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Decolorization of Some Azo Dyes by Immobilized *Geotrichum* sp. Biomass in Fluidized Bed Bioreactor

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Abstract *Geotrichum* sp. strain, which is able to decolorize azo dyes enzymatically, was used in this study for decolorization of synthetics solutions contaminated by toxic azo dyes orange G, trypan blue, azorubine, and methyl red. The biomass of *Geotrichum* sp. was immobilized in calcium alginate and polyacrylamide gels and used for the decolorization of tested azo dyes in fluidized bed bioreactor. The highest specific decolorization rate was obtained when the fungal biomass was entrapped in calcium alginate beads. Immobilized biomass in calcium alginate continuously decolorized azo dyes after eight repeated batch decolorization experiments without significant loss of activity whereas polyacrylamide immobilized biomass retained only 10% of its activity after 4 days of incubation. The effects of some physicochemical parameters such as temperature, pH, and dyes concentration on decolorization performance of isolated fungal strain were also investigated.

Keywords Decolorization · Azo dyes · *Geotrichum* sp. · Immobilization

Introduction

Azo dyes are synthetic organic compounds characterized by the presence of one or more azo bonds (–N=N–) in association with one or more aromatic systems [1]. Azo dyes account for the majority of all textile dyestuffs produced and are currently the most commonly used synthetic dyes in textile, food, paper-making, and cosmetic industries [2]. Release of azo dyes into the environment from the effluents of dye utilizing industries has become a major concern in wastewater treatment because azo dyes may be mutagens or carcinogens [3].

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Considerable research efforts have been devoted to optimizing color removal from effluents. Several physical and chemical treatment methods have been then suggested [4–6], but have not been widely applied because of the high cost and secondary pollution that can be generated by the excessive use of chemicals. Microbial decolorization of dye wastewater has been proposed as less expensive environmentally intrusive alternative.

In the natural environment, azo dyes can be transformed or degraded by a variety of microorganisms, including aerobic and anaerobic bacteria and fungi [7–11]. Bacterial degradation of azo dyes is often initiated by an enzymatic biotransformation step that involves cleavage of azo linkages with the aid of azoreductase using reduced coenzyme as the electron donor [12, 13], whereas fungal degradation of azo dyes was reported to be catalyzed by extracellular enzymes, ligninolytic peroxidases [14–16]. Peroxidases oxidize the phenolic ring of the dyes to produce a carbonium ion on the carbon bearing the azo linkage. Water attacks the carbonium ion to cause hydrolytic cleavage of the azo linkage [17, 18].

The use of fungi is a matter of great concern as these organisms are able to degrade a wide variety of recalcitrant organopollutants, including various types of dyes [19–22]. The major enzymes associated with lignin degradation are laccase, lignin peroxidase (LiP), and manganese peroxidase (MnP). Some white rot fungi produce all three enzymes whereas others produce only one or two of them [23].

Immobilization of living microorganisms has been described by several investigators [24, 25] to be useful in biological wastewater treatment. It is widely known that immobilized cells offer a lot of advantages: reusability of the same biocatalyst, control of reactions, and the noncontamination of products [26].

The purpose of this study was to investigate the potential of *Geotrichum* sp. to decolorize azo dyes [orange G (OG), trypan blue (TB), azorubine (AR), and methyl red (MR)] in fluidized bed bioreactor. The fungal biomass was immobilized in alginate and polyacrylamide gels. The effect of various physicochemical factors such as dye concentration, pH, and temperature on the azo dyes decolorization were determined and compared. Repeated batch decolorization tests were also performed with immobilized fungal biomass.

Materials and Methods

Dyes Used

The azo dyes used in this study were MR, OG, TB, and AR, which were obtained from Sigma-Aldrich Co. (St. Louis, USA). The concentration of each azo dye in samples was determined spectrophotometrically at 430, 475, 600, and 520 nm, respectively, for MR, OG, TB, and AR after centrifugation at 15,000 rpm for 15 min.

Microorganism Used

Geotrichum sp. was isolated from dye-contaminated wastewater from an industrial area in Daegu City (Korea). It was identified based on the visual observation of isolates grown on potato dextrose agar (PDA) plates, micromorphological studies in slide culture [27] at room temperature, and the taxonomic keys described by Hoog and Guarro [28] as well as the compendium of soil fungi [29].

Stock cultures of isolated strain were routinely maintained on a PDA.

Fungus Growth and Decolorization of OG Azo Dye

Precultures of the fungus were prepared by inoculating plugs (diameter 0.5 cm) from the growing zone of fungus on agar plate to 50 ml of potato dextrose broth (PDB). Then, cells were cultivated statically at 25 °C for 3 days. Afterward, the precultures were homogenized aseptically using a potter homogenizer (200 rpm). Aliquots of 1.5 ml of homogenized precultures were used to inoculate volumes of 150 ml of PDB containing 100 mg/l of OG azo dye in 250-ml Erlenmeyer flasks. The cultures were incubated aerobically at 25 °C on a rotary shaker at 150 rpm for 5 days. At several time intervals 3-ml aliquots of fungal cultures were sampled and centrifuged at 15,000 rpm for 15 min. The clear supernatant was analyzed spectrophotometrically at 475 nm to determine the residual concentration of OG and the pellets were dried at 105 °C for 24 h to determine the biomass dry weight. All experiments were carried out in triplicate

Ligninolytic Enzyme Assays

After decolorization of the culture medium of *Geotrichum* sp., the medium was filtrated and centrifuged (15,000 rpm, 30 min) to remove the fungal biomass. The supernatant was then concentrated ten times by ultrafiltration (10 kDa molecular weight cutoff). Activities of extracellular MnP, manganese (Mn)-independent peroxidase, LiP, and laccase in the concentrated supernatant were determined spectrophotometrically at 25 °C by monitoring the absorbance increase at 469 nm for MnP and Mn-independent peroxidase at 310 nm for LiP and at 420 nm for laccase in the reaction mixtures. Manganese peroxidase activity was determined by oxidation of 1 mM 2,6-dimethoxyphenol in citrate buffer (25 mM, pH 4.5) in the presence of 0.1 mM H_2O_2 and 1 mM $MnSO_4$. Manganese-independent peroxidase activity was determined in the absence of Mn. Lignin peroxidase activity was determined with veratryl alcohol as substrate in the presence of 0.1 mM H_2O_2. The assay was performed in citrate buffer (25 mM, pH 4.5). Laccase activity was determined by oxidation of 0.5 mM 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) in citrate buffer (25 mM, pH 4.5). The total volume of each reaction mixture was 1 ml. One unit of enzyme activity was defined as the amount of enzyme that catalyses the oxidation of 1 μmol substrate/min. All measurements were repeated three times.

Fungal Biomass Preparation

Aliquots of 1.5 ml of homogenized preculture of *Geotrichum* sp., prepared as described above, were used to inoculate 250-ml Erlenmeyer flasks containing 150 ml of PDB. The cultures were incubated aerobically at 25 °C on a rotary shaker at 150 rpm for 5 days. After cultivation the fungal biomass was harvested by filtration and then rinsed with Tris–HCl buffer (50 mM, pH 7).

Fungal Biomass Immobilization

Entrapment in Calcium Gel One hundred milliliter of sterile sodium alginate solution (2% w/v) was mixed until homogenous with 2 g of fungal biomass. The mixture was extruded into 150 mM of $CaCl_2$, forming beads of 5 mm in diameter. The beads were allowed to harden in the $CaCl_2$ solution at room temperature for 30 min, and rinsed with Tris–HCl buffer (50 mM, pH 7).

Entrapment in Polyacrylamide Gel Two grams of fungal biomass were mixed with 78 ml of Tris–HCl buffer (50 mM, pH 7), 20 ml acrylamide–bisacrylamide solution (30–0.8% w/v), and 1 ml of ammonium persulfate solution (10% w/v). The polymerization was initiated adding 100 μl of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine. The polyacrylamide gel was then divided into particles of 0.5 cm in diameter and rinsed with Tris–HCl buffer (50 mM, pH 7).

Decolorization of Tested Azo Dyes in Fluidized Bed Bioreactor Using Free and Immobilized Fungal Biomass

The fluidized bed bioreactors are composed of 500-ml conical flasks containing the immobilized fungal biomass (in calcium alginate and polyacrylamide gels) suspended in 200 ml of minimum mineral (MM) medium composed by 0.6 g/l of K_2HPO_4, 0.1 g/l of $MgSO_4$, 0.6 g/l of $SO_4(NH_4)_2$, 0.02 g/l of $CaCl_2$, 0.5 g/l of NaCl, 1.1 mg/l of $MnSO_4$, 0.2 mg/l of $ZnSO_4$, 0.4 mg/l of $CuSO_4$, and 0.14 mg/l of $FeSO_4$ (pH adjusted to 6 with 1 M of HCl) and supplemented by 1% of glucose and 100 mg/l of tested azo dye. The bioreactors were placed in a rotary shaker at 25 °C, and the fluidization was assured by stirring at a rate of 120 rpm. The decolorization rate was followed according to time in all bioreactors. The same bioreactors have been used for studying tested azo dyes decolorization with free cells; thus, 2 g of fungal biomass were suspended in 200 ml of MM with 1% of glucose and 100 mg/l of dye. Decolorization rate was followed according to time in the bioreactors placed in the same conditions previously cited. For each experiment, a control test without fungal biomass was conducted under the same conditions to evaluate the affinity of the dye for used immobilization supports (calcium alginate and polyacrylamide).

At several time intervals, 1-ml aliquots were collected from the bioreactors and centrifuged at 15,000 rpm for 15 min. The supernatants were analyzed spectrophotometrically to determine the amount of dyes. All values and data points presented are the means of three independent assays.

Repeated Batch Operation Decolorization in Fluidized Bed Bioreactor Using Free and Immobilized Fungal Biomass

The longevity of decolorization activity of the immobilized fungal biomass (in calcium alginate and polyacrylamide gels) was investigated in repeated batch decolorization tests. A fresh decolorization medium (MM containing 1% of glucose and 100 mg/l of dye) was first inoculated with immobilized fungal biomass in the fluidized bed bioreactors, described above, and placed at 25 °C in a rotary shaker at 120 rpm. After 24 h, the decolorized medium was discharged and the immobilized fungal biomass were collected, rinsed with sterile MM, and transferred into a fresh decolorization medium for the next cycle of decolorization experiment. Decolorization rates were monitored according to time in all bioreactors. For comparison, the repeated batch experiments were also conducted using free fungal biomass under identical experimental procedures.

Results and Discussion

Preliminary selection of azo dyes decolorizing fungi was based on the decolorization of OG, TB, MR, and AR azo dyes on MM plates. Four fungal isolates, which decolorized

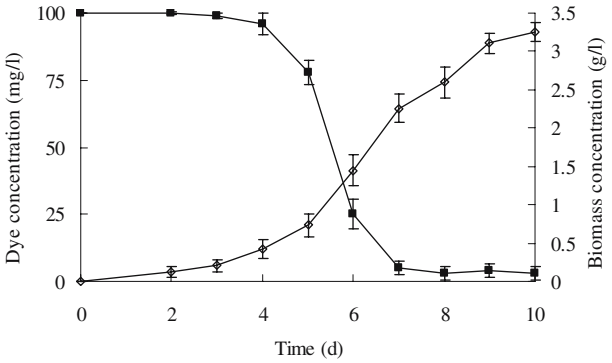

Fig. 1 Decolorization of OG (square) by *Geotrichum* sp. growing (diamond) in PDB containing 100 mg/l of the dye

these azo dyes on MM plates, were isolated from dye-contaminated wastewater. Among these fungi, one fungal strain, which was identified as *Geotrichum* sp., with higher tested azo dyes decolorization potential in MM plates, was selected for further study.

The degradation of OG azo dye during the cultivation of *Geotrichum* sp. in PDB supplemented by 100 mg/l of OG was investigated. The obtained results, presented in Fig. 1, indicated that this isolated fungal strain has a high ability to decolorize 100 mg/l of OG. During the cultivation of *Geotrichum* sp. the decolorization rate of OG was initially low. However, more than 96% of the coloration was removed between the fourth and the seventh day, at which time the fungal biomass began to grow intensively. Conversely, the uninoculated control presented no color removal.

Previous studies reported that the physical adsorption and the enzymatic degradation with the help of some lignin-modifying enzymes (MnP, Mn-independent peroxidase, LiP, and laccase) are major mechanisms involved in the decolorization of dyes by living fungi [9]. In our case, it was observed that the fungal biomass remained clear during the whole cultivation process. This indicates that the physical adsorption of the azo dye on the fungal biomass was negligible. However, as shown in Fig. 2, important activities of Mn-independent peroxidase (0.35 ± 0.05 U/ml), laccase (0.1 ± 0.03 U/ml), LiP (0.05 ± 0.01 U/ml) with major activity of MnP (0.88 ± 0.08 U/ml) were recorded in the culture medium of *Geotrichum* sp. at the end of the decolorization process. These results suggest that the decolorization of OG by *Geotrichum* sp. is essentially done by an enzymatic degradation mechanism.

To investigate the decolorization of OG azo dye by *Geotrichum* sp., the following procedure was implemented. In fluidized bed bioreactor, this fungus was entrapped in both

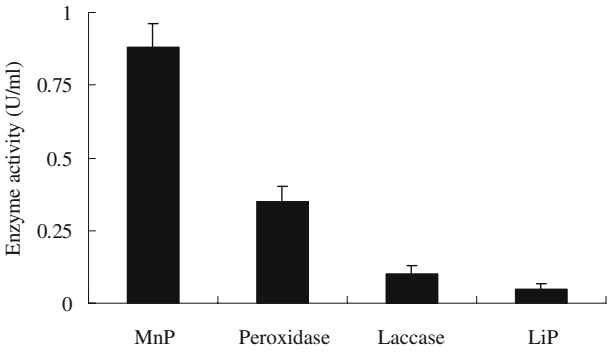

Fig. 2 Enzymatic activities of extracellular ligninolytic enzymes recorded in culture medium of *Geotrichum* sp. MnP = Manganese peroxidase, Peroxidase = Mn-independent peroxidase, LiP = lignin peroxidase

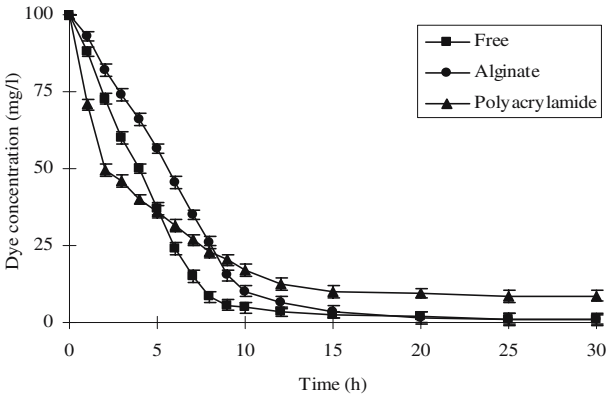

Fig. 3 Decolorization of OG by free and immobilized biomass of *Geotrichum* sp. in fluidized bed bioreactor: free biomass (square), immobilized biomass in alginate (circle), immobilized biomass in polyacrylamide (triangle)

alginate and polyacrylamide gels. The concentration of OG was measured at predefined interval times for immobilized *Geotrichum* sp. and for free fungal biomass. The obtained results were represented in the Fig. 3.

Free and alginate immobilized *Geotrichum* sp. exhibited very similar decolorization patterns for OG. The concentration of the dye decreased progressively until complete decolorization of the reactional media, whereas the obtained profile for polyacrylamide immobilized biomass shows a quick drop in OG concentration exceeding 50% initial dye concentration at the end of 2 h of treatment followed by a much slower decolorization rate phase.

The kinetics of OG decolorization with polyacrylamide immobilized biomass could be related to the extensive capacity of the polyacrylamide gel to fix the dye (data not shown). The fast initial decolorization rate may be attributed to OG adsorption on the polyacrylamide gel, and the subsequent slower decolorization may be attributed to biotransformation of OG by immobilized biomass. For the calcium alginate immobilized biomass, the color removal was conducted by fungal dye decolorization, as the physical adsorption of the dye on the alginate gel was negligible.

Using calcium alginate as entrapment matrix for *Geotrichum* sp. offered a greatest purifying performances with a quasitotal decolorization of reactional media with a specific decolorization rate of 4.6 ± 0.3 mg g^{-1} h^{-1} (Table 1). The immobilization of *Geotrichum* sp. in polyacrylamide gel facilitates the removal of 91% of the color with a specific decolorization rate of 2.1 ± 0.3 mg g^{-1} h^{-1} (Table 1). This limitation of decolorization on the fungal activity may be explained by the existence of an unfavorable microenvironment inside the gel matrix and the presence of residual monomers that leads to a toxicity of the fungal cells [30]. The lower decolorization rates for immobilized biomass compared to free

Table 1 The equilibrium conversion and the specific decolorization rate of OG recorded by free and immobilized biomass of *Geotrichum* sp.

	Equilibrium conversion (%)	Specific decolorization rate (mg g^{-1} h^{-1})
Free biomass	99	5.8 ± 0.4
Alginate immobilized biomass	99	4.6 ± 0.3
Polyacrylamide immobilized biomass	91	2.1 ± 0.3

Table 2 Comparison of equilibrium conversion (T_d) and specific decolorization rate (V_d) of OG recorded by free and immobilized biomass of *Geotrichum* sp. during repeated batch decolorization cycles.

	Free biomass		Alginate immobilized biomass		Polyacrylamide immobilized biomass	
	T_d (%)	V_d (mg g^{-1} h^{-1})	T_d (%)	V_d (mg g^{-1} h^{-1})	T_d (%)	V_d (mg g^{-1} h^{-1})
Cycle 1	99	5.8	99	4.6	91	2.1
Cycle 2	98	5.8	99	4.5	65	1.5
Cycle 3	99	5.7	98	4.7	27	1.1
Cycle 4	97	5.4	99	4.6	10	0.8
Cycle 5	94	4.7	98	4.6	4	0.4
Cycle 6	86	4.1	98	4.5	2	0.2
Cycle 7	75	3.9	99	4.5	–	–
Cycle 8	68	3.6	98	4.3	–	–

biomass can be attributed to the mass transfer restriction arising from fungal biomass entrapment.

To investigate the possibility of the reusability of the same fungal biomass in successive cycles of decolorization, repeated batch experiments were performed. As evident in Table 2, free biomass of *Geotrichum* sp. remained active during all the eight cycles of treatment. However, a progressive decrease in decolorization efficiency was observed over the fourth cycle. After eight cycles of decolorization, the recorded equilibrium conversion and specific decolorization rate using a free biomass dropped to about 68% and 3.6 mg g^{-1} h^{-1}, respectively. The immobilization of *Geotrichum* sp. in calcium alginate gel greatly stabilizes the fungal activity for more than eight cycles. This stability returns to soft polymerization condition of the gel and to a direct role of the calcium in the cells conservation [31]. The use of *Geotrichum* sp. entrapped in polyacrylamide gel in repeated batch fluidized bioreactor has proven not to be interesting. Indeed the decolorization system lost more than 70% of its decolorization efficiency at the fourth cycle of treatment, and practically cancelled at the end of the fifth cycle (Table 2).

The gelling properties of alginate, allowing the entrapment of the fungal biomass under soft and easily realizable conditions while providing a great stability of the fungal activity with high purifying performances, led us to use this gel as matrix for the entrapment of *Geotrichum* sp. to study the influence of certain physicochemical parameters on the decolorization of some azo dyes (OG, AR, TB, and MR) in fluidized bed bioreactor.

To evaluate the influence of dye concentration on the decolorization performances of *Geotrichum* sp., decolorization experiments of OG, TB, AZ, and MR at different concentrations (50, 100, and 200 mg/l) were conducted in a fluidized bed bioreactor. The evolution of the concentration of each dye in the presence of the calcium alginate

Table 3 Equilibrium conversion (T_d) and specific decolorization rate (V_d) recorded by alginate immobilized *Geotrichum* sp. biomass for different concentrations of tested azo dyes.

	OG		AR		MR		TB	
	T_d (%)	V_d (mg g^{-1} h^{-1})	T_d (%)	V_d (mg g^{-1} h^{-1})	T_d (%)	V_d (mg g^{-1} h^{-1})	T_d (%)	V_d (mg g^{-1} h^{-1})
50 mg/l	99	2.8 ± 0.3	98	1.8 ± 0.1	99	3.5 ± 0.5	99	3.2 ± 0.3
100 mg/l	99	4.6 ± 0.3	99	3.2 ± 0.2	97	6.6 ± 0.4	99	6.1 ± 0.4
200 mg/l	98	7.3 ± 0.5	98	5.9 ± 0.4	96.5	11.7 ± 0.6	99	11.3 ± 0.6

Table 4 Equilibrium conversion (T_d) and specific decolorization rate (V_d) recorded by alginate immobilized *Geotrichum* sp. biomass for tested azo dyes at different pH.

	OG		AR		MR		TB	
	T_d (%)	V_d (mg g^{-1} h^{-1})	T_d (%)	V_d (mg g^{-1} h^{-1})	T_d (%)	V_d (mg g^{-1} h^{-1})	T_d (%)	V_d (mg g^{-1} h^{-1})
pH 3	95	2.8 ± 0.3	97	2.9 ± 0.4	95	4.1 ± 0.3	98	4.2 ± 0.3
pH 4	98	4.3 ± 0.2	98	3.1 ± 0.3	97	6.2 ± 0.4	98	6.1 ± 0.5
pH 5	99	4.8 ± 0.4	98	3.7 ± 0.3	98	7.1 ± 0.6	99	6.6 ± 0.5
pH 6	99	4.6 ± 0.3	98	3.2 ± 0.2	98	6.6 ± 0.4	99	6.1 ± 0.4
pH 7	97	3.8 ± 0.4	97	2.8 ± 0.2	97	5.3 ± 0.3	98	5.2 ± 0.4
pH 9	96	1.5 ± 0.1	95	0.7 ± 0.1	94	1.8 ± 0.2	97	2.5 ± 0.2

immobilized fungal biomass was followed according to time. The obtained results plotted in Table 3 testify of the high ability of the isolate fungus to decolorize azo dyes. Indeed, the use of *Geotrichum* sp. enables a quasitotal decolorization of the four dyes for all tested concentrations, but with different specific decolorization rates that vary according to the used dye and its initial concentration. These rates, for the same dye, increase proportionally with its initial concentration. *Geotrichum* sp. decolorizes more quickly the MR compared to the others tested dyes with specific decolorization rates of 3.5 ± 0.5, 6.6 ± 0.4, and 11.7 ± 0.6 mg g^{-1} h^{-1} for MR initial concentrations of 50, 100, and 200 mg/l, respectively (Table 3). The lowest specific decolorization rates have been recorded using the AR dye with 1.8 ± 0.1, 3.2 ± 0.2, and 5.9 ± 0.4 mg g^{-1} h^{-1} for AR initial concentration of 50, 100 and 200 mg/l, respectively (Table 3).

The effect of pH on decolorization of OG, AR, TB, and MR by calcium alginate-immobilized *Geotrichum* sp. was investigated in the range of 3 to 9. From the obtained results (Table 4), it was observed that the fungus could effectively decolorize the four tested azo dyes for all pH values examined with a conversion rates superior to 94%. However, different decolorization rates were obtained depending on initial pH of the reactional media and the used dye. The maximum decolorization rates occurred for all tested dyes at about pH 5 and then declined with further increases in pH with considerable decolorization activity in the range of 4 and 6 (Table 4). At pH 5 the recorded decolorization rates were more than threefold higher than at pH 9, which dropped from 4.8 ± 0.4 to 1.5 ± 0.1 mg g^{-1} h^{-1} for OG, from 3.7 ± 0.3 to 0.7 ± 0.1 mg g^{-1} h^{-1} for AR, from 7.1 ± 0.6 to 1.8 ± 0.2 mg g^{-1} h^{-1} for MR, and from 6.6 ± 0.5 to 2.5 ± 0.2 mg g^{-1} h^{-1} for TB when the pH increased from 5 to 9 (Table 4), generating longer incubation times to reach the maximum conversion rates of these dyes.

The effect of temperature on decolorization efficiency of calcium alginate-immobilized *Geotrichum* sp. for the tested azo dyes was investigated at a temperature range of 25–45 °C. The obtained results are presented in Table 5. It is evident that *Geotrichum* sp. manages to

Table 5 Equilibrium conversion (T_d) and specific decolorization rate (V_d) recorded by alginate immobilized *Geotrichum* sp. biomass for tested azo dyes at different temperature.

	OG		AR		MR		TB	
	T_d (%)	V_d (mg g^{-1} h^{-1})	T_d (%)	V_d (mg g^{-1} h^{-1})	T_d (%)	V_d (mg g^{-1} h^{-1})	T_d (%)	V_d (mg g^{-1} h^{-1})
25°C	99	4.6 ± 0.3	98	3.2 ± 0.2	99	6.6 ± 0.4	99	6.1 ± 0.4
35°C	99	4.9 ± 0.4	99	3.7 ± 0.3	98	6.8 ± 0.3	99	6.7 ± 0.4
45°C	42	1.5 ± 0.1	36	0.9 ± 0.2	61	2.7 ± 0.3	58	2.5 ± 0.2

decolorize the four tested dyes with maximum conversion rates at 25 and at 35 °C. The increase of temperature from 25 to 35 °C appeared to enhance the specific decolorization rates of used dye, which pass from 4.6 ± 0.3 to 4.9 ± 0.4 mg g^{-1} h^{-1} for OG, from 3.2 ± 0.2 to 3.7 ± 0.3 mg g^{-1} h^{-1} for AR, from 6.6 ± 0.4 to 6.8 ± 0.3 mg g^{-1} h^{-1} for MR, and from 6.1 ± 0.4 to 6.7 ± 0.4 mg g^{-1} h^{-1} for TB (Table 5). However, the decolorization efficiency of *Geotrichum* sp. decreased dramatically when the temperatures of incubation reached 45°C, recording conversion rates not exceeding 42% for OG, 36% for AR, 61% for MR, and 58% for TB (Table 5). The reduction of decolorization activity at 45 °C can be attributed to denaturation of the enzymes involved in the degradation of these azo dyes and also to the loss of the fungal cell viability at high temperature (45 °C).

Conclusion

It has been shown that immobilized *Geotrichum* sp. biomass could effectively decolorize at high level azo dyes in fluidized bed bioreactor. The obtained results reveal that this bioprocess facilitates reproducible decolorization of azo dye with high yield.

The use of *Geotrichum* sp. entrapped in polyacrylamide gel in repeated batch fluidized bioreactor has proven not to be interesting because the decolorization system lost more than 70% of its decolorization efficiency at the end of the third cycle of treatment. However the entrapment of *Geotrichum* sp. in alginate offers the greatest purifying performance with quasitotal decolorization of the tested dyes and the greatest bioreactor stability. The alginate gel would be a suitable immobilization matrix for entrapment of the isolated of fungal strain to remove azo dyes from wastewater on an industrial scale.

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