Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi

Pamukkale University Journal of Engineering Sciences

Biological reduction of acid red 14 by elemental sulfur and methanol-based reactors

Asit kırmızı 14 boyasının elementel kükürt ve metanol bazli reaktörlerde biyolojik indirgenmesi

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Abstract

In this study for the first time, biological reduction of the Acid Red 14 in elemental sulfur-based anaerobic reactor was studied. Two up-flow packed bed reactors were investigated in parallel for 95 days. Sulfur and methanol were used as electron donors in these reactors and the performance of the sulfur-based reduction process was investigated in comparison with a conventional electron source - methanol. The reactors were operated in 7 different periods, the influent dye concentration was kept at 5-10 mg/L and HRT was set to 12-24 hours. While influent Acid Red 14 concentration was 10±0.14 mg/L, it was reduced to 1.3±0.2 in the methanol-based reactor and 2.05±0.2 in the sulfur-based reactor. Elemental sulfur was used for the first time in and the effect of sulfur oxidation in dye reduction process on sulfate and alkalinity production was also investigated.

Keywords: Acid red 14, Elemental sulfur, Methanol, Biodegradation, Decolorization

Öz

Bu çalışmada ilk kez elementel kükürt bazlı anaerobik reaktörde Asit Kırmızı 14'ün biyolojik olarak indirgenmesi çalışılmıştır. İki yukarı akışlı paket yataklı reaktör birbirine paralel olarak 95 gün boyunca işletilmiştir. Bu reaktörlerde elektron kaynağı olarak kükürt ve metanol kullanılmış ve kükürt bazlı indirgeme işleminin performansı yaygın bir elektron kaynağı olan metanol ile karşılaştırmalı olarak incelenmiştir. Reaktörler 7 farklı periyotta çalışılmış olup, giriş boya konsantrasyonu 5-10 mg/L'de ve HRT 12-24 sa. olarak ayarlanmıştır. Asit Kırmızı 14 konsantrasyonu girişte 10±0.14 mg/L iken, metanol bazlı reaktörde 1.3±0.2 mg/L'ye, kükürt esaslı reaktörde ise 2.05±0.2 mg/L'ye düşmüştür. Kükürt bu çalışma ile boya indirgeme sürecinde ilk kez kullanılmış ve bu süreçte kükürt oksidasyonunun sülfat ve alkalinite üretimine etkisi ayrıca araştırılmıştır.

Anahtar kelimeler: Asit kırmızı 14, Elementel kükürt, Metanol, Biyolojik bozunum, Renk giderme

1 Introduction

Textile wastewater is one of the wastewaters that are difficult to treat due to their complicated compositions [1]. It is estimated that 200-400 L water is used per kilogram finished product [2]. The most important component in textile wastewater is dyestuffs and their discharge to the receiving environment without treatment can cause significant adverse effects in the receiving environment. According to the water pollution and control regulation published by the Ministry of Environment and Urbanism in Turkey, the color discharge standards of industrial areas are given as 280 and 260 Pt-Co according to 2 hour and 24 hour composite samples.

There are a number of methods for treatment of textile wastewater such as membrane filtration, chemical oxidation, flotation, biodegradation, adsorption, chemical coagulation and photocatalysis [3],[4]. Among these methods, biological decolorization is the preferred method due to its simplicity, not requiring expensive catalyst and not creating secondary pollution.

Biological treatment of dyes in textile wastewaters is basically the dye metabolism that occurs in the presence of different oxidizing or reducing enzymes [5]. Electron sources can be simple organic compounds such as glucose, acetate, ethanol, meat extract, or more complex compounds such as starch, polyvinyl alcohol and carboxymethylcellulose [6],[7]. The substrate used can also affect the reduction process directly or indirectly by attracting specific microorganisms present in the mixed culture [8].

Sulfur is used in many studies as an alternative to organic electron donors, and sulfur-based reactors are an economical and practical method for water treatment [9]. In these reactors, the biological processes can be maintained by adding dissolved sulfur compounds (i.e. thiosulfate) in the feed as well as in the granular form as the reactor filling material. Sulfur used in the reactor also forms a surface area for microorganisms [10]. Hence, there is no need for continuous supply of dissolved or gaseous electron sources to the reactor. Sulfur provides the electron source required for long-term operation [11].

Despite its many advantages, as far as the author's knowledge, the use of sulfur-based autotrophic reactors are limited to the removal of heavy metals [9],[12], bromate [13] perchlorate [13],[14] and mainly nitrate [15] from drinking water due to its clean nature. Therefore, it is necessary to investigate the applicability of elemental sulfur-based reactors to various fields, especially to the textile industry.

Therefore, in this work, the reducibility of Acid Red 14 (AR14) textile dye in an elemental sulfur-based upstream reactor was investigated in comparison with a methanol-based reactor. Reactors were operated with simulated wastewater containing AR14 at various concentrations for 94 days and at the end of the study, the real dye house wastewater was also tested in the reactors.

2 Materials and methods

2.1 Bioreactors

Upflow anaerobic reactors were utilized to investigate AR14 reduction (Figure 1). Sulfur and methanol were utilized to provide necessary electrons in reductive processes. The active volume of the reactors was 500 mL. Sulfur and sand particles (0.5-1 mm) were utilized for reactor filling materials. N_2 gas was utilized to remove dissolved oxygen in the feed by bubbling for about 20 minutes. To minimize microbial contamination, the feed was placed in a refrigerator at 4 °C and reactors were operated in the absence of light.
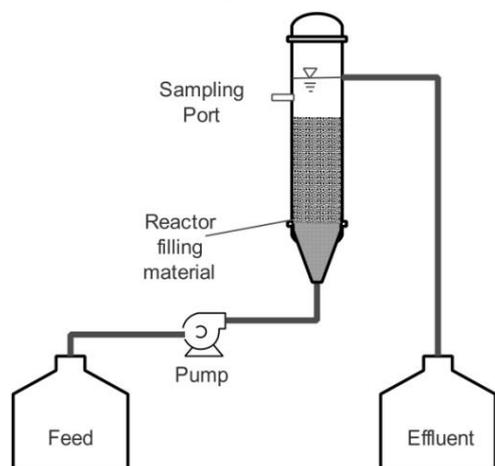

Figure 1: Up-flow anaerobic reactor.

2.2 Operational conditions

The reactors were initially operated under denitrifying conditions for another project. Then the reactors were used for decolorization and influent nitrate was gradually decreased while Acid Red 14 was added to the feed (Table 1). Structure of Acid Red 14 is given in Figure 2. Acid red 14 is a conventional dyestuff and has azo bonds in its chemical structure.
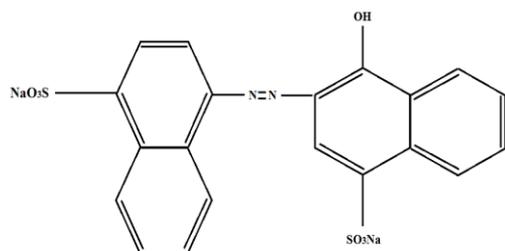

Figure 2: Molecular structure of Acid Red 14 (Molecular formula is $C_{20}H_{12}N_2Na_2O_7S_2$ and CAS no: 3567-69-9).

The reason for the use of denitrification reactors is that microorganisms in these reactors have adapted to sulfur oxidation. These sulfur-oxidizing microorganisms, were forced to reduce AR14 instead of nitrate.

During the first four periods, hydraulic retention time was maintained at 12 h and then it was increased to 24 h for both reactors. The COD in the feed of methanol-based reactor was 100 mg/L. Reactors were monitored for 96 days (Table 1).

Color, sulphate, alkalinity and nitrate analyses were regularly performed from the feed of the sulfur-based reactor. In the methanol-based reactor, COD test was performed in addition to

these analyses. In the effluents of reactors, alkalinity, color, sulfide, sulfate, nitrate and COD analysis were performed.

2.3 Analytical methods

In the first three periods, NO_3^--N, NO_2^--N and SO_4^{2-} were analyzed by suppressed conductivity ion chromatography using a Shimadzu HIC SP system with a DIONEX Ion-Pac AS9-HC column (4 mm x 250 mm). Chemical Oxygen Demand and Alkalinity experiments were carried out as described in Standard Methods [16]. Cord-Ruwisch's spectrophotometric method (480 nm) was used in sulfide quantifying [17]. The concentration of dye in the effluent samples was determined spectrophotometrically at λ_{max}=515 nm and a calibration curve. The samples were filtered with an injector filter (0.45 μm) for the sulfur and ion analyzes

2.4 Batch adsorption tests

Adsorption tests were performed to determine whether the dye removal mechanism is biological or physicochemical. To do this, one gram of sulfur or sand were added to 50 mL 10-100 mg AR14/L solutions and mixed for about 4 hours. Equilibrium dye concentrations were then measured in spectrophotometer.

3 Results and discussion

3.1 Bio-Reduction of AR14

Acid Red 14 concentration in the feed was 5 mg/L except for the period 6 in which AR14 concentration was increased to 10 mg/L. Both the AR14 samples and the actual wastewater were taken from a dye house in Şanlıurfa/Turkey. Nitrate was given 20, 15 and 10 mg NO_3^--N/L in the 1st, 2nd and 3rd periods, respectively, and nitrate was not added in the following periods to observe AR14 reduction. During the 1st period influent nitrate nitrogen and AR14 were 20 and 5 mg/L respectively. During this period, 4% AR14 removal was observed for both reactors (Figure 3). After this 10 day period, the NO_3^--N concentration was reduced to 15 mg/L and acclimation of the reactors to dye reducing conditions was expected.

During the second period, the dye reduction rate was initiated with 8±0.2% for the methanol-based reactor and 12% for the sulfur-based reactor. At the end of the second period, the removal efficiencies for methanol and sulfur-based reactors are 16±0.4 and 10%, respectively. In this period, the performance of the methanol-based reactor increased. However, sulfur-based reactor's performance remained stable (Figure 3).
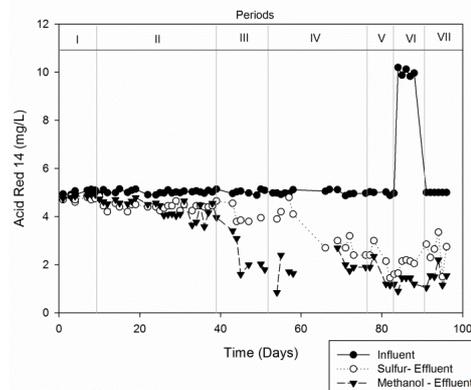

Figure 3: Influent and effluent AR14 variations throughout the study.

Table 1: Operational conditions for the reactors. Methanol fed reactor was fed with 100 mg CH_3OH/L (150 mg/L COD).

Parameters/Periods	P.1	P.2	P.3	P.4	P. 5	P. 6	P.7
Days	1-10	11-39	43-51	53-78	81-83	85-88	91-96
HRT (hour)	12	12	12	12	24	24	24
Dye-AR14 (mg/L)	5	5	5	5	5	10	5*
Nitrate (mg/L)	20	15	10	0	0	0	0

*: In the 7th period, dye house effluent was treated which contained 5 mg/L AR14.

In the 3rd period, nitrate was reduced to 10 mg NO_3^--N/L and more electrons were expected to participate in the reduction of AR14. In this period, a decrease in the concentration of AR14 from the effluent of methanol-based reactor was observed, with an effluent dye concentration of 1.96±0.1 mg/L (62% removal). A similar decrease was also observed in the sulfur-based reactor, but the efficiency was remained at 20%.

In the following periods nitrate was not added to feed and the dye removal performances were monitored only in the dye-reducing conditions of the reactor. In these conditions, the performance of methanol-based reactor remained stable, however, sulfur-based reactors dye reduction rate was increased from 19% to 51%. In order to further increase the dye reducing performances in the reactors, HRT was increased from 12 hours to 24 hours in the following period. Thus, 76 and 65% dye reduction was achieved in the heterotrophic and autotrophic reactors, respectively. The effluent AR14 concentrations in this period were 1.18±0.4 and 1.73±0.6 mg/L for methanol and sulfur-based reactors, respectively. When HRT is increased, dye removal efficiencies may also increase. This could be explained by microorganisms having longer contact times to perform biochemical processes. However, removal rate may decrease as reported in Li et al.'s study [18]. In the 6th period, the AR14 concentration was increased to 10 mg/L in the same HRT and the removal efficiencies were 74±1.1% and 60±0.8% for the methanol-based and sulfur-based reactors, respectively. After this stage, the reactor was fed with real dye house effluent. During the first 6 periods, maximum dye removal rate for methanol and sulfur-based reactors were 3.71 and 2.96 mg/(L.d) respectively.

In the 7th period, effluent from a dye house was added to the feed of the reactors and the real wastewater contained 5 mg/L AR14. A decrease in both reactors performances was observed which may be explained by the impurities in the wastewater such as salinity, sulfate and other chemical constituents. In this period, the removal efficiencies for methanol and sulfur-based reactors were 70±0.7 and 49±0.4%, respectively. The batch tests revealed that AR14 was not adsorbed on sand or elemental sulfur particles.

3.2   Alkalinity

During the first 51 days effluent alkalinity concentrations were depending on the nitrate being reduced. In the methanol-based denitrification process, 3.57 mg $CaCO_3$/NO_3^--N is produced, while in the sulfur-based denitrification process, 4.57 mg $CaCO_3$/NO_3^--N is consumed. Since alkalinity may change according to reduction process, its concentration was monitored during AR14 reducing conditions as well. In the first period, average NO_3^--N reduction rates were 39 and 38.4 mg/(L.d) in methanol and sulfur-based reactors, respectively. In the following periods (period 2 and 3), influent nitrate was decreased and therefore, nitrate related variations

in the effluent alkalinity concentration was also decreased (Figure 4).
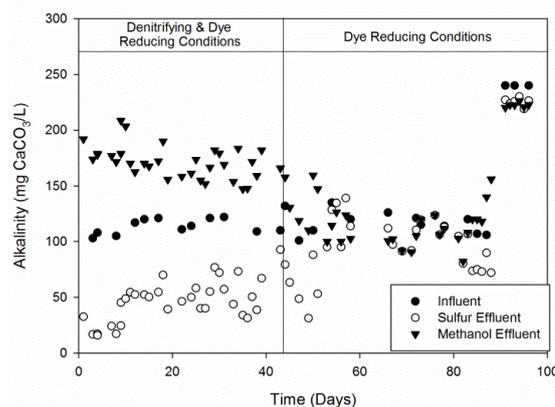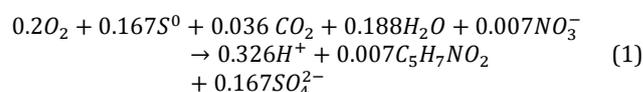

Figure 4: Influent and effluent alkalinity concentrations.

The reactors were fed only with AR14 from day 52, and the effect of dye reduction on alkalinity can be seen in Figure 4. In 4th and 5th periods, feed AR14 concentration was adjusted to 5 mg/L. The average alkalinity in the influent and effluent of sulfur-based reactor in these periods were 120 and 110 mg $CaCO_3$/L, respectively. In the same periods, the methanol-based reactor average effluent alkalinity was 106 mg $CaCO_3$/L. In the 6th period, the feed was contained 10 mg AR14/L and 106 mg $CaCO_3$/L. In the effluents of methanol and sulfur-based reactors alkalinity concentrations were 130 and 77 mg $CaCO_3$/L, respectively. In elemental sulfur-based reactors, alkalinity decrease is a common situation. Several factors could decrease the effluent alkalinity. For example, when oxygen leaks to sulfur-based reactor, 2.54 mg $CaCO_3$/O_2 is consumed (Equation 1).

$$0.2O_2 + 0.167S^0 + 0.036\,CO_2 + 0.188H_2O + 0.007NO_3^- \rightarrow 0.326H^+ + 0.007C_5H_7NO_2 + 0.167SO_4^{2-} \qquad (1)$$

Leakage of oxygen to the reactors during sampling or feeding is unavoidable during reactor operation although some preventions were taken. As in the oxygen and nitrate reduction, dye reduction may also consume alkalinity in elemental sulfur-based reduction. In the 7th period, the influent alkalinity was measured as 240 mg/L. In this period, reactors were supplied with real dye house effluents which contain approximately 5 mg AR14/L. The effluent alkalinity concentrations for methanol and sulfur-based reactors were 222 and 225 mg $CaCO_3$/L, respectively.

3.3   COD, Sulfate and sulfide variations

Methanol was used for nitrate reduction in the early stages of reactor operation and over time it was targeted to reduce the AR14 dye of electrons. Theoretically, 2.47 mg CH_3OH is

necessary to reduce each mg NO_3^--N. To avoid methanol limitation, 100 mg/L methanol (150 mg/L as COD) was added to feed to reduce 20 mg NO_3^--N/L. While nitrate was decreased in the feed, methanol was not decreased and kept at 100 mg/L. Although operational conditions were changed during the reactor operation (Table 1), effluent COD were stable. In the 1st period influent and effluent COD were 150±6 and 17.5±1.8 mg/L. In the periods 2-6 when the reactor was fed with synthetic wastewater, average effluent COD was 15.9±5 mg/L (Figure 5).
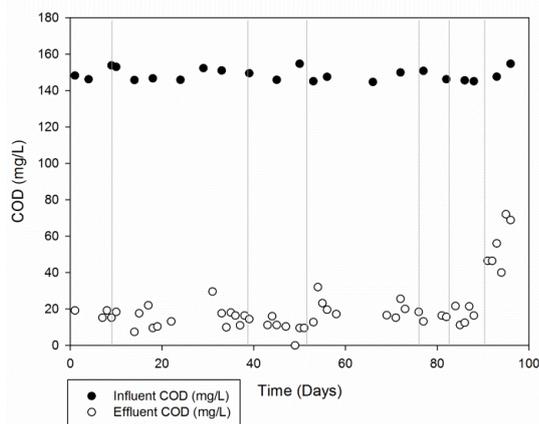

Figure 5: Influent and effluent COD concentrations for methanol-based reactor.

In the 7th period, COD in the effluent increased to 61±10.2 mg/L corresponding to a 30% decrease in COD removal. Influent and effluent COD concentrations are presented in Figure 5 below. The decrease in COD may be explained by various impurities in the wastewater. For example conductivity of wastewater was 3015±22 µS/cm and some salt sensitive-COD oxidizing microorganisms could be affected by the high salinity range in the reactor. It is reported that salinity is strongly related to bacterial community structure and it affects the extracellular enzyme activity [19]. Sulfur has been used as an electron donor in many biochemical reduction processes [9],[12],[20]. The most important advantages of sulfur are the low cost, ease of operation and its utilization as reactor filling material [10].

Depending on the reduction process, it can be converted to sulfate [21]. In some cases, sulfur disproportionation can lead to direct sulfide formation [22]. Therefore, sulfate and sulfide concentrations were monitored regularly (Figure 6). In the first three periods, produced sulfate was compatible with the reduced NO_3^--N reduced (Figure 6). In the following periods, sulfate was produced between 28 and 87 mg/L independent from the influent AR14 concentration.

4 Conclusions

In this study, for the first time, dye removal in sulfur-based reactors was investigated and the results were compared with methanol-based reduction process. Two up-flow packed bed reactors were operated in parallel for 95 days to remove AR 14. In elemental sulfur-based reactor, influent 10±0.14 mg AR14/L was reduced to 2.05±0.2 mg AR14/L, corresponding to 80% removal. In the methanol-based reactor, efficiency was 87% for the same operational conditions. Hence it could be noted that elemental sulfur is comparable with a traditional organic electron donor-methanol in reducing low concentrations of AR14.
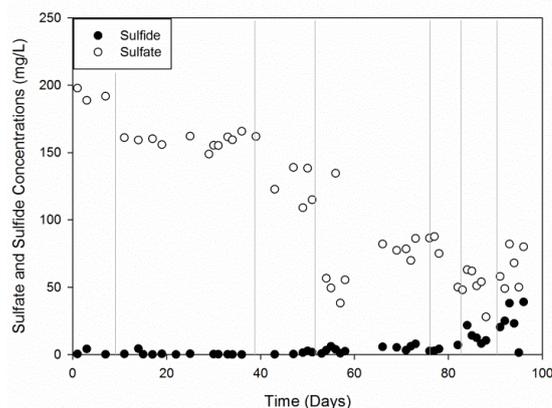

Figure 6: Sulfate and sulfide concentrations for elemental sulfur-based reactor.

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