

## Anaerobic–aerobic sequencing batch reactor treating azo dye containing wastewater: effect of high nitrate ions and salt

Ali Assadi, Morteza Naderi and Mohammad Reza Mehrasbi

### ABSTRACT

In this work, the treatment of wastewater containing azo dye using anaerobic–aerobic sequencing batch reactor (SBR) based on mixed culture for its efficacy in decolorization and reduction in chemical oxygen demand (COD) under different operational conditions has been analyzed. Effects of hydraulic retention time (HRT), salts content and nitrate ion concentration on the rate and extent of color and COD removal through 180 days containing steady-state and acclimation periods were investigated. Solid retention time was kept constant at 20 days in all experiments. Almost complete decolorization could be achieved at dye concentrations between 5 and 500 mg/L, but the removal of COD decreased gradually from 90 to 65% with increasing dye concentration. The results indicated that color was mainly removed under anaerobic conditions and it was almost filled out within 2–3 h of the anaerobic residence time with up to 98% decolorization efficiency. Besides, cutting the cycle time from 24 to 8 h does not have an effect on color removal. Increases in HRT provide enough time for partial mineralization of COD and intermediates in SBR system. The rates of color and COD removals decreased with increasing salt content and nitrate ion concentration in the feed wastewater.

**Key words** | anaerobic–aerobic treatment, azo dye, decolorization, sequencing batch reactor

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### INTRODUCTION

Textile wastewater is a complex and highly variable mixture of various pollutants such as degradable organics, dyes, nutrients, salts, sulfur, toxicants and refractory organics. Azo dyes account for more than 50% of the dyes used in textile processing industries and are the most common synthetic dyes discharged into the environment (Khouni *et al.* 2012). Azo dyes are characterized by the presence of one or more azo bonds ( $-N=N-$ ) connecting aromatic rings. Different substitutions on aromatic nucleus give a structurally diverse and most versatile group of compounds which makes them recalcitrant and xenobiotic (Ali 2010). Wastewaters from the textile industry can produce severe

environmental problems due to their toxicity, mutagenicity and carcinogenicity effects to aquatic life and hence need to be treated before being released into the environment or any reuse program (Tony *et al.* 2009; Iasur-Kruh *et al.* 2010). Hence, there is an urgent need for a technically feasible and cost-effective treatment method (Popli & Patel 2015).

Several physicochemical technologies such as coagulation, electrocoagulation, adsorption, membrane filtration, ion-exchange, irradiation and oxidation have been employed for the removal of azo dyes from wastewaters (Forgacs *et al.* 2004; Singh & Arora 2011). The disadvantages of using these treatment methods are high cost, low efficiency, change of pollutants phase, toxic intermediates and production of hazardous sludge which are hard to dispose of (Singh *et al.* 2012). As a consequence, biological treatment is an environmental friendly and cost effective alternative with

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the ability to produce safe sludge (Baban *et al.* 2010; Champagne & Ramsay 2010; Kolekar *et al.* 2012; Ma *et al.* 2016).

Azo dye removal has been studied using both pure and mixed mediums (Lourenco *et al.* 2001). Although considerable results have been achieved using pure mediums (Ghodake *et al.* 2009; Parshetti *et al.* 2010; Silveira *et al.* 2011), these seem to be not usable at full scale facilities for real textile wastewater treatment. Many works have recommended that mixed medium may be more appropriate for decolorization of azo dyes (Coughlin *et al.* 2003; Guo *et al.* 2007; Koupaie *et al.* 2013b). Thus, the mixed mediums can perform tasks better than or equivalent to that of an individual pure culture without any precautions to prevent contamination (Popli & Patel 2015). Biological treatment can lead to complete mineralization of organic pollutants at low cost. The development of high-rate systems, in which hydraulic retention time (HRT) are uncoupled from solid retention time (SRT), facilitate the removal of dyes from textile processing wastewaters (Forgacs *et al.* 2004).

Sequential or two stage anaerobic–aerobic processes are one of the most accepted technologies for bioremediation of azo dye-containing wastewaters. During the anaerobic stage, decolorization occurs through a microbiological process in which the azo bond is reductively cleaved to aromatic amines (Anjaneyulu *et al.* 2005; Işık & Sponza 2006; Jonstrup *et al.* 2011; Singh & Arora 2011). These resultant aromatic amines are also required to be mineralized during the subsequent aerobic stage. Aromatic amines can be mineralized by means of aerobic treatment of non-specific enzymes through hydroxylation and ring-fission of aromatic compounds. If azo dyes are not reduced and cleaved in the anaerobic stage, they most probably leave the aerobic stage intact. To alleviate this problem an anaerobic–aerobic treatment system has been proposed in which the dead-end toxic byproducts generated under anoxic conditions can be broken down aerobically (Forgacs *et al.* 2004; Supaka *et al.* 2004; Anjaneyulu *et al.* 2005). The extent and rate of anaerobic color removal of azo dyes are influenced by various parameters such as color structure, color concentration, retention times, supplementation with different carbon and nitrogen sources, electron donor, redox mediator and salts (Van der Zee & Villaverde 2005; Ali 2010; da Silva *et al.* 2012; Popli & Patel 2015).

Anaerobic–aerobic sequencing batch reactor (SBR) systems have been widely used in recent works to achieve the desired dye removal with biofilm or suspended growth mode (Van der Zee & Villaverde 2005; Somasiri *et al.* 2008; Koupaie *et al.* 2013b; Franca *et al.* 2015; Popli & Patel 2015). Color removal, especially from textile wastewaters, has been a huge challenge over the last decades, and up to now there is no single and economically attractive treatment that can effectively remove colors. Also, the reuse of water from effluents in the production process or treatment plant leads to a reduction in costs for the textile industry. In a study for possibility of wastewater reuse the authors have shown that the use of advanced oxidation processes (AOPs) can reuse the effluent water by up to 10 times in the dyeing process (Rosa *et al.* 2015). The presence of dye and high concentrations of salt complicates the treatment of textile wastewaters. Many microbial species are able to decolorize some azo dyes anaerobically within a certain limit of salt, but most of them are unable to decolorize azo dyes in high salt conditions (dos Santos *et al.* 2007; Uddin *et al.* 2007).

The primary purpose of this study was to determine the performance of an anaerobic–aerobic SBR technology for the removal of azo dye. Likewise, the effects of some operational parameters such as cycle times, high total dissolved solids (TDS) and nitrate concentration were determined.

## METHODS

### Experimental set-up

A laboratory scale SBR system consisted of a 10 L cylindrical reactor made of plexiglass with an inner diameter of 17 cm (working volume of 8 L). The scheme of the SBR system is shown in Figure 1. The reactor was operated with cycle times of 8, 12 and 24 h and the volume exchange in each cycle was 4 L (volumetric exchange ratio 0.5). The system was first operated under anaerobic conditions with a slight mixing to obtain homogenous conditions. After that, the anaerobic step was completed. The peristaltic pump, mixer, blower and solenoid valve were controlled by a PLC time controller (Omron, Japan). Phase duration and operating condition of the system's working cycles are presented in Table 1.

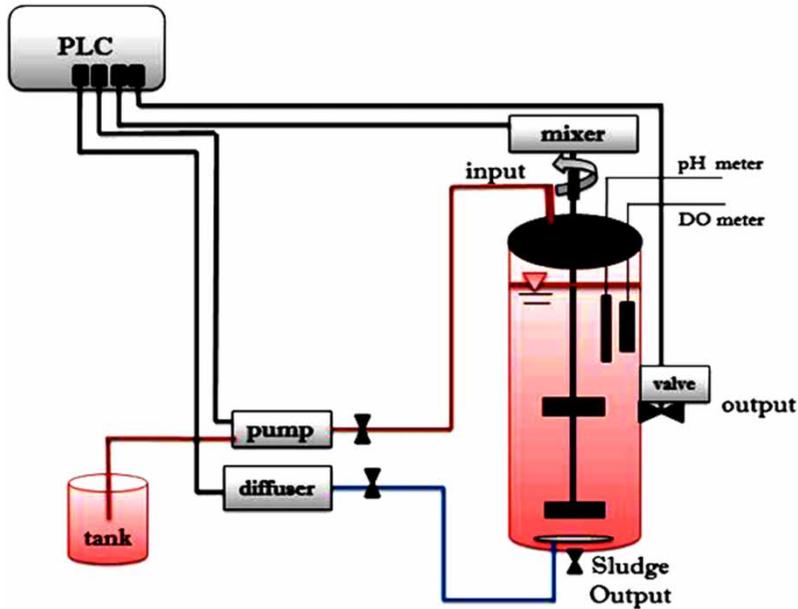


Figure 1 | Diagram of pilot scale of SBR.

Table 1 | Details of working cycles in the SBR system

Cycles	Fill	React			
		Anaerobic	Aerobic	Settle	Decant
24 h	10 min	16 h	6 h, 40 min	60 min	10 min
12 h		6 h	4 h, 40 min		
8 h		2 h	4 h, 40 min		
Feed pump	On	Off	Off	Off	Off
Blower	Off	Off	On	Off	Off
Mixer	Off	On	On	Off	Off
Decant valve	Off	Off	Off	Off	On
Condition	Anoxic	Anaerobic	Aerobic	Anoxic	Anoxic

### Characterization of synthetic wastewater

Synthetic wastewater was prepared with ordinary tap water and dye, and glucose as sources of carbon and energy. Synthetic wastewater used throughout this survey is presented in Table 2. The commercial basic dye used as a pollutant in the present study was C.I. Basic Red 46 (BR46), which was purchased from Alvan Sabet Co. (Hamedan, Iran). This dye is soluble in

Table 2 | Feed wastewater characteristics

Constituents	Concentration (mg/L)
NH <sub>4</sub> Cl	286.61
HBO <sub>3</sub>	0.35
MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.50
ZnCl <sub>2</sub>	0.05
K <sub>2</sub> HPO <sub>4</sub>	84.29
KH <sub>2</sub> PO <sub>4</sub>	65.85
Na <sub>2</sub> SO <sub>3</sub> ·5H <sub>2</sub> O	0.164
(NH <sub>4</sub> ) <sub>6</sub> MO <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O	0.05
CaCl <sub>2</sub> ·2H <sub>2</sub> O	22
MgCl <sub>2</sub>	15
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.038
FeCl <sub>3</sub> ·6H <sub>2</sub> O	5
NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.09
AlCl <sub>3</sub> ·6H <sub>2</sub> O	0.09
CoCl <sub>2</sub> ·6H <sub>2</sub> O	1
Basic Red 46	5–500
Glucose as a COD	1,500

water and belongs to the cationic basic dye group. The chemical structure and other characteristics of BR46 are shown in Table 3. Dye solutions were prepared by dissolving dye in water. Glucose (contributed to chemical oxygen demand

**Table 3** | Commercial dye used in the study

Color index	Basic Red 46	Dye structure
Type	cationic	
Azo group	1	
$\lambda_{max}$	530	
Molecular formula	$C_{18}H_{21}BrN_6$	
Molecular weight	401.3	

(COD) of 1,500 mg/L after start-up) was added into the media to offer a readily biodegradable carbon source, while at the same time it provided the electrons for the reductive cleavage of the BR46 dye. All chemicals were analytical grade (Merck, Germany) and were used without any further purification.

## Experiments

The activated sludge medium obtained from the sludge return line from Zanjan municipal wastewater treatment plant was applied as the seed sludge. At first the sludge was passed through a screen to remove existing gravel. Dilution was performed several times until mixed liquor suspended solids (MLSS) were adjusted to approximately 3,000 mg/L in the reactor. In order to achieve stability conditions, the COD content of the fill wastewater was kept constant at  $1,000 \pm 25$  mg/L glucose as sources of carbon and energy. During the start-up of the reactor, HRT was kept constant for 24 h and SRT was adjusted to 20 d by removing a certain amount of sludge daily. This procedure was performed until the system was able to reach an entirely stable condition and COD removal was over 90%. After the start-up, the inlet COD into the reactor was raised to  $1,500 \pm 25$  mg/L and in order to acclimatize the biomass, BR46 content of between 5 and 500 mg/L was added gradually. This step was continued until the system was able to return to the primary stable condition. The variations of cycle times were studied in the range of 8–24 h in the reactor. The effects of TDS and nitrate on the performance of SBR were also investigated in the range of 1,000–8,500 mg/L and 10–120 mg/L, respectively.

## Analysis

Samples were withdrawn from the sample port of the reactor at predetermined time intervals and were centrifuged

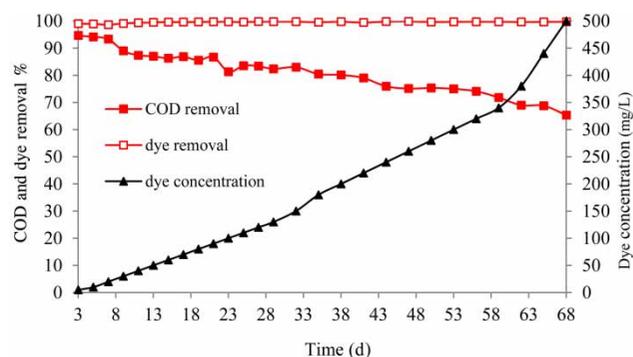
before analyses. The concentration of the dye was determined by measuring the absorption intensity at the maximum absorbent wavelength of BR46 (530 nm) using UV-vis spectrophotometer (DR 4000, HACH, USA). Also, COD, nitrate, total suspended solids (TSS), MLSS and TDS concentrations were determined according to *Standard Methods* (APHA 2005).

## RESULTS AND DISCUSSION

### Dye and COD removal

Start-up with the glucose as growth substrate was quite prompt and high COD removal of up to 90% was achieved at two steps COD increment from 1,000 to 1,500 mg/L. Therefore, after three weeks of stable operating conditions, a biomass concentration of about 3,000 mg/L and SRT of 20 d were obtained. In the next step of study, biomass was acclimated to BR46 and the feed BR46 content was gradually increased from 5 to 500 mg/L for 68 d. The MLSS concentrations were found to vary in the range of 3,400–4,000 mg/L for the operation period. With increasing color concentration, COD of color is added for COD induced by glucose in wastewater. Figure 2 shows the detail of acclimation.

The results demonstrated that activated sludge from municipal wastewater treatment plants have high capability in color and COD removal from synthetic wastewater. As with increasing color concentration, almost all of the color was removed in the operation period. However, the removal of COD decreased gradually from 90 to 65% with increasing color concentration. The anaerobic phase provides

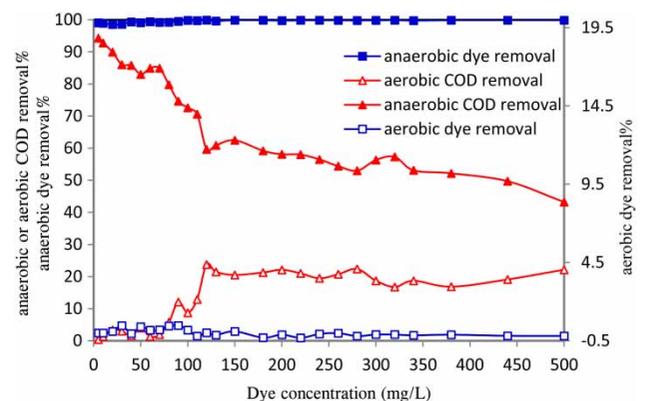
**Figure 2** | Effects of initial dye concentration on dye and COD removal by SBR.

significant COD reduction. However, aeration promoted the COD removal. No significant difference was obtained between the repeated experimental conditions. [Kapdan & Oztekin \(2006\)](#) found that about 70% of COD was removed in the first 5 h of the anaerobic phase using anaerobic/aerobic SBR (including 12 h anaerobic and 11 h aerobic phase) and glucose as growth substrate. Compared to the anaerobic phase, the much lower COD removal rate in the aerobic phase could be because of the insufficient amount of readily biodegradable substrate that remained after the anaerobic stages. The main carbon source of the reactor was glucose with an attribution of 1,500 mg/L COD. Since the anaerobic phase was the first stage of the SBR, the glucose was rapidly reduced in the anaerobic stage by anaerobic organisms to serve as an electron donor and carbon source, thereby causing higher COD removal in the first 2 h of the anaerobic stage. This result is in agreement with previous studies. COD removal efficiency in the anaerobic phase of SBR was found to depend on dyestuff type, amount of initial COD concentration and anaerobic cycle time ([Çınar \*et al.\* 2008](#); [da Silva \*et al.\* 2012](#); [Hakimelahi \*et al.\* 2012](#); [Cirik \*et al.\* 2013](#); [Franca \*et al.\* 2015](#)). According to other research, only about 81 and 91% of the COD removal occurred in the wastewater containing 500 and 1,000 mg/L of Acid Red 18 using an anaerobic SBR/moving bed sequencing batch biofilm reactor and combined anaerobic/aerobic granular activated carbon-sequencing batch biofilm reactor (GAC-SBBR), respectively ([Hosseini Koupaie \*et al.\* 2012](#); [Koupaie \*et al.\* 2013a](#)). Similarly, overall COD removal values in the range of 81–90% for RB5 concentrations in the range of 500–100 mg/L were obtained for sequential anaerobic–aerobic reactor ([Bonakdarpour \*et al.\* 2011](#)). Considering the obtained results, the application of anaerobic–aerobic SBR is comparable for azo dye BR46 containing wastewater in the present study.

Approximately total color removal occurs in the anaerobic phase and sequencing aerobic phase which causes further reduction of COD. A similar relation between dye concentrations and its removal efficiencies was reported by another study group ([Işık & Sponza 2004](#); [Hakimelahi \*et al.\* 2012](#); [Wang \*et al.\* 2014](#)). Therefore, at high concentrations, the almost whole color removal takes place in the early hours of the anaerobic phase. In low concentrations (less than 100 mg/L) the sequencing aerobic phase causes

only a small amount of color removal (less than 1%), however in higher concentrations of 100 mg/L not only does this phase not help with the color removal, but it also causes very slight color formation (approximately 1%). This may be formed due to the recolorization or intermediate oxidation of azo dye BR46. To explain the possible reasons for formation of color the mechanisms responsible for color change in the aerobic phase of an anaerobic–aerobic process should be looked at. According to previous work the major azo dye removal mechanism during aerobic biological processes is adsorption to the biomass ([Panswad \*et al.\* 2001](#)). On the other hand, aerobic color formation can occur as the result of the autooxidation of dye reduction metabolites ([da Silva \*et al.\* 2012](#); [Wang \*et al.\* 2014](#)). Moreover, oxygen is a more effective electron acceptor than azo dyes, which justifies the low decolorisation rates under aerobic conditions ([dos Santos \*et al.\* 2007](#)).

These findings are in agreement with previously published works where the majority of color was removed in the anaerobic stage; in the aerobic stage in some runs no change in color was observed ([Sponza & Işık 2005](#); [Çınar \*et al.\* 2008](#); [Bonakdarpour \*et al.\* 2011](#); [da Silva \*et al.\* 2012](#)). The color and COD removal have obtained 90–99 and 80% respectively for a combined SBR treating 100 mg/L of azo dye ([Albuquerque \*et al.\* 2005](#)). It is noteworthy that the high color concentration of 500 mg/L in the influent of the present study was finally reduced to less than 5 mg/L in the reactor effluent. Variation of color and COD removal in the reactor at anaerobic and aerobic phases is depicted in [Figure 3](#).



**Figure 3** | Fraction of anaerobic and aerobic phases in dye and COD removal by SBR.

With increasing color concentration to 500 mg/L, the anaerobic phase contribution in COD removal decreased from 94 to 43% and the aerobic phase contribution increased from 10 to more than 22%. This is probably because aromatic compounds resulting from azo bond breaking have more toxic effects on the biomass and for this reason anaerobic phase contribution in the COD removal reduces very significantly. The azo dyes, however, are reduced and hence decolorized when acting as electron acceptors for the microbial electron transport chain, then a source of labile carbon is required (Supaka *et al.* 2004; Cirik *et al.* 2013). COD from the soluble microbial products (SMP) through substrate metabolism and biomass decay should be considered because the effluent soluble COD could be mostly SMP (50–90%) (Işık & Sponza 2006). The restrictions in single step anaerobic decolorization processes are low COD removal and formation of toxic aromatic amines as a result of azo dye degradation. However, an aerobic unit sequential to anaerobic treatment provides enhanced COD and toxic substances removal from the anaerobic unit effluent, rather than decolorization. It has been shown that an aerobic unit after anaerobic decolorization is necessary in order to obtain the effluent discharge permit (Shaw *et al.* 2002; Kapdan & Oztekin 2006). Hence, from the presented results, post-treatment is necessary not only to reduce residual color and COD from anaerobic effluent but also to mineralize the aromatic amines generated, which are potentially carcinogenic and mutagenic (Işık & Sponza 2006; Pandey *et al.* 2007; da Silva *et al.* 2012). Probably the main reason for reduction in COD removal is aromatic compounds resulting from the breakdown of BR46 color, and the biomass of the aerobic phase does not have the ability to degrade them

completely. As a result, the aromatic compound discharges as COD in the effluent. Aeration degrades these intermediates. Their mineralization is desirable to reduce toxicity (Tan *et al.* 2000; Jonstrup *et al.* 2011). In a study using granular sludge with SBR (Franca *et al.* 2015), COD removal efficiency was affected neither by the presence of the azo dye nor by that of its breakdown metabolites and high COD removal was produced within the 80–90% range, with 55%, on average, occurring during the anaerobic phase.

### Effect of different cycle times on color and COD removal

There is a direct relationship between the HRT of the anaerobic biological treatment unit and the color removal efficiencies (Jonstrup *et al.* 2011; da Silva *et al.* 2012; Popli & Patel 2015). The results of the color and COD removal for total cycle times of 8, 12 and 24 h in the SBR reactor are presented in Figure 4. The initial color concentration was kept constant at 200 mg/L, while the SRT was 20 d. The results showed that total color (~100%) was removed in the first hours of the anaerobic phase and reducing cycle times from 24 to 8 h did not have an effect on color removal. Hence, the reason for using a long anaerobic phase time was to obtain the most COD removal opportunity that was exerted due to biodegradation of BR46 and glucose. It can be concluded that the contribution of aerobic phase to decolorization was almost none. A cycle of 8 h caused 81% removal of COD and residual of the anaerobic and aerobic phase in cycles 12 and 24 h had no effect on reactor effectiveness in the COD removal. Most of the COD removal occurred on the anaerobic stage of the reactor for all operational conditions. It was found that a small

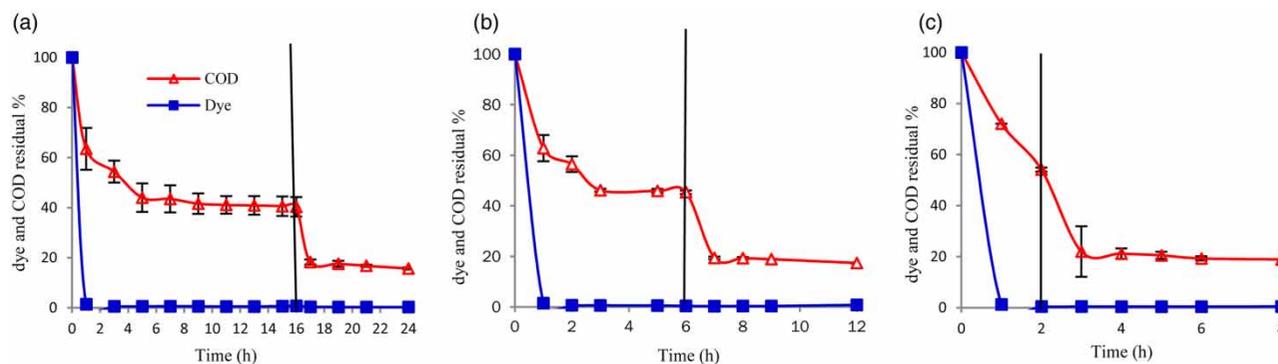


Figure 4 | Dye and COD removal rates in SBR with three different total cycle times: (a) 24 h, (b) 12 h and (c) 8 h.

portion of COD removal was achieved in the aerobic cycle of the SBR. The pH is a good indicator to monitor the stability of the reactor. The pH did not change much at the different cycle times studied and was always in the range of 7–8. These results are in agreement with earlier works (Lourenço *et al.* 2006; Çınar *et al.* 2008). Moreover, they found that the effluent degradation is vastly improved by the HRT in the activated sludge system. The application of an anaerobic (HRT of 4 h)/aerobic (HRT of 20 h) SBR system to treat a synthetic Remazol Rot-containing wastewater (70 mg/L) with glucose as carbon source have shown the same results as other researchers. They found COD removal of 50% during the anaerobic phase and reached 80% after aerobic treatment. However, as the anaerobic residence time was increased, the contribution of the aerobic phase of COD removal was negligible. The main reason for this result could be the toxic effect of metabolites of the anaerobic phase. Due to batch operation in SBR, they accumulated in the system and long term exposure of the cultures to these metabolites may have inhibited the activity of aerobic organisms. Moreover, transition between anaerobic to aerobic phases may have not enhanced the growth of aerobic organisms and they were eliminated from the system (Kapdan & Oztekin 2006). They have also indicated that the minimum HRT in the anaerobic phase should be 6 h for efficient color removal. The SBR system requires long aeration periods to decrease the effluent COD concentration to less than 100 mg/L. The results of Albuquerque *et al.*'s (2005) study have shown 49–71% COD removal in 10.5 h anaerobic reaction phases. In the present study, the outstanding capacity of the SBR to remove COD under anaerobic conditions in much shorter cycles was demonstrated, a feature which is of much importance for performance optimization in wastewater treatment.

It may also be necessary to combine AOPs with biological processes to achieve the required degree of treatment of dye containing wastewaters so that regulatory standards can be met (Popli & Patel 2015). With the high potential for decolorization and application for reuse of treated textile wastewater effluent, AOPs are viable and competitive treatment alternatives when compared with conventional processes for effluent treatment. In addition, conventional bioreactors may be ineffective in treating sludge formation

and the large number of aromatic rings present in organic dye compounds (Rosa *et al.* 2014, 2015; Azizi *et al.* 2015).

### Effect of TDS on reactor stability

Textile effluents contain various acids, alkalis, salts, or metal ions as impurities. Thus, microbial species capable of tolerating salt stress will be important for treating such wastewaters (Ali 2010).

Figure 5 shows the SBR performance during 43 days of operation in which the TDS ranged from 1,000 to 8,500 mg/L at HRT of 12 h. The initial color concentration was kept constant at 200 mg/L, while the SRT was 20 d. The reactor was kept stable during the whole performance, maintaining the pH of the effluent between 7 and 8. As with an increasing TDS concentration of feed wastewater, the efficiency of color and COD removal reduces to 77.6 and 53.4%, respectively. In the first step (0–29 days of operation), increasing TDS to 5,800 mg/L does not have much consequence on the reactor performance in terms of color and COD removal and the reactor can remove more than 91% of dye and 70% of COD. In the second step (29–43 days of operation), with increasing TDS up to 8,500 mg/L, the reactor effectiveness decreases greatly in color and COD removal.

In recent years, several studies have been focused on halophilic and halotolerant microorganisms and their abilities for decolorization of azo dyes (Amoozegar *et al.* 2011; Meng *et al.* 2012). Due to the complexity of substrate and processes in real textile wastewater, mixed culture appears more effective than pure cultures in degradation of azo dyes. The pure culture may attack the dye molecule at different positions or may use decomposition products produced

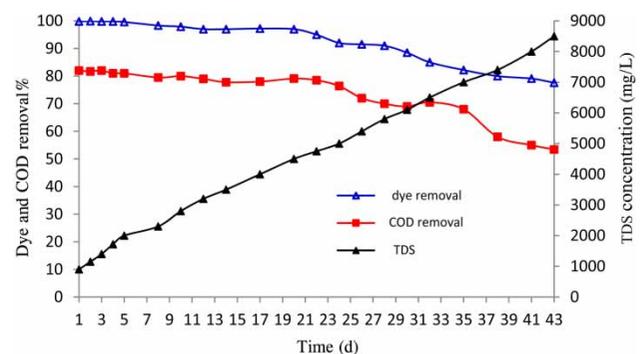


Figure 5 | Effects of TDS concentration on dye and COD removal rates by SBR.

by another strain for further decomposition (Forgacs *et al.* 2004). The findings of the present work indicate that pre-adaptation of the activated sludge cultures to the azo dye can improve the rate and the extent of biodegradation in high salt conditions. This offers further support to the earlier supposition that the capability of biomass in degrading bio-refractory compounds is limited by the induction of the synthesis of the enzymes able to develop specific metabolic pathways; this process is favored by dynamic conditions that are typical of periodic systems (Shaw *et al.* 2002; Van der Zee & Villaverde 2005; Lourenço *et al.* 2006; Movahedyan *et al.* 2008).

The solid content of the reactor was monitored throughout the study of the TDS effect to measure the viability of the suspended biomass during SBR operation with a TSS of effluents (Figure 6). The MLSS were found to vary in the range of 2,200–3,600 mg/L during reactor operation at constant SRT and dye concentration. With the increase in TDS concentration, the MLSS content slowly decreased, indicating the inhibitory nature of the effluent feed on biomass growth. Reducing the biomass concentration while increasing TDS resulted in a considerable decrease (from 99 to 77%), of the color removal efficiency. This finding can be explained by the fact that the accumulation of toxic intermediates of the dye biodegradation in an anaerobic reactor may have adversely affected the microbial growth as well as causing difficulties in complete mineralization at the high salt conditions. This result is in agreement with those reported by other researchers (Kapdan & Alparslan 2005; Uddin *et al.* 2007; Azizi *et al.* 2015). Besides, the lower

COD removal efficiency of the reactor may be due to the higher production of toxic intermediates and these findings were also confirmed by the results of MLSS concentration in the reactor (Figure 5). Very little wash out of the reactor solids occurred during the operation period in term of TSSe and turbidity. The suspended solid concentration varied between 18 and 43 mg/L in the effluent of the reactor. Sludge volume index (SVI) is one of the most important parameters to measure the suitability of sludge in any biological suspended growth system. The SVI had a trifling variation in the range of 19–24 mL/g during acclimation of biomass. Similarly, the results of a study showed that the biomass in the combined SBR supplemented with azo dye (Acid Red 14) maintained its granular structure and low SVI values up to the end of the operational period with stable dye removal above 90% in the anaerobic phase (Franca *et al.* 2015). In another study, the average of SVI numbers for SBR treating Acid Red 18 were in the range of 35–45 mL/g. These data showed good settling properties and increasing the initial dye concentration from 0 to 280 mg/L resulted in no significant variations in the average of SVI numbers (Hakimelahi *et al.* 2012). The good sludge settleability could be explained by the feast and famine conditions prevailing in the reactor which favored floc forming organisms (Lourenço *et al.* 2006; Movahedyan *et al.* 2008). High removal efficiency and good settling characteristics of sludge makes an anaerobic–aerobic SBR suitable for enhancing the microorganism potential for biodegradation of inhibitory compounds (Basheer & Farooqi 2012; Franca *et al.* 2015). The variation of SVI is presented in Figure 6.

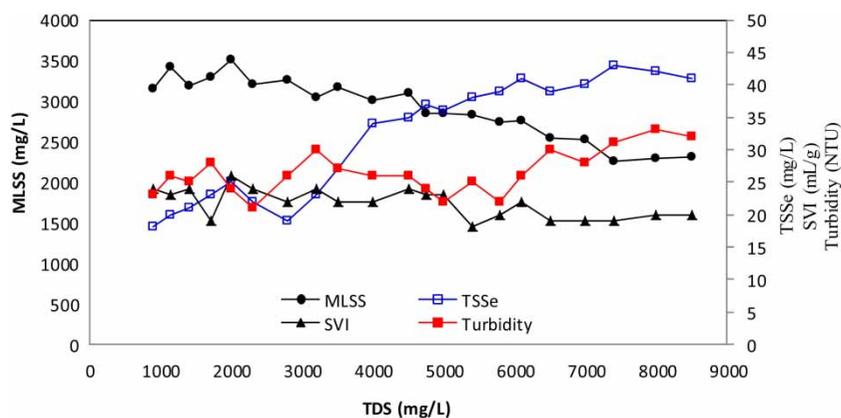


Figure 6 | Variation of solids and sludge settleability with increasing initial TDS concentration.

### Effect on nitrate on color and COD removal

Nitrate was a typical salt species included in dye baths for promotion of dye fixation to the textile fibers and commonly found in saline textile wastewaters (Carliell *et al.* 1998; Varsha & Seema 2013). Therefore, it is necessary to examine their effects on azo dye decolorization. Figure 7 shows the SBR performance during 27 days of operation in which the nitrate concentrations increased up to 120 mg/L. As shown in Figure 7, near complete color removal could be obtained at 12 h cycle times under typical conditions. The initial color concentration was kept constant at 200 mg/L, while the SRT was 20 d. The presence of high nitrate concentrations had a very low effect on the decolorization performance of biomass, which could even completely remove BR46. However, gradual inhibition on COD removal was observed in nitrate above 30 mg/L and COD removal was decreased to 62.3% with increasing nitrate concentration. This provides further backing to the earlier supposition that reduction of azo dyes is an oxidation-reduction reaction in which the dye acts as an electron acceptor (Popli & Patel 2015). The presence of an alternative electron acceptor (nitrate) may compete with the azo dye for reducing equivalents in the anaerobic phase of SBR fed with simulated textile wastewater (Lourenco *et al.* 2001; Franca *et al.* 2015). The presence of nitrate was shown to slow down decolorization (Van der Zee & Villaverde 2005; Khalid *et al.* 2008). In agreement with the present study, nitrate was not found to inhibit azo dye decolorization by *Shewanella* strain J18 143 (Pearce *et al.* 2006). In contrast, Cirik *et al.* (2013) compared anaerobic color removal rate constants for Remazol Brilliant Violet 5R in the presence and absence of nitrate. The authors noted a decrease in

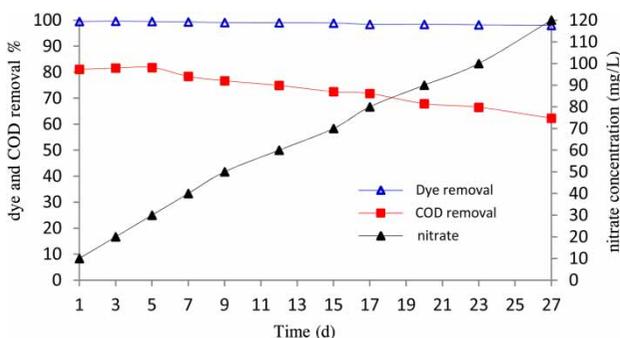


Figure 7 | Effects of nitrate concentration on dye and COD removal by SBR.

decolorization from 93 to 63% with an increase in concentration of nitrate from 0 to 113 mg/L. Similarly, the results of the present study confirm the need for more acclimation time in order to develop a more capable population in the aerobic phase, degrading residual COD of effluent in the presence of nitrate. Another study on the removal of azo dye Reactive Red 141 under anaerobic conditions has proved that nitrate delays the onslaught of decomposition while sulfate did not influence the biodegradation process (Carliell *et al.* 1998).

### CONCLUSIONS

The outcome of this work indicated that an anaerobic-aerobic SBR system has proven to be successful and robust in achieving the complete decolorization of azo dye. Color removal occurs in the anaerobic phase and the sequencing aerobic phase is needed to further reduce the COD effluents. A reduction of HRT from 24 to 8 h does not affect color removal. Increases in HRT provide enough time for mineralization of residual COD in the SBR system. The rates of color and COD removals decreased with increasing salt content and nitrate ion concentration in the feed wastewater. Likewise, operating SBR with an optimized cycle time may help the formation of sludge with good settleability and retain comparable removal of color and COD. It could be reasoned that this type of reactor configuration has potential in treating textile wastewater that varies in both flow and concentration, allowing wastewater recycling.

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