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The effects of reductant and carbon source on the microbial decolorization of azo dyes in an anaerobic sludge process

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Abstract

The effects of a reductant and carbon source on the decolorization of the disazo dye C.I. Reactive Black 5 and real textile wastewater were investigated in an anaerobic sludge system. The color removal without sulfide in this process was about 94% at 35 °C during 72 h. The intermittent addition of sulfide (10 mg/L) led to an increase in the microbial decolorization of more than 9% during 48 h in comparison with the case where no sulfide was added. The LC/MS and spectrophotometric analysis indicated that the microbial decolorization of the azo dye with anaerobic sludge was attributed to the reduction of the azo bonds. The decolorization rates were about 40% without an additional substrate and about 20% with methanol (10^{-3} M), which were about 2- to 3-fold lower than that obtained with the favorite C-source, glucose. In the treatment of real textile wastewater, the unsatisfactory removal of COD (57.8%) and color was obtained during 12 h, due to the lack of readily degradable COD for the azo dye decolorization and the toxic effect of the wastewater.

Keywords: Azo dye; Decolorization; Anaerobic; Textile wastewater; Sulfide; C.I. Reactive Black 5

1. Introduction

Much attention has been focused on the careful reduction of the dyes discharged from the dyeing and textile industries, since some of these dyes or their metabolites may be mutagenic or carcinogenic [1,2]. Furthermore, color has been recognized as a harsh contaminant in these industries and has to be removed before discharging waste into the environment [3–5].

The majority (60–70%) of industrially synthesized dyes are azo compounds [6,7] which can be reduced and decolorized not only by bacteria under anaerobic conditions, but also by reductants such as sodium dithionite and sodium sulfide [8–12].

An anaerobic process with subsequent aerobic treatment can be used to decolorize wastewaters containing dyes and improve their biodegradability [13,14]. It was observed that the decolorization of azo dyes was far superior under strictly anaerobic conditions although it occurred under semi-anaerobic conditions [15]. It has also been reported that the carbon source fed to a bacterial culture could affect the decolorization process and that most of the reduction of azo to amine occurred during active bacterial growth [10,16]. The chemical reduction of the azo dye, C.I. Reactive Orange 96, can be enhanced by sulfide S^{2-} (H_2S, HS^-, S^{2-}), which can be produced via sulfate respiration by sulfate reducing bacteria (SRB), under the exclusion of O_2, with the reaction proceeding according to first order kinetics with respect to both the dye and sulfide concentration [12].

The purpose of this work was to evaluate the feasibility of a combined biotic and abiotic process, using anaerobic sludge and sulfide. The effects of the sulfide concentration and various carbon sources on the decolorization of the popular azo

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dye, C.I. Reactive Black 5, in wastewater were investigated. Based on the results obtained with the wastewater, the decolorization of complex dye compounds in real textile wastewater was also studied.

2. Materials and methods

2.1. Inoculum and basal medium characteristics

Anaerobic sludge was collected from an anaerobic digester in the Ansan Sewage Treatment Plant (Kyunggi province, Korea) which receives wastewaters from an industrial complex. Anaerobic sludge, adapted to the azo dye for the previous 2 months, was used in all experiments. The basal medium contained (g/L) $Na_2HPO_4 \cdot 2H_2O$ 3.5; KH_2PO_4 1.0; $(NH_4)_2SO_4$ 0.5; $MgCl_2 \cdot 6H_2O$ 0.1; $Ca(NO_3)_2 \cdot 4H_2O$ 0.05; Resazurin 0.001, a redox potential dependent indicator for only preculture; 10^{-3} M of glucose, and 1 mL/L of trace element solution containing (g/L) $ZnSO_4 \cdot 7H_2O$ 0.1; $MnCl_2 \cdot 4H_2O$ 0.03; H_3BO_3 0.3; $CoCl_2 \cdot 6H_2O$ 0.2; $CuCl_2 \cdot 2H_2O$ 0.01; $NiCl_2 \cdot 6H_2O$ 0.02; $NaMoO_4 \cdot 2H_2O$ 0.03. After sterilizing it at 121 °C for 30 min, the basal medium was flushed for 15 min with sterile N_2 gas to remove the dissolved oxygen.

2.2. Anaerobic reactor and chemical decolorization

The anaerobic reactor used in this study was a glass vessel with a capacity of 4 L, equipped with a mechanical stirrer with two-stage impellers, a pH controller, and a temperature controller. The temperature was kept at 35 °C and the pH was maintained at 7.0 by the addition of 0.1 N HCl and 0.1 N NaOH solutions. After acclimating the anaerobic sludge by preculture in a 1 L Erlenmeyer flask for 2 months, it was inoculated into an anaerobic reactor with inoculation cell concentrations of less than 200 mg of protein (300 mg of dry cell weight) per liter, in order to minimize the decolorization caused by the biosorption of azo dye which might be dependent on the biomass concentration. $Na_2S \cdot 9H_2O$ (10–5000 mg/L of sulfide) was used as a reductant for the chemical decolorization and was added into phosphate buffer solution containing 7.0 g/L of $Na_2HPO_4 \cdot 2H_2O$ and 2.0 g/L of KH_2PO_4.

2.3. Dye and textile wastewater

The reactive dye, C.I. Reactive Black 5 (C.I. number 20505, Aldrich Chem. Co., U.S.A.) was used as a target dye for the synthetic wastewater [17]. The initial concentration of azo dye was fixed at about 100 mg/L. The real textile wastewater was collected from a neutralization tank in the wastewater treatment plant located in the textile dyeing industrial complex in the vicinity of the Ansan Sewage Treatment Plant.

2.4. Analysis

The Color was measured spectrophotometrically with a UV–vis spectrophotometer (Optizen 2120UV, Mecasys, Korea) based on the maximum absorbance at 595 nm in the visible range. For this purpose, after centrifuging the sample at 6000 revolutions per minute (rpm) for 8 min and diluting it up to an absorbance of less than 1.0 in 0.1 M phosphate buffer, the supernatant was immediately measured to prevent autoxidation, which is the transformation of aromatic amines into colored aromatic compounds when they are exposed to oxygen [6,18]. The dye and its derivatives formed by microbial decolorization were analyzed by LC/MS (1100 LC/MSD trap classic, Agilent, California, U.S.A.) using a mass spectrometer equipped with a diode array detector (DAD). Separation was achieved using a Symmetry® C18 column (3.9 × 150 mm, 5 μm particle size; Waters Inc., Massachusetts, U.S.A.), which was operated under gradient conditions with a mobile phase consisting of distilled water and acetonitrile at a ratio of 0.95/0.05 at the start, 0.7/0.3 at 3 min, 0.65/0.35 at 5 min, 0.55/0.45 at 10 min, and 0.4/0.6 at 20 min, at a flow rate of 5 μL/min.

The dry cell mass was measured by filtering 10 mL of cell broth on preweighted GF/C filter (Whatman, U.K.). The filtered cells were rinsed twice with 10 mL of distilled water and then dried at 100 °C. The protein concentration was determined by the CBB (Coomassie brilliant blue) method as described by Bradford [19]. Glucose was determined spectrophotometrically using the method described by Miller [20]. COD, sulfide, and nitrate were analyzed according to Standard Method [21]. The oxidation–reduction potential (ORP) was measured with an ORP meter (420A, Orion Research, Georgia, U.S.A.).

3. Results and discussion

3.1. Effects of the sulfide dose on the chemical decolorization of C.I. Reactive Black 5

In order to investigate the chemical decolorization of azo dye by sulfide as a chemical reductant, various concentrations of sulfide in the range from 10 to 5000 mg/L were added to a dye solution containing 100 mg/L of C.I. Reactive Black 5. As shown in Fig. 1(a), the color removal rates increased with increasing sulfide concentration. The color removal rates of C.I. Reactive Black 5 at the sulfide doses of 10, 100, 1000, and 5000 mg/L were 1.3, 3.4, 6.7, and 27.9 mg/L h, respectively. Because cell viability can be inhibited at a high dose of sulfide in a combined biotic and abiotic process, chemical decolorization by the intermittent addition of a moderate sulfide concentration was investigated. Fig. 1(b) shows the 1-day time courses of dye removal for the cases of initial and intermittent additions. The initial addition case shows the 1-day time course of dye removal at an initial concentration of sulfide (10 mg/L) and without any addition of Na_2S afterward, while the intermittent addition case shows the 1-day variation at the same initial concentration with the addition of sulfide (10 mg/L) at 4 and 10 h. The removal efficiency of the dye afforded by intermittent addition was 12.4% higher than that obtained with only initial addition.

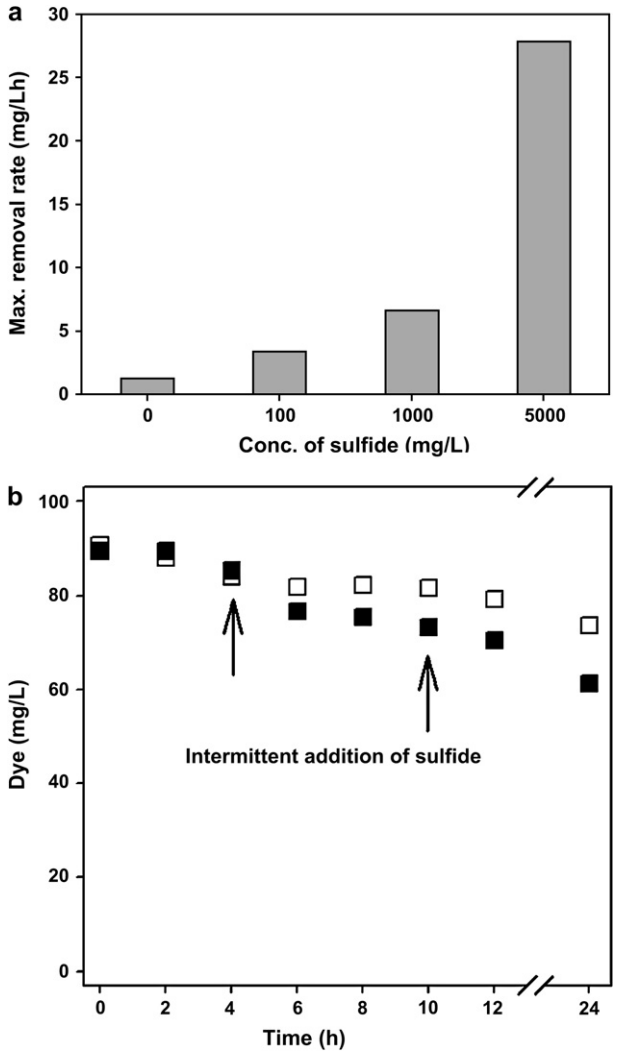


Fig. 1. (a) Effect of sulfide concentration on the chemical decolorization rate of C.I. Reactive Black 5; (b) effect of intermittent additions of sulfide (10 mg/L) on the chemical decolorization of C.I. Reactive Black 5. The initial sulfide concentration was 10 mg/L for both cases. Without intermittent addition (□); with intermittent addition (■).

3.2. Characteristics of the microbial decolorization of C.I. Reactive Black 5 with anaerobic sludge

In the experiments on the microbial decolorization, anaerobic sludge, previously adapted to the dye for a period of 2 months, was introduced into a 4 L bioreactor at an inoculation concentration of less than 200 mg/L of protein (300 mg/L of dry cell weight), in order to minimize the decolorization caused by the biosorption. The initial concentration of C.I. Reactive Black 5 was fixed at 100 mg/L in all experiments. As shown in Fig. 2, the removal rates of the dye were 4.4 mg/L h at 10 °C, 4.9 mg/L h at 20 °C, 12.6 mg/L h at 30 °C, and 23.5 mg/L h at 35 °C, with the maximum removal rate of the dye being the one obtained at 35 °C.

Fig. 3 shows the time courses of the microbial decolorization of C.I. Reactive Black 5, cell growth, glucose concentration, and ORP at 35 °C. The color removal rate was about 50%

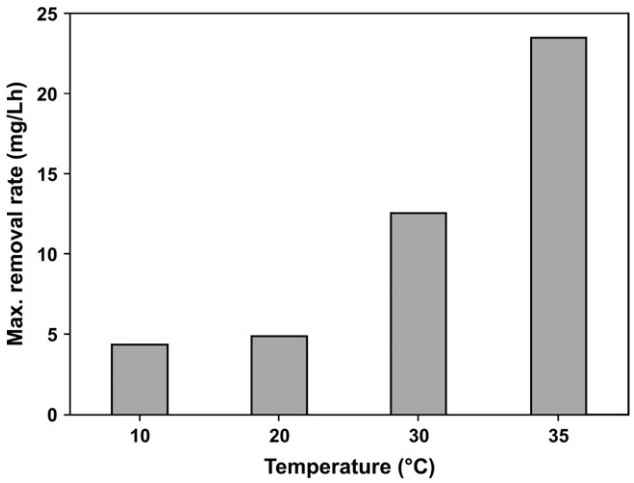


Fig. 2. Effect of temperature on the microbial decolorization rate of C.I. Reactive Black 5.

at 12 h, but the color rate and cell growth rate decreased after 12 h. This seems to be caused by the toxicity of the dye and its derivatives, which resulted in the inhibition of nucleic acid synthesis and cell growth by the dye itself as well as its derivatives such as those containing sulfonic acid groups, which act as a detergent, on the aromatic rings of the azo dye [22]. During the whole period of 72 h, the color removal was about 94% and 964 mg/L of glucose was consumed. The ORP (oxidation–reduction potential) variation was negligible being below −500 mV, due to the operation of the bioreactor under strictly anaerobic condition.

3.3. LC/MS analysis and spectrophotometric analysis

Fig. 4 shows the LC/MS analysis results of the filtered sample at 0, 6, 12, 24, and 48 h, corresponding to the photographs showing the color change of the sample solutions. The dark blue color of C.I. Reactive Black 5 at 0 h changed to pale violet at 6 h and was almost decolorized after 48 h (Fig. 4(a)). As seen in Fig. 4(b), the chromatograms show

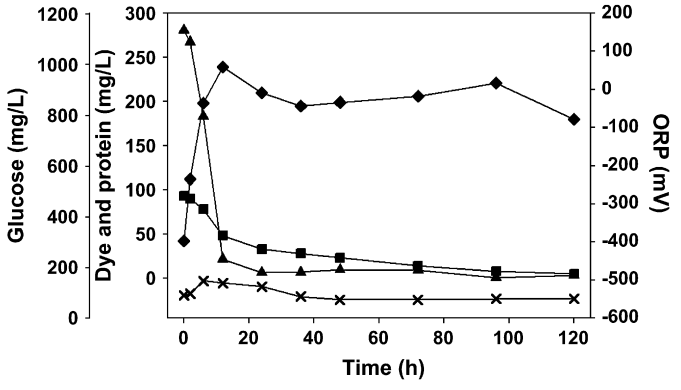
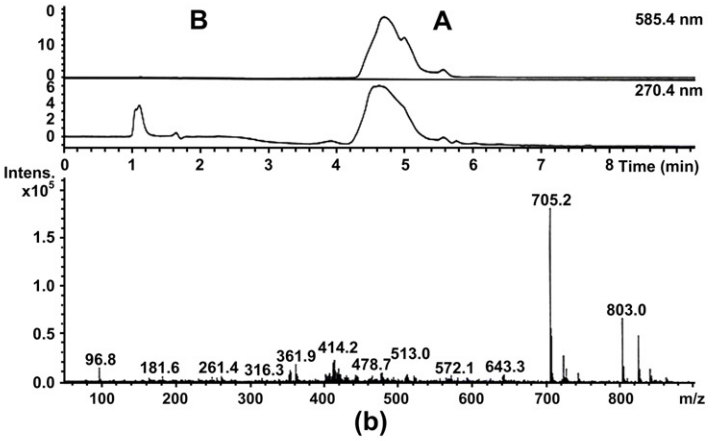
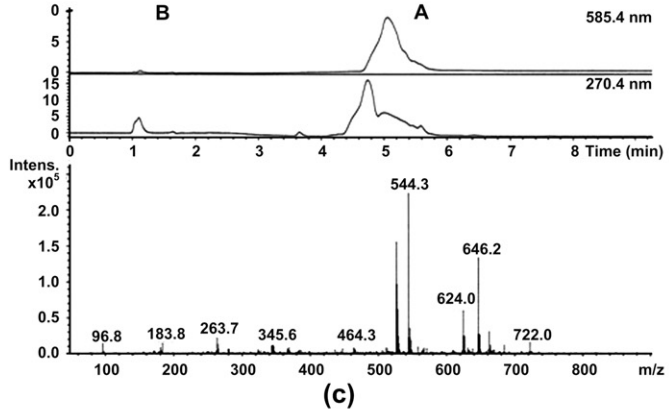


Fig. 3. Variation in dye, protein, glucose, and ORP in the anaerobic bioreactor at 35 °C. Dye (■); glucose (▲); protein (◆); ORP (×).

(a)



(b)



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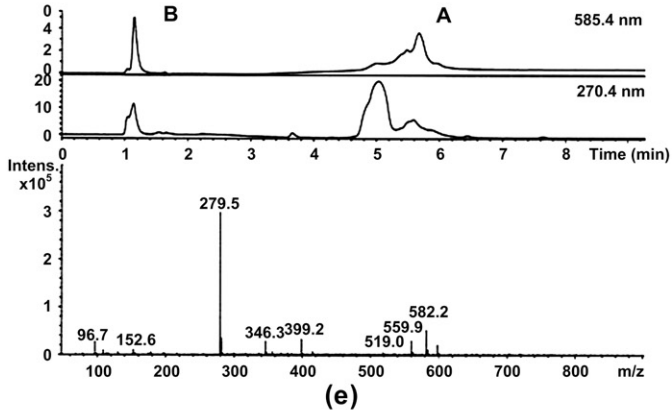

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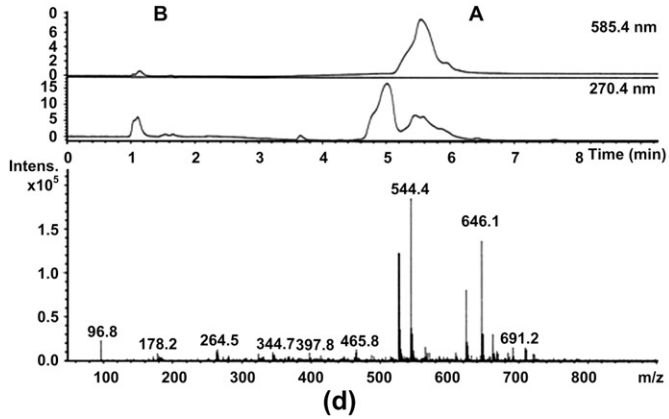
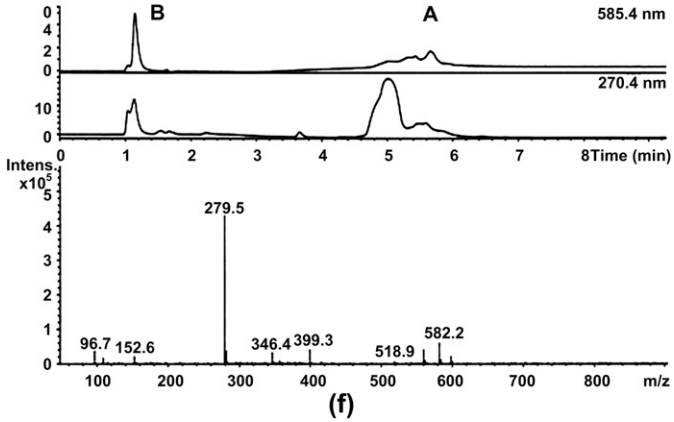


(d)



(f)

Fig. 4. Photograph showing the color change of the sample solution (a) and LC/MS chromatograms of samples collected at different culture time; (b) 0 h, (c) 6 h, (d) 12 h, (e) 24 h, and (f) 48 h.

two major peaks (A and B) at wavelengths of 585.4 nm (positive) and 270.4 nm (negative), respectively. It seemed that the dye and dye intermediates of high molecular weight could not be well separated in the spectrum, resulting in their overlapping in peak A. As the microbial decolorization proceeded, the intensity of peak A decreased, while those of other peaks increased, and azo dye with a molecular weight of about 705 or 803 was decomposed to a decolorized metabolite with a molecular weight of 297.5 via intermediates with molecular weights of 544 and 646.

Fig. 5 shows the visible spectra of the centrifuged samples taken after they were chemically decolorized with sulfide (100 mg/L) and microbiologically decolorized with the anaerobic sludge for different lengths of time. In both cases, as the decolorization proceeded, the maximum absorbance at 595 nm decreased and showed a shift to about 550 nm, which could be explained by the structural modification of the dye molecule [10]. The reduction of C.I. Reactive Black 5 having two azo groups can theoretically yield four aromatic amines [23]. As the two azo groups (chromophores) were slowly transformed into the corresponding amine groups in sequence, it seemed that the color of the dye solution changed from dark blue (max. abs. at 595 nm) to colorless via pale violet (max. abs. at 550 nm), which corresponded to the shift in the maximum absorbance wavelength. It is known that, in the adsorption of an azo dye, all of the peak heights decrease approximately in proportion to each other. In contrast, if the dye removal is attributed to biodegradation, either the maximum absorbance peak will completely disappear or it will shift to another wavelength, resulting in the formation of new peaks [22].

From the above results, it could be concluded that the microbial decolorization of the azo dye by anaerobic sludge was attributed to the reduction of the azo bonds, and that the biosorption by the biomass was nearly negligible.

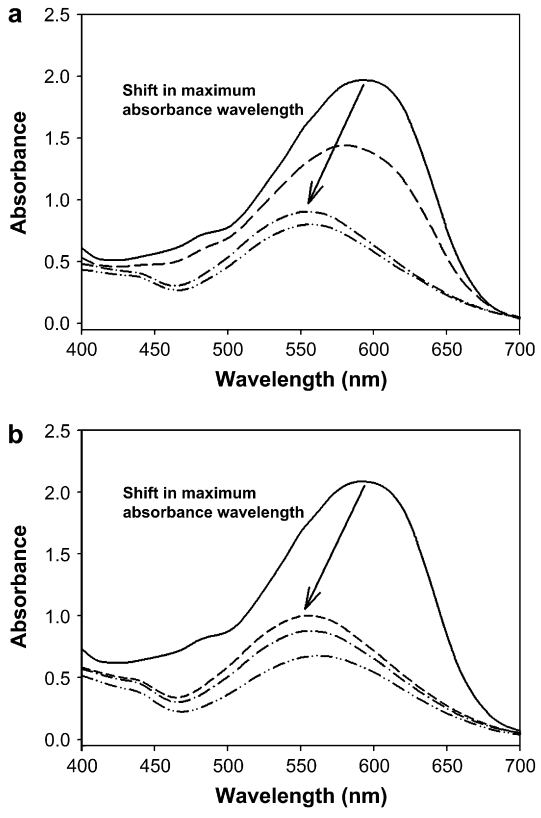


Fig. 5. Spectrophotometric analysis of samples collected at different time. (a) Chemical decolorization with addition of sulfide (100 mg/L); (b) microbial decolorization with anaerobic sludge: 0 h (—), 6 h (– – –), 12 h (—·—), and 24 h (—··—).

3.4. Effects of sulfide on the cell viability and microbial decolorization

Fig. 6 indicates the effects of the sulfide concentration on the cell growth represented as the protein concentrations. The cell growth was extensively inhibited at a sulfide concentration of more than 100 mg/L. Free hydrogen sulfide is presumed to be the most toxic form of sulfide because it can easily diffuse through the lipid cell membrane into the bacterial cytoplasm [24]. Yamaguchi et al. [25] pointed out that the level of undissociated sulfide should be kept below 100 mg/L of H_2S for satisfactory COD removal in a UASB (upflow anaerobic sludge blanket) system. In subsequent experiments, therefore, 10 mg/L of sulfide was intermittently added to the anaerobic reactor in this study. Fig. 7 shows the decolorization of C.I. Reactive Black 5 in the anaerobic reactor with and without the intermittent addition of sulfide. In the case where sulfide (10 mg/L) was intermittently added at 4, 12, and 24 h, the color removal showed an increase of more than 9% during 48 h.

3.5. Effects of carbon sources on the dye removal

Fig. 8 shows the cell growth and substrate uptake patterns obtained with glucose as the carbon source (fermentative substrate) in the absence and presence of C.I. Reactive Black 5. There were no differences in the glucose uptake patterns in

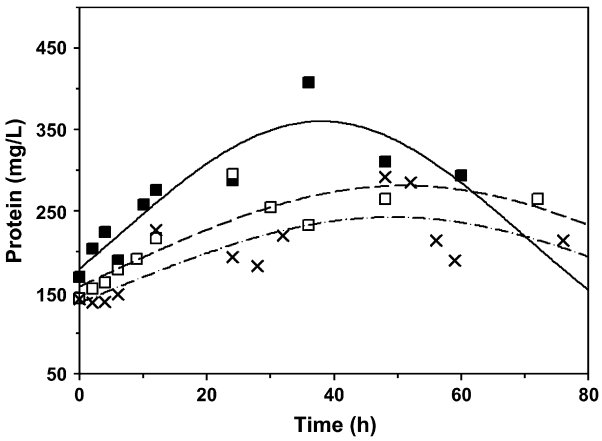


Fig. 6. Effect of sulfide concentration on the cell growth in the anaerobic bioreactor; 100 mg/L (—■—), 200 mg/L (—□—), and 300 mg/L (—·—×—·—).

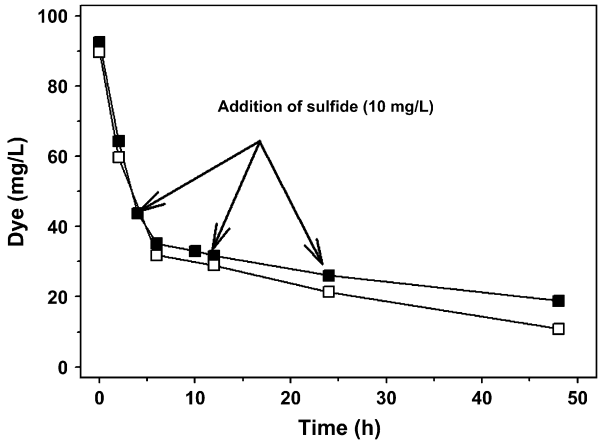


Fig. 7. Decolorization of C.I. Reactive Black 5 in the anaerobic bioreactor with and without intermittent addition of sulfide (10 mg/L) at 4, 12, and 24 h. Intermittent addition of sulfide (□); no addition of sulfide (■).

these two cases, but the cell growth was substantially inhibited by the dye and dye derivatives.

Azo dyes as a non-growth substrate are decolorized by anaerobic consortia through the cleavage of the azo bonds during the course of which reducing equivalents from various carbon sources are transferred to the dye. In anaerobic consortia, acidogenic bacteria convert the soluble substrates, such as carbohydrates, to volatile organic acids or alcohols, such as acetic acid and methanol, which are competitive substrates for methanogenic, sulfate reducing, and acetogenic bacteria [26–27]. Amongst these substrates, methanol deserves particular attention since it is widely used as a cost-effective electron donor for the biological treatment of wastewater. As described by Dos Santos et al. [28], in the presence of acetic acid and methanol, similar decolorization rates of the azo dye, C.I. Reactive Red 2, were found at the temperatures of 30 °C and 55 °C, suggesting that acetoclastic and methylotrophic methanogens could have been involved in the mechanism used for the generation of the reducing equivalents used to cleave the azo bonds. In the subsequent experiments, therefore, decolorization with and without a methanogenic substrate (methanol) was investigated.

As shown in Fig. 9, the decolorization rates were about 40% without the additional substrate and about 20% with methanol (10^{-3} M), which were about 2- to 3-fold lower than the rates obtained with the anaerobic culture in the presence of glucose as a fermentative substrate. These findings

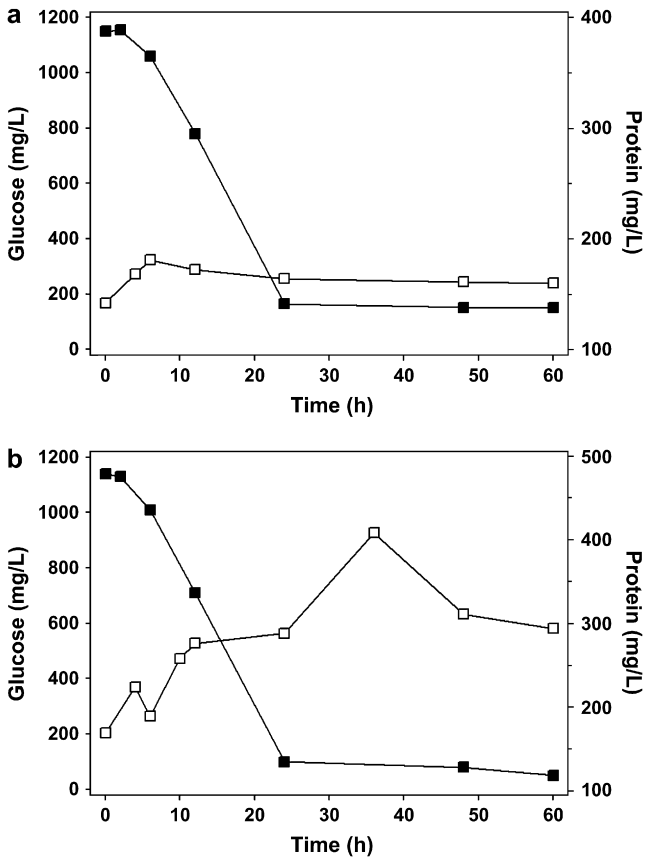


Fig. 8. Growth pattern of cells in the presence of glucose in the cases with and without 100 mg/L of C.I. Reactive Black 5. (a) without dye; (b) with dye. Glucose (■); protein (□).

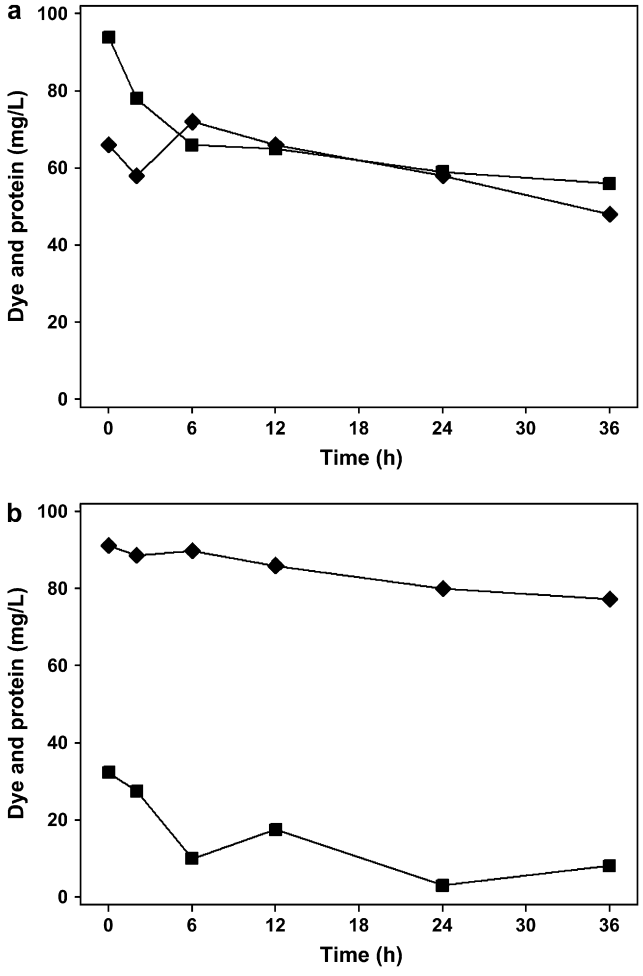


Fig. 9. Effect of carbon sources on the microbial decolorization and cell growth in the anaerobic bioreactor. (a) No substrate; (b) methanol (10^{-3} M). Dye (■); protein (◆).

could be explained by postulating that endogenous respiration occurred in the absence of the substrate and that the anaerobic consortia used in this experiment were insufficiently acclimated to methanol.

3.6. Real textile wastewater

Based on the above results concerning the microbial decolorization of synthetic wastewater, real textile wastewater, containing 1084 mg/L of COD, 394 mg/L of sulfate, 100 mg/L of sulfide, and 0.3 mg/L of nitrate, was fed to the anaerobic reactor without any additional carbon source or nutrient sources. Figs. 10 and 11 show the removal performance and spectrophotometric analysis obtained in the treatment of the real textile wastewater by the anaerobic sludge. As seen in Fig. 11, unsatisfactory removal performance of COD (57.8%) was obtained during 12 h.

Moreover, the absorbance of the centrifuged samples in the visible range showed unexpectedly increasing patterns after inoculation. This seems to be caused by the insufficient amounts of COD available for the decolorization of the dyes and the cell lysate released by the toxic effect of the real textile wastewater. As reported by Dulekgurgen et al. [29], the results of the respirometric analysis of real textile wastewater showed that about 29% of the total soluble COD (965 mg/L) was readily biodegradable COD fraction (280 mg/L), indicating that there was a lack of COD available for the decolorization of the dyes. Moreover, studies on the anaerobic treatment of real textile wastewater indicated that decolorization could be rendered possible with the supplementary addition of an external carbon source in the form of glucose (2 g/L). No color removal and low COD removal (27–59%) were observed during the first 24 days, which is considered to be the necessary acclimation period without any additional carbon and nutrient source [30]. These results suggest that an additional carbon source such as glucose and bioaugmentation with specific bacterial strains preacclimated to real textile wastewater are needed to enhance the color removal in the treatment of real textile wastewater by anaerobic sludge.

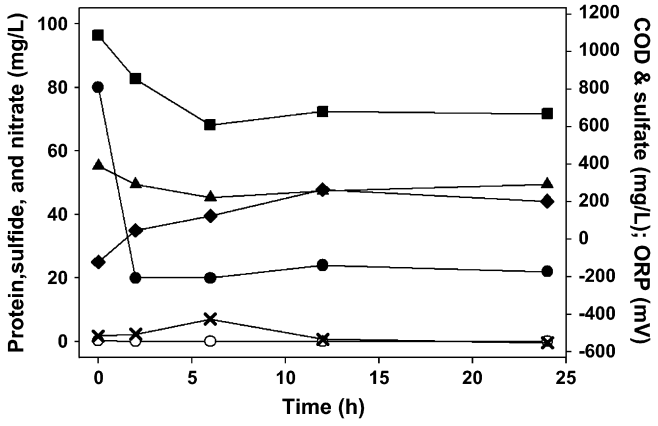


Fig. 10. Variation of COD (■), sulfate (▲), protein (◆), sulfide (●), ORP (×), and nitrate (○) of the real textile wastewater in the anaerobic sludge process.

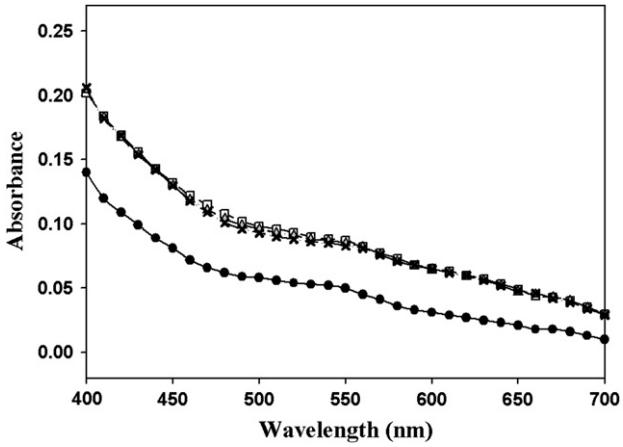


Fig. 11. Spectrophotometric analysis of the centrifuged samples taken from the anaerobic sludge process during the treatment of the real textile wastewater; 0 h (—), 6 h (– – –), 12 h (–·–), and 24 h (–··–).

4. Conclusions

The color removal rates of C.I. Reactive Black 5 at sulfide doses of 10, 100, 1000, and 5000 mg/L were 1.3, 3.4, 6.7, and 27.9 mg/L h, respectively. The removal efficiency of the dye afforded by the intermittent addition with the addition of sulfide (10 mg/L) at 4 and 10 h was 12.4% higher than that observed in the case of only the initial addition of sulfide (10 mg/L). The removal rates of the dye in the anaerobic sludge process were 4.4 mg/L h at 10 °C, 4.9 mg/L h at 20 °C, 12.6 mg/L h at 30 °C, and 23.5 mg/L h at 35 °C, with the maximum removal rate being obtained at 30 °C. The color removal was about 50% at 12 h, after which the cell growth and color removal rates decreased due to the toxic effects of the dye metabolite. During the whole period of 72 h, the color removal was about 94% and 964 mg/L of glucose was consumed.

LC/MS and spectrophotometric analysis indicated that the microbial decolorization of the azo dye with anaerobic sludge could be attributed to reduction of the azo bonds, whereas the biosorption by the biomass was nearly negligible. The microbial decolorization with the intermittent addition of sulfide (10 mg/L) at 4 h and 12 h showed an increase of more than 9% during 48 h in comparison with the case without sulfide. Decolorization rates were about 40% without an additional substrate and about 20% with methanol (10^{-3} M), which were about 2- to 3-fold lower in comparison with the anaerobic culture in the presence of glucose.

In the treatment of real textile wastewater, unsatisfactory removal performance of COD (57.8%) was obtained during 12 h and the absorbance of the centrifuged samples in the visible range showed unexpectedly increasing patterns after inoculation due to the insufficient availability of COD required for

the reduction of the azo group and the toxic effect of the wastewater.

Acknowledgements

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