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Penicillium janthinellum 균체를 이용한 생물흡착에 의한 염료의 제거

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Removal of Dyes by the Biosorption Using Biomass of *Penicillium janthinellum*

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A biosorption of azo and reactive dyes into the intact and modified biomass of *Penicillium janthinellum* were investigated. Initial pH of medium affected the initial adsorption rate and decolorization. The initial optimum pH was found to be 2.0, and the maximum adsorption rates of dyes were 40℃. The reactive dyes called Apollocion Red 7EB, Apollofix Red SF-3B and Apollocion Red H-E3B showed the high initial adsorption rates as 0.06, 0.086 and 0.079 mg/g · min, respectively. A mixture of dyes containing azo and reactive dyes was adsorbed to the biomass of *Pen janthinellum* and revealed that the initial adsorption rate was 0.084 mg/g · min. Both percent decolorization and the initial adsorption rate with the addition of detergents were lower than control. Furthermore, the addition of ions had no influence on the dye adsorption rate. Modified biomass of *Pen janthinellum* was also investigated for the dye adsorption and the superior dye loading performance was observed compared with the ion-exchange/chelating resins used for removal of Apollocion Red 7EB.

Key words : biosorption, *Pen janthinellum*, dye, modified biomass, decolorization

Introduction

Dyes are released into the environment in industrial wastewater from two major sources, textile and dyestuff industries(1-3). Dyes cause the harmful effect, e.g. carcinogenic effect to the human and other organisms. The degradants like amine are colourless but can be toxic and carcinogenic to the organisms(3, 4). In fact, there is the case that dye in itself doesn't have the toxicity but its degradant is carcinogen.

Dyes are difficult to degrade with the conventional wastewater treatment, e.g the activated sludge(3, 5), and cause the serious environmental pollutant problems. Because dye molecules are stable against the temperature, pH and the sunlight due to the development of dyeing process, it is very difficult to remove dyes in the wastewater treatment process. Many approaches to solve the pollutional problems related to dyes have been performed Azo dyes and reactive dyes were degraded within 30 hours ~ 2 weeks by the microorganisms(3, 6-10). Adsorption method has been used for the removal of the various heavy metals from the environment(11-15). Adsorption of pollutant is considered to be better than the degradation of that, in the aspects of the time, the cost and safety. The purpose of the dye wastewater treatment is the removal of dye-toxicity as well as that of dyes from sea and river. It is possible that biosorption is applied to the removal of dyes in the wastewater treatment system. Also the use of dead biomass or modified biomass eliminates the problem of lowering the adsorbability by the contamination as well as trouble with maintenance and nutrient supply to living cell(23, 24).

Dyes usually have the electric charge that occurs in the interaction between the charge of dye and that of textile. It is possible that the various pretreatments of microorganisms improve the properties of the electric charges on the surfaces of the microorganisms(16). In this report, an adsorption of dyes was performed with *Pen. janthinellum* in view of removal of dyes from the wastewater

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Materials and Methods

Preparation of Microorganism for Biosorption

Penicillium janthinellum isolated from soil and drain water was used throughout experiments *Pen janthinellum* was cultivated in the liquid media containing starch, 5.0g/l; NH_4Cl, 0.4g/l; KCl, 0.2g/l; $MgSO_4 \cdot 7H_2O$, 0.14g/l; KH_2PO_4, 0.4g/l; NaCl, 0.4g/l; $CaCl_2$, 0.2g/l at pH 7. Cultures(100ml) for mycelium growth in 250ml conical flasks were incubated at 30℃ and shaken at 100 rpm.

After 5 days of incubation of *Pen janthinellum*, the mycelium were filtered with Whatmann No.3. Then, the mycelium were washed twice with distilled water and the mycelium were inactivated using 1% formaldehyde and dried at 70℃ for 24 hours. For the biosorption studies, a weighed amount of dry cell was homogenized in a mixer to destroy cell aggregates and used as the modified biomass.

Chemicals

The following detergents were used : Tween 20, Tween 80, Triton X-100, Triton X-114, SDS(Sodium Dodecyl Sulfate). For the comparison to different adsorbents, adsorbents used in the biosorption study are as following. Activated carbon(20-60 mesh), DOWEX-5W, XAD-2, XAD-4, XAD-7, XAD-16, Amberlite IR-1, Chitosan. All chemicals were purchased from Sigma (USA). Azo and reactive dyes were used. Acid.Brill.Red 3BL (monoazo), Appolo Nylon Fast Red(diazo); Nylon Fast Red MPG(diazo); Apollocion Red 7EB(reactive dye), Apollofix Red SF-3B(reactive dye); Appolocion Red H-E3B(reactive dye); Mixed dye(a mixture of all above 6 dyes). All dyes provided by a textile company were in liquid preparations of 1%(w/v).

Biosorption Process

The concentration of dyes for all experiments was fixed to 0.001g/l. Equal quantities of weighed dried mycelium were added to Erlnmayer flasks containing dye of known concentrations. The initial pH was adjusted by using dilute NaOH and H_2SO_4. The flasks were agitated at 150 rpm in the shaker for 2 days.

Analysis of Dyes

Samples were centrifuged at 10,000rpm for 1 min and the supernatant retained was analysed for residual dye concentration with the visible spectra of the dyes using Hitach 220 UV-Visible spectrophotometer(Tokyo, Japan). Percentage decolorization was estimated from the absorption values of the spectrum-peaks obtained in comparison to the initial (0h) values.

Results And Discussion

The Effect of pH and Temperature

The effect of pH on the biosorption was investigated with intact and modified biomass. The adsorption rate of dye on the modified biomass increased up to 0.064 mg/g · min with lowering the pH to 2.0. The increase of initial adsorption rate(r_i) of dye and percent decolorization were more efficient as pH decreased in the medium(Table 1). With intact biomass, the initial adsorption rate(r_i) at pH 2.0 was about 7 folds higher than that observed at pH 6.0. The similar result was obtained with the modified biomass, although the initial adsorption rate with intact pellet was higher than that of modified biomass at the same pH indicating that pH affects the adsorption rate of microorganisms due to the adsorption mechanisms onto microorganism surfaces reflecting the nature of the physico-chemical interaction of both ion in solution and nature of the cell adsorption sites(21).

According to Kuhn *et al* and Gadd *et al*(18,19), the adsorption of pollutant on the microorganism is caused by the electric charge on the surface of the microorganisms. The various pH of medium affects the initial adsorption rate of dyes because it changes the electricity and quantity of charges on the surfaces of microorganisms. However, microorganisms have different electricity and quantity of charges. In the case of the biosorption of Copper(II) and Iron with *R.arrhizus*(11, 20), the highest initial adsorption rate was reported at the pH 1~2. The removal of Copper(II) and Iron(III) from the aqueous solution was more efficient with increasing pH and the optimum pH was 1~2. It has also revealed that pH range showed the highest initial adsorption rate at pH 4 for the adsorption of chromium(20). In the studies for adsorption of lead with *Z.ramigera*(21), optimal initial adsorption occurred at the pH 4~5. Thus, the difference in the pH range for the optimal adsorption rate was due to the electric charges and quantities on the surface of the biomass. Also, the optimal pH ranges are various depending on the target substances(16, 21).

The result obtained as the highest initial adsorption rate at pH 2 might be due to the fact that the amino acids on the surface of *Pen janthinellum* had the negative charges. Another explanation might be that carboxyl and phosphate groups on the surface of *Pen. janthinellum* caused the negative charge on the cell surface leading the highest adsorption rate at the strong acid range, pH 2.0.

Table 1. The effect of pH on the biosorption of dye at 30℃.

pH	Intact biomass		Modified biomass	
	*r_i(mg/g · min)	% Decolorization	*r_i(mg/g · min)	% Decolorization
pH 2	0.071	81.8	0.064	99
pH 3	0.055	45.4	0.029	99
pH 4	0.046	36.2	0.005	92
pH 5	0.014	34.4	0.0016	41
pH 6	0.011	25.1	0.001	8.4

* r_i referrs to the initial adsorption rate.

The effect of temperature on the biosorption with *Pen. janthinellum* was investigated. Initial adsorption rate(r_i) of reactive dye, Apollocion Red H-E7B, with modified biomass became more efficient as temperature increased. While the intact biomass showed 14% of percentage decolorization at 40℃, the modified biomass showed 0.136 mg/g · min for the initial adsorption rate and 99% for the percent decolorization at 40℃. These results were not due to the increase in the metabolic reaction since the cell had already autoclaved before the adsorption experiment commenced. In the study of biosorption of lead and copper with modified biomass of *R arrhizus* and *Z. ramigera*, the temperature for the optimal initial adsorption rate was reported to be 35℃~45℃ (20, 21). The temperature is not conferred as biological parameter because biomass used in biosorption research was dead, but means as a parameter of physico-chemical reaction rate.

Table 2. The effect of temperature on the biosorption of dye.

Temperature	Intact biomass		Modified biomass	
	*r_i(mg/g · min)	% Decolorization	*r_i(mg/g · min)	% Decolorization
10℃	0.082	7.6	0.073	50.6
20℃	0.075	10.2	0.085	72
30℃	0.072	12.5	0.126	98
40℃	0.071	14.0	0.136	99

The initial pH of samples was fixed to pH 2.0 as shown the best dye-adsorbability by using dilute H_2SO_4.
*r_i referrs to the initial adsorption rate

Biosorption Profile of Dyes

The modified biomass could adsorb the dyes such as azo and reactive dye which are used currently in the textile industries as shown in Table 3. Among dyes used in this study, Acid.Brill Red 3BL(monoazo dye) showed the highest initial adsorption rate of 0.134 mg/g · min. Also, the reactive dye, although it is difficult to remove in the conventional wastewater treatment, showed the high initial adsorption rate. Initial adsorption rates for Apollocion Red 7EB, Apollofix Red SF-3B and Apollocion Red H-E3B were 0.06, 0.086 and 0.079 mg/g · min, respectively. The values obtained are higher than the initial adsorption rate of di-azo dyes.

Table 3. Biosorption of several dyes on the biomass of *Pen. janthinellum*

Name of dyes	*r_i(mg/g · min)
Acid.Brill.Red 3BL(monoazo)	0.134
Apollo Nylon Fast Red S2B(diazo)	0.015
Nylon Fast Red M-PG(diazo)	0.015
Apollocion Red 7EB(reactive red)	0.06
Apollofix Red SF-3B(reactive red)	0.086
Apollocion Red H-E3B(reactive red)	0.079
Mixed dye(all above dyes)	0.084

*r_i referrs to the initial adsorption rate

A mixture of dyes containing azo and reactive dyes was also adsorbed with the biomass of *Pen. janthinellum* and the initial adsorption rate was 0.084 mg/g · min indicating that the biomass of *Pen janthinellum* could be used for the treatment of wastewater containing mixed dyes from textile industries.

Effect of Detergents on the Biosorption

The initial dye adsorption rate(r_i) and percent decolorization were inhibited by the addition of detergents in the medium. As shown in Figure 1, percent decolorization measured after 10 hours was 97~99% without detergent. However, the initial adsorption rate was lower than control when detergent was added to the medium(data not shown). There was report that uranium-adsorption rates with *Pseudomonas aeroginosa* was decreased on the medium containing detergents(22). Comparing with this report, the effect of detergents on the dye-adsorption showed similar effect as the adsorption of uranium. The surface of biomass has the several functional groups for electrical charges. The charged dyes could be removed by adsorption on the surface of biomass. But, it is likely that the addition of detergents decreases the dye-adsorbability of biomass because the charged parts of the surfaces are surrounded with detergents.

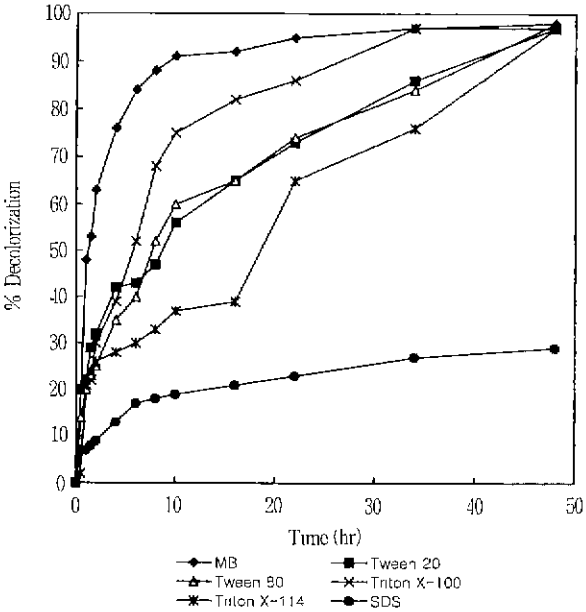

Figure 1. The effect of detergents on the biosorption of dye. MB; Modified Biomass, SDS; Sodium Dodecyl Sulfate.

Effect of Ions on the Biosorption

The effect of various ions on the biosorption of dye was studied and summarized in Table 4. The initial adsorption rates were 0.008 mg/g · min and 0.019 mg/g · min with the addition of Fe^{3-} and Mn^{2-}, respectively. This is a little lower than the control although the different results were reported with other pollutant adsorption studies(22). The reason that no significant inhibition effects were found with the presence of the several ions is likely that *Pen janthinellum* biomass has the specific binding site of dye, Apollocion Red 7EB, independent of the several metal ions. These results are extremely useful in the treatment of wastewater containing dyes because dye wastewater contains various components and many kinds of salts. Michael *et al* (22) have investigated the effect of addition of several ions on the uranium-biosorption. Al^{3-}, Cr^{3-}, Cu^{2-} and Fe^{2-} showed 53.1%, 24.4%, 26% and 58.7% of inhibition to uranium-adsorption, respectively. Because the attraction between surface of biomass and uranium is due to the electric interaction, the addition of ions decreases the electric charges on the surface of biomass and competes with dye for adsorption to surface. However, the addition of several ions has no influence on the dye adsorption rate. Addition of Ca^{2-}, Fe^{3-}, Al^{3-}, Mn^{2-}, Cu^{2-}, Fe^{2-}, and Ba^{2-} did not decrease the dye-adsorption rate, rather showed a little increase in some cases.

Table 4 Effect of the several ions on the biosorption of dye at pH 2 and 30℃.

Ions	Biosorption	
	*r_i(mg/g · min)	% Decolorization
Control	0.021	97.8
Ca^{2+}	0.014	98.2
Fe^{3-}	0.008	95.9
Al^{3-}	0.014	98.2
Cu^{2+}	0.013	98.2
Fe^{2-}	0.016	99
Ba^{2+}	0.02	99.3
Mn^{2-}	0.019	97.4

*r_i referrs to the initial adsorption rate

Comparison between Pen. janthinellum and Other Adsorbents

Comparing modified biomass of *Pen. janthinellum* with ion-exchage/chelating resins for the adsorption of dye(Apollocion Red 7EB), the modified biomass of *Pen. janthinellum* showed superior dye loading performance as shown in Fig 2. Several adsorbents except the activated carbon reported are lower than *Pen janthinellum* biomass in dye adsoption rate. Dye was removed by 0.1g/100ml with activated carbon within about 30 minites after the beginning of the reaction. The removal of dye by modified biomass of *Pen. janthinellum* was completed at 24 hours. The percentage decolorization of dye was shown to be 65.7% for XAD-16 and 44% for XAD-17, respectively. Other adsorbents including DOWEX-5W and Amberlite IR-1 had low dye adsorption rate or no adsorbability. Chitosan had also low dye-adsorbability compared with modified biomass of *Pen janthinellum*.

The compositions of hyphal walls in fungi are glucan, chitin and cellulose as the principal structural polysaccharides. And the layer containing proteins and short fiber exists below polysaccharides layer(25). During modified processing of biomass, the protein layer explosured to dye. The ionic state of ligands such as carboxyl, phosphate and amino groups on proteins will promote reaction with the dye(16).

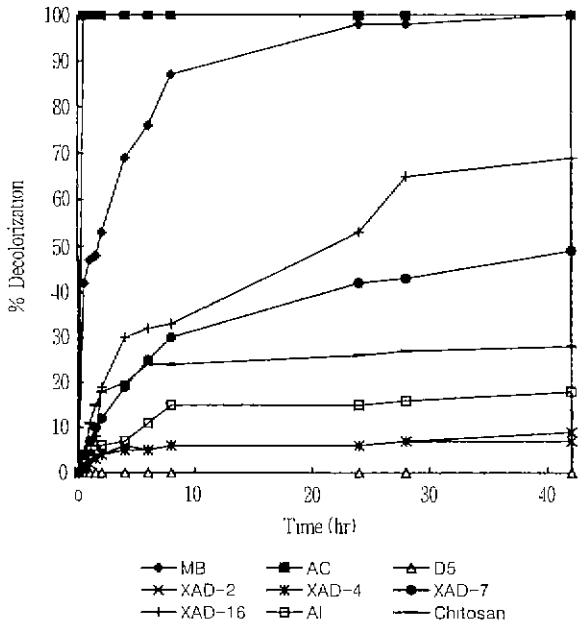

Figure. 2. The comparison between the *Pen. janthinellum* and other adsorbents. MB: Modified Biomass, AC, Activated Carbon, D5, DOWEX-5W, AI: Amberlite IR-1.

요 약

*Penicillium janthinellum*의 생균체와 변형 균체를 사용한 azo와 reactive계 염료의 생흡착에 대한 연구가 수행되었다. 반응액의 pH가 초기 흡착율과 탈색에 영향을 주어, 최적 pH는 2.0이었으며, 최적 온도는 40℃이었다. Reactive계 염료인 Apollocion Red 7EB와 Apollofix Red SF-3B와 Apollocion Red HE-3B는 각각 초기 흡착율이 0.06, 0.086, 0.07 mg/g · min이었다. Azo와 reactive계 염료들을 함유한 혼합염료도 *Pen. janthinellum* 균체에 흡착되어 0.084mg/g · min의 초기 흡착율을 보였다. Detergent 첨가시 탈색 백분율과 초기 흡착율

은 대조군의 경우보다 낮았다. 또한 이온의 첨가는 염료의 흡착율에 영향을 미치지 않았다. *Pen. janthinellum*의 변형 균체도 염료의 흡착율을 보였고, Apollocion Red 7EB의 흡착에 있어 이온교환수지보다 월등한 흡착능을 보였다.

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