ПРИКЛАДНАЯ БИОХИМИЯ И МИКРОБИОЛОГИЯ, 2011, том 47, № 1, с. 66–72

UDC 570

DECOLORIZATION OF THE ANTHRAQUINONE DYE CIBACRON BLUE 3G-A WITH IMMOBILIZED *Coprinus cinereus* IN FLUIDIZED BED BIOREACTOR

© 2011 A. Moutaouakkil*, **, M. Blaghen**

*Unit of Radio-Immuno-Analyse, Division of Life Science Applications, National Center of Energy, Sciences and Nuclear Techniques (CNESTEN), 10001 Rabat, Marocco

e-mail: moutaouakkil@cnesten.org.ma

**Unit of Bio-industry and Molecular Toxicology, Laboratory of Microbiology, Biotechnology and Environment, Faculty of Sciences Aïn Chock, University Hassan II – Aïn Chock, 20100 Casablanca, Morocco

e-mail: blaghen@facsc.ac.ma

Received January 16, 2010

Coprinus cinereus, which was able to decolorize the anthraquinone dye Cibacron Blue 3G-A (CB) enzymatically, was used as a biocatalyst for the decolorization of synthetic solutions containing this reactive dye. *Coprinus cinereus* was immobilized in both calcium alginate and polyacrylamide gels, and was used for the decolorization of CB from synthetic water by using a fluidized bed bioreactor. The highest specific decolorization rate was obtained when *Coprinus cinereus* was entrapped in calcium alginate beads, and was of about 3.84 mg g^{-1} h^{-1} with a 50% conversion time ($t_{1/2}$) of about 2.60 h. Moreover, immobilized fungal biomass in calcium alginate continuously decolorized CB even after 7 repeated experiments without significant loss of activity, while polyacrylamide-immobilized fungal biomass retained only 67% of its original activity. The effects of some physicochemical parameters such as temperature, pH and dye concentration on decolorization performance of isolated fungal strain were also investigated.

Synthetic organic dyes are extensively used in the textile industry. During manufacturing, an estimated 10–15% of these dyes are lost in the effluents, rendering them highly colored [1, 2]. Release of these dyes into the environment has become a major concern in wastewater treatment since some of them or their metabolites may be mutagens or carcinogens [3, 4].

Cibacron Blue 3G-A (C.I. 61211), also called Procion Blue H-B or Reactive Blue 2, is one of the most reactive synthetic dyes used in the textile industry [5]. In addition to the triazine group acting as reactive group, the cibacron Blue 3G-A (CB) presents an anthraquinone group as chromophore (Fig. 1) dyeing natural fibers, such as cellulose, through covalent bonds [6]. Moreover, this polynuclear monochlorotriazine dye was shown to bind most types of proteins [7, 8].

The major environmental problem associated with the use of this type of reactive dyes is due to their inefficient fixation to the fibers. Therefore, significant losses occur during the manufacture and processing with dyes being discharged as effluents into publicly owned water treatment plants.

Considerable research efforts have been devoted to optimize color removal from effluents of the textile industry. Most of the chemical and physical methods proposed for treating dye wastewater have been not widely applied because of the high cost and the secondary pollution that can be generated by the excessive use of chemicals [9–11]. This has led many workers to search for the use of microorganisms, such as bacteria or fungi, for the degradation of these compounds in wastewater treatment systems. Most biological degradations of textile dyes are carried out by anaerobic bacteria [12–14]. Generally, textile dyes are resistant to attack by bacteria under aerobic conditions [15–17]. Furthermore, bacterial enzymes involved in the dye degradation have been shown to be very specific [18, 19].

White-rot fungi are however efficient in biodegradation of recalcitrant compounds like xenobiotics, lignin and dyestuffs by their extracellular ligninolytic enzyme system [20–22]. White-rot fungi offer significant advantages over bacteria. Their extracellular enzyme systems including Mn-dependent peroxidase (MnP), Mn-independent peroxidase (P), lignin peroxidase (LiP) and laccase (L) being non-specific can attack a wide variety of complex aromatic dyestuffs [23, 24]. Since the enzymes are extracellular, the substrate diffusion limitation into the cell, generally observed in bacteria, is not encountered. White-rot fungi do not require preconditioning to particular pollutants, because enzyme secretion depends on nutrient limitation, nitrogen or carbon, rather than presence of pollutant. The extracellular enzyme system also enables white-rot fungi to tolerate high concentrations of pollutants [25].

Immobilization of living microorganisms has been described as useful in biological wastewater treatment

[26–28]. Immobilization of bacteria, yeast cells and fungi has been carried out by various techniques. Entrapment process within natural or synthetic matrices is the most conventional method of immobilization [29]. It is widely known that immobilized cells offer many advantages: enhance the stability, mechanical strength and reusability of the biocatalyst [30, 31].

The aim of this work was to evaluate the potential of the white-rot fungus *Coprinus cinereu*s to decolorize the reactive anthraquinone dye CB in fluidized bed bioreactor. *Coprinus cinereus*, which was isolated from dye-contaminated sludge, has been immobilized in both calcium alginate (natural gel matrix) and polyacrylamide (synthetic polymer matrix). Effects of various physicochemical parameters such as temperature, pH and dye concentration on the kinetics of CB decolorization were investigated. Repeated batch decolorization tests were also performed with both free and immobilized fungal biomass to justify repetitive use. Decolorization rates obtained were compared. The information obtained from this study is expected to become a useful reference for the development of practical decolorization processes using immobilized fungal biomass as the biocatalyst.

MATERIALS AND METHODS

Microorganism and culture media. *Coprinus cinereus* used in this study was isolated from dye-contaminated sludge collected from an industrial area in Casablanca city (Morocco). It was identified based on the visual observation of isolates grown on potato dextrose agar (PDA) plates, micro-morphological studies in slide culture [32] at room temperature, and the taxonomic keys described by De Hoog et al. [33] as well as the compendium of soil fungi [34].

Two media were used in this study: one was potato dextrose (Topley House, Bury, England) used for routine transfer and fungus cultivation, and the other was minimum medium (MM) containing (g l^{-1}): K_2HPO_4 – 0.6, $MgSO_4$ – 0.1, $(NH_4)_2SO_4$ – 0.1, NaCl – 0.5; (mg l^{-1}): $CaCl_2$ – 20, $MnSO_4$ – 1.1, $ZnSO_4$ – 0.2, CuSO4 – 0.2 and $FeSO_4$ – 0.14 (pH adjusted to 7.0 with 1 M HCl), used to monitor the decolorization.

Measurement of dye concentration. The anthraquinone dye used in this study was CB (Fig. 1), which was obtained from BDH Chemicals Ltd Poole (England). The concentration of anthraquinone dye in samples was determined after centrifugation at 15000 g for 15 min by measuring the absorbance of the supernatant at 612 nm using a Jenway 6405 UV/Visible spectrophotometer (USA).

Fungus growth and decolorization of CB. Precultures of *Coprinus cinereus* were prepared by inoculating plugs (diameter 0.5 cm) from the growing zone of fungus on PDA plate to 50 ml of PDB. Then, cells were cultivated statically at 25°C for 3 days. Afterwards, the precultures were homogenized aseptically using a potter homogenizer (200 rpm). Aliquots of 2 ml of homogenized precultures were used to inoculate volumes of 200 ml of MM containing 1% (w/v) of glucose and 200 mg l^{-1} of CB in 500 ml Erlenmeyer flasks. The cultures were incubated aerobically at 25°C on a rotary shaker at 100 rpm for 10 days. At several time intervals 3 ml aliquots of fungal cultures were sampled and centrifuged at 15000 g for 15 min. The clear supernatant was analyzed spectrophotometrically to determine the residual concentration of CB and the pellets were dried at 105°C for 24 h to determine the biomass dry weight. All experiments were carried out in triplicate.

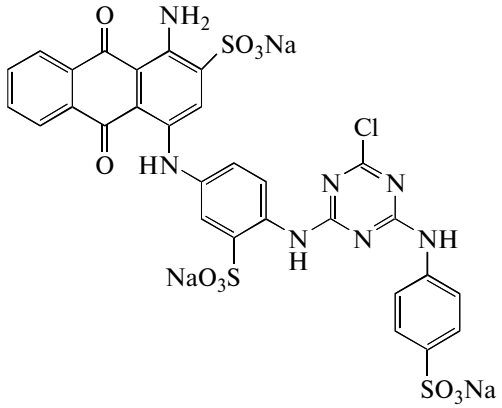

Fig. 1. Chemical structure of Cibacron Blue 3G-A (CB, C.I. 61211).

Ligninolytic enzyme assays. After decolorization of the culture medium of *Coprinus cinereus*, the medium was filtrated and centrifuged (15000 g for 30 min) in order to remove the fungal biomass. The supernatant was then concentrated 10 times by ultrafiltration (10 kDa MW cutoff). Activities of extracellular Mn-dependent peroxidase (MnP), Mn-independent peroxidase (P), lignin peroxidase (LiP) and laccase (L) in the concentrated supernatant were determined spectrophotometrically at 25°C by monitoring the absorbance increase at 469 nm for Mn-dependent peroxidase and Mn-independent peroxidase, at 310 nm for lignin peroxidase and at 420 nm for laccase in the reaction mixtures [21, 22]. Mn-dependent 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 $MnSO_4$. Mn-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 of substrate per minute. All measurements were repeated three times.

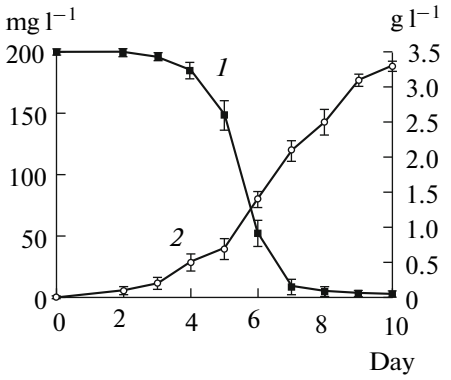

Fig. 2. Decolorization of CB (1, mg l^{-1}) by *Coprinus cinereus* growing (2, biomass g l^{-1}) in minimum medium (MM) containing 1% (w/v) of glucose and 200 mg l^{-1} of the dye.

Fungus immobilization methods. ***Entrapment in calcium alginate gel.*** One hundred milliliters of sterile sodium alginate solution (2%, w/v) were mixed with 2 g (wet weight) of fungal biomass obtained by filtration of a stirred culture of *Coprinus cinereus* on PDB. The mixture was extruded through a needle (3.0 mm, i.d.) into 150 mM $CaCl_2$, forming beads of 5.0 mm diameter. The beads were allowed to harden in the $CaCl_2$ solution at room temperature for 30 minutes, and were rinsed with Tris-HCl buffer (50 mM, pH 7.0).

Entrapment in polyacrylamide gel. Two grams (wet weight) of fungal biomass were mixed with 78 ml of Tris-HCl buffer (50 mM, pH 7.0), 20 ml acrylamide-bisacrylamide solution (29.2–0.8%, w/v) and 1 ml ammonium persulfate solution (10%, w/v). The polymerization was initiated by adding 100 μl of N,N,N',N'-tetramethylethylenediamine. The polyacrylamide gel was then divided into 5.0 mm cubes, which were rinsed with Tris-HCl buffer (50 mM, pH 7.0).

Decolorization of CB in fluidized bed bioreactor using free and immobilized fungus. The fluidized bed bioreactors are composed with 500 ml conical flasks containing the immobilized fungus in different supports (calcium alginate and polyacrylamide) suspended in 200 ml of MM with 0.1% (w/v) of glucose and 200 mg l^{-1} of CB (decolorization medium). The bioreactors were placed in a rotary shaker at 25°C and the fluidization is assured by stirring at a rate of 100 rpm. Decolorization rate was followed according to time in all bioreactors. The same bioreactor has been used for studying CB decolorization with free fungus; thus, 2 g (wet weight) of fungus obtained by filtration of stirred fungal culture on PDB was suspended in 200 ml of MM with 0.1% (w/v) of glucose and 200 mg l^{-1} of CB. Decolorization rate was followed according to time in the bioreactor placed in the same conditions cited previously. For each experiment, a control test without fungus was carried out in the same conditions in order to evaluate the affinity of CB for used immobilization supports (calcium alginate and polyacrylamide).

At several time intervals, 1 ml aliquots were collected from bioreactors and centrifuged at 15000 g for 15 min. The supernatants were analyzed spectrophotometrically to determine the amount of CB.

Effects of some physicochemical parameters on CB decolorization. To evaluate the effects of operation and environmental factors on the efficiency of color removal, the batch decolorization experiments were carried out at different temperatures (20–55°C), pH values (3.0–9.0) and initial dye concentrations (50–1600 mg l^{-1}). The above experiments were performed under stirring (100 rpm).

Repeated-batch operations of decolorization with free and immobilized fungus. The performances of fluidized bed bioreactors used continuously were evaluated by following the decolorization rates during 7 cycles. The immobilized fungal biomasses (in calcium alginate and in polyacrylamide) were placed into a decolorization medium containing 200 mg l^{-1} of CB. The resulting solution (typically pH 7.0) was then incubated at 25°C under stirring (100 rpm) for decolorization. After complete color removal, the immobilized-fungus particles were collected, rinsed twice with sterile deionized water and transferred into a fresh decolorization medium for the second decolorization experiments. The same procedures were repeated 7 times. For comparison, the repeated batch experiments were also conducted using free fungal biomass under identical experimental procedures.

RESULTS AND DISCUSSION

Isolation of CB decolorizing fungus. Preliminary selection of CB decolorizing fungi was based on the decolorization of CB on MM plates. Four fungal isolates, which decolorized this anthraquinone dye (CB), were isolated from dye-contaminated sludge. Among these fungi, one fungal strain, which was identified as *Coprinus cinereus*, with higher CB decolorization potential in MM plates was selected for further study.

Decolorization of CB by *Coprinus cinereus* growing in liquid medium. The decolorization of CB during the cultivation of *Coprinus cinereus* in MM containing 1% (w/v) of glucose and 200 mg l^{-1} of CB was investigated. The obtained results, presented in Fig. 2, indicated that this isolated fungal strain has a high ability to decolorize 200 mg l^{-1} of CB. During the cultivation of *Coprinus cinereus*, the decolorization rate of CB was initially low. However, more than 95% of the coloration was removed between the 4^{th} and the 7^{th} day, at which time the fungal biomass began to grow intensively. Conversely, the uninoculated control presented no color removal.

Ligninolytic enzyme activities. Previous studies reported that the physical adsorption and the enzymatic degradation with the help of some lignin modifying enzymes (Mn-dependent peroxidase, Mn-independent peroxidase, lignin peroxidase and laccase) are major mechanisms involved in the decolorization of dyes by living fungi [20, 21]. In our case, it was observed that the fun-

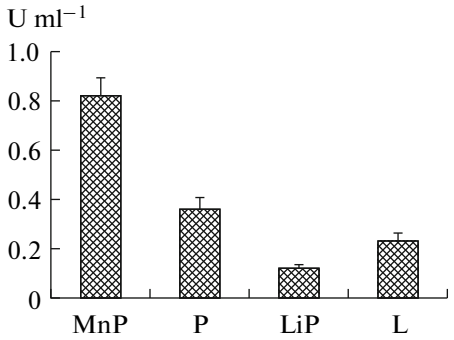

Fig. 3. Enzymatic activities (U ml^{-1}) of extracellular lignolytic enzymes recorded in culture medium of *Coprinus cinereus*. MnP – Mn-dependent peroxidase; P – Mn-independent peroxidase; LiP – lignin peroxidase; L – laccase.

gal biomass remained clear during the whole cultivation process. This indicates that the physical adsorption of the anthraquinone dye on the fungal biomass was negligible. However, and as shown in Fig. 3, important activities of Mn-independent peroxidase (0.36 ± 0.05 U ml^{-1}), lignin peroxidase (0.12 ± 0.02 U ml^{-1}), laccase (0.23 ± 0.04 U ml^{-1}) with major activity of Mn-dependent peroxidase (0.82 ± 0.08 U ml^{-1}) was recorded in the culture medium of *Coprinus cinereus* at the end of the decolorization process. These results suggest that the decolorization of CB by *Coprinus cinereus* is essentially done by an enzymatic degradation mechanism.

Decolorization of CB with free and immobilized fungus. *Coprinus cinereus* was entrapped in both calcium alginate and polyacrylamide gels. The concentrations of CB were measured spectrophotometrically at various times for immobilized fungus, as well as for free fungus.

Figure 4 (a and b) demonstrate typical residual dye profiles for decolorization with free and immobilized fungus. The trends for free and calcium alginate-immobilized fungus were similar, as the dye concentration dropped almost linearly until decolorization was completed, while the profile for polyacrylamide-immobilized fungus exhibited an extra phase (indicated by different slopes) during the early stage of decolorization. The different residual dye profile for polyacrylamide-immobilized fungus may correlate with CB adsorption capacities of this immobilization support (data not shown), since the early drop in the dye concentration (Fig. 4b) may be do to a matrix adsorption effect [35], while the second slope resulted from biotransformation by immobilized *Coprinus cinereus*. For calcium alginate-immobilized fungus, decolorization was primarily contributed by fungal decolorization, as the adsorption effect was less important.

The specific decolorization rate, determined from Fig. 4, was 5.58, 3.84 and 1.46 mg g^{-1} h^{-1}, respectively, for free fungus and immobilized fungus in calcium alginate and in polyacrylamide (Table 1). The lower decolorization rate for immobilized fungus compared to free fungus can be attributed to the mass transfer restriction arising from fungus entrapment.

From the two supports used for the immobilization of *Coprinus cinereus*, calcium alginate showed the greatest purifying performance with a specific decolorization rate of 3.84 mg g^{-1} h^{-1} and an equilibrium conversion of 99% (Table 1). All the more that the hydrodynamic behaviour and mechanical beads properties of calcium alginate make this polymer a matrix of choice for the utilisation in fluidised bed bioreactor [36, 37]. The immobilization of *Coprinus cinereus* in polyacrylamide gel allows a specific decolorization rate of about 1.46 mg g^{-1} h^{-1} (Table 1). This limitation of the decolorization activity is probably due to the existence of an unfavourable microenvironment inside the gel matrix and the presence of residual monomer that leads to a toxicity of the fungal biomass [38].

Effects of some physicochemical parameters on CB decolorization. Over the range of 20 to 45°C, the specific decolorization rate of free and immobilized *Coprinus cinereus* increased as the temperature increased (Fig. 5a); the final conversion maintained above 98%. However, for free and immobilized fungus, decolorization operations at temperatures higher than 45°C led to a significant loss in viability of the fungus and a sharp decrease in the decolorization activity (Fig. 5a). Therefore, to ensure the stability for long-term operation, decolorization with

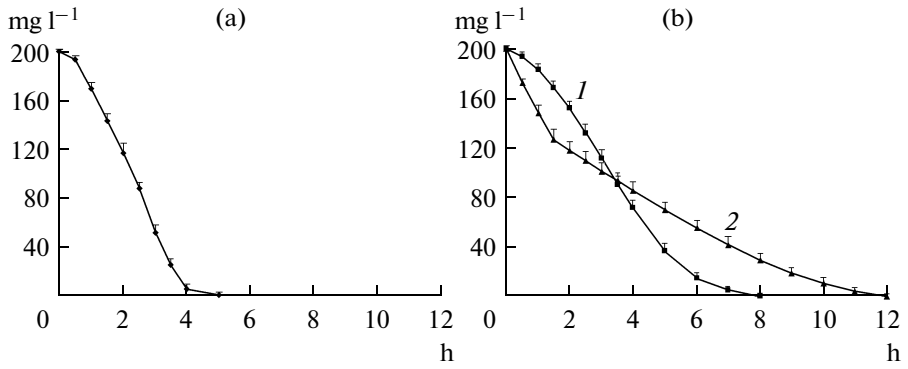

Fig. 4. Decolorization of CB by *Coprinus cinereus* in fluidized bed bioreactor; (a) free fungus; (b) immobilized fungus in both alginate (*1*) and polyacrylamide gels (*2*).

free and immobilized *Coprinus cinereus* should not be undertaken at temperature higher than 45°C.

Free fungus exhibited better decolorization rates at pH 5.0, as the specific decolorization rate enhanced twofold for a pH decrease from 9.0 to 5.0 (Fig. 5b). In contrast to free fungus, decolorization with the immobilized fungus was less sensitive to pH with less than 16% deviation over the pH range of 3.0–9.0. This seems to suggest that the mass transfer barrier in the immobilized fungus may impede the transport of H^+ ions into the fungus embedded within the polymeric matrices, resulting in a less significant pH effect for the immobilized fungus. A similar effect has been shown for immobilized bacterial cells of *Pseudomonas luteola* [30].

Figure 6 shows the dependence of CB concentration on the decolorization rate for free and immobilized *Coprinus cinereus*. The kinetic trends were interpreted according to a conventional Michaelis-Menten model, but for immobilized fungus, especially in polyacrylamide gel, the model only represents the apparent rather than intrinsic kinetics, because of the mass transfer barrier and adsorption effect of the immobilization matrix. Although the adsorption effect played a role in the decolorization dynamics primarily during the early stage, enzymatic transformation by *Coprinus cinereus* would eventually dominate and lead to a complete color removal. The optimal kinetic parameters (V_{max} and K_m) were estimated from the experimental data shown in Fig. 6. The V_{max} of free fungus was 30.4 mg g^{-1} h^{-1}, similar to that for polyacrylamide-immobilized fungus (32.1 mg g^{-1} h^{-1}), but much higher than that for fungus immobilized in calcium alginate gel (16.8 mg g^{-1} h^{-1}). In contrast to V_{max}, K_m values varied less significantly for free and calcium alginate-immobilized fungus. The K_m values for free and calcium alginate-immobilized fungus were 374 and 367 mg l^{-1}, respectively, while polyacrylamide-immobilized fungus had a much larger K_m value of 942 mg l^{-1}, which seems to indicate a low affinity between the dye (CB) and polyacrylamide-immobilized fungus.

Table 1. The specific decolorization rate of Cibacron Blue and the equilibrium conversion recorded by free and immobilized *Coprinus cinereus* in both alginate (Al) and polyacrylamide (Pa) gels

Variant	Specific decolorization rate, mg g^{-1} h^{-1}	Equilibrium conversion, B, %
Free fungus	5.58 ± 0.32	100
Al-immobilized fungus	3.84 ± 0.29	99
Pa-immobilized fungus	1.46 ± 0.25	98

Repeated-batch decolorization of CB. Repeated-batch experiments were performed to examine the reusability of the free and immobilized *Coprinus cinereus* in dye decolorization. After 7 cycles, the specific decolorization rate of free and polyacrylamide-immobilized fungus dropped to below about 55 and 67%, respectively, while the specific decolorization rate remained over 98% for calcium alginate-immobilized fungus (Table 2). Thus, calcium alginate-immobilized fungus appeared to exhibit better reusability. Evaluation of $t_{1/2}$ (the time required for 50% color removal) shows that $t_{1/2}$ varied only slightly (2.60–2.65 h) for calcium alginate-immobilized fungus during 7 repeat-

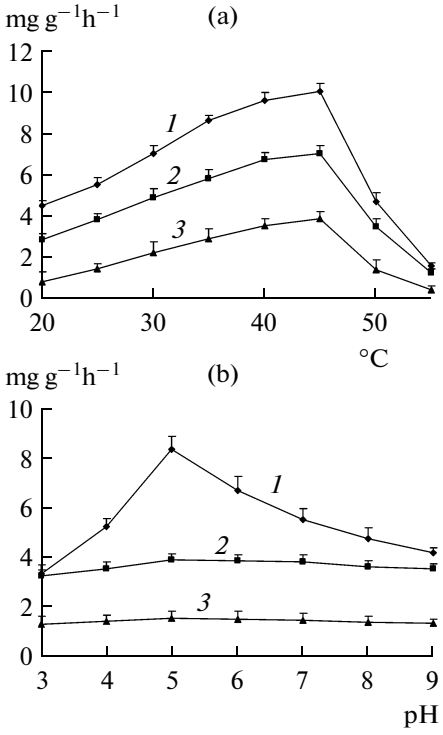

Fig. 5. Effect of temperature (a) and pH (b) on specific decolorization rate (mg g^{-1} h^{-1}) of CB recorded by free (*1*) and immobilized *Copinus cinereus* in both alginate (*2*) and polyacrylamide (*3*) gels.

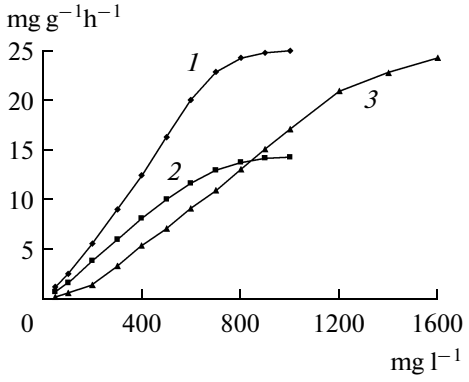

Fig. 6. Effect of initial concentration of CB on specific decolorization rate (mg g^{-1} h^{-1}) recorded by free (*1*) and immobilized *Copinus cinereus* in both alginate (*2*) and polyacrylamide (*3*) gels.

Table 2. Comparison of relative specific decolorization rate (V_d) and time required for 50% conversion ($t_{1/2}$) recorded by free and immobilized *Coprinus cinereus* in alginate (Al) and in polyacrylamide (Pa) during repeated batch decolorization cycles

Cycle	Free fungus		Al-immobilized fungus		Pa-immobilized fungus	
	V_d, %	$t_{1/2}$, h	V_d, %	$t_{1/2}$, h	V_d, %	$t_{1/2}$, h
1	100	1.79	100	2.60	100	6.82
2	95	1.88	100	2.60	98	6.96
3	85	2.10	99	2.62	95	7.09
4	79	2.26	100	2.60	89	7.71
5	74	2.41	99	2.62	82	8.42
6	67	2.67	99	2.62	76	8.80
7	55	3.25	98	2.65	67	9.46

ed cycles (Table 2). This indicates that calcium alginate, used as an entrapment gel in a continuous fluidized bed bioreactor, offers a great stability of the fungal activity. This stability may be attributed to sweet polymerization conditions of the calcium alginate gel and direct role that plays the calcium in the cell conservation [39]. However, in the first run of experiments, $t_{1/2}$ for free and polyacrylamide-immobilized fungus were 1.79 and 6.82 h, respectively (Table 2). After 7 runs, the $t_{1/2}$ increases and reaches 3.25 and 9.46 h for free and polyacrylamide-immobilized fungus, respectively (Table 2).

***

It has been shown that high-efficiency decolorization of water contaminated by the anthraquinone dye CB using immobilized *Coprinus cinereus* in a fluidized bed bioreactor is feasible. The obtained results show that this technology allows specific and reproducible decolorization of CB with high yield.

The specific decolorization rate obtained by the free fungus is superior to those obtained by immobilizing this fungus in calcium alginate or polyacrylamide gels. However, the entrapment of *Coprinus cinereus* in calcium alginate gel offers the greatest purifying performances as well as the biggest stability of the bioreactor. All the more, the hydrodynamic behaviour of the beads, in a fluidized bed bioreactor, is very satisfactory. The high specific decolorization rate obtained and the simplicity of the immobilization method mean that alginate would be a suitable immobilization matrix for using fungi to remove anthraquinone dyes from wastewater on an industrial scale.

ACKNOWLEDGMENTS

The authors would like to thank Professor Yahia Koulali (University Hassan 1st of Settat, Morocco) for his assistance and helpful suggestions in the fungus identification.

REFERENCES

1. Vaidya, A.A. and Datye, K.V., *Colourage*, 1982, vol. 14, pp. 3–10.
2. Boer, C.G., Obici, L., Souza, C.G.M., and Piralta, R.M., *Bioresour. Technol.*, 2004, vol. 94, pp. 107–112.
3. Anliker, A., *Ecotoxicol. Environ. Safety*, 1979, vol. 3, pp. 59–74.
4. Chung, K.T. and Cerniglia, C.E., 1992, *Mutation Res.*, vol. 277 pp. 201–220.
5. Ingamells, W., Colour for Textiles – a User's Handbook, London: Staples Printers Rochester Ltd, 1993.
6. Zollinger, H., Colour in Chemistry – Syntheses, Proprieties and Applications of Organic Dyes and Pigments, New York: VCH Publications, 1991.
7. Haff, L.A. and Easterday, R.L., Cibacron Bblue-Sepharose: A Tool for General Affinity Chromatography, Eckstein, F., and Sundaram, P.V., Eds, New York: Academic Press, 1978.
8. Liu, Y.C. and Stellwagen, E., *J. Biol. Chem.*, 1987, vol. 262, pp. 583–588.
9. McKay, G., Geundi, M.E., and Nassar, M.M., *Water Res.*, 1988, vol. 22, pp. 1527–1533.
10. Vandevivere, P.C., Bianchi, R., and Verstraete, W., *J. Chem. Technol. Biotechnol.*, 1998, vol. 72, pp. 289–302.
11. De Moraes, S.G., Freire, R.S., and Duran, N., *Chemosphere*, 2000, vol. 40, pp. 369–373.

12. Rafii, F. and Cerniglia, C.E., *Appl. Environ. Microbiol.*, 1993, vol. 59, pp. 1731–1734.
13. Carliell, C.M., Barclay, S.J., Naidoo, N., Buckley, C.A., Mulholland, D.A., and Senior, E., *Water SA*, 1995, vol. 21, pp. 61–69.
14. Zissi, U. and Lyberatos, G., *Water Sci. Technol.*, 1996, vol. 34, pp. 495–500.
15. Sarnaik, S. and Kanekar, P., *Appl. Microbiol. Biotechnol.*, 1999, vol. 52, pp. 251–254.
16. Chang, J.S. and Kuo, T.S., *Bioresour. Technol.*, 2000, vol. 75, pp. 107–111.
17. Walker, G.M. and Weatherley, L.R., *Environ. Pollution*, 2000, vol. 108, pp. 219–223.
18. Zimmermann, T., Kulla, H.G., and Leisinger, T., *Eur. J. Biochem.*, 1982, vol. 129, pp. 197–203.
19. Moutaouakkil, A., Zeroual, Y., Dzayri, F.Z., Talbi, M., Lee, K., and Blaghen, M., *Arch. Biochem. Biophys.*, 2003, vol. 413, pp. 139–146.
20. Fu, Y. and Viraraghavan, T., *Bioresour. Technol.*, 2001, vol. 79, pp. 251–262.
21. Shah, V. and Nerud, F., *Can. J. Microbiol.*, 2002, vol. 48, pp. 857–870.
22. Wesenberg, D., Kyriakides, I., and Agathos, S.N., *Biotechnol. Adv.*, 2003, vol. 22, pp. 161–187.
23. Maximo, C., Pessao-Amorim, M.T., and Costa-Ferreira, M., *Enzyme Microbiol. Technol.*, 2003, vol. 32, pp. 145–151.
24. Selvam, K., Swaminathan, K., and Chae, K.S., *Bioresour. Technol.*, 2003, vol. 88, pp. 115–119.
25. Knapp, J.S., Zhang, F., and Tapley, N.K., *J. Chem. Technol. Biotechnol.*, 1997, vol. 69, pp. 289–296.
26. Chibata, I. and Tosa, T., *Annu. Rev. Biophys. Bioeng.*, 1981, vol. 10, pp. 197–216.
27. Hyde, F.W., Hunt, G.R., and Errede, L.A., *Appl. Environ. Microbiol.*, 1991, vol. 57, pp. 219-222.
28. Zeroual, Y., Moutaouakkil, A., and Blaghen, M., *Curr. Microbiol.*, 2001, vol. 43, pp. 322–327.
29. Monsan, P., Les méthodes d'immobilisation d'enzymes, Durand, G., and Monsan, P., Eds, Paris: Gauthier-Villards, 1982.
30. Chang, J.S., Chou, C., and Chen, S.Y., *Process Biochem.*, 2001, vol. 36, pp. 757–763.
31. Moutaouakkil, A., Zeroual, Y., Dzayri, F.Z., Talbi, M., Lee, K., and Blaghen, M., *Curr. Microbiol.*, 2004, vol. 48, pp. 124–129.
32. Riddell, R.W., *Mycologia*, 1950, vol. 42, pp. 265–270.
33. De Hoog, G.S., Guarro. J., Gene, J., and Figueras, M.J., Atlas of Clinical Fungi, 2nd Ed., Utrecht/Reus: Centraalbureau Voor Schimmelcultures / Univeesitat Rovira I Virgili, 2000.
34. Domsch, K.H., Gams, W., and Anderson, T.H., Compendium of Soil Fungi, London: Academic Press, 1980.
35. Philips, C.R. and Poon, Y.V., Immobilization of Cells, New York: Springer-Verlag, 1988.
36. Smidsrod, O. and Skjak, B.G., *Trends Biotechnol.*, 1990, vol. 8, pp. 71–78.
37. Badalo, A., Gomez, E., Gomez, J.L., Bastida, J., Maximo, M.F., and Diaz, F., *Process Biochem.*, 1991, vol. 26, pp. 349–353.
38. Mosbach, K. and Mosbach, R., *Acta Chem. Scand.*, 1966, vol. 20, pp. 2807–2810.
39. Tamponnet, C., Matsumara, M., and Veliky, I.A., *Appl. Microbiol. Biotechnol.*, vol. 32, pp. 211–217.