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

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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 fulfilled 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

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. 2014). 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 2013).

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
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 2013). 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; Išık & 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.
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 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...
(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 ± 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 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

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.

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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 (Çinar 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 RB3 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 (İsk & 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 & İşik 2005; Çinar 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 5.

![Figure 3](https://iwaponline.com/jwrd/article-pdf/8/2/251/240606/jwrd0080251.pdf) | 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. 2015). 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%) (İşık & 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 & Öztekin 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 (İşık & 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](https://iwaponline.com/jwrd/article-pdf/8/2/251/240606/jwrd0080251.pdf)
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 & Öztekin 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](https://iwaponline.com/jwrd/article-pdf/8/2/251/240606/jwrd0080251.pdf)
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 biorefractory 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 TSS$_e$ 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](https://iwaponline.com/jwrd/article-pdf/8/2/251/240606/jwrd0080251.pdf)

**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 2008). 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. 2013). 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 145 (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 decolorization from 95 to 63% with an increase in concentration of nitrate from 0 to 115 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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REFERENCES

Albuquerque, M., Lopes, A., Serralheiro, M., Novais, J. & Pinheiro, H. 2005 Biological sulphate reduction and redox mediator effects on azo dye decolourisation in anaerobic–
Promoting aerobic or anaerobic sequencing batch reactors. Enzyme Microb. Technol. 36, 790–799.

Ali, H. 2010 Biodegradation of synthetic dyes – a review. Water. Air Soil Pollut. 213, 251–273.

Amoozegar, M. A., Hajighasemi, M., Hamedi, J., Asad, S. & Ventosa, A. 2011 Azo dye decolorization by halophilic and halotolerant microorganisms. Ann. Microbiol. 61, 217–230.

Anjaneeyulu, Y., Chary, N. S. & Raj, D. S. S. 2005 Decolorization of industrial effluents – available methods and emerging technologies – a review. Rev. Environ. Sci. Biotechnol. 4, 245–273.

APHA 2005 Standard Methods for the Examination of Water and Wastewater, 15th edn. APHA American Public Health Association, Washington, DC, USA.

Azizi, A., Moghaddam, M. A., Maknoon, R. & Kowsari, E. 2015 Comparison of three combined sequencing batch reactor followed by enhanced Fenton process for an azo dye degradation: bio-decolorization kinetics study. J. Hazard. Mater. 299, 343–350.

Baban, A., Yediller, A., Avaz, G. & Hostede, S. 2010 Biological and oxidative treatment of cotton textile dye-bath effluents by fixed and fluidized bed reactors. Bioresour. Technol. 101, 1147–1152.

Basheer, F. & Farooqi, I. H. 2012 Development of aerobic granules in sequencing batch reactor with p-nitrophenol as sole carbon source. J. Water Reuse Desalin. 2, 22–32.

Bonakdarpour, B., Vyrides, I. & Stuckey, D. C. 2011 Comparison of the performance of one stage and two stage sequential anaerobic–aerobic biological processes for the treatment of reactive-azo-dye-containing synthetic wastewaters. Int. Biodeter. Biodegrad. 65, 591–599.

Carfi, C., Barclay, S., Shaw, C., Wheatley, A. & Buckley, C. 1998 The effect of salts used in textile dyeing on microbial decolorisation of a reactive azo dye. Environ. Technol. 19, 1133–1137.

Champagne, P.-P. & Ramsay, J. 2010 Dye decolorization and detoxification by laccase immobilized on porous glass beads. Bioresour. Technol. 101, 2230–2235.

Çınar, Ö., Yaşar, S., Kertmen, M., Demiöröz, K., Yigit, N. Ö. & Kitis, M. 2008 Effect of cycle time on biodegradation of azo dye in sequencing batch reactor. Process Saf. Environ. Prot. 86, 455–460.

Çirik, K., Kitis, M. & Çinar, O. 2013 The effect of biological sulfate reduction on anaerobic color removal in anaerobic–aerobic sequencing batch reactors. Bioprocess BioEng. 36, 579–589.

Coughlin, M. F., Kinkle, B. K. & Bishop, P. L. 2005 High performance degradation of azo dye Acid Orange 7 and sulfanilic acid in a laboratory scale reactor after seeding with cultured bacterial strains. Water Res. 37, 2757–2763.

da Silva, M. E. R., Firmino, P. I. M., de Sousa, M. R. & dos Santos, A. B. 2012 Sequential anaerobic/aerobic treatment of dye-containing wastewaters: colour and COD removals, and ecotoxicity tests. Appl. Biochem. Biotechnol. 166, 1057–1069.

dos Santos, A. B., Cervantes, F. J. & van Lier, J. B. 2007 Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology. Bioresour. Technol. 98, 2369–2385.

Forgacs, E., Cserhati, T. & Oroš, G. 2004 Removal of synthetic dyes from wastewaters: a review. Environ. Int. 30, 953–971.

Franca, R. D., Vieira, A., Mata, A. M., Carvalho, G. S., Pinheiro, H. M. & Lourenço, N. D. 2005 Effect of an azo dye on the performance of an aerobic granular sludge sequencing batch reactor treating a simulated textile wastewater. Water Res. 35, 327–336.

Ghodake, G., Jadhav, S., Dawkar, V. & Govindwar, S. 2009 Biodegradation of diazo dye Direct brown MR by Acinetobacter calcoaceticus NCIM 2890. Int. Biodeterior. Biodegrad. 63, 433–439.

Guo, J., Zhou, J., Wang, D., Tian, C., Wang, P., Uddin, M. S. & Yu, H. 2007 Biocatalyst effects of immobilized anthraquinone on the anaerobic reduction of azo dyes by the salt-tolerant bacteria. Water Res. 41, 426–432.

Hakimelahi, M., Moghaddam, M. R. A. & Hashemi, S. H. 2012 Biological treatment of wastewater containing an azo dye using mixed culture in alternating anaerobic/aerobic sequencing batch reactors. Biotechnol. Bioprocess Eng. 17, 875–880.

Hosseini Koupaei, E., Alavi Moghaddam, M. R. & Hashemi, S. H. 2012 Investigation of decolorization kinetics and biodegradation of azo dye Acid Red 18 using sequential process of anaerobic sequencing batch reactor/moving bed sequencing batch biofilm reactor. Int. Biodeterior. Biodegrad. 71, 43–49.

Iasur-Kruh, L., Hadar, Y., Milstein, D., Gasith, A. & Minz, D. 2010 Microbial population and activity in wetland microcosms constructed for improving treated municipal wastewater. Microb. Ecol. 59, 700–709.

Işık, M. & Sponza, D. T. 2004 Monitoring of toxicity and intermediates of CI Direct Black 38 azo dye through decolorization in an anaerobic/aerobic sequential reactor system. J. Hazard. Mater. 114, 29–59.

Işık, M. & Sponza, D. T. 2006 Biological treatment of acid dyeing wastewater using a sequential anaerobic/aerobic reactor system. Enzyme Microb. Technol. 38, 887–892.

Jonstrup, M., Kumar, N., Murto, M. & Mattiasson, B. 2011 Sequential anaerobic–aerobic treatment of azo dyes: decolourisation and amine degradability. Desalination 280, 339–346.

Kapdan, I. K. & Alparslan, S. 2005 Application of anaerobic–aerobic sequential treatment system to real textile wastewater for color and COD removal. Enzyme Microb. Technol. 36, 273–279.

Kapdan, I. K. & Oztekin, R. 2006 The effect of hydraulic residence time and initial COD concentration on color and COD removal performance of the anaerobic–aerobic SBR system. J. Hazard. Mater. 136, 896–901.

Khalil, A., Arshad, M. & Crowley, D. E. 2008 Decolorization of azo dyes by Shewanella sp. under saline conditions. Appl. Microbiol. Biotechnol. 79, 1053–1059.

Khouni, I., Marrot, B. & Amar, R. B. 2012 Treatment of reconstituted textile wastewater containing a reactive dye in
an aerobic sequencing batch reactor using a novel bacterial consortium. Sep. Purif. Technol. 87, 110–119.
Kolekar, Y. M., Nemade, H. N., Markad, V. L., Adav, S. S., Patole, M. S. & Kodam, K. M. 2012 Decolorization and biodegradation of azo dye, reactive blue 59 by aerobic granules. Bioreour. Technol. 104, 818–822.
Koupaie, E. H., Moghaddam, M. A. & Hashemi, S. 2013a Successful treatment of high azo dye concentration wastewater using combined anaerobic/aerobic granular activated carbon-sequence batch biofilm reactor (GAC-SBBR): simultaneous adsorption and biodegradation processes. Water Sci. Technol. 67, 1816–1821.
Koupaie, E. H., Moghaddam, M. A. & Hashemi, S. 2013b Evaluation of integrated anaerobic/aerobic fixed-bed sequencing batch biofilm reactor for decolorization and biodegradation of azo dye Acid Red 18: comparison of using two types of packing media. Bioreour. Technol. 127, 415–421.
Lourenço, N., Novais, J. & Pinheiro, H. 2001 Effect of some operational parameters on textile dye biodegradation in a sequential batch reactor. J. Biotechnol. 89, 163–174.
Lourenço, N. D., Novais, J. M. & Pinheiro, H. M. 2006 Kinetic studies of reactive azo dye decolorization in anaerobic/aerobic sequencing batch reactors. Biotechnol. Lett. 28, 733–739.
Ma, H., Bonnie, N. A., Yu, M., Che, S. & Wang, Q. 2016 Biological treatment of ammonium perchlorate-contaminated wastewater: a review. J. Water Reuse Desalin. 6, 82–107.
Meng, X., Liu, G., Zhou, J., Fu, Q. S. & Wang, G. 2012 Azo dye decolorization by Shewanella aquimarina under saline conditions. Bioreour. Technol. 114, 95–101.
Movahedyan, H., Assadi, A. & Amin, M. 2008 Effects of 4-chlorophenol loadings on acclimation of biomass with optimized fixed time sequencing batch reactor. Iran. J. Environ. Health Sci. Eng. 5, 225–234.
Pandey, A., Singh, P. & Iyengar, L. 2007 Bacterial decolorization and degradation of azo dyes. Int. Biodeterior. Biodegrad. 59, 73–84.
Panswad, T., Techovanich, A. & Anotai, J. 2001 Comparison of dye wastewater treatment by normal and anoxic+ anaerobic/aerobic SBR activated sludge processes. Water Sci. Technol. 43, 355–362.
Parshetti, G., Telke, A., Kalyani, D. & Govindwar, S. 2010 Decolorization and detoxification of sulfonated azo dye methyl orange by Kocuria rosea MTCC 1532. J. Hazard. Mater. 176, 503–509.
Pearce, C. I., Christie, R., Boothman, C., von Canstein, H., Guthrie, J. T. & Lloyd, J. R. 2006 Reactive azo dye reduction by Shewanella strain J18 143. Biotechnol. Bioeng. 95, 692–703.
Popli, S. & Patel, U. D. 2015 Destruction of azo dyes by anaerobic/aerobic sequential biological treatment: a review. Int. J. Environ. Sci. Technol. 12, 405–420.
Rosa, J. M., Tambourgi, E. B., Santana, J. C. C., de Campos Araujo, M., Ming, W. C. & Trindade, N. 2014 Development of colors with sustainability: a comparative study between dyeing of cotton with reactive and vat dyestuffs. Text. Res. J. 84, 1009–1017.
Rosa, J. M., Fileti, A. M., Tambourgi, E. B. & Santana, J. C. 2015 Dyeing of cotton with reactive dyestuffs: the continuous reuse of textile wastewater effluent treated by Ultraviolet/Hydrogen peroxide homogeneous photocatalysis. J. Clean. Prod. 90, 60–65.
Shaw, C., Carliell, C. & Wheatley, A. 2002 Anaerobic/aerobic treatment of coloured textile effluents using sequencing batch reactors. Water Res. 36, 1993–2001.
Silveira, E., Marques, P. P., Macedo, A. C., Mazzola, P. G., Porto, A. L. F. & Tambourgi, E. B. 2011 Decolorization of industrial azo dye in an anoxic reactor by PUF immobilized Pseudomonas oleovorans. J. Water Reuse Desalin. 1, 18–26.
Singh, K. & Arora, S. 2011 Removal of synthetic textile dyes from wastewaters: a critical review on present treatment technologies. Crit. Rev. Environ. Sci. Technol. 41, 807–878.
Singh, P., Iyengar, L. & Pandey, A. 2012 Bacterial decolorization and degradation of azo dyes. In: Microbial Degradation of Xenobiotics (S. N. Singh, ed.) Springer, Berling, Heidelberg, pp. 101–133.
Somasiri, W., Li, X.-F., Ruan, W.-Q. & Jian, C. 2008 Evaluation of the efficacy of upflow anaerobic sludge blanket reactor in removal of colour and reduction of COD in real textile wastewater. Bioreour. Technol. 99, 3692–3699.
Sponza, D. T. & Isik, M. 2005 Toxicity and intermediates of CI Direct Red 28 dye through sequential anaerobic/aerobic treatment. Process Biochem. 40, 2735–2744.
Supaka, N., Untongjin, K., Damronglerd, S., Delia, M.-L. & Strehaiano, P. 2004 Microbial decolorization of reactive azo dyes in a sequential anaerobic-aerobic system. Chem. Eng. J. 99, 169–176.
Tan, N., Borger, A., Slenders, P., Svitekaya, A., Lettinga, G. & Field, J. 2000 Degradation of azo dye Mordant Yellow 10 in a sequential anaerobic and bioaugmented aerobic bioreactor. Water Sci. Technol. 42, 337–344.
Tony, B. D., Goyal, D. & Khanna, S. 2009 Decolorization of textile azo dyes by aerobic bacterial consortium. Int. Biodeterior. Biodegrad. 63, 462–469.
Uddin, M. S., Zhou, J., Qu, Y., Guo, J., Wang, P. & Hong Zhao, L. 2007 Biodecolorization of azo dye acid Red B under high salinity condition. Bull. Environ. Contam. Toxicol. 79, 440–444.
Van der Zee, F. P. & Villaverde, S. 2005 Combined anaerobic-aerobic treatment of azo dyes – a short review of bioreactor studies. Water Res. 39, 1425–1440.
Varsha, G. & Seema, S. 2013 Physico-chemical analysis of textile effluents of dye and printing clusters of Bagru region, Jaipur, India. J. Environ. Res. Dev. 8, 11.
Wang, X., Cheng, X., Sun, D., Ren, Y. & Xu, G. 2014 Fate and transformation of napthylaminesulfonic azo dye Reactive Black 5 during wastewater treatment process. Environ. Sci. Pollut. Res. 21, 5713–5723.

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