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Anthraquinone compounds as redox mediators for enhanced continuous-flow anaerobic biotransformation of reactive dyes under hypersaline conditions

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ABSTRACT

The effect of anthraquinone disulfonate (AQDS) on the decolorization of the azo dye Reactive Red 198 (RR198) and the anthraquinone dye Reactive Blue 4 (RB4), as well as the effect of RB4 on the decolorization of RR198, both AQDS and RB4 serving as redox mediators, was assessed. An anaerobic, continuous-flow, fluidized-bed bioreactor with an immobilized halotolerant mixed culture was used in this study. All tests were performed at a reactor temperature of 35°C, a hydraulic retention time of 6.1 h, pH 7.2±0.1, with glucose used as the carbon and electron source. AQDS at relatively low influent concentrations (10–150 μM) enhanced the extent of RR198 reduction, but had a negligible effect on the biodecolorization of RB4. At an equal concentration of 50 μM, RB4 and AQDS had a similar enhancing effect on the biodecolorization of the azo dye RR198. Therefore, anthraquinone dyes can serve as redox mediators in mixed, spent textile dyebaths potentially leading to enhanced reductive biodecolorization rate and extent of other types of dyes (i.e., azo dyes).

Keywords: Textile dyes; Decolorization; Redox mediators; Fluidized-bed bioreactor; Salt

1. Introduction

Reactive dyes, which are used to dye cellulosic fibers (i.e., cotton), are an important class of textile dyes. The most widely used reactive dyes are azo and anthraquinone dyes [1,2]. Under typical reactive dyeing conditions (pH ≥ 10, temperature ≥ 60°C, salt 60–100 g/L), up to 50% of the initial dye remains in the spent dyebath in its hydrolyzed form [2,3]. The hydrolyzed dye no longer has an affinity for the fabric, and therefore cannot be reused in the dyeing process, resulting in colored effluent [2,4]. Discharge of dyes into the environment should be avoided, not only for aesthetic reasons, but also because many reactive dyes and their breakdown products are toxic [5,6]. In addition to the presence of dye, the high salt concentration further complicates the management of spent reactive dyebaths.

Reactive dyes are persistent under aerobic conditions, but under anaerobic conditions they undergo reductive microbial decolorization [4,7–9]. Given the relatively high molecular weight of the dyes, their reductive biodecolorization has been attributed to a microbially mediated process that channels reducing equivalents from a biodegradable carbon and energy source to the dyes, which serve as electron acceptors in a non-energy yielding, and thus non-growth-related, process [10–12]. The reductive decolorization of azo and anthraquinone dyes takes place by the two-step reductive cleavage of the azo bond and formation of the corresponding amines [13,14]

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and the two-step reduction of the anthraquinone moiety to dihydroxyanthracene, respectively [15,16]. Although a substantial number of reports have documented the microbial reductive decolorization of reactive azo and anthraquinone dyes, very limited research dealing with microbial decolorization under hypersaline conditions has been conducted.

Successful, batch microbial reductive decolorization of reactive azo and anthraquinone dyebaths by a suspended-growth, halotolerant enrichment culture under hypersaline conditions has been reported [4,9,14,17]. However, application of the suspended-growth culture for the treatment of spent reactive dyebaths at a commercial scale is limited because it requires biomass separation before reusing the decolorized solution. Biofilm-based reactors, such as continuous-flow anaerobic fluidized bed reactors (AFBR) have several significant advantages over batch suspended-growth reactors, such as higher decolorization rates and lower effluent biomass concentration, thus leading to a higher quality of decolorized effluent which can then be considered for reuse in the dyeing operation.

Consistent with previous reports [18,19], work conducted in our laboratory has shown that reductive biodecolorization of reactive dyes with triazinyl reactive groups is slow and requires relatively long residence times [11,12,17]. In order to increase the rate and extent of reductive decolorization, various redox mediators, which shuttle reducing equivalents from external electron donors to dyes, and which in turn serve as electron acceptors, have been applied [10,19–24]. A recent review summarizes the beneficial effect of redox mediators for the reductive (bio)transformation of organic and inorganic contaminants, among them dyes [25]. When a continuous-flow AFBR was used, the decolorization efficiency of the azo dye Reactive Red 2 (RR2) at an influent concentration of 1,000 mg/L increased from 83.4 to 99.8% with increasing concentrations from 0 to 50 μM of anthraquinone disulfonate (AQDS), a known redox mediator [17].

Although azo dyes represent approximately 60% of all reactive dyes used by the textile industry, other classes of reactive dyes, namely anthraquinone and to a lesser degree phthalocyanine dyes, are extensively used either as primary or secondary dyes in commercial di- and trichromatic dyeing formulations [1,26]. Thus, it is expected that spent commercial dyebaths, and therefore textile wastewaters, contain two or more classes of reactive dyes. However, the potential beneficial effect of dye mixtures on the decolorization efficiency of commercial dyebaths and wastewaters has not been explored sufficiently. In the present study, when the continuous-flow AFBR was operated at a high influent azo dye concentration and short hydraulic retention time (HRT) values, the use of redox mediators was evaluated for possible enhancement of the decolorization of reactive dyes with a triazinyl reactive group. The objective of the research reported here was to investigate the use of AQDS as a redox mediator for the decolorization enhancement of the anthraquinone dye Reactive Blue 4 (RB4) and the azo dye Reactive Red 198 (RR198), as well as the effect of RB4, serving as a redox mediator, on the biodecolorization of RR198.

2. Materials and methods

2.1. Chemicals

Two commercial quality dyes were used in this study: a) RR198, a monoazo, bifunctional dye with both a vinyl sulfonyl and a monochlorotriazinyl reactive group; and b) RB4, an anthraquinone dye with a dichlorotriazinyl reactive group (Fig. 1). Both dyes were obtained from a textile dyeing plant (Washington Manufacturing, Washington, GA, USA) and used without any purification. 9,10-anthraquinone-2,6-disulfonic acid disodium salt (AQDS) was obtained from Sigma-Aldrich Chemical (Milwaukee, WI, USA) and used as a redox mediator model compound without additional purification. Spectrophotometric scanning of dilute dye solutions was performed and absorbance maxima were identified. The pH, chemical oxygen demand (COD), and carbon content of dye and AQDS solutions were determined (Table 1). Before use, solutions of both dyes were hydrolyzed (i.e., reacted) and then used after pH adjustment to neutral. Preparation of hydrolyzed dyes was based on the simulation of typical textile dyebath conditions as previously reported [11,27].

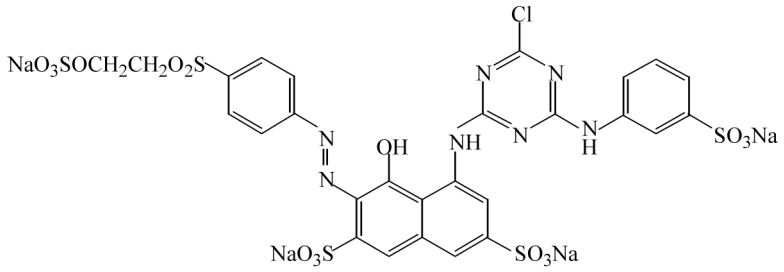
2.2. Continuous-flow bioreactor

A continuous-flow, up-flow AFBR system, developed using a suspended-growth halotolerant mixed culture as inoculum was used in this study. The AFBR was fed continuously with an inorganic feed and an organic (i.e., glucose) feed. The inorganic salt feed solution contained culture media [4], 4 mg/L yeast extract, 3.75 g/L $NaHCO_3$, and 100 g/L NaCl. The reactor was maintained at 35°C with a HRT value of 6.1 h. The glucose and salt loading rate was 776 mg glucose/L-d and 393 g NaCl/L-d, respectively. The specific biomass concentration in the AFBR after over three years of operation under anaerobic hypersaline conditions was 58.7 ± 2.9 mg volatile solids/g support media. Details on the development and operation of the AFBR system have been reported before [4,9,14,17].

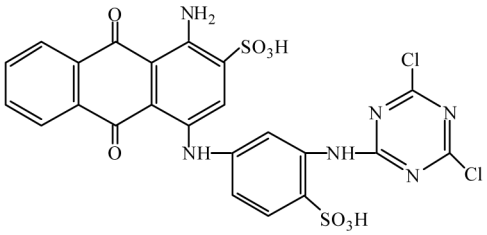
2.3. Redox mediators and decolorization runs

After the long-term operation of the AFBR without any dye and AQDS, in order to assess any potential inhibition by RB4, the reactor was operated with an influent RB4 concentration of 1,000 mg/L for at least five HRTs until the reactor reached steady-state (control run). Then, the effect of AQDS on the decolorization of RB4 at an influent dye concentration of 1,000 mg/L was tested with AQDS at an influent concentration ranging from 10 to 150 μM.

C.I. Reactive Red 198 (RR198)



C.I. Reactive Blue 4 (RB4)



9,10-Anthraquinone-2,6-Disulfonic Acid Disodium Salt (AQDS)

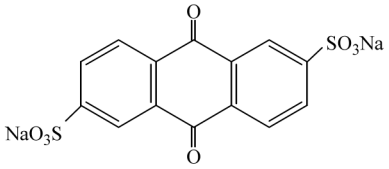


Fig. 1. Chemical structures of the two reactive dyes and AQDS used in this study.

Table 1
Results of characterization of the selected reactive dyes and AQDS

Parameter	RR198	RB4	AQDS
Molecular weight[a]	967.5	637.4	412.3
λ_{max} (nm)	516	598	326
Normalized absorbance (mA units per mg/L dye)	14.9	8.6	14.1
pH[b]	5.6	6.6	6.1
COD (mg/L)[c]	610	849	NM[e]
Carbon content (% dry weight)	11.7[d] (33.5)[a]	29.6[d] (43.3)[a]	(40.8)[a]

[a] Based on the following formulae: RR198, $C_{27}H_{18}O_{15}N_7S_5ClNa_4$; RB4, $C_{23}H_{14}O_8N_6S_2Cl_2$; AQDS, $C_{14}H_6O_8S_2Na_2$
[b] Unhydrolyzed, 50 mg/L dye solution
[c] Chemical oxygen demand value of a 1,000 mg/L unhydrolyzed dye solution
[d] Measured experimentally
[e] NM, not measured

In order to test the effect of anthraquinone compounds as redox mediators on the decolorization of the azo dye RR198 at an influent dye concentration of 300 mg/L, the reactor influent was amended with 10 and 50 μM AQDS, and then with RB4 at an influent concentration of 30, 100, and 300 mg/L, corresponding to 50, 167, and 500 μM of hydrolyzed RB4 (MW = 600.5 g/mole) [27]. Between each set of runs with RB4 or with RR198, the AFBR was operated for at least ten HRTs at the baseline condition (HRT of 6.1 h, 776 mg glucose/L-d, 393 g NaCl/L-d, and 35°C) without any redox mediator amendments until the reactor's performance was stabilized and reached steady-state. For all runs, the reactor was operated for at least four HRTs until the reactor reached a new steady-state condition. In all runs, when the inorganic salt media and glucose were mixed in the reactor influent line, the resulting glucose concentration in the reactor feed was 196 mg/L. The feed glucose, inorganic constituents and yeast extract loading rate (see section 2.2, above) were kept constant during all runs in order to achieve similar culture activity levels under all conditions.

Dye, decolorized dye products, glucose, acetate, and dissolved organic carbon (DOC) concentrations in the reactor effluent, as well as pH and oxidation-reduction potential (ORP) of the reactor contents were monitored. The ORP values were reported directly as measured by

the instrument (platinum electrode with a Ag/AgCl reference electrode). To obtain ORP values with reference to the standard hydrogen electrode (SHE), a correction factor of +220 mV must be added to the reported ORP values. For all runs, glucose was not detected in the AFBR effluent and the only detected volatile fatty acid (VFA) resulting from glucose fermentation was acetic acid. GC and HPLC analyses confirmed that there was no production of citrate, malate, pyruvate, succinate, lactate, fumarate, propionate, butyrate, valerate, methanol, and ethanol via conversion of the glucose in the AFBR. Methane production in the AFBR was not observed and the total gas production was negligibly low.

2.4. Analytical methods

ORP, pH, volatile solids (VS), and volatile suspended solids (VSS) analyses were carried out in accordance with Standard Methods [28]. VFAs, DOC, glucose, total gas production, carbon dioxide and methane analyses were performed following previously reported procedures [11,12]. All spectrophotometric analyses were carried out using an HP 8453 UV-Visible, diode array spectrophotometer. All color data were recorded as an absorbance at the characteristic wavelength (598, 516, 485, and 362 nm for RB4, RR198, reduced RB4, and reduced RR198, respectively)[11]. Non-reduced dye concentrations are based on dye calibrations (i.e., absorbance at the maximum wavelength versus mass concentration correlations). The absorbance of dye mixtures was approximately the additive absorbance of the individual dyes and their decolorization product(s). The concentrations of RR198, RB4 and their decolorizatiion products (i.e., reduced RB4 and reduced RR198) were calculated by the use of a system of simultaneous linear equations based on absorbance measurements at the characteristic maximum absorbance wavelengths corrected for interferences.

3. Results and discussion

3.1. Effect of AQDS on the extent of RB4 decolorization

At an influent RB4 concentration of 1,000 mg/L, the steady-state RB4 concentration in the AFBR effluent was 528±1, 520±8, 584±5, and 636±4 mg/L at an influent AQDS concentration of 0, 10, 50, and 150 μM, respectively (Fig. 2). Therefore, the extent of RB4 decolorization was 47.2% without AQDS amendment and 48.0% at an influent AQDS concentration of 10 μM. Further increase in the influent AQDS concentration to 50 and 150 μM adversely affected the RB4 decolorization and resulted in a RB4 decolorization extent of 41.6 and 36.4%, respectively. The effluent RB4 reduction product(s) measured at 485 nm did not vary significantly (between 1.4 and 1.5 AU) as the influent AQDS concentration increased (Fig. 2). Therefore, addition of AQDS to the AFBR influent did not improve the extent of RB4 decolorization, but rather resulted in a slight decrease. It should be noted that the capacity of the microbial consortium to reduce AQDS was not measured prior to the decolorization runs. However, the observed enhancement of RR198 decolorization with increasing influent AQDS concentrations (see Section 3.2, below) is at least partially attributed to reduction of AQDS to anthrahydroquinone disulfonate (AHQDS), which in turn served as electron donor.

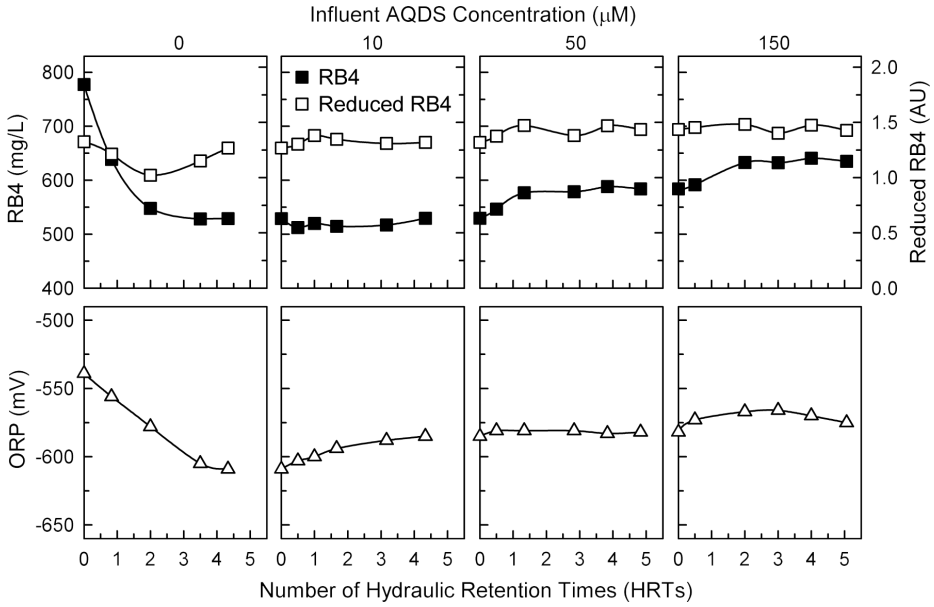


Fig. 2. Effect of influent AQDS concentration on the extent of RB4 decolorization by the AFBR (Influent RB4 concentration, 1,000 mg/L; HRT, 6.1 h)(The reactor was maintained for over four HRTs at each influent AQDS concentration).

The AFBR pH was 7.2±0.2 at all influent AQDS concentrations, but the ORP value increased from –597±17 to –570±5 mV as the influent AQDS concentration increased from 0 to 150 μM. It is noteworthy that the ORP values in the AFBR at all influent AQDS concentrations were slightly more positive than the reported AQDS midpoint potential (SHE) of –384 mV at 25°C (equal to –604 mV with Pt vs. Ag/AgCl)[29], indicating incomplete reduction of AQDS occurred in the AFBR. Therefore, the presence of AQDS in the reactor influent created a less reduced environment in the AFBR. Similar to our results, Lazlo [30] observed an increase in the ORP value of a *Burkholderia cepacia* culture amended with increasing anthraquinone-2-sulfonate (AQS) concentrations up to 2.5 mM. The observed decrease in the extent of RB4 decolorization with increasing influent AQDS concentration is more likely the result of the observed increase in the reactor ORP. These results indicate that an increase of the influent AQDS concentration in the presence of a relatively high concentration of an anthraquinone dye did not enhance the extent of decolorization.

As shown in Table 2, in the absence of AQDS in the reactor influent, the influent total DOC was 329.6 mg/L and increased with increasing AQDS influent concentrations. The extent of DOC removal was 47.8, 47.6, 45.9, 41.4% at an influent AQDS concentration of 0, 10, 50, and 150 μM, respectively. Therefore, a decrease of DOC removal was observed with increased influent AQDS concentration, indicating that AQDS was not biodegraded under the anaerobic hypersaline conditions of the AFBR system. Acetate was the only fermentation product measured, and on a COD basis corresponded to 22.7, 22.3, 22.2, and 23.0% of the influent glucose at influent AQDS concentrations of 0, 10, 50, and 150 μM, respectively. Therefore, the amount of glucose converted to acetate was almost the same at all influent AQDS concentrations, suggesting that the glucose fermentation pattern was not altered with increasing influent AQDS concentrations up to 150 μM.

3.2. *Effect of AQDS on the extent of RR198 decolorization*

The effect of influent AQDS concentration on the decolorization of the azo dye RR198 at an influent dye concentration of 300 mg/L under hypersaline conditions was investigated at influent AQDS concentrations of 0, 10, and 50 μM. The pH in the reactor was 7.2±0.1 at all influent AQDS concentrations. The reactor performance at each of the influent AQDS concentrations in terms of effluent RR198 and absorbance at 362 nm of reduced RR198 products, as well as the reactor ORP, are shown in Fig. 3. The corresponding mean RR198 extent of decolorization at influent AQDS concentrations of 0, 10, and 50 μM was 86.1±0.9, 90.0±0.9, and 91.5±0.3%, respectively. The absorbance of the RR198 reduction product(s) at 362 nm significantly increased from 1.10±0.06 to 1.97±0.11 AU when the influent AQDS concentration increased from 0 to 50 μM. Therefore, in contrast to the results obtained with the anthraquinone dye RB4, as the influent AQDS concentration increased, the RR198 decolorization extent increased. The ORP value in the AFBR decreased from –493±4 to –547±1 mV as the influent AQDS concentration increased from 0 to 50 μM, respectively (Fig. 3). Therefore, similar to our previous study with the azo dye RR2 [17], the presence of AQDS in the reactor influent created a significantly more reduced environment in the AFBR, which is not consistent with the above-discussed results on the effect of AQDS on the ORP and the decolorization of the anthraquinone dye RB4 at 1000 mg/L. Therefore, a relatively small increase of the AQDS concentration in the absence of any other anthraquinone compounds resulted in an enhancement of the azo dye reduction. RR198 spectra in the media feed solution and in the AFBR effluent at an influent AQDS concentration of 0 and 50 μM showed a shift of the visible absorbance maxima to between 340 and 380 nm, which is attributed to the respective dye decolorization product(s), and the absorbance at this wavelength region increased with increased influent AQDS concentration (Fig. 4). Therefore, the use of the redox mediator AQDS significantly increased the extent of azo dye decolorization in the continuous flow AFBR system.

The role of redox mediators during the reduction of azo dyes has been studied by several researchers. Similar to the present study, Beydilli [17] reported that the aniline concentration in the AFBR effluent resulting from the reduction of azo dye RR2 increased when the

Table 2
Effect of influent AQDS concentration on DOC removal by the AFBR operated at an influent RB4 concentration of 1,000 mg/L

AQDS (μM)	Influent DOC (mg/L)			Effluent DOC (mg/L)		DOC Removal (%)
	RB4[a]	Glucose	Total	Acetate	Total	
0	251.2	78.4	329.6	17.8	172.0	47.8
10	261.3	78.4	339.7	17.5	177.9	47.6
50	267.0	78.4	345.4	17.4	186.8	45.9
150	282.8	78.4	361.2	18.0	211.8	41.4

[a] Values of the inorganic feed solution with RB4 and amendments of AQDS

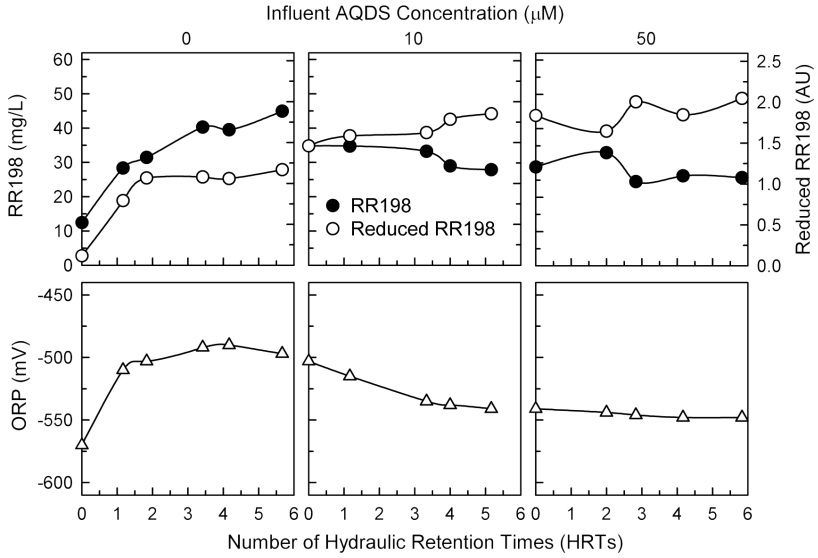


Fig. 3. Effect of influent AQDS concentration on the extent of RR198 decolorization by the AFBR (Influent RR198 concentration, 300 mg/L; HRT, 6.1 h)(The reactor was maintained for over five HRTs at each influent AQDS concentration).

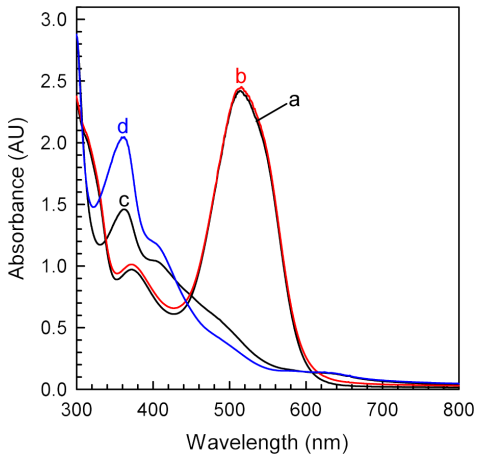


Fig. 4. Spectra of RR198 in the media feed solution (a and b) and in the AFBR effluent (c and d) at different influent AQDS concentrations (Spectra a and c without AQDS amendment; spectra b and d with an influent AQDS concentration of 50 μM) (Influent RR198 concentration, 300 mg/L; HRT, 6.1 h).

influent AQDS concentration was increased. Kudlich and co-workers [10] investigated the effect of different redox mediators on the reduction of the azo dye amaranth by whole cells of *Sphingomonas* sp. strain BN6. A 6- to 10-fold increase in the azo reduction rate was observed with the addition of 100 μM of 2-anthraquinone sulfonate (AQS), AQDS, and 2-hydroxy-1,4-naphthoquinone in solutions containing 0.5 mM amaranth. The enhancement of azo dye decolorization rate by the addition of AQDS in a lab-scale continuous-flow UASB reactor was reported by van der Zee et al. [19]. AQDS addition at an influent concentration of 19 μM resulted in a rapid improvement of the reactor performance and the RR2 decolorization efficiency increased to 85%, with a methanogenic VFA conversion efficiency of 95%. A summary of studies conducted on the beneficial effect of redox mediators on dye decolorization was recently published by van der Zee and Cervantes [25].

The effect of influent AQDS concentration on DOC removal is summarized in Table 3. At all influent AQDS concentrations, glucose was not detected in the reactor effluent and acetate was the only fermentation product detected in the reactor effluent. At an influent RR198 concentration of 300 mg/L and 50 μM AQDS, only 4.3% of the electron equivalents available in the form of glucose were utilized for the observed degree of RR198 decolorization, while 24.0% of the available electron equivalents were converted to acetate. Therefore, the influent glucose concentration in the AFBR was much higher than that required for the complete decolorization of 300 mg/L RR198 at an influent AQDS concentration up to 50 μM. The mean DOC removal at influent AQDS concentration of 0, 10, and 50 μM was 40.2, 41.5, and 42.3%, respectively. Therefore, DOC removal was almost the same at all influent AQDS concentrations.

3.3. *Effect of RB4 on the extent of RR198 decolorization*

Given the observed enhancement of decolorization of the azo dye RR198 by the AQDS, the effect of the anthraquinone dye RB4 as a possible redox mediator for the decolorization of RR198 by the AFBR was further investigated at influent RB4 concentrations of 30, 100, and 300 mg/L. The influent RR198 concentration and HRT were 300 mg/L, and 6.1 h, respectively, during the course of this set of runs and the reactor pH was 7.1±0.1 at all

Table 3
Effect of influent AQDS or RB4 concentration on DOC removal by the AFBR operated at an influent RR198 concentration of 300 mg/L

Redox mediator	Influent DOC (mg/L)			Effluent DOC (mg/L)		DOC removal (%)
	RR198[a]	Glucose	Total	Acetate	Total	
None	37.2	78.4	115.6	18.9	69.1	40.2
AQDS (10 μM)	37.5	78.4	115.9	18.7	67.8	41.5
AQDS (50 μM)	37.8	78.4	116.3	18.8	67.2	42.3
RB4 (30 mg/L)	40.1	78.4	118.6	19.2	67.4	43.2
RB4 (100 mg/L)	50.4	78.4	128.9	19.9	74.8	41.9
RB4 (300 mg/L)	90.4	78.4	168.8	19.6	102.4	39.3

[a] Values of the inorganic feed solution with RR198 and amendments of AQDS or RB4.

influent RB4 concentrations. Fig. 5 shows the spectra in the media feed solution and in the AFBR effluent at an influent RB4 concentration of 0 and 100 mg/L.

The reactor performance at each influent RB4 concentration in terms of RR198, RB4, reduced RR198 and reduced RB4 products in the effluent is shown in Fig. 6. The extent of RR198 decolorization was 86.6±0.2% when the reactor influent did not contain RB4 and reached 92.6±0.2% at an influent RB4 concentration of 30 mg/L, which corresponds to 50 μM as hydrolyzed RB4. Therefore, AQDS and RB4 at the same influent concentration of 50 μM resulted in a comparable extent of RR198 decolorization (91.5 and 92.6%, respectively), demonstrating that RB4 has a redox mediating effect comparable to that of AQDS. A further increase in the influent RB4 concentration to 100 and 300 mg/L resulted in a RR198 decolorization extent of 93.1±0.2 and 89.2±0.1%, respectively. Therefore, RB4 up to 100 mg/L significantly enhanced

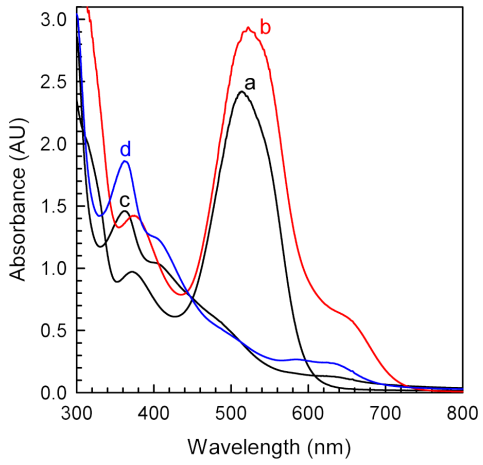


Fig. 5. Spectra of the media feed solution (a and b) and the AFBR effluent (c and d) at different influent RB4 concentrations (Spectra a and c without RB4 amendment; spectra b and d with an influent RB4 concentration of 100 mg/L)(Influent RR198 concentration, 300 mg/L; HRT, 6.1 h).

the RR198 decolorization process by serving as a redox mediator. However, at an influent RB4 concentration of 300 mg/L, corresponding to 500 μM RB4, a decrease in the RR198 decolorization extent was observed due to the possible toxic effect of anthraquinone compounds at relatively high concentrations [11,19,31,32]. The RB4 decolorization efficiency was 49.9, 74.5, and 76.2% at an influent RB4 concentration of 30, 100, and 300 mg/L, respectively. The ORP value in the AFBR decreased from –534±1 to –545±2 mV, as the influent RB4 concentration increased from 30 to 300 mg/L, which correspond to 50 and 500 μM as hydrolyzed RB4, respectively. Therefore, similar to the AQDS, the presence of a relatively low concentration of RB4 in the reactor influent created a more reduced environment in the AFBR and led to an enhancement of the extent of RR198 decolorization. It should be noted that aromatic amines produced as a result of the azo bond cleavage of the azo dye RR198 may also have contributed to the observed decolorization of RR198. van der Zee et al. [33] reported that the aromatic amine 1-amino-2-naphthol, which was the reduction product of the monoazo dye Acid Orange 7 (AO7) accelerated the reduction process perhaps by mediating the transfer of reducing equivalents, a process that they termed autocatalysis.

The effect of increasing influent RB4 concentration along with RR198 at 300 mg/L on DOC removal is summarized in Table 3. The mean DOC removal at influent RB4 concentration of 30, 100, and 300 mg/L was 43.2, 41.9, and 39.3%, respectively. Therefore, a decrease of DOC removal was observed with increased influent RB4 concentrations, which is only expected to undergo reductive transformation without further biodegradation of the RB4 reduction products. The acetate concentration at an influent RR198 concentration of 300 mg/L was almost the same at all influent RB4 concentrations (Table 3). At influent concentrations of 300 mg/L RR198 and 300 mg/L RB4, only 5.1% of the electron equivalents available in the form of glucose were utilized for the observed degree of decolorization of both RR198 and RB4. Therefore, the

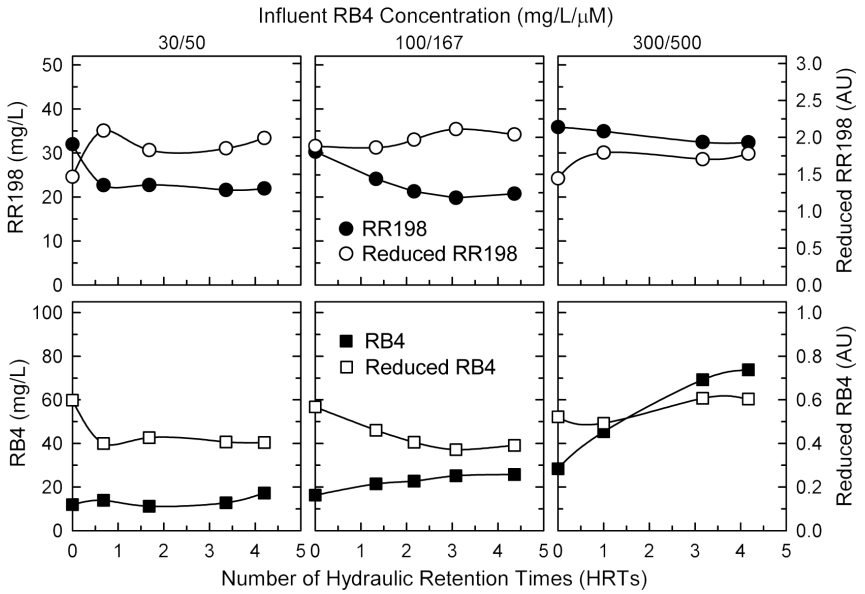


Fig. 6. Effect of influent RB4 concentration on the extent of RR198 decolorization by the AFBR (Influent RR198 concentration, 300 mg/L; HRT, 6.1 h)(The reactor was maintained for over four HRTs at each influent RB4 concentration).

influent glucose concentration was much higher than that required for the reductive decolorization of 300 mg/L RR198 and RB4 up to 300 mg/L.

Very few reports exist on the beneficial effect of anthraquinone dyes on the decolorization of azo dyes. Wong and Yu [34] investigated the laccase-catalyzed decolorization of mixed dyes (anthraquinone dye Acid Green 27, azo dye Acid Violet 7, and indigo carmine dye) and reported that the very slow degradation (0.2– 0.3 mg dye/L-h) of mixed dyes by laccase without the anthraquinone dye was dramatically increased to 35–40 mg dye/L-h at an anthraquinone dye concentration of 33 μM. Therefore, the anthraquinone dye served as a mediator and was also decomposed gradually along with the other dyes. From a practical standpoint, the use of trace levels of an anthraquinone dye for the biological reductive decolorization of spent reactive azo dyebaths is beneficial because of the higher azo dye decolorization efficiency achieved via electron shuttling.

4. Conclusions

The effect of redox mediators on the extent of dye decolorization by the AFBR operated at a high influent dye concentration, short HRT and hypersaline conditions was evaluated. At an influent RB4 concentration of 1,000 mg/L, an HRT of 6.1 h, and reactor temperature of 35°C, the extent of RB4 decolorization decreased from 47.2 to 36.4% with increased influent AQDS concentration from 0 to 150 μM. Therefore, the addition of AQDS to the AFBR did not improve the extent of decolorization of the anthraquinone dye RB4. In contrast, in the case of the azo dye RR198 at an influent concentration of 300 mg/L, an HRT of 6.1 h, and reactor temperature of 35°C, the extent of decolorization increased from 86.1 to 91.5% as the influent AQDS concentration increased from 0 to 50 μM. Shifts of visible absorbance maxima observed in spectrophotometric scans of reactor effluent confirmed that the observed decolorization was the result of non-reversible microbial reductive RR198 transformation. Similar to the effect of AQDS, the anthraquinone dye RB4 enhanced the extent of decolorization of the azo dye RR198 at relatively low influent RB4 concentrations, but a higher influent RB4 concentration led to a decreased extent of RR198 decolorization. Given the fact that mixtures of different classes of dyes are often found in spent textile dyebaths and wastewater, the results of the present study show that anthraquinone dyes, even at very low concentrations, can serve as redox mediators potentially leading to an enhanced rate and extent of reductive decolorization of other dyes (i.e., azo dyes).

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