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Research article

Biosorption and biodegradation of a sulfur dye in high-strength dyeing wastewater by *Acidithiobacillus thiooxidans*

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ABSTRACT

The ability of the bacterial strain *Acidithiobacillus thiooxidans* to remove sulfur blue 15 (SB15) dye from water samples was examined. This bacterium could not only oxidize sulfur compounds to sulfuric acid but also promote the attachment of the cells to the surface of sulfidic particles, therefore serving as an efficient biosorbent. The biosorption isotherms were better described by the Langmuir equation than by the Freundlich or Dubinin-Radushkevich equation. Also, the biosorption process followed the pseudo-second-order kinetics. At pH 8.3 and SB15 concentrations up to 2000 mg L^{-1} in the biomass/mineral salt solution, the dye removal and decolorization were 87.5% and 91.4%, respectively, following the biosorption process. Biodegradation was proposed as a subsequent process for the remaining dye (250–350 mg L^{-1}). A central composite design was used to analyze independent variables in the response surface methodology study. Under the optimal conditions (i.e., initial dye concentration of 300 mg L^{-1}, initial biomass concentration of 1.0 g L^{-1}, initial pH of 11.7, and yeast extract dose of 60 mg L^{-1}), up to 50% of SB15 was removed after 4 days of biodegradation.

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1. Introduction

Sulfur dyes play an important role in textile dyeing industries. Their dyeing processes use sodium sulfide (Na_2S) for reduction purposes which is cheaper and applied more popularly than the other dyeing processes, causing serious environmental pollution (Nguyen and Juang, 2013). The sulfur-containing aromatic heterocyclic units occur in the structures of sulfur dyes (Cai et al., 2012). By using the reducing agent, sulfur dyes are converted from the insoluble form to a soluble form. This traditional dyeing process produces harmful residues and effluents (Nguyen and Juang, 2013). These effluents contain high concentrations of toxic components, such as sulfides (S^{2-}), thiosulfate ($S_2O_3^-$), and massive release of hydrogen sulfide (H_2S), which can cause harm to microorganisms in wastewater treatment plants. Alternative treatment methods for removing sulfur dyes from wastewater have been studied, including physicochemical and biological methods (Nguyen and Juang, 2013). Among these methods, biological methods have attracted a considerable amount of attention because such systems are often considered to be more cost effective and environmentally friendly than physicochemical ones and because they produce less sludge. Biological decolorization of sulfur dye-containing solutions has been reported using bacterial strains, including *Stenotrophomonas maltophilia* AAP56, *Galactomyces geotrichum*, *Bacillus* sp. VUS, and *Bacillus circulans* GC subgroup B.

The *Thiobacillus* species isolated from the activated sludge is observed commonly and has specific sulfur oxidation activity (Lee et al., 2000). The abilities of *Thiobacillus thiooxidans* to oxidize liberated hydrogen sulfide (H_2S), sulfides (S^{2-}), thiosulfate ($S_2O_3^-$), elemental sulfur (S^0), and sulfur compounds have been surveyed previously (Khan et al., 2012; Midha and Dey, 2008; Pepper and Miller, 1978). They can adapt to live well in the environments that contain sulfur compounds and inorganic carbons are utilized as carbon sources under aerobic conditions. Sulfates (SO_4^{2-}) and sulfuric acid (H_2SO_4) are readily produced by these oxidation processes. Besides, these bacteria can be chosen appropriately for biological sulfide oxidation because of their high resistances in sulfide-rich environments and simple nutrient inputs, inorganic carbons are utilized as carbon sources. Although *Acidithiobacillus thiooxidans* (*A. thiooxidans*) is a strictly autotrophic bacterium, it is

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able to assimilate and metabolize organic substances such as glucose, acetate, and glycerol (Butler and Umbreit, 1966). In this sulfur oxidation process, this strain is not bound with their growth when oxygen or nitrate is a primary electron acceptor (Kuenen and Robertson, 1993). The autotrophic sulfur bacteria can live within the wide pH range of 2.0–8.0 (Lee et al., 2000); however, a more suitable environment will be acidic due to their fast reproduction (Shan and Zhang, 1998).

The attachment occurs with *A. thiooxidans* on the surfaces of sulfur and sulfidic particles (Liu et al., 2003). The mechanism of this attachment can relate to many factors such as the effect of electric charges, surface irregularity, cell membrane characteristics or the excretion of extracellular polymeric substances. A recent study reported that the polymers on the surface of *A. thiooxidans* and the solution pH have a significant effect on the adhesion of bacteria onto mineral surfaces and that the adhesion force of this bacteria increases as the solution pH decreases (Diao et al., 2014). Carboxyl, amino, and phosphodiesters groups are present at this cell surface and an electrical charge balance of bacteria can lead to a balance of these groups.

The emissions of hydrogen sulfide and the discharge of wastewater containing sulfides and high color into the drainage system are not allowed by the environmental protection regulations. The cost-effectiveness of biological methods for the treatment of toxic substances is concerned seriously. To meet the requirements and to improve the treatment of sulfur dyes and sulfides in high-strength dyeing wastewater, the biomass *A. thiooxidans* was introduced. The aim of this study was to evaluate the ability of *A. thiooxidans* to remove sulfur dye from synthetic dyeing solution and to determine the optimal conditions for the bioremoval of sulfur dye from synthetic dyeing solution.

2. Materials and methods

2.1. Microorganisms

2.1.1. Growth of A. thiooxidans

A. thiooxidans (BCRC 80191) of a local strain from Taiwan obtained from the Food Industry Research and Development Institute (FIRDI), Hsinchu, were used in this work. The so-called 828 culture medium contained the following components (per liter of distilled water): 2.0 g of $(NH_4)_2SO_4$, 3.0 g of KNO_3, 3.0 g of KH_2PO_4, 0.5 g of $MgCl_2 \cdot 6H_2O$, 0.25 g of $CaCl_2 \cdot 2H_2O$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 5.0 g of $Na_2S_2O_3 \cdot 5H_2O$, 0.3 mg of $Na_2MoO_4 \cdot 2H_2O$, 0.1 g of yeast extract, 10.0 mg of bromophenol blue, and 1.0 g of sulfur (powder). These chemicals were obtained from Sigma Chemical Co., St. Louis, USA.

The sterilization of sulfur element was conducted separately by autoclaving for 60 min at 105 °C. This step was done repeatedly in 3 successive days. The pH of the medium was adjusted to 4.0–4.6 with sulfuric acid. In an incubator shaking at 150 rpm, *A. thiooxidans* readily grew in the 828 culture medium containing pre-sterilized elemental sulfur at 30 °C. The oxidation of this element sulfur took place during 10 days of incubation and the pH was decreased to 1.4. The cells were counted based on the optical density (OD) at 440 nm (Khan et al., 2012), which was monitored using a Jasco V-630 spectrophotometer. Fig. 1 shows that the growth of *A. thiooxidans* exhibits an exponential phase during days 9 and 10.

2.1.2. Cell harvest

In the exponential phase (after the first 9–10 days of incubation), the biomass was collected in centrifuge tubes after centrifugation at 10,000×g for 20 min, and washed three times with distilled water prior to use in the subsequent experiments (Chang et al., 2008).

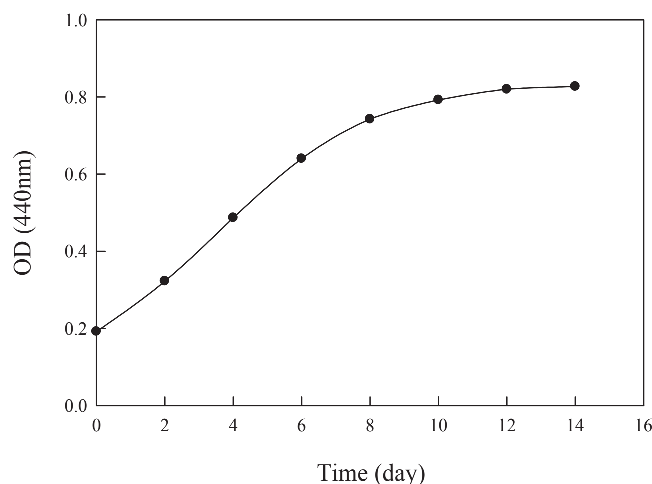

Fig. 1. Growth curve of *A. thiooxidans* in the 828 culture medium with an initial pH of 4.6 at 30 °C.

2.2. Sulfur dye and dyeing

The dye used was sulfur blue 15, SB15 (Sky blue CV B-15, C.I. 53540, CAS 1327-69-1). This dye was purchased from Ningbo New Dragon International Co., Ltd. The sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) was obtained from Alfa Aesar, Co., which was used as reducing agent for the dyeing process of SB15 (the weight ratio of SB15 and sodium sulfide being 0.4).

2.3. Analytical methods

A spectrophotometric method was applied for measuring the absorbance or the concentration of different types of dyes, such as cyanine dye (Kunzler et al., 2011; Penzkofer et al., 1987), dye indicator (Shah et al., 2013), food dye (Sigmann and Wheeler, 2004), disperse dye (Golob and Tušek, 1999; Şahin et al., 2007), and acid dye (Alarfaj et al., 2008). The concentration of SB15 was determined using a Jasco V-630 spectrophotometer with the deionized water as a blank at a wavelength of 298 nm where the absorbance of SB15 reached a maximum value in this work. Color was measured by HACH DR/2000 spectrophotometer. The samples were filtered using filter papers prior to the color assay. This filter paper, which was purchased from Toyo Roshi Kaisha Ltd., retains large crystalline particles, coarse and gelatinous precipitates, and suspended solids.

The biomass concentration during the experiments was determined using the dry cell weight (DCW) method (Aremu et al., 2010; Bandyopadhyay et al., 2013; Loc and Nhat, 2013). Basically, this method is based on the difference between the weights of fresh cells before and after drying at 50 °C to a constant weight.

The surface morphology of the biomass and dye solution during the adsorption process was examined by scanning electron microscopy (SEM) (Jeol JSM 5600). Before SEM measurements, the samples were dried at 65 °C to a constant weight. FTIR spectroscopy was applied to analyze the chemical structure of the dye solution collected from independent biodegradation and adsorption experiments. A FTIR spectrometer (BRUKER TENSOR 27, Germany) was used to record these spectra. Each spectrum was collected over the range 4000–400 cm^{-1} with a 4 cm^{-1} resolution and 32 scans. The collected dye solutions were dried to a constant weight at 65 °C, ground, and mixed with potassium bromide (KBr) to form pellets.

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