Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2013.819148

52 (2014) 6694–6702
October

Taylor & Francis
Taylor & Francis Group

Discoloration of aqueous textile dyes solution by *Phanerochaete chrysosporium* immobilized in modified PVA matrix

Ani Idris[a,*], Mohd Suardi Suhaimi[a], Nor Azimah Mohd Zain[b], Roslina Rashid[a], Norasikin Othman[c]

[a]*Department of Bioprocess Engineering, Institute of Bioproduct Development (IBD) Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*
Email: ani@cheme.utm.my
[b]*Faculty of Biosciences and Bioengineering, Department of Industrial Biotechnology, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia*
[c]*Faculty of Chemical Engineering, Department of Chemical Engineering, Institute of Bioproduct Development (IBD) Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

Received 16 February 2012; Accepted 3 June 2013

ABSTRACT

This study investigates the performance of immobilized *Phanerochaete chrysosporium* in modified PVA matrix in removing a mixture of textile dyes consisting of Yellow FG, Red 3BS, Orange 3R, Blue RSP, Black B, and remazol turquoise blue from aqueous solution. The effects of agitation and temperature were thoroughly investigated in determining the optimum operating condition of dye discoloration using central composite design. Optimum condition was determined as 100 rpm and 35°C. Evidently, adsorption only plays a minor role in removing these dyes as it contributes to about 8% of color removal. The rest of color removal could be attributed to fungal enzymatic biodegradation, particularly the highly regarded manganese peroxidase. Manganese peroxidase assay revealed that a maximum enzyme activity of 174 U/l was achieved in the third cycle of repeated use. The reusability test also revealed that the immobilized fungus could be used for up to six cycles with a peak of 82% of discoloration, where discoloration started to fluctuate until the fifth cycle before a sharp decrease in dye discoloration was observed. The incidence of aromatic ring breakage was confirmed by FTIR analysis as indicated by the occurrence of certain corresponding peaks.

Keywords: Immobilized *Phanerochaete chrysosporium*; Modified PVA matrix; Dye discoloration; Manganese peroxidase; FTIR

1. Introduction

Dyes are available commercially in the form of powders, granules, pastes, and liquid dispersions, with concentrations of active ingredients ranging typically from 20 to 80%. Most of the dyes used in the textile industry are synthetic, usually derived from coal tar and petroleum-based intermediates. Dyes are applied to textiles to add color and esthetic appearance, hence, increasing product value. The dyeing process itself employed various types of dyestuffs, techniques, and equipment. According to a report by EPA [1], among the main phases that greatly

*Corresponding author.

contribute to the generation of effluent are the wet processing stages with a ratio of 12–20 gallons/pound of product [2]. These stages that also include the application of dyes onto fabric, produce dye bath and wash water that contains approximately 10–15% of residual dyes [2]. In addition, textile effluent may also contain certain pollutants including certain by-products, oxalic acid and auxiliary chemicals. Improper disposal or ineffective treatment of such large volume of generated wastewater would undoubtedly harm the environment due to the wide variety of contaminants' contents.

Various chemical, physical, and biological methods have been proposed since the past three decades in the treatment of textile industry effluent [3]. No single method could work effectively in isolation to achieve high discoloration rate due to the limitations of each method in terms of practicality [4]. Therefore, a combination of certain methods would be a reasonable option. Adsorption has been recognized as an outstanding physical separation technique in the removal of several types of water pollutants, predominantly those that are recalcitrant to biological treatment including a wide range of dyes [3]. This is due to the fact that it is easy to operate, versatile, and does not form hazardous substances [5].

Biological techniques that employ certain microorganisms such as a number of potent bacteria and fungi have been reported in various publications [6,7]. Among the tested microorganisms, fungi have been identified as the most effective microorganisms in treating textile industry effluent [8,9]. These findings are often associated with their inherent ability to produce extracellular enzymes with low specificity towards substrates that are capable of hydrolyzing various types of recalcitrant organic compounds [8]. This has led to the extensive investigation of various fungi strains in search for the most potent fungi strain of all in the dye discoloration application.

Among the kingdom of fungi, the white-rot fungi were recognized as the most capable fungi in degrading a wide range of synthetic dyes [9]. *Phanerochaete chrysosporium*, a strain that belongs to this class of fungi, has been thoroughly studied by previous researchers for its potential in several recalcitrant pollutants' degradation [10]. Ever since its isolation, *P. chrysosporium* has been demonstrated as a fungus that holds great prospective in the biodegradation of various dyes owing to its ability to secrete a range of extracellular oxidative enzymes [11]. Manganese peroxidase, which is among the extracellular oxidative enzymes secreted by the fungus, has been identified as the most potent enzyme in depolymerization of lignin, pulp biobleaching, and effluent discoloration [12,13]. It has also been shown to efficiently decolorize Poly R-478, a dye known for its recalcitrance, *in vitro* [14].

For almost four decades, the utilization of immobilized fungi for certain purposes has gained vast interest and significance [15]. These advances could be attributed to a range of advantages associated with immobilization, primarily reusability, ease in biomass reuse, easier separation, and reduction of clogging in continuous-flow systems [16,17]. Moreover, immobilized cells also exhibit much higher activity and immobilization itself may also protect cells from potential hostile environments in the surroundings [18]. The use of immobilized fungi on certain supports could be viewed as a combination of physical and biological methods, as both the support matrix and fungal biomass could play their own role in dye discoloration.

This study focused on the discoloration of six different textile dyes widely used in Batik industry in Malaysia, namely Yellow FG, Turquoise Blue, Red 3BS, Orange 3R, Blue RSP, Black B, and Remazol Turquoise Blue, via the use of *P. chrysosporium* immobilized in modified PVA–Alginate matrix. The general structures of the chromophores of the aforesaid dyes are depicted in Figs. 1 and 2. These dyes are regarded as recalcitrant organic pollutants that are also toxic, thus improper discharge may lead to serious environmental and health hazards. The use of *P. chrysosporium* immobilized in the modified PVA–Alginate matrix in this particular application has never been reported yet. To the best of our knowledge, the use of our PVA–alginate flat-sheet matrix modified from our patented PVA–alginate beads has never been reported before in the discoloration of this particular mixture of dyes [19].

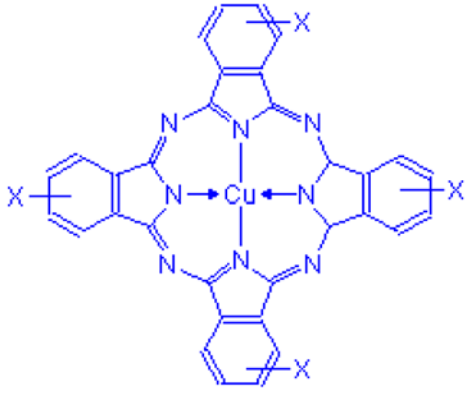

Fig. 1. General structure of phthalocyanine dye chromophore [9].

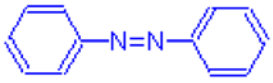

Fig. 2. General structure of Azo dye chromophore [9].

2. Materials and methods

2.1. Fungal strain

P. chrysosporium (ATCC 24725) was purchased from the Deutsche Sammlung von Mikroorganism und Zekulturen and maintained at 4°C on 2.5% malt extract agar slant. Subculturing was done every month.

2.2. Immobilization procedures

The modified PVA–Alginate matrix was prepared according to our previous work with slight modification [20]. The matrix takes the form of flat-sheet matrix instead of beads using petri dish as the mold. The flat-sheet matrix has a dimension of 6 cm in diameter and 1 mm in thickness with an average weight of 0.5 g. *P. chrysosporium* was grown under stationary condition on Potato dextrose agar (OXOID Ltd., UK) at 37°C. Fungal biomass was harvested after 5 days of incubation to prepare spore suspension. Ten milliliters of spore suspension were mixed with 90 ml of PVA solution containing 12% v/w of PVA. Then, 6 ml of the resulting mixture was poured into petri dishes and left to settle. A solution of 6.5% w/v boric acid and 2% w/v was then poured into each of the petri dishes and stored at 4°C for 24 h. The solution was then replaced with 0.5 M sodium sulfate after 24 h and then the matrices were kept at 4°C for further use.

After the immobilization, the immobilized spores of *P. chrysosporium* were germinated with 2% w/v malt extract and incubated for four days. The resulting biomass was washed with distilled water prior to freeze drying. The dry weight of the immobilized fungal biomass was determined by weighing the PVA–alginate flat-sheet matrix, after freeze drying, before and after fungal growth.

2.3. Dye preparation

The powder of each of the six dyes (each with a concentration of 300 mg/L), courtesy: Razali Batik Kota Bharu, Malaysia, was dissolved in distilled water and made into a stock solution. The stock solution was then mixed together to yield the emulated batik effluent with six different concentrations ranging from 50 to 300 mg/l.

2.4. Dye characteristics and discoloration determination

The characteristics of the dye solution mixture are as follows: pH, 4.95; λ_{max}, 386 nm; and color, dark purple. Discoloration was determined by measuring the decrease in absorbance at 386 nm. The percentage of dye discoloration could be expressed as:

$$\text{Decolorization}\,(\%) = \frac{A_0 - A_t}{A_0} \quad (1)$$

where A_o is the initial absorbance and A_t final absorbance at time t.

2.5. Enzyme assay

Manganese peroxidase assay was performed spectrophotometrically according to the method explained by Bergmeyer [21] at 436 nm and 25°C. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the conversion of one μmole of H_2O_2 per minute under the assay condition.

2.6. Optimization of operating condition using central composite design

A central composite design experiment was conducted to investigate the relationship between the considered operating parameters (agitation and temperature) and the process performance measured in terms of discoloration percentage (%). This particular experiment would also serve as a mean to determine the optimum operating condition. A total of 13 experiments as shown in Table 1 were generated by Design Expert Software using face-centered cubic as the design. Later, a total of three confirmation runs were performed to validate the determined optimum condition. The selection of parameters with their corresponding levels was narrowed down from literature. In a study that investigated the discoloration of 14 structurally different dyes, Knapp et al. [22] set the temperature at 27°C, while Pazarlioglu [10] fixed the temperature at 39°C. Nevertheless, neither report justified the selection of the temperature value although both reported a decent discoloration %.

2.7. Discoloration of textile dyes by immobilized *P. chrysosporium*

PVA–alginate flat-sheet matrix innoculated with *P. chrysosporium* spores was introduced into shake flasks containing growth medium that contains 2% w/v malt extract and incubated at 37°C for four days. After that, the growth medium was replaced

Table 1
Decolorization% according to each run

Std. run no.	Factors		Response variable decolorization (%)
	Agitation (rpm)	Temperature (°C)	
1	50	30	32
2	150	30	38
3	50	40	29
4	150	40	33
5	50	35	51
6	150	35	51
7	100	30	57
8	100	40	61
9	100	35	76
10	100	35	75
11	100	35	72
12	100	35	77
13	100	35	75

aseptically with nitrogen-limiting medium (NLM) containing the mixture of the six dyes with concentration of 300 mg/l. The NLM contains the following per liter of distilled water: 15 g glucose, 400 mg malt extract, 0.3 mg $MnCl_2$, 4 mg $FeSO_4 \cdot H_2O_2$, 40 mg $MgSO_4 \cdot 7H_2O_2$, and 20 mmol 3,3-dimethylglutarate [23]. The control experiment was also performed without the addition of the NLM to investigate the extent of adsorption in discoloration. Sampling was performed daily by withdrawing 3 ml of aliquots to determine reduction in absorbance and enzyme activity.

2.8. SEM images

The surface and cross-section images of immobilized *P. chrysosporium* were obtained by using FESEM. The PVA–alginate flat-sheet matrices were patted dry with tissues and then placed on a stand, and the image of the surface was obtained using FE-SEM (Model Zeiss SUPRA 35 VP FE-SEM). To obtain the cross-section image, the dried PVA–alginate flat-sheet matrix was first cut using a surgical knife before it was placed on the stand.

2.9. Reusability test

Repeated batch experiments under the same conditions mentioned previously were performed to determine the reusability of immobilized *P. chrysosporium* in decolorizing the dye. Fresh media was added to the shake flasks during each cycle.

2.10. FTIR spectroscopy analysis

Treatment of dye solution would result in breaking of certain bonds of the dyes due to fungal enzymatic degradation. FTIR spectroscopy was used to confirm such phenomenon using transmittance mode. The dye solutions were prepared by filtering the untreated and treated dye solutions using 0.45 μm nylon. The untreated dye solution was used as control. FTIR spectra of dye solutions were then determined by direct transmittance.

Spectra were recorded by using Perkin-Elmer spectrum one FTIR spectrophotometer. All spectra were measured at a spectral resolution in the range of 370–4,000 cm^{-1} and the samples were scanned at a rate of 16 scans/min. The FTIR spectra were smoothened and corrected to the baseline correction and the major vibration bands were associated with chemical groups [23].

3. Results and discussion

3.1. Optimization

Optimization experiment via CCD revealed that the optimum operating conditions for the discoloration of the mixture of dyes occurred when the agitation and temperature was set at 100 rpm and 35°C, respectively. Table 1 lists the discoloration% according to each run. A marked decrease in discoloration was observed beyond this particular operating condition, possibly due to the excessive shear stress and heat rendering the MnP activity diminishing to a significant extent. This observation was validated through confirmation runs with three experiments under three different operating conditions as described in the methodology. The contour plot for the optimization experiment is depicted by Fig. 3, while the ANOVA is summarized by Table 2.

Results tabulated in Table 2 reveal that only the suggested model and the square of each predictor, i.e. both the agitation and temperature are significant, while interaction effect and the linear model are insignificant. This supposition is also substantiated by the associated $Prob > F$ value which was less than 0.05. The lack-of-fit test also revealed a desirable observation as the associated $Prob > F$ value is well above 0.05, implying that the model is fit. This would imply that discoloration varies solely on the square of both the agitation and temperature yielding the following quadratic curve:

$$\text{Discoloration}\,(\%) = 75.28 + 1.33A - 0.67B - 0.40AB - 15.98A^2 - 16.97B^2 \quad (2)$$

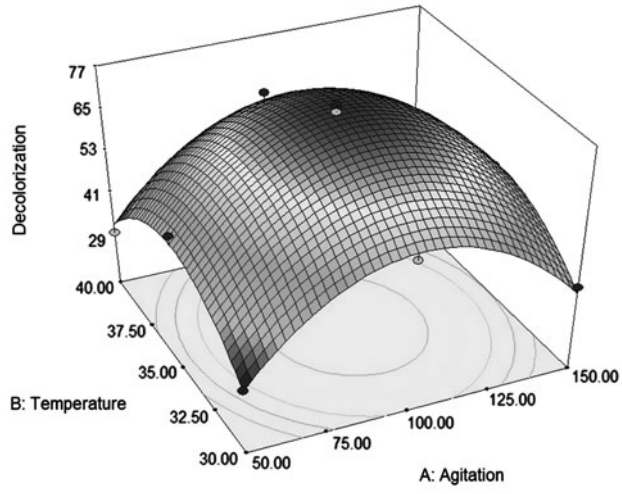

Fig. 3. Three-dimensional illustration of decolorization percentage response upon variation in temperature (°C) and agitation (rpm).

where A represents the agitation (rpm) and B represents the temperature (°C).

Insignificant lack-of-fit on the other hand, signifies the desirable fit of the model. This implies that the model is adequate to represent the behavior of discoloration upon varying both parameters.

Confirmatory experiment with a total of three runs was conducted to validate the model developed by the optimization experiment. The results are tabulated in Table 3. The treatment combinations of the confirmation runs consisted of the temperature and agitation setting that was used previously in the optimization. The predicted values of discoloration percentages at these three different settings were calculated using Eq. (2). Upon comparison, it is clear that the quadratic model generated by RSM corresponds reasonably well with the experimental value (<5% errors). This shows that the quadratic model obtained from the optimization experiment is certainly adequate as reproducible results could be obtained from confirmation runs.

3.2. Reusability of immobilized fungus in membranes

Reusability test that follows after optimization revealed that the immobilized fungus could be used for up to five cycles with discoloration of well above 70% before a marked decrease in performance could be observed as depicted in Fig. 4.

From Fig. 4, it is also clear that the trend of discoloration variation seems to increase for the first three cycles before a noticeable decrease could be observed. The increase in the first three cycles could possibly result from the acclimatization of the immobilized fungus to the new environment where there is a difference in the nutrient content. The fungus started to adapt to the new environment by slowly adjusting its metabolism. The marked decrease in the discoloration percentage on the other hand could be attributed to the entrance of fungus growth phase from stationary to the death phase. Death phase that could be characterized as the gradual decrease in the number of viable cells could result in slower metabolism,

Table 2
ANOVA table (partial sum of square) for response surface quadratic model

Source	Sum of square	DF	Mean square	F	Prob > F	Remarks
Model	4006.50	5	801.30	120.82	<0.001	Significant
A	16.67	1	16.67	2.51	0.157	
B	2.67	1	2.67	0.040	0.546	
A^2	1721.43	1	1721.43	259.56	<0.001	
B^2	794.96	1	794.96	119.86	<0.001	
AB	1.00	1	1.00	0.15	0.709	
Residual	46.43	7	6.63			
Lack-of-fit	32.43	3	10.81	3.09	0.152	Not significant
Pure error	14.00	4	3.50			
Cor. Total	4502.92	12				
SD	2.58	R^2		0.989		
Mean	55.92	Adj. R^2		0.980		
C.V.	4.61	Pred. R^2		0.916		
PRESS	341.30	Adeq. precision		25.014		

Table 3
Confirmation runs results (predicted response vs. observed response)

Agitation (rpm)	Temperature (℃)	Calculated value (%) (predicted response)	Actual value (%) (observed response)	Errors (%)
50	30	42.33	40.86	3.60
100	35	75.28	73.67	2.19
150	40	42.33	44.12	4.06

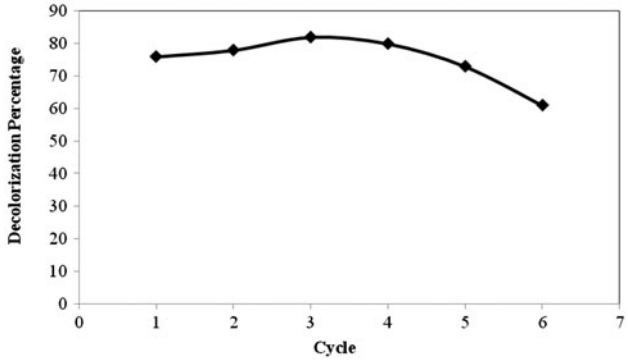

Fig. 4. Decolorization percentage for each subsequent decolorization cycle.

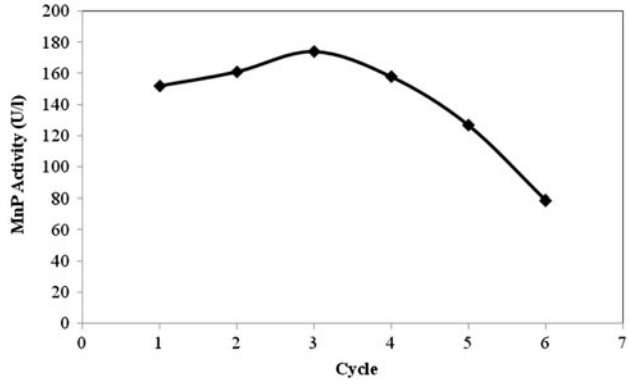

Fig. 5. MnP activity from cycle to cycle.

hence the production of certain enzymes that could be responsible for dye molecules degradation which in turn lead to a noticeable decrease in discoloration. This supposition is confirmed by the observed MnP activity from cycle to cycle as depicted in Fig. 5. Evidently, the MnP activity seems to correspond to the discoloration percentage suggesting the involvement of this enzyme in dye discoloration.

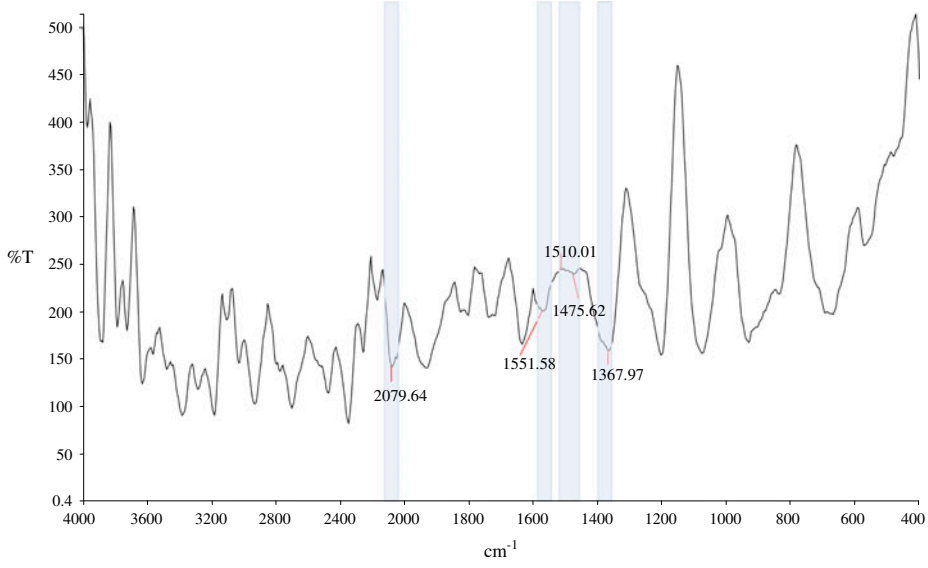

Fig. 6. FTIR spectra of synthetic waste before treatment.

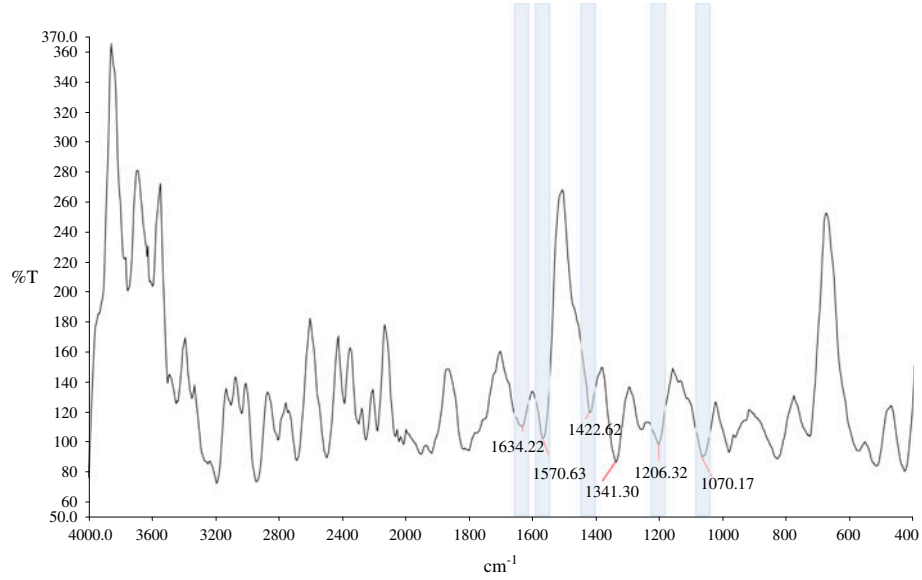

Fig. 7. FTIR spectra of synthetic waste after treatment.

3.3. Characterization of the dye solution and PVA alginate matrix

FTIR analysis also supports the involvement of enzymatic degradation of the dye. The bonds that originally exist in the structure of each dye molecule, particularly the chromophore, have been broken down after the synthetic waste has been subjected to immobilized fungal treatment. This would eventually lead to a change in color intensity as the chromophore that is responsible for giving each dye its characteristics color underwent a change in the structure.

Fig. 6 illustrates FTIR spectra of synthetic waste before treatment. Prior to treatment, characteristic peaks of N=N are observed at 1510.01 and 1475.62 cm^{-1}. The peak at 1510.01 and 1475.62 cm^{-1} corresponds to the azo linkages that are present in *cis*-aromatic azo and aromatic azoxy compounds, respectively [24]. The incidence of these peaks is highly likely since the synthetic waste consists of five azo dyes. The peak at 1,551.58 cm^{-1} meanwhile signifies the occurrence of C=N that possibly belongs to the benzimidazoles, imidazoles or imine functional group [24]. In the perspective of this study, the bond most likely belongs to benzimidazoles or imidazoles functional group as Turquoise Blue contains the chromophore that is related to both functional groups.

Fig. 7 on the other hand shows the FTIR spectra of synthetic waste following treatment. The characteristics peak at 1,341.30 cm^{-1} corresponds to the N=N bonds in aliphatic azoxy compounds [25]. This observation shows that the aromatic ring of the azo dyes have been broken down most probably via biodegradation. Similarly, the observed peaks at 1,570.63 and 1,634.22 cm^{-1} also indicates the occurrence of aromatic rings rupture as these particular peaks correspond to the N=N bonds of alkyl azo and diazoketone compounds [25]. The peak at 1206.32 cm^{-1} on the other hand shows the incidence of 2-monosubstituted furans which could result from the biodegradation of Turquoise Blue [24].

The mechanisms of dye discoloration, namely, adsorption, biosorption and enzymatic degradation, were investigated thoroughly by conducting the discoloration tests both by immobilization matrix and fungal biomass as well as enzyme assay. Control experiment that serves as a comparison was conducted simultaneously. This helps to elucidate the extent adsorption role in discoloration. From Fig. 8,

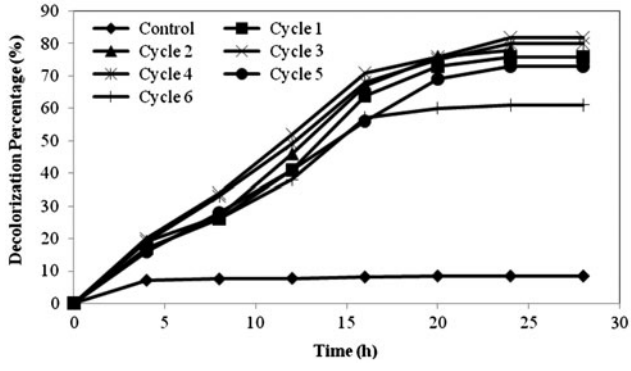

Fig. 8. Comparison of decolorization for each run vs. time.

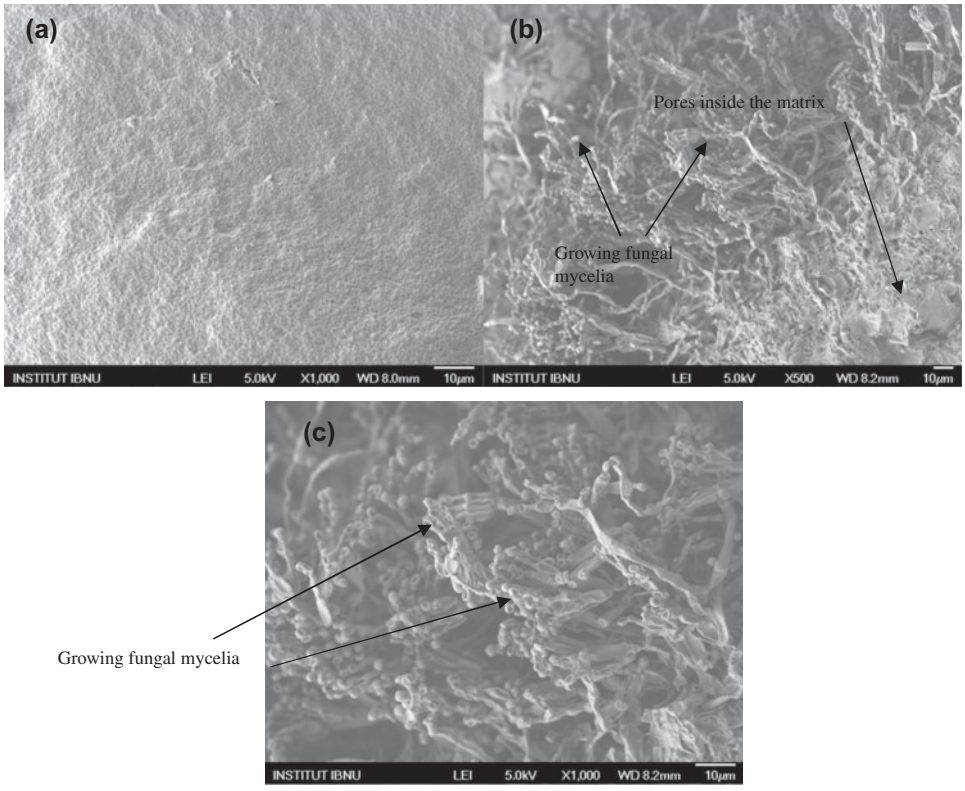

Fig. 9. (a) Surface of uninnoculated PVA–alginate that was used as control, (b) cross-section image of innoculated PVA–alginate with *P. chrysosporium* mycelia protruding from the inside of the matrix, and (c) surface of inoculated PVA–alginate flat-sheet matrix that has been fully colonized by *P. chrysosporium* mycelia.

apparently, adsorption only accounts for approximately 8.4% of total discoloration. This observation suggests that both immobilization matrix and fungal biomass are poor adsorbents. The poor adsorption capacity is consistent with the finding by Prigione et al. [25] that fungal biomass is a poor sorbent of dye molecules, while no previous study reported the adsorption capacity of the immobilization matrix since this is the first time it ever been used in dye discoloration study.

The images of uninoculated and inoculated PVA matrix surface at 1,000× magnification are shown in Fig. 9(a) and (c). At 500× and 1,000× magnification, the budding mycelia of the immobilized *P. chrysosporium* could be clearly observed as revealed in Fig. 9(b) and (c), respectively. Judged against the uninoculated matrix, the surface of inoculated matrix has been fully colonized by the budding fungal mycelia. This condition would probably hinder the transport of dye molecules onto the surface of the flat-sheet matrix and consequently render the adsorption of dye molecules to be predominated by fungal biomass which is also a poor adsorbent.

4. Conclusion

Results revealed that the immobilized *P. chrysosporium* displayed a great potential in the discoloration of the synthetic waste for up to six consecutive cycles. Discoloration steadily increased from the first cycle at 76% until a maximum dosage of discoloration (82%) was achieved in the third cycle. From the third cycle onwards, the discoloration percentage started to decrease steadily until a sharp dip was observed on the sixth cycle, where it dropped significantly to 61%. There was also an obvious correlation between dye discoloration percentage and MnP activity indicating the involvement of enzymatic biodegradation in dye discoloration. The comparison of discoloration percentage with the control run revealed that adsorption only plays a minor role in decolorizing the dye, thus most of the discoloration percentage could attributed to biodegradation. This supposition was confirmed by the FTIR spectroscopy, where certain bonds were broken down after the synthetic waste has been subjected to the treatment by immobilized *P. chrysosporium*.

Acknowledgments

Financial support from the Ministry of Science, Technology and Environment through the FRGS funding 78673 and short-term grant 77370 is gratefully acknowledged.

References

[1] Environmental Protection Agency, EPA Office of Compliance Sector Notebook Project: Profile of the Textile Industry, 401 M St., SW, Washington, DC (1997).
[2] L.J. Snowden-Swan, Pollution prevention in the textile industries, in: H.M. Freeman (Ed.), Industrial Pollution Prevention Handbook, McGraw-Hill, New York, 1995, p. 837.
[3] G. Crini, Kinetics and equilibrium studies in the removal of cationic dyes from aqueous solution by adsorption onto a cyclodextrin polymer, Dyes Pigm. 77 (2008) 415–426.
[4] A. Kunz, V. Reginatto, N. Duran, Combined treatment of textile effluent using the sequence *Phanerochaete chrysosporium*-ozone, Chemosphere 44 (2001) 281–287.
[5] G. Crini, Non-conventional low-cost adsorbents for dye removal: A review, Bioresour. Technol. 97 (2006) 1061–1085.
[6] Y. Wang, J. Zhang, J. Yin, Progress of enzyme immobilization and its potential application, Desalin. Water Treat. 1 (2009) 157–171.
[7] E.K. Hassib, Y. Yousseff, J.J. Godon, M. Hamdi, Enhancement of textile wastewater decolourization and biodegradation by isolated bacterial and fungal strains, Desalin. Water Treat. 2 (2009) 310–316.
[8] P. Kaushik, A. Malik, Fungal dye decolourization: Recent advances and future potential, Environ. Int. 35 (2009) 127–141.
[9] S.R. Couto, Dye removal by immobilised fungi, Biotechnol. Adv. 27 (2009) 227–235.
[10] N.K. Pazarlioglu, R.O. Urek, F. Ergun, Biodecolourization of direct blue 15 by immobilized *Phanerochaete Chrysosporium*, Process Biochem. 40 (2005) 1923–1929.
[11] M. Sedighi, A. Karimi, F. Vahabzadeh, Involvement of ligninolytic enzymes of *P. chrysosporium* in the textile effluent containing Astrazon Red FBL in a packed-bed bioreactor, J. Hazard. Mater. 96 (2009) 88–93.
[12] D.K. Dahoo, R. Gupta, Evaluation of ligninolytic microorganisms for efficient discoloration of a small pulp and paper mill effluent, Process Biochem. 40 (2005) 1573–1578.
[13] M. Hofrichter, Review: Lignin conversion by manganese peroxidase, Enzyme Microb. Technol. 30 (2002) 454–466.
[14] D. Moldes, S.R. Couto, C. Cameselle, M.A. Sanroman, Study of the degradation of dyes by MnP of *P. chrysosporium* produced in a fixed-bed bioreactor, Chemosphere 51 (2003) 295–303.
[15] J.G. Anderson, Immobilized cell and film reactor system for filamentous fungi, In: J.E. Smith, D.R. Berry, B. Kristiansen (Eds.), Fungal technology. The Filamentous Fungi, Edward Arnold, London, pp. 146–170, 1975.
[16] M.Y. Arica, F.A. Sharif, N.G. Alaeddinoglu, N. Hasirci, V. Hasirci, Covalent immobilization of *Aspergillus niger* on phema membrane – application to continuous flow reactors, J. Chem. Technol. Biotechnol. 58 (1993) 281–285.
[17] Y.P. Tieng, G. Sun, Use of polyvinyl alcohol as a cell entrapment matrix for copper biosorption by yeast cells, Chem. Technol. Biotechnol. 75 (2000) 541–546.
[18] M. Shin, T. Nguyen, J. Ramsay, Evaluation of support materials for the surface immobilization and decoloration of amaranth by *Trametes versicolor*, Appl. Microbiol. Biotechnol. 60 (2002) 218–323.
[19] A. Idris, N.A. Zain, M.S. Suhaimi, An immobilization of enzymes using an improved preparation technique of PVA-Alginate matrix. Malaysian Patent PI 20081825 (2008).
[20] A. Idris, N.A. Zain, M.S. Suhaimi, Immobilization of Baker's yeast invertase in PVA-alginate matrix using innovative immobilization technique, Process Biochem. 43 (2008) 331–338.
[21] H.U. Bergmeyer, Methods of Enzymatic Analysis 1, 2nd ed., Academic Press, New York, p. 495, 1974.
[22] J.S. Knapp, P.S. Newby, L.P. Reece, Discoloration of dyes by wood-rotting basidiomycete fungi, Enzyme Microb. Technol. 17 (1995) 664–668.
[23] H.S. Mansur, C.M. Sadahira, A.N. Souza, A.A. Mansur, FTIR spectroscopy characterization of poly (vinyl alcohol) hydrogel with different hydrolysis degree and chemically crosslinked with glutaraldehyde, Mater. Sci. Eng. 28 (2008) 539–548.
[24] S. George, Infrared and Raman Characteristic Group Frequencies: Tables and Charts, 3rd ed., John Wiley, Chichester, 2001.
[25] V. Prigione, G.C. Varese, L. Casieri, V.F. Marchisio, Biosorption of simulated dyed effluents by inactivated fungal biomasses, Bioresour. Technol 99 (2008) 3559–3567.