characteristics of C.I. Acid Red 18 (AR18).

Parameter	Value
Molecular formula	$C_{20}H_{11}N_2Na_3O_{10}S_3$
Molecular weight	604.5 (g/mol)
COD of 1 g-AR18/L	597 ± 17 (mg/L)
λ_{max}	507 (nm)
Chemical structure	

2.3. Operation of lab-scale treatment systems

Granulated anaerobic sludge was obtained from a full scale UASB reactor treating a dairy factory wastewater (Pegah Dairy Company, Tehran, Iran) and used as seed in An-SBRs. The MB-SBBRs were inoculated with the activated sludge taken from a municipal wastewater treatment plant (Zargandeh, Tehran, Iran).

A 24 h operation cycle of both anaerobic and aerobic reactors consisted of five phases including filling, reaction, settling, draw and idle which was controlled by a digital timer. In the filling phase, 2 liters of new SWW were supplied to each An-SBR and the effluent of An-SBRs was passed to the MB-SBBRs by gravity. The complete mixing condition in the anaerobic reactors was provided by a low speed (100 rpm) gear motor driving two paddle-shaped impellers. In MB-SBBRs, an electromagnetic air pump (RESUN; ACO-006, China) was used for supplying air and keeping dissolved oxygen concentration above 3 mg/L during the reaction phase. The main operating parameters of the laboratory treatment systems are listed in Table 2.

2.4. Analytical methods and procedures

2.4.1. Dye and COD analysis

The concentration of the dye was determined by measuring the absorbance of the test samples at the maximum absorbent wavelength of AR18 (λ_{max}: 507 nm) using UV–Vis spectrophotometer (DR 4000, HACH, USA). The limit of detection (LOD) for UV–Vis analysis was 0.3 mg AR18/L. Before the analysis, the samples withdrawn from the treatment systems were centrifuged at 6000 rpm for 10 min. The qualitative information related to the decolorization of AR18 as well as formation of the dye metabolic intermediates was determined by scanning of complete spectrum from 200 to 800 nm. Chemical oxygen demand (COD) analysis was carried out using colorimetric method according to the Standard Methods [32]. All experiments were conducted at room temperature (22 ± 2 °C).

2.4.2. Abiotic adsorption test

To investigate the contribution of abiotic conditions to decolorization, the anaerobic sludge was sterilized in autoclave (20 min at 121 °C and 0.103 MPa). The autoclaved sludge was added to the 500 mL of SWW solution containing 100 mg-AR18/L plus other auxiliary substrates similar to the composition of feed solution in An-SBRs. The mixture was anaerobically incubated for 24 h with

Table 2
Operating parameters of the applied treatment systems.

Parameter	Treatment system 1		Treatment system 2		Treatment system 3	
	An-SBR1	MB-SBBR1	An-SBR2	MB-SBBR2	An-SBR3	MB-SBBR3
Influent dye (mg AR18/L)	100	–	500	–	1000	–
Influent COD (mg/L)	3040 ± 90	–	3337 ± 75	–	3620 ± 105	–
OLR (mg COD/L.d)	1105 ± 33	–	1213 ± 27	–	1316 ± 38	–
Influent BA[a] (mg $CaCO_3$/L)	1163 ± 96	–	1280 ± 107	–	1148 ± 83	–
Effective volume (L)	5.5	5.5	5.5	5.5	5.5	5.5
Fill (min)	20	20	20	20	20	20
Reaction (h)	21	22.5	21	22.5	21	22.5
Settling (h)	2	0.5	2	0.5	2	0.5
Draw (h)	0.5	0.5	0.5	0.5	0.5	0.5
Idle (min)	10	10	10	10	10	10
Hydraulic retention time (d)	2.75	2.75	2.75	2.75	2.75	2.75
Temperature (°C)	35 ± 0.2	22 ± 2	35 ± 0.2	22 ± 2	35 ± 0.2	22 ± 2

[a] BA, bicarbonate alkalinity.

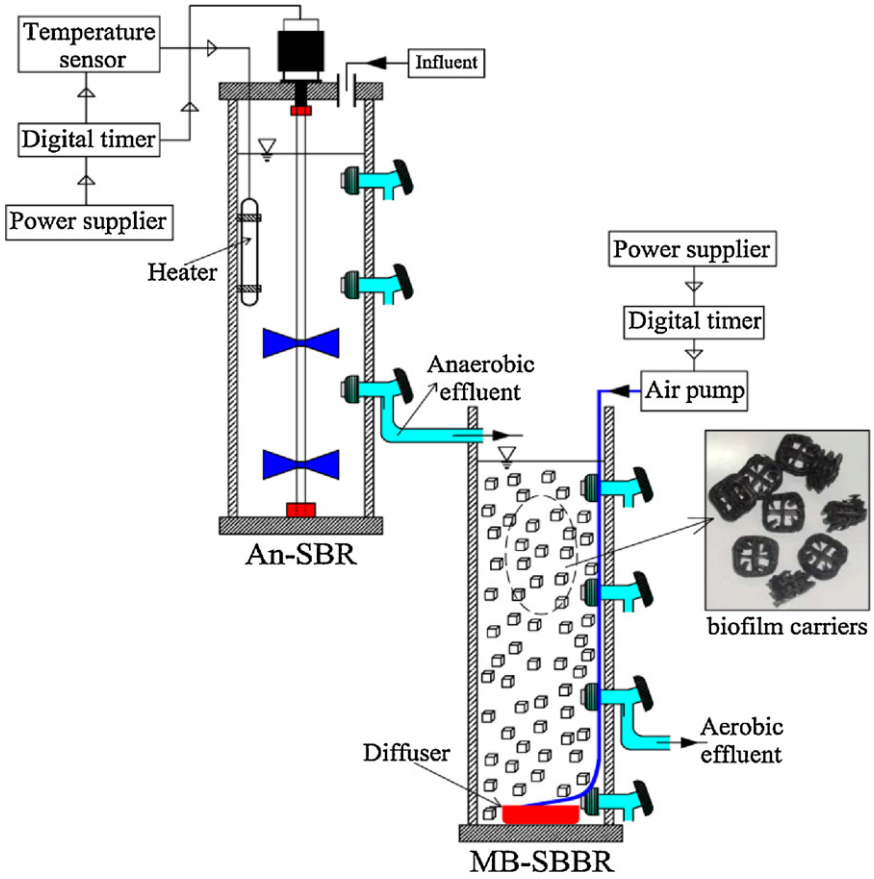


Fig. 1. Schematic of the experimental anaerobic–aerobic treatment systems used in this study.

a constant mixing speed of 100 rpm at 35 °C. After the incubation period, the UV–Vis absorption was used as the measure of abiotic decolorization.

2.4.3. Respiration–inhibition study

The respiration–inhibition test was performed to assess the inhibitory effect of An-SBRs effluent containing the dye intermediates on the activity of aerobic microorganisms in MB-SBBRs. For this purpose, the specific oxygen uptake rate (SOUR) was measured for 400 mL of the mixed liquor samples withdrawn immediately after the start of the aeration phase in MB-SBBRs. The SOUR tests were accomplished according to the procedure outlined in the Standard Methods [32].

2.4.4. Biofilm mass measurement

The mass of attached-growth biofilm in MB-SBBRs was determined after detachment of the biofilm from the surfaces of sample moving carriers (15 pieces) and weighing the dried biomass. The total attached-growth biofilm mass in MB-SBBRs was calculated as:

$$\text{Attached} - \text{growth biofilm mass (mg)} = \frac{n}{15}(m_1 - m_2) \quad (1)$$

where m_1 and m_2 are the mass of the sample carriers before and after the biofilm washout (mg), respectively and n is the total number of the biofilm carriers in each MB-SBBR. All the moving carriers were freely immersed in the whole effective volume of the reactors under complete mixing conditions during the aeration phase.

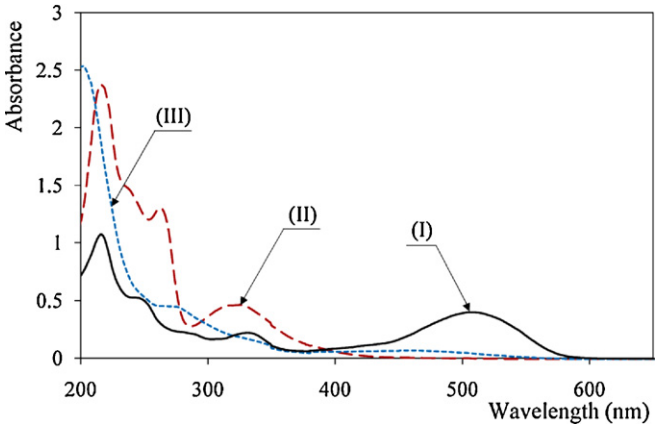


Fig. 2. UV–Vis spectral analysis: An-SBR3 influent (I); An-SBR3 effluent (II) and MB-SBBR3 effluent (III).

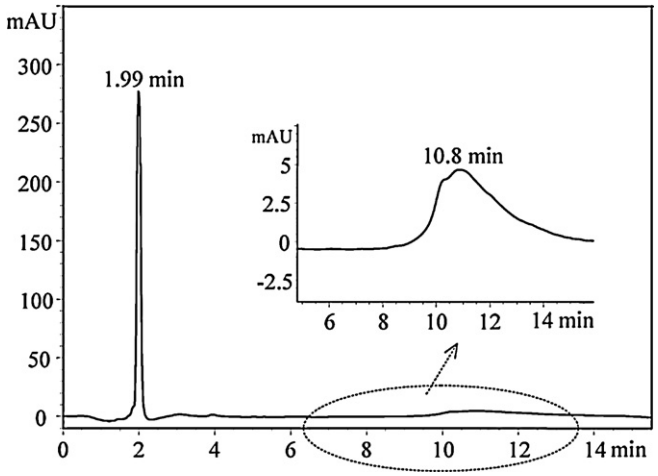


Fig. 3. HPLC chromatogram of the standard solution.

Therefore, the distribution of the attached-growth biofilm and the suspended biomass were uniform in the MB-SBBRs.

2.4.5. High performance liquid chromatography (HPLC)

HPLC analysis was carried out using an Agilent 1200 chromatograph equipped with a variable wavelength detector (190–600 nm). A 5 μm C18 column (1.6 mm × 250 mm) was connected for analytical, reverse-phase separation. The mobile phase was a gradient started with 91% water, 5% acetonitrile and 4% methanol. The gradient changed linearly to 69% water, 27% acetonitrile and 4% methanol over 25 min. The detection was performed at 254 nm. The limit of detection for monitoring the concentration of 1-naphthylamine-4-sulfonate (1N-4S) was 0.06 mg 1N-4S/L.

2.4.6. Scanning electron microscopy (SEM)

In order to observe the attached-growth biofilm morphology, the micrograph images were taken by a digital scanning electron microscope (Philips-XL30; Holland) applying 25 kV accelerating voltage.

3. Results and discussion

3.1. Anaerobic–aerobic degradation of AR18

The general experimental data including the effluent and the bio-sludge properties of the reactors are summarized in Table 3.

According to Table 3, up to 98% of the AR18 decolorization and more than 80% of the COD removal occurred in the An-SBRs. To realize whether the differences observed among three An-SBRs in decolorization and COD removal efficiency are meaningful or not, the experimental data were compared using one-way ANOVA (95% confidence interval). The results (not presented) showed that there was no statistically significant difference among three An-SBRs in the case of decolorization ($P = 0.885$) as well as the COD removal efficiency ($P = 0.136$). Since the AR18 decolorization and also the COD removal efficiency were not affected by the initial dye concentration, it can be inferred that the dye or its breakdown metabolites had no inhibition effects on performance of the An-SBRs.

The complete biological removal of azo dyes occurs in a two-stage anaerobic–aerobic process. As shown in Eq. (2), the first stage (anaerobic) involves the reductive cleavage of the azo bond resulting in production of aromatic compounds which are expected to be mineralized in the second stage (aerobic) [16].

$$4H^+ + 4e^- + R_1 - N = N - R_2 \rightarrow R_1 - NH_2 + R_2 - NH_2 \quad (2)$$

Fig. 2 shows the UV–Vis spectrum of the samples taken from treatment system 3 in which due to the higher initial dye concentration, the peaks are more visible than those of treatment system 1 and 2.

The disappearance of the absorbance peak at 507 nm indicates the complete decolorization of AR18. Considering a selective absorbance decrease only in the visible region and the change of UV–Vis absorption pattern after the treatment process (Fig. 2), it can be concluded that the anaerobic decolorization was caused by the azo bond degradation. The biodegradation of AR18 was also confirmed by the abiotic adsorption test which demonstrated that the dye removal efficiency has been only 5–7% due to the adsorption of the dye into the inactivated anaerobic cells. It is noteworthy that the anaerobic effluents exhibited two strong absorbance peaks at around 263 and 325 nm (Fig. 2), while, the intensity of these peaks was significantly diminished after the aerobic treatment in MB-SBBRs. This indicates the possible ability of the applied biofilm process to decompose the anaerobically formed the dye aromatic metabolites.

3.2. Monitoring of the dye metabolites through HPLC

The HPLC chromatogram of the standard solution containing AR18 (100 mg/L) and 1N-4S (100 mg/L) is presented in Fig. 3. Two peaks are respectively observed at retention times (RT) of 1.99 and 10.8 min related to 1N-4S and AR18.

The results of HPLC analysis of the samples extracted from treatment system 1, 2 and 3 are presented in Figs. 4–6, respectively. It should be noted that in addition to the reactors effluents, the HPLC analysis was also carried out on the samples extracted immediately after the complete anaerobic decolorization of AR18. According to the kinetic study tests, complete anaerobic decolorization was obtained after 8, 13 and 16 h from the start of anaerobic phase in An-SBR1, 2 and 3, respectively. All the HPLC chromatograms of the samples taken from the An-SBRs (Figs. 4a, 5a and 6a) showed a peak with the retention time almost similar to 1.99 min (2.02 min). Accordingly, 1N-4S was identified as one of the dye intermediates formed during the anaerobic AR18 degradation.

The appearance of different peaks in the HPLC chromatograph of the An-SBRs effluent (Figs. 4b, 5b and 6b) as well as the high AR18 decolorization efficiency achieved anaerobically (Table 3) shows that the influent dye was significantly degraded to its metabolic intermediates. In other words, the applied anaerobic process in An-SBRs improved the biodegradability of AR18 for further aerobic treatment by transforming the original dye AR18 to its metabolites that could be mineralized aerobically. The results reported by Sponza and Işik [33] and An et al. [34] also prove the positive effect

Table 3
The general experimental data of the applied treatment systems.

Experimental data[a]	Treatment system 1		Treatment system 2		Treatment system 3	
	An-SBR1	MB-SBBR1	An-SBR2	MB-SBBR2	An-SBR3	MB-SBBR3
Effluent COD (mg/L) ($n=8$)	532 ± 51	17 ± 3	625 ± 31	84 ± 17	672 ± 40	210 ± 30
COD removal (%)	82.5 ± 1.7	16.9 ± 0.1	81.3 ± 0.9	16.1 ± 0.5	81.4 ± 1.1	12.8 ± 0.8
AR18 decolorization (%) ($n=8$) [b]	98.1 ± 1.4	<LOD	98.0 ± 0.6	<LOD	97.9 ± 0.4	<LOD
MLSS (mg/L) ($n=8$)	7646 ± 318	1614 ± 151	8289 ± 301	1567 ± 215	8763 ± 418	1318 ± 249
MLVSS/MLSS (%) ($n=8$)	74.9 ± 3.1	79.3 ± 1.4	72.0 ± 3.3	79.0 ± 1.7	72.5 ± 4.0	76.2 ± 2.2
Effluent pH ($n=8$)	7.5 ± 0.4	8.7 ± 0.2	7.3 ± 0.4	8.6 ± 0.1	7.3 ± 0.3	8.6 ± 0.1
Effluent BA [c] (mg $CaCO_3$/L) ($n=4$)	1113 ± 41	420 ± 32	1246 ± 55	350 ± 42	1088 ± 62	350 ± 54

[a] The average values were determined from the steady-state data obtained during the last 30 days of the operation period.
[b] LOD, limit of detection.
[c] BA, bicarbonate alkalinity.

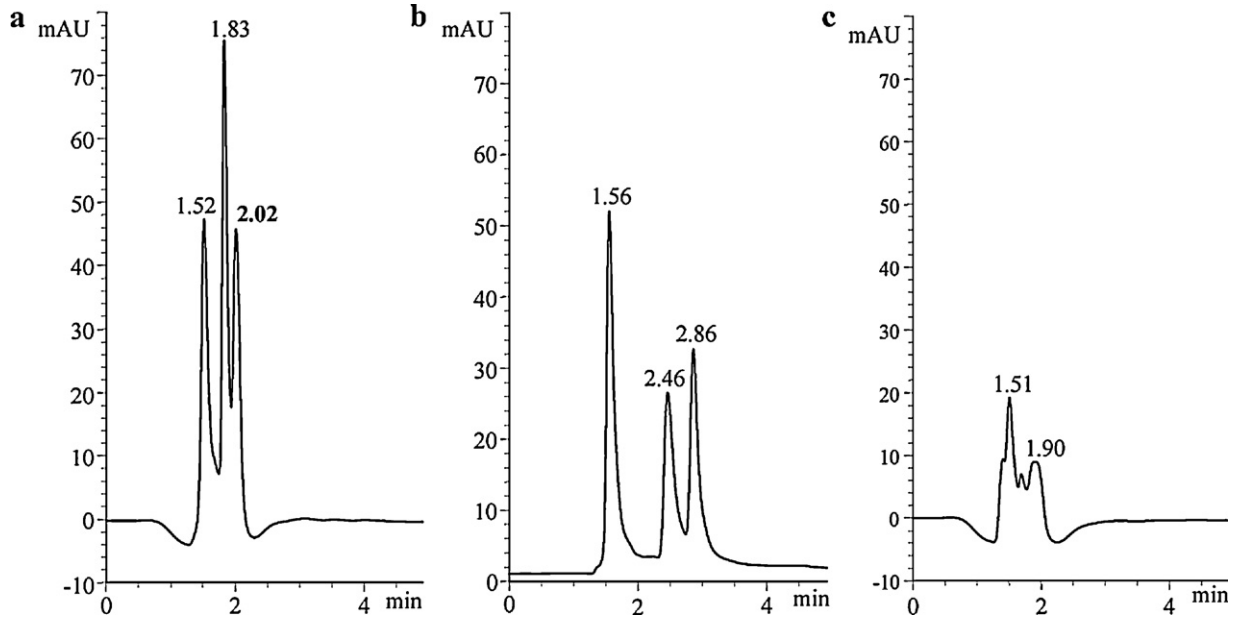


Fig. 4. HPLC chromatograms: (a) 8 h after the start of anaerobic phase; (b) An-SBR1 effluent and (c) MB-SBBR1 effluent.

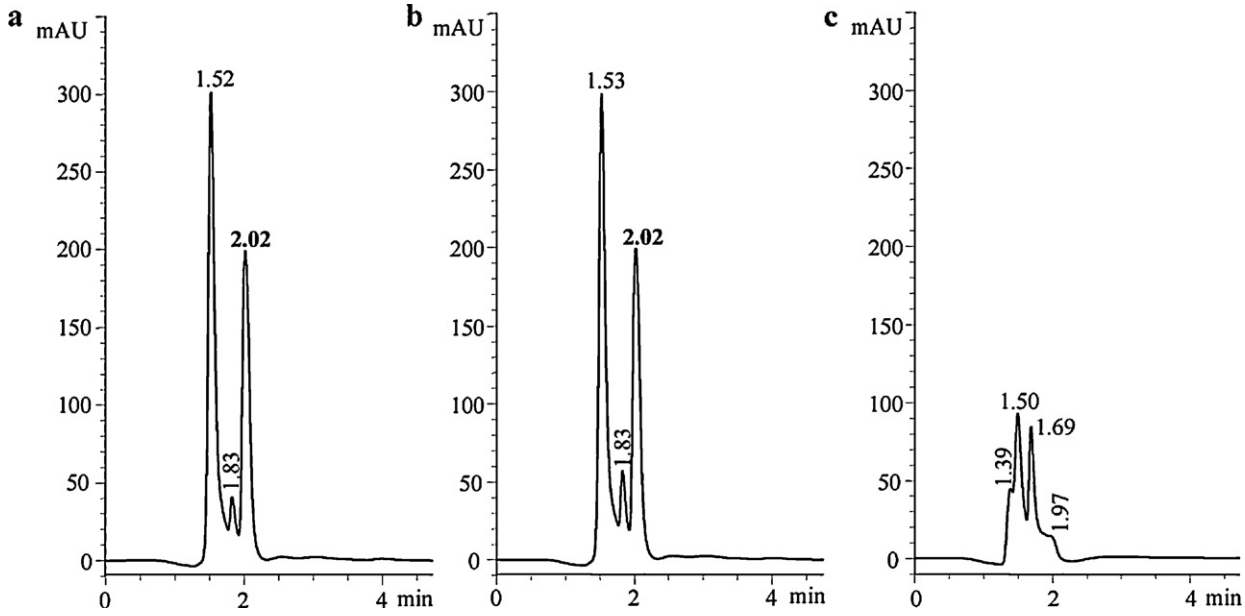


Fig. 5. HPLC chromatograms: (a) 13 h after the start of anaerobic phase; (b) An-SBR2 effluent and (c) MB-SBBR2 effluent.

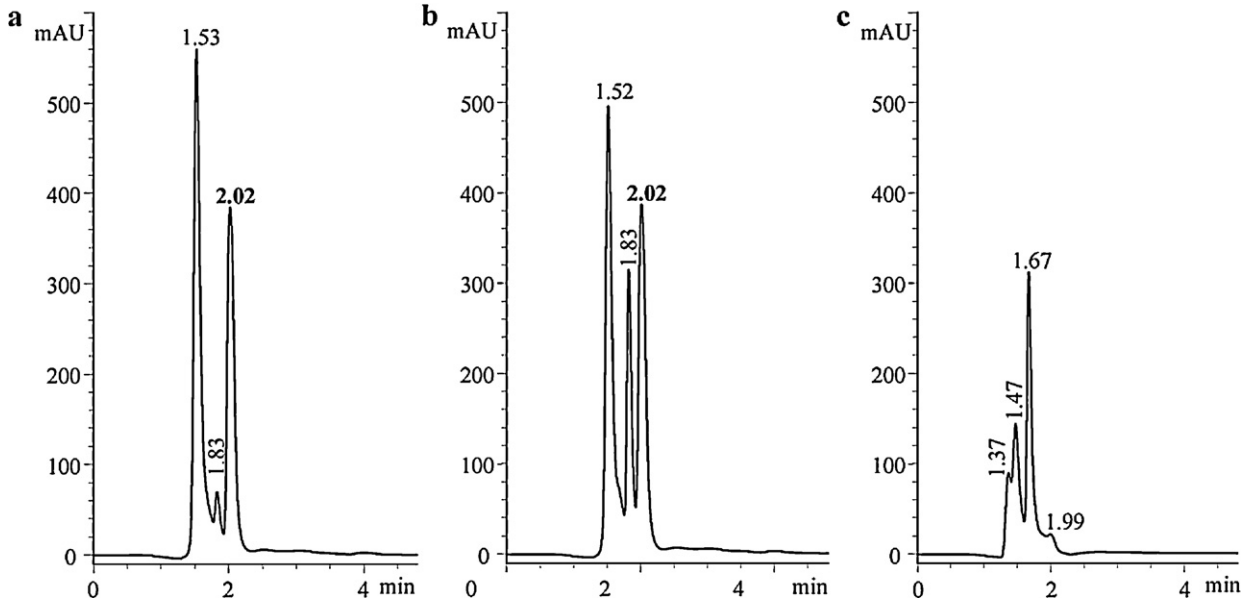


Fig. 6. HPLC chromatograms: (a) 16 h after the start of anaerobic phase; (b) An-SBR3 effluent and (c) MB-SBBR3 effluent.

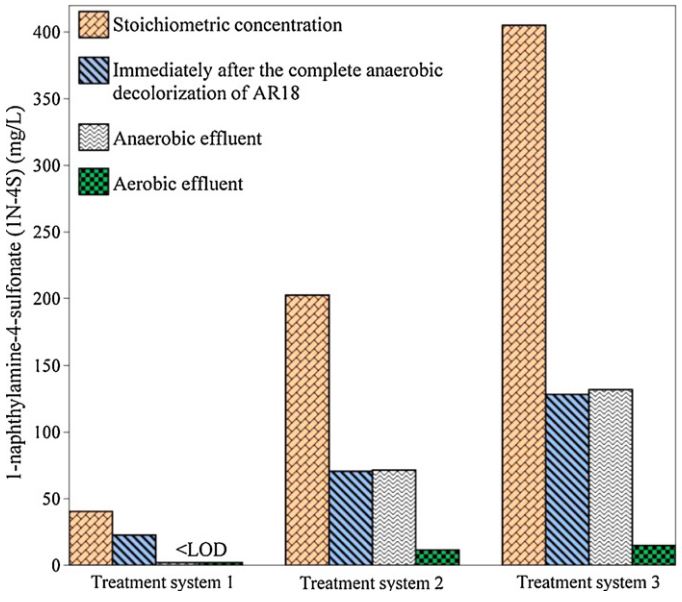


Fig. 7. Change in 1N-4S concentration during the applied anaerobic–aerobic treatment systems.

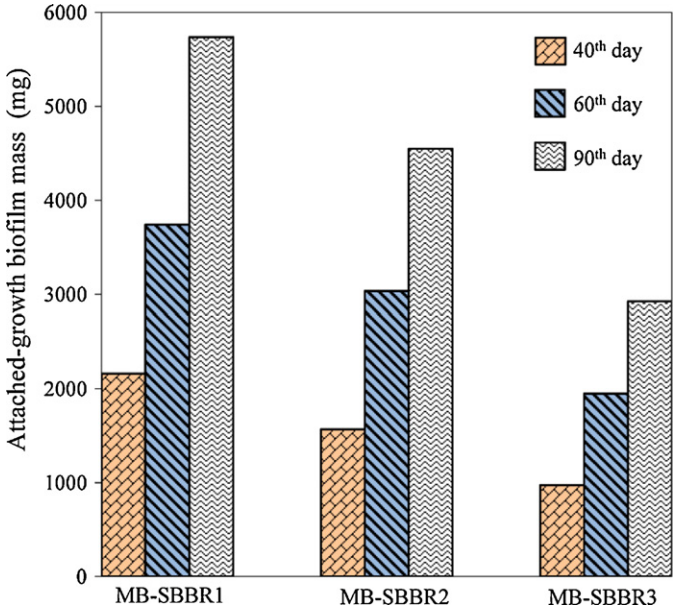


Fig. 8. Attached-growth biofilm mass in MB-SBBRs.

of the anaerobic process on the aerobic biodegradability of some azo dyes.

The similarity of the chromatographic peak area corresponding to 1N-4S (RT: 2.02 min) in Fig. 5a and b and also in Fig. 6a and b, reveals that the anaerobic process had no effect on further biodegradation of 1N-4S in An-SBR2 and An-SBR3. However, in the case of An-SBR1 with the lower influent dye concentration (100 mg/L), 1N-4S was no longer present after the complete anaerobic cycle (Fig. 4b). The anaerobic removal of 1N-4S in An-SBR1 could be due to the almost long anaerobic hydraulic retention time (HRT = 2.75 day) and low concentration of 1N-4S in An-SBR1 compared to those of An-SBR2 and An-SBR3. The HPLC analysis also reveals that after the aerobic treatment, the chromatographic peak area decreased and shifted to the lower retention times (Figs. 4c, 5c and 6c). This finding indicates the formation of less aromatic and more polar compounds during the treatment process in MB-SBBRs. The similar results has also been reported by other study groups [11,19].

Stoichiometrically, the complete reduction of the azo bond with the assumption of no anaerobic degradation of aromatic amines, leads to the accumulation of 40.5, 202.6 and 405.3 mg/L of 1N-4S in An-SBR1, 2 and 3, respectively. The change in 1N-4S concentration during the anaerobic–aerobic treatment systems which was determined from the developed area–concentration curve is shown in Fig. 7.

Fig. 7 proves that the MB-SBBRs were dramatically able to mineralize 1N-4S. The concentration of 1N-4S in the effluent of MB-SBBR1, 2 and 3 were below than detection limit (0.06 mg/L), 11.5 mg/L and 14.9 mg/L resulting in almost 100, 83.9 and 88.7% of 1N-4S removal efficiency, respectively. Contrary to the several studies which reported difficulties with the conventional aerobic decomposition of sulfonated aromatic amines [3,10,35], in this

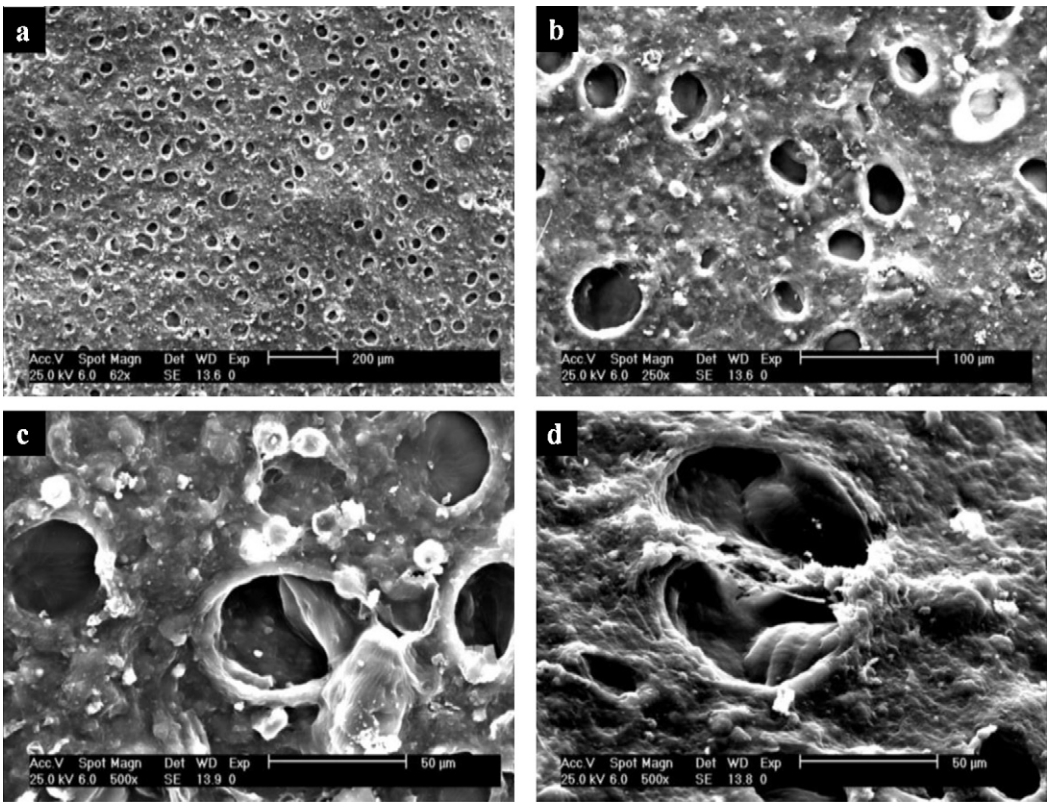


Fig. 9. SEM micrographs of 80-day old biofilm grown on surfaces of the biofilm carriers.

study, a considerable removal efficiency of 1N-4S was obtained even at high initial dye concentration (1000 mg/L).

Since the authentic standard was available just for 1N-4S, the other anaerobically formed intermediates could not be quantified through the HPLC analysis. However, as described by Libra et al. [17], the overall mineralization of the azo dyes metabolites can be followed by using lumped parameters such as dissolved organic carbon (DOC), total organic nitrogen (TON), etc. In this regard, considering the average COD concentration of the MB-SBBRs effluent and the COD concentration of AR18 (597 mg-COD/L for 1000 mg/L of the dye solution), it is inferred that at least 71.5, 71.9 and 64.8% of the dye metabolites were completely mineralized using treatment system 1, 2 and 3, respectively. Comparison of the overall peak area among the HPLC chromatogram of the An-SBRs effluent to that of the MB-SBBRs effluent, reveals that almost 59.9, 51.7 and 50.1% of the total dye metabolites remaining in the effluent of An-SBRs were removed in MB-SBBR1, 2 and 3, respectively.

3.3. Bioaccumulation of attached-growth biofilm

The attached-growth biofilm mass in MB-SBBRs which was evaluated in the 40th, 60th and 90th day of the operation are compared in Fig. 8. It is evident that as the reactors operation progressed, the biofilm mass increased in all three MB-SBBRs. It is also obvious that the biofilm mass decreased with increasing the initial dye concentration. The ratio of the attached-growth biofilm concentration to the suspended biomass concentration in the MB-SBBRs was also increased as the reactors operation progressed and reduced as the initial dye concentration increased. This ratio was 0.76, 0.7 and 0.51 at 90th day of the operation in MB-SBBR1, 2 and 3, respectively.

Since all the parameters except the initial dye concentration were the same for all three treatment systems, the reduction in biofilm growth is most likely due to the toxicity effects of the dye metabolites which was increased with increasing the initial AR18 concentration. This finding is in agreement with those reported by Asad et al. [2] in which the reduction in cell growth was observed during the decolorization of azo dye Remazol Black B. In another study carried out by Renganathan et al. [12], the inhibitory effect of AR18 at higher dye concentrations on growth and final concentration of White rot fungus S. has been observed. In our study, the inhibitory effect of the dye metabolites on the microorganisms' activity was also confirmed by the respiration–inhibition test. The SOUR values were determined 31.9 ± 3.6, 18.0 ± 4.9 and 16.9 ± 2.7 mg O_2/g VSS/h for the mixed liquor withdrawn from MB-SBBR1, 2 and 3, respectively.

3.4. Biofilm morphology

Biofilm usually contains three-dimensional structures composed of interstitial voids, channels, cell clusters and extracellular polymeric substances (EPS) [36,37]. The SEM photographs of the 80-day old attached-growth biofilm are presented in Fig. 9 where the micro pores and channels (15–50 μm in diameter) are clearly visible. Areal porosity of a biofilm section is defined as the ratio of the porous area to the total area of the section [38]. In our study, the areal porosity of the biofilm was determined 15–25% with an image analysis of Fig. 9a. As noted by Villena et al. [37], the advantage of a channeled structure in comparison with the non-porous one is that it allows fluids to pass through, enhancing mass transfer [37]. Therefore, one reason for significant removal efficiency of the dye intermediates achieved in MB-SBBRs could be the high percentage of porosity present in the biofilm structure (Fig. 9).

4. Conclusions

In this study, up to 98% of AR18 decolorization and more than 80% of COD removal efficiency occurred anaerobically. Based on statistical analysis, there was no significant difference among three

An-SBRs in the case of AR18 decolorization as well as the COD removal efficiency. The aerobic moving bed biofilm process applied in this study has shown to be efficient in elimination of aromatic amines formed through the anaerobic degradation of AR18. More than 80% of the anaerobically formed 1N-4S was removed in all three MB-SBBRs. The COD analysis results showed that at least 71.5, 71.9 and 64.8% of the dye total metabolites were completely mineralized through the treatment system 1, 2 and 3, respectively. The attached-growth biofilm mass measurement as well as the respiration–inhibition test proved the inhibitory effect of the dye metabolites on the microorganisms' activity in MB-SBBRs.

The outcome of present study showed that the applied moving bed sequencing batch biofilm reactor was highly efficient in the removal of aromatic amines formed during the anaerobic degradation of azo dye AR18.

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