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Semicontinuous Decolorization of Azo Dyes by Rotating Disc Contactor Immobilized with *Aspergillus sojae* B-10

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Abstract *Aspergillus sojae* B-10 was immobilized and used to treat model dye compounds. The model wastewater, containing 10 ppm of azo dyes such as Amaranth, Sudan III, and Congo Red, was treated with cells attached to a rotating disc contactor (RDC). Amaranth was decolorized more easily than were Sudan III and Congo Red. Decolorization of Amaranth began within a day, and the dye was completely decolorized within 5 days of incubation. Both Sudan III and Congo Red were almost completely decolorized after 5 days of incubation. Semicontinuous decolorization of azo by reusing attached mycelia resulted in almost complete decolorization in 20 days. This experiment indicated that decolorization was successfully conducted by removing azo dyes with *Aspergillus sojae* B-10.

Keywords: azo dye, decolorization, rotating disc contactor, *Aspergillus sojae*

Dyes are released into the environment through industrial wastewater from two major sources: textiles and dyed materials [1]. Major classes of synthetic dyes include azo, anthraquinone and triaryl methane dyes. Many of these are toxic, or even carcinogenic, compounds with long turnover times [2,3]. Moreover, they are considered to be a pollution problem, due to their wide dispersal in the environment. Among these substances, azo dyes are the largest class of dyes, with the greatest variety of colors [4,5]. Azo dyes are also structurally heterogeneous, thus, as a group, they are not uniformly susceptible to microbial binding [5]. Therefore, they are not readily degradable, and, typically, can not be removed from water by conventional wastewater treatment systems. Although most azo dye is eliminated from waste streams by adsorption to sludge, it typically remains undegraded under aerobic conditions [6,7], but degrades under anaerobic conditions [8]. In addition to biological treatments, physical and chemical methods are also used to remove colored dye substances from wastewater. Physical methods mainly involve adsorption by active carbons. Chemical methods often involve coagulation of dye substances, followed by precipitation of the chemical sludge, or oxidation *via* ozone [9]. In hybrid physical-chemical or physical-biological processes, ionizing irradiation and ultra-filtration have proven to be useful methods of pretreatment. Nevertheless, both physical and chemical methods have their shortcomings [10,11]. These challenges provide obvious opportunities for the development of new biotechnological treatment methods, and viable alternatives for treating dye-containing wastewater. Although azo dyes are generally considered to be nondegradable under aerobic conditions, *Basidiomycetes* could be reasonably expected to be effective in degrading these dyes [12-15]. Recent results in a number of laboratory experiments have shown that the ability of this organism to degrade such an array of chemical compounds is due to its lignin-degrading system, which is activated in response to nutrient-deficient conditions [16].

The lignin-degrading system comprises a number of peroxidases which are excreted by the fungus under conditions of nutrient limitation [16]. These peroxidases have the ability to catalyze the decolorization of azo dye, as well as induce the initial oxidation of a wide variety of other compounds [17]. On the other hand, *Pseudomonas* strains, which decolorized the azo dye, excreted azoreductase [18]. These results provided little information with respect to the effectiveness of *Pseudomonas* strains in dye color degradation. The authors isolated and identified the azo dye decoloring microbe from the wastewater, and identified the degradation pathways utilized by the microbe [19]. The model wastewater was treated, therefore, by a rotating disc contactor (RDC), with disc attached to *Pseudomonas cepacia* [20,21]. Rotating disc contactor (RDC) are attached-growth biological reactors containing a series of closely spaced circular discs, made of plastic media, and mounted on horizontal shafts. Microorganisms adhere to and grow on the rotating disc, forming biofilms which degrade organic matter in wastewater [22,23]. It is expected that wastewater clarification technology will soon be developed, for removing dyes and other organic substances in a single operation, using dye microbials. In the previous report, the decolorization of azo dyes using *Aspergillus sojae* B-10 was advanced as a clear example of color removal through microbial degradation of the color substances [24]. We hypothesized

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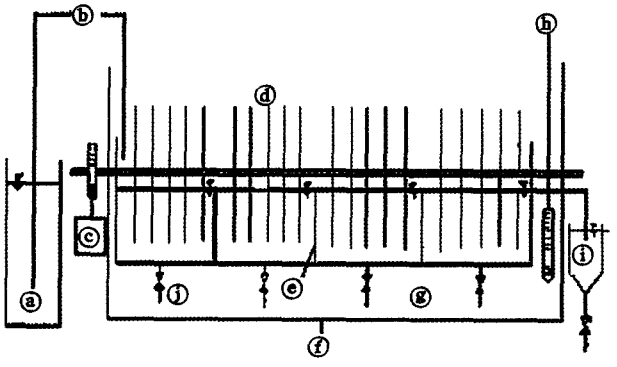

Fig. 1. Schematic diagram of rotating disc contactor.
ⓐ Storage tank, ⓑ Contactor, ⓒ Peristaltic Pump, ⓓ Water Bath, ⓔ Reduction, ⓕ Heater, ⓖ Disks, ⓗ Clarifier, ⓘ Baffle, ⓙDrain Valve.

that a decoloring system of non-specific nature could be achieved, and ultimately, could be an effective degrader for a variety of dyes. This report demonstrates the semi-continuous biodegradation of azo dyes by a RDC attached to *Aspergillus sojae* B-10.

Aspergillus sojae B-10 [24] was allowed to grow for 5 days, and was then poured into the RDC, where it could attach to the disc. The liquid medium used in this experiment contained 2.0% glucose, 0.06% $NaNO_2$, 0.1% KH_2PO_4, and 0.5% $MgSO_4 \cdot 7H_2O$, pH 5.0. On day 5, one of the dyes was added to each culture. All dyes were prepared in water at a concentration of 1.0 mg/mL and were added to achieve a final concentration of 10 ppm for Amaranth, Sudan III, and Congo Red. Chemical dyes were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA).

Decolorization in the extracellular fluid was monitored by assaying the absorbance of the wavelength for each dye at selected intervals during the incubation period. Some of the dyes remained bound in the fungal mycelia. In an attempt to solubilize any bound dye, the mycelial mat was homogenized for 10 seconds in 10 mL of methanol in a Potter-Elvehjem tissue homogenizer. During this process, azo dyes were still stable for decolorization. The homogenate was centrifuged at 5,000×g for 10 min, and the mycelial pellet was suspended in an additional 5 mL of methanol, and recentrifuged. The two resulting supernatants were then combined, and the absorbance of the supernatant was determined. The wavelengths, in nanometers, used for the absorbance ratios of Amaranth, Sudan III, and CongoRed were A_{520}/A_{332},A_{500}/A_{351}, and A_{496}/A_{351}, respectively, and the amount of dye associated with the mycelial mat was calculated [14].

Each rotating polymethyl methacrylate disc, 15 cm in diameter and 1.0 mm thick, was covered with a disc sheet, and the circumference was fixed with 10 mm-wide rubber tape (Fig. 1). On the 5th day, cultivated cell suspension (300 mL) was run on the contactor. The volume and surface area of the spores that adhered to each disc were 110.4 cm^3 and 130.6 cm^2, respectively.

The RDC discs can be rotated by a motor and drive system on each shaft, or by an air drive system, with a coarse bubble diffuser at the bottom of the tank, supplying air which is caught by air cups attached to the discs. Individual RDC units are placed in series to maximize treatment efficiency. Baffles are placed to separate the shafts into a series of completely mixed bioreactors, each one referred to as a stage. These baffles are moveable, allowing the number of stages, as well as their size, to be adjusted in response to variations in the loading process. The rotation of the discs imparts a shear force to the biofilm, keeping its thickness relatively consumed by the substrates. Rotation of the discs also serves to provide oxygen required for the growth of biomass and substrate degradation. A settling tank is normally required to remove the biomass from the effluent. A cover made of fiberglass is generally provided over RDC units, in order to provide protection against inclement weather, freezing, and sunlight.

Results from the laboratory scale unit, after treating the model wastewater, show decolorization characteristics which are consistent with classical biodegradation in rotating disc contactors (RDC). In these experiments, 800 mL of culture media in 3 L RDC was used for decolorization under optimal cultural conditions. Decolorization by *Aspergillus sojae* B-10 occurred at pH 5.0, at 37°C, gradually after 1 day of incubation in culture media containing 2.0% glucose, 0.06% sodium nitrate, 0.1% potassium pyrophosphate, and 0.5% magnesium sulfate. As shown in Fig. 2(a), the RDC trials all included a period during which almost complete removal occurred. The retention time was defined as the period from the 1st stage, when the solution was poured, to the 4th stage. The rate of decolorization of azo dyes was kept stable under the RDC conditions. The rates increased as the solution moved from the 1st stage, through the 2nd, 3rd, and 4th stages. The rates in the 1st and 2nd stages were lower in the RDC system on day 1 than on day 2. The rates in the 3rd and 4th stages were approximately 85% in both systems.

This indicates that the retention time in the treatment of the wastewater was as short as possible, because the wastewater was treated in multiple stages. The final rates of the RDC system were approximately equal. However, the amount of decolorization on day 1 was close to double that of day 2. It was suggested that *Aspergillus sojae* B-10, as well as fungal transformation, reduced the intensity of the azo dyes in solution. In order to confirm the decolorization, it was necessary to measure the soluble dye absorbance at various wavelengths.

The model wastewater containing 10 ppm Amaranth, was treated with *Aspergillus sojae* B-10 attached to an RDC. As shown in Fig. 3, the UV spectra of the Amaranth decreased in fluids containing Amaranth. Decolorization of Amaranth began within 1 day and was nearly complete by the 5th day of incubation [7]. As shown in Figs. 2(b) and (c), Sudan III and Congo Red were also treated with the RDC system for 5 days. The Sudan III and Congo Red were not as easily decolorized as was the Amaranth. The decolorization rate of Sudan III was initially high, but decreased with longer operation time. In

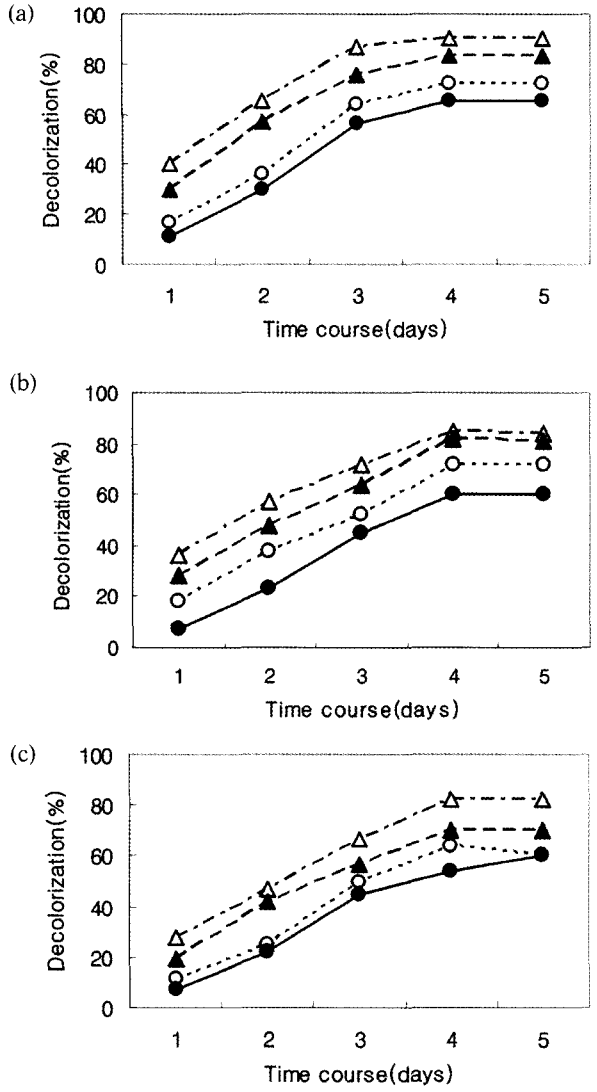

Fig. 2. Time course of decolorization of (a) Amaranth, (b) SudanIII, and (c) Congo Red by *Aspergillus sojae* B-10 using rotating disc contactor. Stages of rotating disc contactor ; 1st: ●, 2nd: ○, 3rd: ▲, 4th: △.

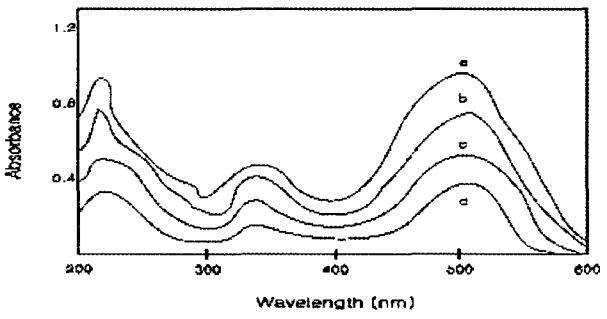

Fig. 3. Absorption spectra of decolorized Amaranth in each stage of rotating disc contactor. Stages of rotating disc contactor ; 1st: a , 2nd: b , 3rd: c , 4th: d.

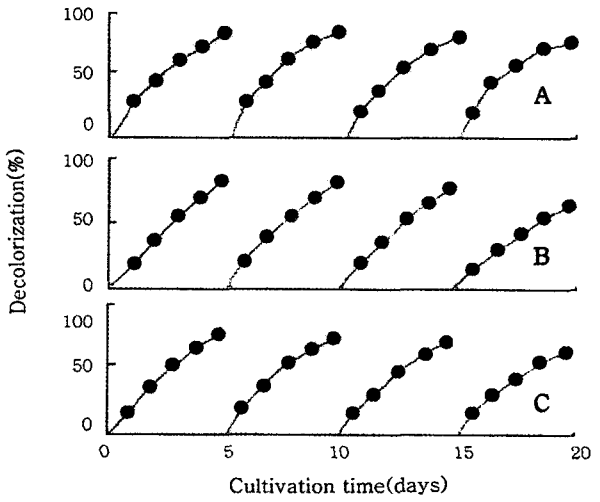

Fig. 4. Successive decolorization of azo dyes by immobilized attached mycelia system. A; Amaranth, B; Sudan III, C; Congo Red.

the case of Congo Red, decolorization rate was lower than that of both Amaranth and Sudan III. It was clear from the result that the different decolorization rates of Sudan III and Congo Red were due to factors such as enzyme physical and chemical properties. The decolorization rates of azo compounds may be affected by different chemical structures, most notably those of azo, hydroxy, and sulfate groups. It takes longer for Sudan III and Congo Red to be decolorized than the time required to enhance the ratio of removal of chemical structures. It is also clear that the majority of color was removed in the aerobic stage. All of these observations suggest that substantial biodegradation of three azo dyes occurred in the *Aspergillus sojae* B-10 culture. The effectiveness of *Aspergillus sojae* B-10 in decolorizing these dyes may vary, depending on the structure and complexity of the dye, and whether or not the fungus is in idiophase. It appears that a number of enzymes, or enzyme systems other than lignin peroxidase [24,25] must be involved in the break down of these dyes, and are perhaps responsible for the initial step in the degradation of azo dyes.

In recent years, the practical importance of immobilized cells has been successfully applied to the decolorization of dyes, using RDC [23]. In order to improve the azo dye decolorization process, *Aspergillus sojae* B-10 mycelia were grown at 37°C for 5 days, and mycelia were attached to the RDC system discs. Attached mycelia were reused for successive cultivation, and the fresh culture medium was replaced every 5 days. As shown in Fig. 4, the RDC system was more effective in decolorization during longer operations. The azo dyes (Amaranth, Sudan III and Congo Red) were almost completely decolorized with nitrogen-limited culture, over 20 days of successive cultivation. After 20 days, decolorization of all three azo dyes slightly decreased, by about 10%, under the same conditions. Therefore, a longer retention time may be required for the RDC system, in order to enhance

the rate of elimination of those dyes. The attached mycelia discs were maintained to decolorize the three azo dyes for successive operations. The RDC system exhibited decolorization activity during four substrate replacements. It appears that the low decolorization rates of Amaranth, Sudan III, and Congo Red are due to this factor. This experiment demonstrated that the RDC system, with attached *Aspergillus sojae* B-10, was an effective protocol for removing azo dyes. Further research on azo dye decolorization will yield practical benefits in the treatment of azo dyes from wastewater.

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