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Efficient decolorization of an anthraquinone dye by recombinant dye-decolorizing peroxidase (rDyP) immobilized in silica-based mesocellular foam

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ABSTRACT

A recombinant dye-decolorizing peroxidase (rDyP) produced from *Aspergillus oryzae* was immobilized in synthesized silica-based mesocellular foam (MCF: average pore size 25 nm) and used for decolorization of the anthraquinone dye, Remazol Brilliant Blue R (RBBR). The adsorption yields of rDyP immobilized in MCF increased as the pH decreased from 6 to 3. However, the activity yields of the immobilized rDyP decreased with decreasing pH. The overall efficiency, defined as adsorption yield × activity yield, reached its maximum of 83% at pH 5. In repeated dye-decolorization tests, 20 batches of RBBR could be decolorized by the MCF-immobilized rDyP. MCF showed significantly better performance for rDyP immobilization in term of retaining enzyme activity and dye-decolorization ability compared to previous studies using other mesoporous materials.

1. Introduction

Dye-contaminated effluents from the textile, paper and leather industries are known to cause environmental problems. Such dyes are likely to be carcinogenic and are known to have potent acute and/or chronic effects on exposed organisms [1], including strong detrimental effects on the growth of microorganisms [2,3]. Moreover, dyes are highly visible to the human eyes, and can cause aesthetic contamination even at very low concentrations. Among the 12 classes of chromogenic dyes, the most common is the azo group, which comprises nearly 70% of all textile dyestuff produced. The next most common is the anthraquinone dye group [4].

Considerable research has focused on the use of enzymes in colored wastewater treatment [5]. Previously, a novel dye-decolorizing peroxidase (DyP) was isolated from *Thanatephorus cucumeris* strain Dec 1 (former name, *Geotrichum candidum* strain Dec 1) [6]. The Dec 1 was deposited at National Institute of Technology and Evaluation, Japan with deposition number: FERM P-15348 (http://www.nbrc.nite.go.jp/npmd/e/). The *dyp* gene of strain DeC 1 was transformed to *Aspergillus oryzae* RD005, and rDyP production was enhanced more than 3000-fold [7,8].

Immobilization of this enzyme in appropriate supports that retain its enzyme activity and even enhance catalytic efficiency in repeated-batch mode has been sought because all carriers tested for immobilization of rDyP were not successful. Among the supports tested for enzyme immobilization, mesoporous materials are particularly efficient candidates because they have uniform and adjustable pore sizes, large surface areas and large pore volumes [9]. We previously succeeded in immobilizing rDyP on two mesoporous materials, FSM-16 and AlSBA-15, which have pore sizes in the range of 6–10 nm. However, the small pore size and two-dimensionality of materials resulted in mass transfer limitations and low activity yields [10].

Recently, the synthesis of silica-based mesocellular foam (MCF) and its successful application in enzyme immobilization have opened up a new era in enzymes applications [11,12]. MCFs possess 3D cage-like structures with spherical pores having diameters of 20–40 nm, interconnected by windows around 10 nm in size. The relatively large size of entrance pores and windows allows MCF to host bulky enzymes with molecular masses as high as 200 kDa [2]. Moreover, enzyme adsorption in MCFs having eight different pore sizes, revealed that the larger the pore diameter, the faster the adsorption rates. This showed that the pore diameter should be 4–5-fold larger than the enzyme diameter to allow free access of the enzyme [13]. As the longest dimension of rDyP is 6.6 nm, the pore diameter of a mesoporous host to immobilize rDyP should be around 26 nm.

Here, we report the immobilized rDyP in a synthesized MCF (pore size around 25 nm), followed by examination of its use in decolorizing RBBR as a representative anthraquinone dye.

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Additionally, this activity was compared with that of rDyP immobilized on a commercially available 3-aminopropyl functionalized silica gel (APSG) possessing a positive charge on its surface.

2. Materials and methods

2.1. Chemicals

The swelling agent, 1,3,5-trimethylbenzene (TMB), was purchased from Acros Organic (Belgium). The non-ionic triblock copolymer, poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (Pluronic 123, molecular weight 5800, $EO_{20}PO_{70}EO_{20}$), tetraethyl orthosilicate (TEOS), ammonium fluoride (NH_4F) and 3-aminopropyl functionalized silica gel (APSG: pore diameter 6–9 nm) were purchased from Sigma–Aldrich (USA). The Remazol Brilliant Blue R (RBBR) and reactive blue 5 (RB5) were purchased from Nippon Kayaku Co. Ltd. (Tokyo, Japan). These chemical reagents were of guaranteed reagent grade.

2.2. MCF synthesis

MCF was synthesized based on the previously described method [12]. Four grams of the triblock copolymer, Pluronic 123 were dissolved in 75 ml of 1.6 M HCl, and then 3.4 ml of TMB was added dropwise. The resulting solution was heated at 40 °C with vigorous stirring for 2 h. Then, 9.2 ml of TEOS was added, the mixture was stirred for 5 min, and the solution was aged at 40 °C for 20 h under static condition. NH_4F (46 mg) in water (5 ml) was added to the solution, which was then transferred to an autoclave for aging at 100 °C for 24 h. The resulting precipitate was filtered through filter paper, washed with ethanol and water, and then air-dried. This material was extracted with alcohol and called MCF-Ex. The MCF-Ex was calcined at 550 °C in air for 6 h to remove the remaining organic fraction, and the prepared material was called MCF.

2.3. Characterization of the MCFs

Nitrogen adsorption–desorption isotherm measurements of MCF were conducted at 77 K using a Belsorp 28 SA sorptometer (Bell Japan). The specific surface area was calculated with the Brunauer–Emmett–Teller (BET) method using adsorption data in the BET region (P/P_0 = 0.0 to 0.3). The total pore volume was determined from the adsorbed amount of nitrogen at the relative pressure of P/P_0 0.95. The pore sizes and morphology of MCF were obtained using field-emission scanning transmission electron microscopy (FE-SEM) (Hitachi S-5200, Japan). Elemental analysis was conducted using CHN analyzer (YANACO, CHN Corder MT-5, Japan) to calculate the percentages of carbon and hydrogen in MCF and MCF-Ex.

2.4. Production of rDyP

The utilized rDyP was produced by a 5-day cultivation of *A. oryzae* RD005 carrying *dyp* gene isolated from strain Dec 1, in a 10-l jar fermentor using wheat bran powder as the carbon source. The rDyP was purified using the previously reported method [6]. The culture supernatant (4000 ml) was passed through gauze to remove *A. oryzae* pellets. Then, the filtered supernatant, which contained 14 U/ml of rDyP, was centrifuged at 4 °C for 30 min, and then concentrated to 85 ml by ultrafiltration with a 10-kDa membrane. The concentrated enzyme was precipitated in ammonium sulfate (70% saturation) at 4 °C, centrifuged at 4 °C for 30 min, and then dissolved in 25 mM piperazine buffer (pH 5.5). Ammonium sulfate (1.5 M) was added to 40 ml of the enzyme solution and the mixture was applied to a butyl-toyopearl column. Gradient elution was carried out from 25 mM piperazine buffer (pH 5.5) containing 1.5 M ammonium sulfate to ammonium sulfate-free 25 mM piperazine buffer (pH 5.5). The rDyP was eluted at around 0.8 M ammonium sulfate. The collected fractions were used for determination of the activity and specific activity of the produced rDyP, which had a molecular mass of 58 kDa [6], and monomer dimensions of approximately 6.2 nm × 6.6 nm × 4.8 nm [18].

2.5. Immobilization of rDyP in mesoporous materials and measurement of activity

Solutions of 10 mM citrate-buffered rDyP (initial activity of approximately 300 U/ml) at pH values of 3–6 were used for immobilization. The immobilization material (MCF-Ex, MCF or APSG: 30 mg each) was placed in a 1.5-ml microtube containing 1 ml of citrate-buffered rDyP solution (equivalent to 10,000 U rDyP per gram of each mesoporous material), and the mixture was stirred overnight at 4 °C. The mesoporous materials containing immobilized rDyP were recovered by centrifugation and the supernatant was used for determination of rDyP activity. The remaining mesoporous materials were washed twice with 10 mM citrate buffer at the same pH as used for immobilization, and resuspended in the same buffer for determination of rDyP activity. Adsorption yield (%) was defined as $((A_i - A_r)/A_i) \times 100$, where A_i is the initial rDyP activity in the buffered rDyP solution before immobilization and A_r is the residual rDyP activity in the supernatant after immobilization.

The activity of the immobilized rDyP is of critical interest for its use in dyes decolorization. Therefore, we defined activity yield as (measured rDyP activity/expected rDyP activity) × 100 (%). The expected rDyP activity was defined as $(A_i - A_r)$/concentration of the carrier. Overall efficiency (%) was defined as adsorption yield (%) × activity yield (%), thereby reflecting the overall activity performance of the immobilized rDyP.

Leaching of the immobilized rDyP from MCF was assessed by storing it in 10 mM citrate buffer at 4 °C for 10 days, with sequential daily changes of buffer. At each buffer exchange, the activities of the immobilized rDyP and the free rDyP in the supernatant were measured.

2.6. Assay of rDyP activity

To determine the activity of immobilized and free rDyP, RB5 decolorization was monitored at its maximum absorbance of 600 nm by spectrophotometer (UV-2400PC; Shimadzu, Kyoto, Japan) [6]. Citrate buffer (2920 μl, 25 mM pH 3.2), RB5 (15 μl, 25 mM) and immobilized rDyP (50 μl; containing 0.1–0.3 mg mesoporous material with immobilized rDyP) or free rDyP (50 μl) were added to a 3 ml cuvette. The reaction was initiated by the addition of 15 μl of 40 mM H_2O_2. One unit (U) of rDyP activity was defined as the amount of enzyme required to decolorize 1 μmol of RB5 in 1 min.

2.7. Repeated-batch RBBR decolorization by rDyP immobilized in MCF

Decolorization of RBBR was carried out in 1.5-ml vials containing 1 ml of 10 mM citrate-buffered solution (pHs 3 and 4), 150 mg/l (0.24 mM) RBBR and 600 U/l rDyP immobilized in MCF. The mixture was incubated at 30 °C with shaking at 100 strokes per minute (spm), and the decolorization reaction was initiated by addition of 0.24 mM of H_2O_2. When the decolorization ratio (%) reached more than 90%, the reaction mixture was centrifuged at 4 °C at 15,000 rpm for 10 min, the decolorized dye solution was withdrawn, and new dye solution was added for the next round of decolorization.

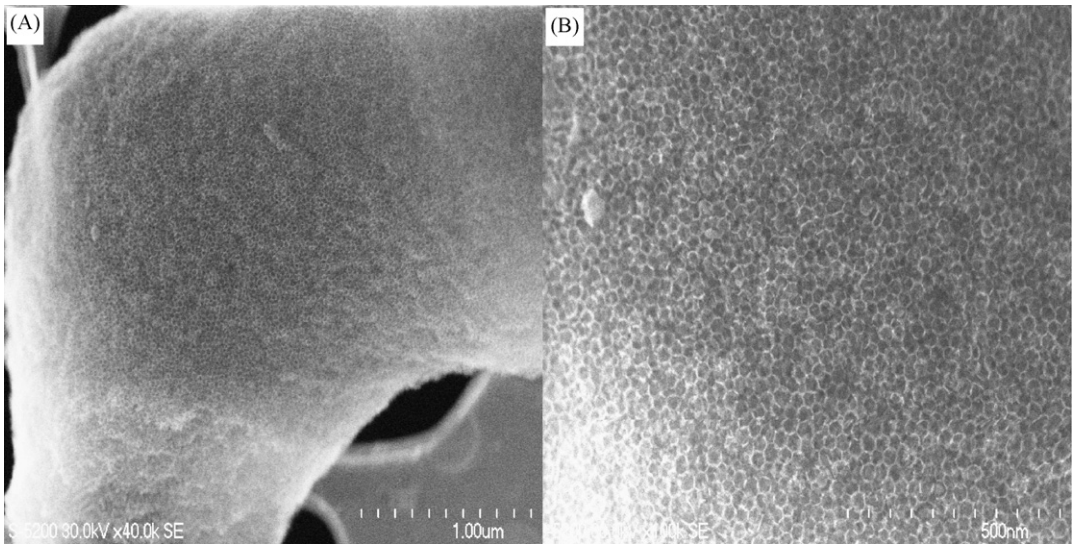

Fig. 1. Image of MCF (A) and higher resolution image of MCF (B) observed by FE-SEM.

The RBBR concentration was determined by spectrophotometrically measuring its maximum absorbance at 593 nm, and then converting absorbance to dye concentration using a calibration curve. The decolorization ratio (%) was calculated as $(C_i - C_t)/C_i \times 100$, where C_i and C_t are the initial dye concentration and the dye concentration at time t, respectively. Residual rDyP activity (%) was defined as $(A_t/A_i) \times 100$, where A_i and A_t are the initial activity of rDyP and the activity of rDyP at time t, respectively.

3. Results

3.1. Characteristics of the MCF material

The SEM and STEM images of MCF are shown in Fig. 1. The synthesized MCF possessed well-defined pores and high porosity. The average pore diameter observed by STEM was 25 nm. The specific pore volume and surface area values for MCF, which were obtained from gas adsorption–desorption isotherms (not shown), were 0.81 cm^3/g and 532 m^2/g, respectively.

3.2. Effect of pH on adsorption yield and activity yield of rDyP immobilized in MCF

The immobilizations of rDyP in MCF and MCF-Ex were conducted in the pH range of 3–6. As rDyP adsorption to MCF-Ex was possible only at pH 3 and adsorption yield and activity yield were 94% and 4%, respectively, the overall efficiency was 3.8%. When MCF, the calcined product of MCF-Ex, was used for rDyP immobilization, immobilization occurred at all pHs tested (Table 1). The rDyP adsorption yield for MCF was highest of 97% at pH 3, but the maximum measured activity of rDyP immobilized in MCF was 8330 U/g-MCF at pH 5. Thus, the maximum and minimum overall efficiencies were 83% and 34% at pH 5 and pH 3, respectively.

To examine the possibility of rDyP immobilization on a support possessing a positive surface charge, rDyP immobilization on commercially available APSG was conducted. The results are given in Table 2. rDyP adsorption yield increased with increases in pH and the maximum rDyP adsorption yield was 90% at pH 6. However, the rDyP activity was only 0.81%. Thus, the maximum measured rDyP activity and overall efficiency in APSG were only 150 U/g-silica gel and 1.48%, respectively.

3.3. Leaching of immobilized rDyP from MCF

In order to test the stability of rDyP immobilization in MCF, the leaching of rDyP from MCF was investigated by repeated washing of the immobilized rDyP with buffer solution daily for 10 days at 4 °C. The activity of rDyP immobilized in MCF dropped only by 6% and 12% at pH 3 and pH 4, respectively, but the activity decreased by 35% and 70% at pH 5 and pH 6, respectively (Fig. 2). We previously showed that rDyP sustained its activity in pHs from 3 to 6 [10]. Therefore, the decrease in activities of rDyP immobilized in MCF

Table 1
Results of 300 U rDyP immobilization on 30 mg MCF at different pHs.

pH	Adsorption yield (%)	Expected activity (U/g-carrier)	Measured activity (U/g-carrier)	Activity yield (%)	Overall efficiency (%)
3	97	9700	3400	35	34
4	91	9100	7330	80	73
5	86	8600	8330	97	83
6	63	6300	6400	101	64

Table 2
Results of 300 U rDyP immobilization on 30-mg 3-aminopropyl functionalized silica gel (APSG).

pH	Adsorption yield (%)	Expected activity (U/g-carrier)	Measured activity (U/g-carrier)	Activity yield (%)	Overall efficiency (%)
3	1.6	160	150	93	1.48
4	55	5500	60	1	0.55
5	70	7000	60	0.85	0.59
6	90	9000	73	0.81	0.72

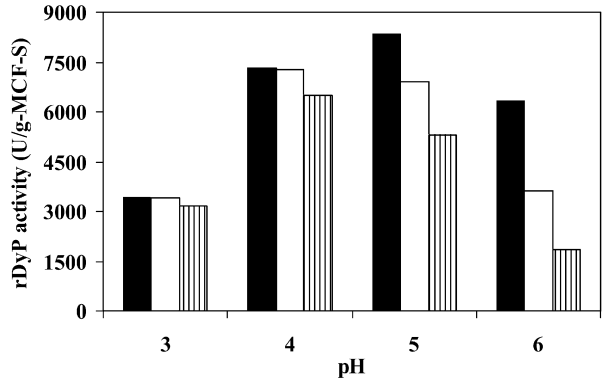

Fig. 2. Activity change of MCF-immobilized rDyP, after daily leaching treatment under different pH conditions. *Bars*: day 1, black; day 5, white; and day 10, stripe.

was mainly due to leaching active rDyP from MCF-rDyP, not due to low pH of buffer.

3.4. Repeated-batch decolorization of RBBR

Repeated decolorization of RBBR by rDyP immobilized in MCF was examined at pH 3 and pH 4 (Fig. 3), due to the relatively significant leaching of rDyP from MCF at pH ≥5 (Fig. 2). At pH 3, only one batch of RBBR decolorization was possible, and decolorization did not occur thereafter (Fig. 3A). At pH 4, however, decolorization continued for 20 batches of RBBR (Fig. 3B). The residual activity of MCF-immobilized rDyP withdrawn from the decolorized solution after the twentieth batch was 10% that of the initial immobilized rDyP.

Although MCF has high surface area, less than 5% dye was adsorbed when RBBR and MCF were mixed (data not shown). This indicates negligible contribution of RBBR adsorption on MCF in dye decolorization.

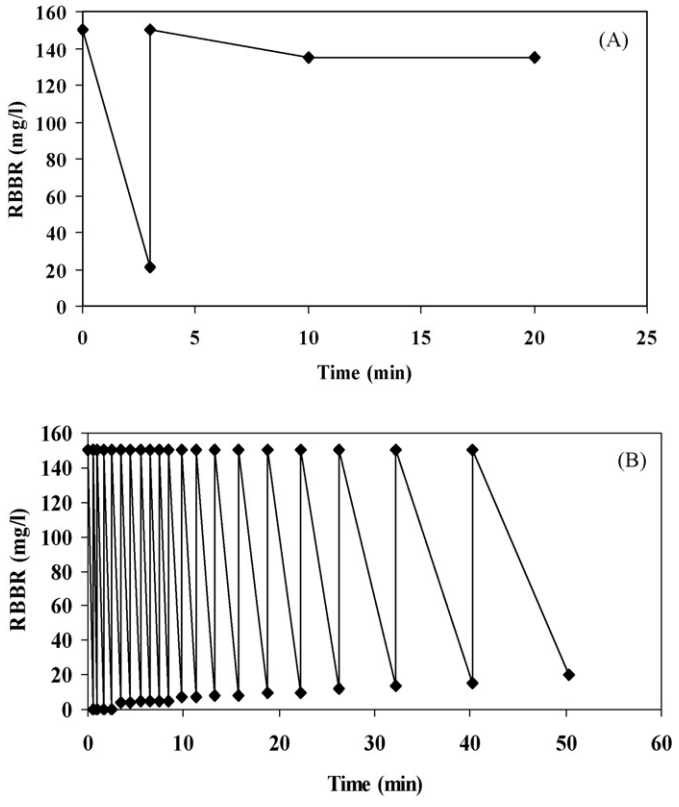

Fig. 3. Repeated-batch RBBR decolorization by rDyP immobilized in MCF at pH 3 (A) and pH 4 (B).

4. Discussion

rDyP immobilized in MCF materials retained almost all of its activity (Table 1), compared to the activity decreases previously associated with immobilization of rDyP in APSG and other mesoporous materials [10].

The MCF synthesized in this study possessed relatively large (25 nm) pores that appeared to be cage-like structures interconnected by windows of nearly 10 nm [14]. These windows were smaller than the diameter of the cage itself, yet big enough to host an enzyme such as rDyP. These characteristics allowed efficient entrapment of the enzyme, while the relatively small window size minimized enzyme leaching [12].

The surface charges of the enzymes and silica materials are important in enzymes immobilization [10,15]. Silica has a net negative charge above pH 2 because its isoelectric point (pI) is 2 [16]. As pI of rDyP is 4.2 [6], the rDyP is positively charged at pH values below 4.2 and negatively charged at pH above this point. Thus, there was an electrostatic attraction between the positively charged rDyP and the negatively charged MCF at pH range of 2–4.2, resulting in a higher adsorption yield at these pHs (Table 1). At pHs above 4.2, the surfaces of the rDyP became progressively negatively charged, causing lower levels of rDyP adsorption on the negatively charged MCF. Complete release of the immobilized rDyP was achieved by transferring the MCF-immobilized rDyP from buffer solution at pH 3 to buffer solution at pH 7, and the released rDyP recovered the original activity of free rDyP (data not shown). This proves relatively lower adsorption of rDyP in MCF at pH 7. Similar changes in the activity of enzymes immobilized under different pH conditions have been reported for chloroperoxidase (CPO) [17], and lipase [18,19]. Despite the increased adsorption yield at lower pH values, the activity yield of the immobilized rDyP decreased at these lower pH values. This is consistent with previous findings when rDyP was immobilized in other mesoporous materials, such as AlSBA-15 and FSM-16 [10]. One possibility might be because the enhanced electrostatic interaction between the rDyP and the MCF surface changed the enzyme conformation [17], causing some of the enzyme's active sites to become improperly oriented for the reactions [10,17].

Unlike the trend observed for MCF (Table 1), the adsorption yield of rDyP to positively charged APSG increased as the pH increased from 3 to 6 (Table 2). This is likely because the negative charge density on the surface of rDyP was enhanced with increases in pH, resulting in a stronger attraction with the positively charged APSG. This led to increase in adsorption yield of rDyP. Despite the high adsorption yield (90% at pH 6), the overall efficiencies were less than 2%, suggesting that the rDyP may have been immobilized on the positively charged APSG through the enzyme's active site, resulting in almost complete inactivation of rDyP. Therefore, even though positively charged carriers have a high adsorption yield, they are not suitable candidates for rDyP immobilization.

When using mesoporous materials, it is important that the enzyme is immobilized inside the pores, as this minimizes leaching. To confirm that the rDyP was immobilized inside the MCF pores and not weakly immobilized on the external surfaces of the material, rDyP immobilization on MCF-Ex was conducted. We found that rDyP was immobilized on MCF-Ex only at pH 3, whereas immobilization in MCF took place at all tested pH values. To clarify the remarkable differences in rDyP adsorption yield, we conducted elemental C and H analysis of MCF and MCF-Ex. We found that MCF-Ex contained 1.5% H and 8.4% C, whereas, MCF only showed 0.28% H, which existed in the silanol group and no C was detected. We speculated from this result that the existence of organic fraction

Table 3
Repeated-batch decolorization of 150 mg/l RBBR by rDyP immobilized in MCF, compared with previous reports for rDyP immobilized in FSM-16 and AlSBA-15 [10].

	Number of repeated-batch RBBR decolorization		
	pH 3	pH 4	pH 5
AlSBA-15	1	2	–
FSM-16	4	8	5
MCF	1	20	–

(–) was not conducted.

in MCF-Ex blocks the introduction of enzyme into the inside pores and thus the enzyme was adsorbed on the surface of the MCF-Ex. At pH 3 high ionic interaction between MCF-Ex and the enzyme, rDyP can be adsorbed on multilayer on MCF-Ex. However, at pH higher than 3, almost no enzyme was adsorbed on the MCF-Ex due to weak ionic interaction. This leads to significantly low overall efficiencies of MCF-Ex. In MCF, the strong ionic interaction can lead to rDyP adsorption inside MCF and maintain high overall efficiency.

Immobilization of an enzyme on a carrier often decreases the enzyme's native activity by more than 50% [20,21]. When we previously immobilized rDyP in FSM-16 and SBA-15 under similar conditions as those described herein for MCF, the maximum overall efficiency was only 29% for FSM-16 [10]. However, overall efficiencies of rDyP immobilized in MCF reached 73–83% at pHs 4 and 5. The ability of MCF to retain higher rDyP activity mainly arises from the large pore size and efficient mass transfer of MCF materials. Similar results were reported for the immobilization of CPO in Cs^+-impregnated SBA-16 [22].

MCF-immobilized rDyP could decolorize only one batch of RBBR at pH 3, whereas 20 batches could be decolorized at pH 4. This was because rDyP was inactivated by H_2O_2 much more quickly at pH 3 than that at pH 4 [10]. Comparisons of repeated-batch RBBR decolorization by 600 U/l rDyP immobilized in MCF versus that by 400 U/l rDyP immobilized in FSM-16 and AlBA-15 [10] are shown in Table 3. The decolorization of 20 batches of RBBR by rDyP immobilized on MCF was significantly better than the number of batches decolorized by rDyP immobilized on other materials, largely because of the cage-like pores and high porosity of MCF, which facilitate mass transfer. The fact that inter-pore windows are smaller than the pore size may decrease enzyme leaching.

Precursors for synthesis of MCF are relatively inexpensive and when rDyP-MCF after 20 repeated decolorizations is transferred to high pH ≥7, rDyP is released and then MCF can be used for immobilization of new batch of rDyP. These advantages of MCF will give a possibility of use in wastewater treatment.

5. Conclusions

We showed efficient immobilization of rDyP in MCF and superiority of MCF in immobilization of rDyP to other mesoporous materials is mainly because of its larger pore size (25 nm) and inter-pore windows and subsequently better mass transfer. Overall efficiency of rDyP adsorption in MCF which was defined as adsorption yield multiplied by activity yield and dye decolorization were strongly pH-dependent. The overall efficiency was highest at pH 5, but the 20 batches of RBBR were possible at pH 4 mainly because of significant leaching of rDyP from MCF at pH ≥5. As mesoporous and mesocellular materials are the only carriers available for immobilization of rDyP, MCF-immobilized rDyP can be a promising biocatalyst for use in enzyme-based colored wastewaters treatment.

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References

[1] M.A. Brown, S.C. De Vito, Crit. Rev. Environ. Sci. Technol. 23 (1993) 249.
[2] Y.M. Slokar, M. Le Marechal, Dyes Pigm. 37 (1998) 335.
[3] A.F. Strickland, W.S. Perkins, Text. Chem. Color 27 (1995) 11.
[4] P.C. Vandevivere, R. Binachi, W. Verstraete, J. Chem. Technol. Biotechnol. 72 (1998) 289.
[5] J. Karam, J. Nicell, J. Chem. Technol. Biotechnol. 69 (1997) 141.
[6] S.J. Kim, M. Shoda, Appl. Environ. Microbiol. 65 (1999) 1029.
[7] Y. Sugano, R. Nakano, K. Sasaki, M. Shoda, Appl. Environ. Microbiol. 66 (2000) 1754.
[8] M. Shakeri, Y. Sugano, M. Shoda, J. Biosci. Bioeng. 103 (2007) 129.
[9] H.P. Yiu, P.A. Wright, J. Mater. Chem. 15 (2005) 3690.
[10] M. Shakeri, M. Shoda, J. Mol. Catal. B: Enzymatic 54 (2008) 42.
[11] P. Schmit-Winkel, P.P. Lukens, D. Zhao, P. Yang, B.F. Chmelka, G.D. Stucky, J. Am. Chem. Soc. 121 (1999) 254.
[12] Y. Han, S.S. Lee, J.Y. Ying, Chem. Mater. 18 (2006) 643.
[13] J.A. Bosley, J.C. Clayton, Biotech. Bioeng. 43 (1994) 934.
[14] J.S. Lettow, Y.J. Yong, P. Schmit-Winkel, P. Yang, D. Zhao, G.D. Stucky, J.Y. Ying, Langmuir 16 (2000) 8291.
[15] J.F. Diaz, K.J. Balkus, J. Mol. Catal. B: Enzymatic 15 (1996) 115.
[16] D. Moelans, P. Cool, J. Baeyens, E.F. Vansant, Catal. Commun. 6 (2005) 307.
[17] Y.J. Han, J.T. Watson, G.D. Stucky, A. Butler, J. Mol. Catal. B: Enzymatic 17 (2002) 1.
[18] A. Salis, I. Svensson, M. Monduzzi, V. Solinas, P. Adlercreutz, Biochim. Biophys. Acta 1646 (2003) 145.
[19] R.M. Blanco, P. Treros, M. Ternandes-Perez, C. Otero, G. Diaz-Gonzalez, J. Mol. Catal. B: Enzymatic 30 (2004) 83.
[20] J. Bryjak, B.N. Kolarz, Biochemistry 33 (1998) 409.
[21] C. Lei, Y. Shin, J.K. Magnuson, G. Fryxell, L.I. Lasure, D.C. Elliott, J. Liu, E.J. Ackerman, Nanotechnology 17 (2006) 5531.
[22] J. Aburto, M. Ayala, I. Bustos-Jamies, C. Montiel, E. Terres, J.M. Dominguez, E. Torres, Micropor. Mesopor. Mater. 83 (2005) 193.