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Statistical analysis of immobilized *Phanerochaete chrysosporium* in PVA–alginate–sulfate beads for textile wastewater treatment

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ABSTRACT

A two-level fractional factorial design was used to assess the ability of immobilized *Phanerochaete chrysosporium* in polyvinyl alcohol (PVA)–alginate–sulfate beads to treat textile effluents. The analysis from scanning electron microscope micrographs reveals that the fungus is successfully immobilized in this type of matrix. Based on the statistical analysis, it was found that pH, nitrogen concentration and beads loading are the significant factors affecting the production of ligninolytic enzymes: manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase (Lac). Analysis of variance reveals that coefficient determination R^2 of MnP and LiP was 0.8695 and 0.8607, respectively, and the coefficient determination of Lac was 0.9078 and reflects that model fits well to the data. The results also revealed that textile dye discoloration (associated with producing a large set of ligninolytic enzymes) occurred best at pH 7 with 10 g of the beads loaded containing *P. chrysosporium* (approximately 3.4067×10^7 spores) into textile wastewater without addition of nitrogen. The maximum discoloration percentage was up to 65.5%. Additionally, immobilized cells can be reused for up to five cycles as a wastewater treatment.

Keywords: Statistical analysis; Textile wastewater treatment; Ligninolytic enzymes; PVA–alginate–sulfate beads; *Phanerochaete chrysosporium;* Immobilized cells

1. Introduction

In this ever-changing environment, many products have been developed or modified in order to meet the global demands. There are over 100,000 types of dyestuffs used in textile industry and they can be characterized by their colors and the molecular structures. The use of these different dyes generates more than 7×10^5 tons of waste is generated per year worldwide [1–5].

The dye effluents notably are able to cause water pollution, and they are able to discharge highly concentrated contaminants that can cause harm or damage to the environment [2]. With the increase in annual production and use; synthetic dyes could lead to environmental pollution and health problems [6]. Moreover, the untreated effluents are being used in the field of agriculture and they have damaging impacts on the ecosystem and human health [7].

The disposal of untreated wastewater into water bodies can increase toxicity of both water and the soil, directly affecting the water supply, aquatic life and soil quality [8,9]. As for the synthetic dyes, they can form a thin layer of emulsion on the surface of the water bodies and this will minimize the light penetration. Without a proper light penetration, this would prevent the aquatic flora from getting enough light source for the activities of photosynthetic. Consequently, it will interrupt and disturb the food chain

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system in the aquatic habitats. Additionally, the presence of synthetic dyes is able to threat the life of the aquatic fauna by reducing the amount of dissolved oxygen in the water. Therefore, as a result of such reduction of the dissolved oxygen, it can increase the biochemical oxygen demand of the contaminated water [9].

Through years, there are various treatments that have been developed to minimize these impacts. For example, such treatment namely is the process of the photochemical oxidation by using H_2O_2–UV, which is the activated carbon by the use of adsorption and ozonation using the ozone gas through the oxidation reaction. Unfortunately, most of the treatments are not cost-effective and eco-friendly. For a better alternative, the textile waste treatment by using the *Phanerochaete chrysosporium* is proved to be effective in the discoloration of all molecular weight fractions of the textile effluents in the environment-friendly way [10]. The *P. chrysosporium* is a type of white-rot fungi. It can produce the extracellular ligninolytic enzymes such as (i) manganese peroxidase (MnP), (ii) lignin peroxidase (LiP) and (iii) Laccase (Lac) [11]. The LiP is used to degrade xenobiotics compounds from wastewater by converting it into CO_2 and H_2O, while the MnP is the main enzyme that participates in lignin depolymerization, which helps in effluent discoloration and the biobleaching of pulp [10].

Some studies show that the performances of immobilized fungi are better than the free fungi [12]. The various immobilization matrices have been used to immobilize fungi, for example: the use of alginate, κ-carrageenan, polyvinyl alcohol (PVA), etc. PVA–alginate–sulfate beads offer several advantages over other immobilization matrices. A study shows that the enzymes immobilized in PVA indicates a higher activity, thermal stability even higher possibility to be reused [12]; thus, it increases the biosynthetic processes in terms of economic value. In this present research, an attempt has been made to immobilize *P. chrysosporium* in PVA–alginate-sulfate beads. Based on a previous study [13], it is hoped that this kind of immobilization matrix will be able to preserve the viability of *P. chrysosporium* and maximize the rate of discoloration [12].

The effect of different nitrogen concentration on the growth of *P. chrysosporium* was also studied. The amount of nitrogen that is required for the fungal growth was investigated by varying nitrogen amount within a certain range, while the concentration of carbon source is kept constant. This will enable the determination of the best ratio of carbon and nitrogen in optimizing the composition of culture medium.

2. Materials and methods

2.1. Chemicals

The PVA 60,000 MW and boric acid are purchased from the Merck Schuchardt OHG, Darmstadt, Germany. Meanwhile, the sodium alginate is obtained from the FlukaChemie GmbH, Buchs; the sodium sulfate is from the GCE Laboratory Chemicals, Malaysia and the calcium chloride is from the R&M Marketing, Essex, UK. The textile effluents are obtained from Razali Batik Kota Bharu, Kelantan, Malaysia.

2.2. Microorganisms and inoculum preparation

The *P. chrysosporium* ATCC24725 is obtained from the Faculty of Chemical Engineering, Universiti Teknologi Malaysia (UTM), Malaysia. The fungi suspension is grown in potato dextrose agar at 37°C for 6 d. The hemocytometer-light microscope is used to determine the existence of spore's concentration in the suspension.

2.3. Immobilization of P. chrysosporium

The inoculum suspension (10 mL) is mixed with 12% w/v of PVA and 1% w/v sodium alginate solution. The mixture is dropped into a solution mixture which contains 5% w/v boric acid and 2% w/v calcium chloride solution to form a spherical beads and it is stirred for 30–50 min. The beads which are formed were kept at 4°C in the sterilized distilled water for 24 h. Then, the beads are stirred for 30 min in 10% w/v boric acid solution before it being replaced with 0.5 M sodium sulfate solution and again, it is stirred for another 30 min [14].

2.4. Germination of immobilized fungal spores

The malt extract medium (2%) is used to germinate growth of immobilized spores as a starting culture. The beads are incubated at 37°C until the growing biomass enters the idiophasic growth. After 4 d of the incubation, the growth medium will be replaced aseptically with the textile effluents and nitrogen limited medium (1.5% glucose, 0.04% w/v malt extract, 174 μM $MnSO_4.H_2O$, 0.0004% w/v $MgSO_4.7H_2O$, 20 mM 3,3-dimethylglutrate) [15,16].

2.5. Scanning electron microscopy (SEM) analysis

The immobilized cells are dried by using the filter paper and they are cut out to obtain the cross-section of the beads. Samples are coated by using the platinum coating with the Auto Fine Coater JFC-1600 (Jeol, USA Inc., USA) before they are been observed through the scanning electron microscope (Model JSM-6390) [17].

2.6. Experimental design

A fractional 2^4 factorial design is performed to determine the significant factors that influenced the ability of immobilized *P. chrysosporium* to treat the textile effluents. Table 1 summarizes these factors and shows their respective levels. The production of ligninolytic and Lac enzymes is screened in order to study the ability of immobilized cells to treat the

Table 1
Factors and levels used in the 2^4 fractional factorial design study

Factors	Levels	
	(–)	(+)
A – pH	7	11
B – Temperature (°C)	34	37
C – Nitrogen concentration (mM)	0	22
D – Beads loading (g)	1	10

textile effluents. The interaction between the enzymes and the discoloration is also been studied. The ranges selected are based on the literature review and the statistical calculations (*t* tests, *F* tests, analysis of variance (ANOVA) and multiple regressions). They are performed using the software packages of Design Expert 6.0.4.

2.7. Analytical methods

2.7.1. American Dye Manufacturer's Institute (ADMI) removal

The percentage of ADMI removal is measured by using HACH DR/4000U spectrophotometer. The discoloration rate is calculated as follows:

$$\% \text{ of discoloration} = A_0 - A_t/A_0 \quad (1)$$

where A_0 is initial absorbance and A_t is absorbance at time '*t*'.

2.7.2. Manganese peroxidase activity

The MnP activities are determined by UV/Vis spectrophotometer at 431 nm. The reaction mixtures contain the MnP (1 μg/mL), H_2O_2 (0.01–0.1 mM), $MnSO_4$ (10× concentration H_2O_2) in 50 mM sodium tartrate at pH 4.5 [18].

2.7.3. Lignin peroxidase activity

The LiP activity is determined based on the oxidation of azure B. 1 mL of 125 mM sodium tartarate buffer pH 3, 0.5 mL azure B, 0.5 mL culture filtrate and 0.5 mL hydrogen peroxidase are used. The reaction is initiated by adding the hydrogen peroxidase and one unit of the enzyme activity is expressed as an optical density decrease of units per min per mL of the culture filtrate at 651 nm [19].

2.7.4. Laccase activity

The Lac activity is tested using 2 mM of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 0.1 M sodium citrate buffer (pH 3) and culture filtrate [20]. ABTS oxidation is measured at 420 nm.

2.8. Reusability test

The reactions are initiated by adding 5 g of immobilized cells into the textile effluents and nitrogen limited medium mixture at 37°C, 150 rpm. After each cycle of (24 h), the immobilized beads are drained and rinsed by using distilled water before the ADMI and enzymes activities are evaluated as mentioned.

3. Results and discussion

3.1. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) analysis is carried out to verify the existence of *P. chrysosporium* in the PVA–alginate–sulfate beads. Fig. 1(a) acts as the control with no cells entrapped. Meanwhile, Fig. 1(b) represents the existence of *P. chrysosporium* inside the PVA–alginate–sulfate bead with 10,000× magnification. It can be seen clearly that the matrix successfully immobilizes the fungus.

3.2. ANOVA analysis: interaction between enzymes activities and discoloration rate

In order to ensured the validity of the model suggested, several analysis such as the significance of the regression model, the test of significance on individual model coefficient and the test of lack of fit are evaluated. The value of Prob > 0.05 in Table 2 for the model is less than 0.05, which indicates that the model is significant, which is desirable as it indicates that the terms in the model have a significant effect on the response. In a similar manner, for MnP production (Table 3), nitrogen concentration is the most significant factor with *p*-value less than 0.0001, which follows by the amount of beads loading and pH, which both showed the *p* values of 0.009 and 0.0161, respectively. However, the *p*-value of temperature is 0.1273 and it can be regarded as not significant since $p > 0.050$.

Table 4 shows the *p* values less than 0.0001 for pH, the nitrogen and beads loading show that these factors are highly significant in the production of LiP. Results also show that the interaction between AB which is pH and temperature are not significant exhibiting $p > 0.050$. The insignificant terms can be removed and may result in an

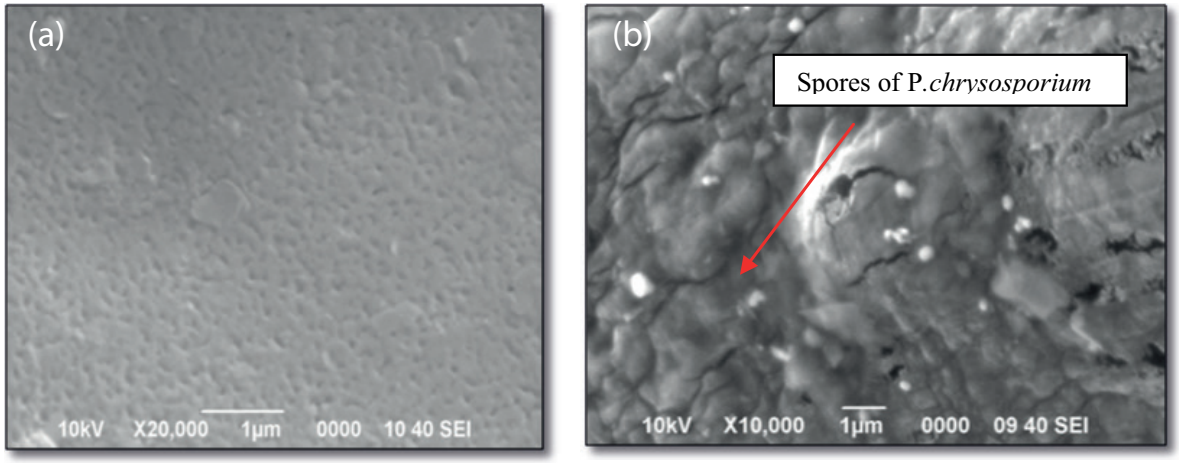

Fig. 1. SEM image: (a) plain beads and (b) beads with entrapped cells.

Table 2
Results of the ligninolytic enzymes, laccase enzyme and discoloration percentage of the fractional 2^4 factorial designs for the textile wastewater treatment

Run	A	B	C	D	MnP (U/mg)	MnP(p)[a] (U/mg)	LiP (U/mg)	LiP(p)[a] (U/mg)	Lac (U/mg)	Lac(p)[a] (U/mg)	Discoloration (%)	Discoloration(p)[a] (%)
1	–1	–1	–1	–1	0.18	0.18	0.27	0.30	0.29	0.28	0.45	0.45
2	1	–1	–1	1	0.27	0.10	0.33	0.10	0.20	0.17	0.59	0.53
3	–1	1	–1	1	0.25	0.09	0.45	0.15	0.27	0.08	0.66	0.47
4	1	1	–1	–1	0.34	0.23	0.28	0.20	0.32	0.21	1.01	0.67
5	–1	–1	1	1	0.11	0.11	0.29	0.32	0.21	0.21	0.52	0.52
6	1	–1	1	–1	0.10	0.27	0.12	0.32	0.17	0.20	0.53	0.59
7	–1	1	1	–1	0.09	0.26	0.18	0.42	0.08	0.27	0.47	0.66
8	1	1	1	1	0.17	0.16	0.15	0.17	0.15	0.15	0.50	0.50

[a]MnP(p), LiP(p), Lac(p) and discoloration(p) represent the responses results predicted from Eqs. (1), (2), (3) and (4), respectively.

Table 3
Analysis of variance for manganese peroxidase

Manganese peroxidase					
Source	Sum of squares	Degrees of freedom	Mean square	*F* value	*p*-Value (Prob > *F*)
Model	0.11	5	0.021	23.99	<0.0001
A – pH	6.208E–3	1	6.208E–3	7.05	0.0161
B – Temperature	2.251E–3	1	2.251E–3	2.56	0.1273
C – Nitrogen concentration	0.083	1	0.083	94.21	<0.0001
D – Beads loading	0.014	1	0.014	15.64	0.0009
AB	4.475E–4	1	4.475E–4	0.51	0.4850
Residual	0.016	18	8.804E–4	–	–
Lack of fit	2.666E–4	2	1.333E–4	0.14	0.8731
Pure error	0.016	16	9.738E–4	–	–
Corr. total	0.12	23	–	–	–

improved model. As for the Lac production, the nitrogen concentration and the combination of pH and amount of beads loading are highly significant (Table 5). Table 6 shows the ANOVA for the discoloration percentage. The results show that the significant factors for the discoloration rate are: the nitrogen concentration, the combination factor of pH and amount of beads loading which each factors showed *p* values less than 0.05. The equations of the model are generated based on the regression analysis which is provided as follows:

$$\text{MnP} = -0.55636 + 0.059141 \times \text{pH} - 5.34427\text{E}{-003} \times \text{Nitrogen conc} + 5.32214\text{E}{-003} \times \text{Beads loading} \quad (2)$$

$$\text{LiP} = 0.38559 + 0.013597 \times \text{pH} - 5.61867\text{E}{-003} \times \text{Nitrogen conc} + 0.027460 \times \text{Beads loading} - 8.47268\text{E}{-004} \times \text{pH} \times \text{Temperature} \quad (3)$$

$$\text{Lac} = 2.72952 - 0.22845 \times \text{pH} - 0.068875 \times \text{Temperature} - 0.012916 \times \text{Nitrogen conc} + 0.022697 \times \text{Beads loading} + 6.25747\text{E}{-003} \times \text{pH} \times \text{Temperature} + 9.76316\text{E}{-004} \times \text{pH} \times \text{Nitrogen conc} - 2.23078\text{E}{-003} \times \text{pH} \times \text{Beads loading} \quad (4)$$

$$\text{DP} = -1.93014 + 0.21149 \times \text{pH} + 0.058458 \times \text{Temperature} + 1.75472\text{E}{-003} \times \text{Nitrogen conc} + 0.049779 \times \text{Beads loading} - 6.47364\text{E}{-004} \times \text{pH} \times \text{Nitrogen conc} - 5.13542\text{E}{-003} \times \text{pH} \times \text{Beads loading} \quad (5)$$

In determining the significance of the suggested model, the conformational runs are done by comparing the predicted responses with the experimental responses from the adjusted R^2 and predicted R^2 values. Table 6 shows that the R^2 values of the MnP, LiP, Lac and the discoloration rate and it is proved that this model is sufficient to assess the response of the discoloration and enzymes' activity.

Table 4
Analysis of variance for lignin peroxidase

Lignin peroxidase					
Source	Sum of squares	Degrees of freedom	Mean square	*F* value	*p*-Value (Prob > *F*)
Model	0.25	5	0.014	22.24	<0.0001
A – pH	0.061	1	0.061	26.93	<0.0001
B – Temperature	–	–	–	–	–
C – Nitrogen concentration	0.092	1	0.092	40.20	<0.0001
D – Beads loading	0.083	1	0.083	36.25	<0.0001
AB	0.013	1	0.013	5.63	0.0289
AD	4.988E–3	1	4.988E–3	2.19	<0.1564
Residual	0.041	18	2.281E–3		–
Lack of fit	1.135E–3	2	5.675E–4	0.23	0.7991
					Not significant
Pure error	0.040	16	2.495E–4	–	–
Corr. total	0.29	23	–	–	–

Table 5
Analysis of variance for laccase

Laccase					
Source	Sum of squares	Degrees of freedom	Mean square	*F* value	*p*-Value (Prob > *F*)
Model	0.096	7	0.014	22.49	<0.0001
A – pH	5.898E–3	1	5.898E–3	9.63	0.0068
B – Temperature	8.515E–3	1	8.515E–3	13.90	0.0018
C – Nitrogen concentration	0.050	1	0.050	80.81	<0.0001
D – Beads loading	3.337E–3	1	3.337E–3	5.45	0.0330
AB	8.458E–3	1	4.599E–3	13.80	0.0019
AC	0.011	1	0.011	18.07	0.0006
AD	9.674E–3	1	6.127E–3	15.79	<0.0011
Pure error	9.803E–3	16	6.127E–4	–	–
Corr. total	0.11	23	–	–	–

3.3. Analysis of the main effects and interaction plots LiP, MnP, Lac and discoloration

Based on the obtained results (Table 2), the interaction between number of beads loading, pH, the nitrogen concentration and the temperature can affect the secretion of the ligninolytic enzymes and the rate of discoloration. For example, the enzymes secretion by LiP enzymes is optimum when the number of beads loading and pH are set at the higher level. In the mean time, the enzymes secretions are tend to be higher when the temperature and nitrogen concentration are set at the lowest level.

Additionally, the Lac productions are affected by the nitrogen concentration and the interaction between the nitrogen concentration and pH. There is some very slight indication of inequality of the variance shown by the small decreasing funnel shape in the plot of residuals vs. predicted.

On the other hand, the amount of beads which are loaded will affect the discoloration percentage. As the loaded amount of beads is higher, the concentration of cells will be higher to produce more enzymes to degrade the dyes. However, it is not true because other conditions may affect the viability of cells. In this experiment, a small amount of beads at high pH can increase the discoloration percentage. In contrast, the high amount of beads can maximize the discoloration percentage at lower pH.

3.4. Comparison between immobilized and free cells

3.4.1. Rate of dye discoloration

Fig. 2 represents the rate of dye removal by the immobilized and free cells during the 7 d incubation period. The discoloration rate in the immobilized *P. chrysosporium*

increases gradually to 10% during the initial 20 h of the experiment because all the associated enzymes are produced at a slower rate. During the next 50 h period, a further increase in the discoloration was observed due to the presence of the associated enzymes. Between 72 and 112 h, a noticeable reduction in the discoloration rate was observed which coincide with an increase in the activity of all the three enzymes. Subsequent increase in discoloration rate was only observed around 40 h after. A sharp increase in the discoloration rate was observed with a percentage of 20.9% at 120 h.

In comparison, the highest discoloration by free cells is only 7.5% at 72 h. After this period, the discoloration rates by free cells tend to decrease. It is noticed that the maximum discoloration percentage for the immobilized fungus is up to 18.64%, which is around 2.5 times larger than the free cells. Lower discoloration percentage in case of free cells could be due to denaturation of enzymes by the surrounding bulk liquid. The discolorations by the immobilized beads continue to increase steadily due to the protection provided by the immobilization matrix to the cells and enzymes from the surrounding settings.

3.4.2. Laccase activity

Fig. 3 represents the Lac activity of the immobilized and free cells. The Lac activity in the free cells system is higher on the first day, but drops drastically due to the occurrence of spores' germination. On day two, the spores allow the vegetative growth but at a slower rate due to inhibition caused by excessive nitrogen that was present in the environment. Once the nitrogen is completely consumed on day three, the extracellular enzyme was released in large quantity. Consequently, the Lac activity raised immediately to its peak (0.0646 U/mg), higher than that of the immobilized system on day five. However, it dropped slightly during the consecutive days. Previous study also shows that the free cells system give maximum enzyme activity during earlier days of experiment and then they decrease rapidly afterward compared with that of the immobilized cells [21].

For the immobilized system, it was found that the Lac activity was slightly lower on the first day but increases gradually on day two. Nevertheless, it starts to produce spores and the Lac activity reduced to approximately 0.0033 U/mg. The vegetative growth allows the mycelium and hyphal to

Table 6
Analysis of variance for discoloration percentage

Discoloration percentage					
Source	Sum of squares	Degrees of freedom	Mean square	*F* value	*p*-Value (Prob > *F*)
Model	0.15	7	0.021	20.17	<0.0001
A – pH	0.015	1	0.015	14.20	0.0017
B – Temperature	0.015	1	0.015	15.06	0.0013
C – Nitrogen concentration	0.048	1	0.048	46.85	<0.0001
D – Beads loading	6.159E–3	1	6.159E–3	5.99	0.0263
AB	4.599E–3	1	4.599E–3	4.47	0.0504
AC	4.868E–3	1	4.868E–3	4.74	0.0448
AD	0.051	1	0.051	49.89	<0.0001
Pure error	0.016	16	1.028E–3	–	–
Corr. total	0.16	23	–	–	–

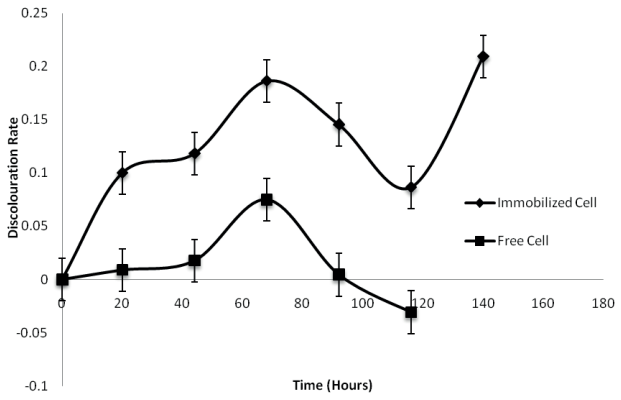

Fig. 2. Discoloration rate by immobilized and free cells systems.

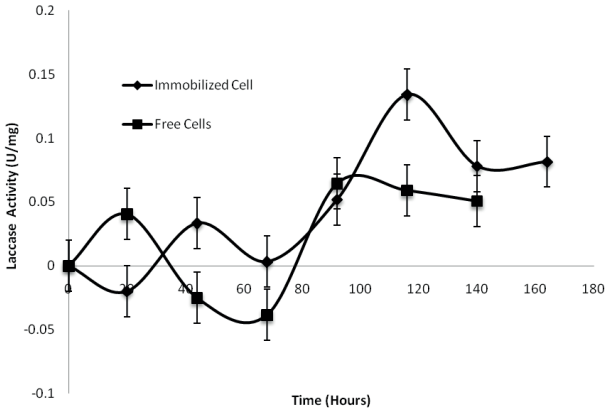

Fig. 3. Lac activity of immobilized and free cells systems.

grow on the following day as the enzyme was produced. The enzyme activity reaches its highest peak at 0.134 U/mg on day five. However, it has drastically decreased to 0.078 U/mg and then the enzyme activity increased again on the final day of the experiment to 0.0816 U/mg.

3.4.3. Lignin peroxidase activity

Fig. 4 represents the comparison of LiP activity between immobilized and free cells. LiP activities in both of the immobilized and free cell systems showed a sharp increase on the first day, with the immobilized cells recorded enzyme activity about 1.25-fold higher than the free cells. Higher LiP activity in the immobilization system could be due to faster nutrients intake rate as the immobilized cells are much more stable. However, it is gradually increased on the day two and drops to 0.225 U/mg due to the spores' germination. Later, it produces mycelium and starts to secrete LiP. Thus, secreted enzymes reach the highest peak of activity at 0.303 U/mg on day five. On the contrary, the LiP activity in free cells was stagnant after the second day.

3.4.4. Manganese peroxidase activity

Fig. 5 shows that MnP activity in the immobilized system increases steadily during first 50 h period. However, on day two, it undergoes the first generation of spores germination leading to enzymes activity drop to 0.023 U/mg on day three. According to Xiong et al. [22], 2 d are needed for the nitrogen to be completely depleted. Depletion of nitrogen could stimulate MnP activity as evidenced by an increase in MnP activity on the following day and achieved its highest activity on day five at 0.088 U/mg. For the free cells, the trend of the MnP activity is found to be similar to the Lac activity.

Previous study shows that the ligninolytic enzyme systems are mainly activated during the secondary metabolic phase of the fungus and are often triggered by nitrogen depletion [22]. With less nitrogen in the medium, lesser time is required to deplete the nitrogen. The enzymes will be secreted during this time to yield the highest enzyme activity.

However, according to Gao et al. [24], the MnP cannot be produced in the absence of nitrogen. This is because the mycelium pellet will not be produced in a nitrogen-free environment due to the absence of inducer for the mycelium production. The Lac production is also found to be similar to MnP. If the nitrogen concentrations are excessive, the highest value of Lac and MnP activity can be measured during the exhaustion of nitrogen [23]. On the other hand, the LiP will not be produced in this case, except under the carbon limitation.

In short, the immobilized *P. chrysosporium* increases the discoloration percentage by approximately 2.8-fold compares to the free cell system. As for the production of LiP, Lac and MnP, the enzymes activity are up to 1.4-, 2- and 2.1-fold, respectively, in comparison to that of the free cells system.

3.5. Reusability test

The screened condition identified previously is used for each cycle in the textile effluent discoloration reusability tests. Fig. 6 shows the discoloration results of reusability test that

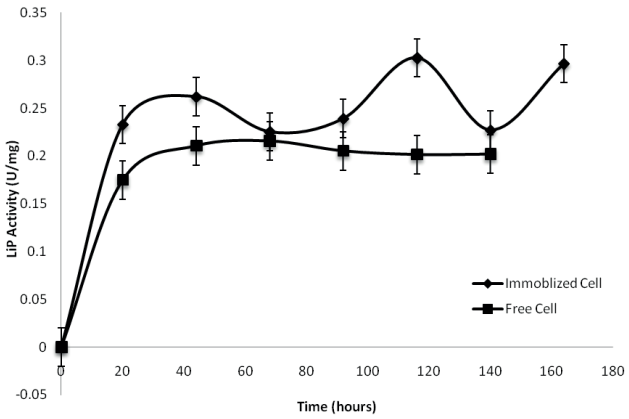

Fig. 4. LiP activity of immobilized and free cells systems.

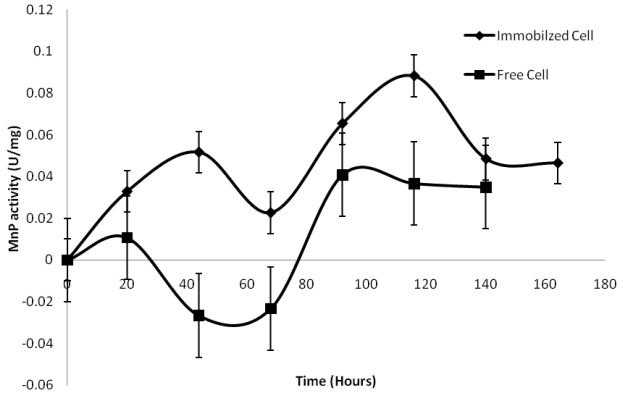

Fig. 5. MnP activity of immobilized and free cells systems.

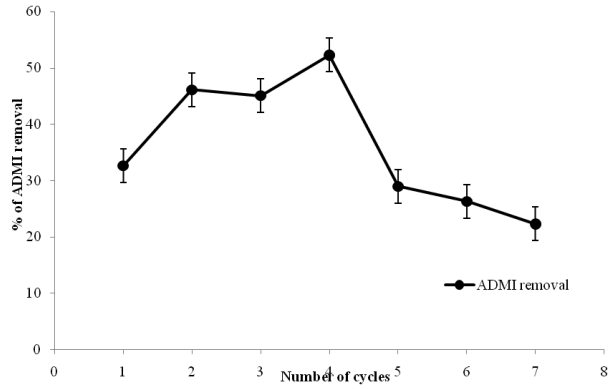

Fig. 6. Number of bead cycles.

was performed for every 24 h. Apparently, the immobilized cells can be reused for up to seventh cycle with maximum discoloration of 52.29% on the fourth cycles. The previous studies [24,25] have shown that the PVA–alginate gels possess excellent reusability properties; where they can be recycled for up to seven times. The compression test results show that the PVA beads are more elastic, rubbery, porous and spongy [24]. The PVA–alginate beads are formed by cross-linking of

the borate ions $B(OH)_4^-$ with the alcohol groups on adjacent chains [26], thus providing a sparse but rather flexible and uniform bead matrix which allows for good mass transfer.

4. Conclusion

Investigation of significant factors affecting discoloration of textile wastewater has been performed in this study. ANOVA reveals that discoloration is correlated to the production of MnP, LiP and Lac enzymes. These are the enzymes that constitute ligninolytic enzymes that are involved in textile wastewater discoloration. Such correlation suggests their role in degrading molecules that are present in textile wastewater leading to discoloration. Peak activity of each enzyme is found to occur at the same time (at t = 120 h) with Lac and MnP show greater fluctuation compared with LiP. An increase in discoloration percentage of up to 20.9% for immobilized cells was also observed. This could be attributed to the advantage of immobilization as discussed earlier. Another advantage of immobilized cells is that it could be reused for up to four times before a marked decrease in discoloration was observed.

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References

[1] V. Faraco, C. Pezella, G. Giardina, A. Piscitelli, S. Vanhullec, G. Sanniaa, P. Pezella, Decolourization of textile dyes by the white-rot *Phanerochaete chrysosporium* and *Pleurotus ostreatus*, J. Chem. Technol. Biotechnol., 84 (2009) 414–419.
[2] K.C. Chen, J.Y. Wu, J. Liou, S.C. Hwang, Decolorization of the textile dyes by newly isolated bacterial strains, J. Biotechnol., 101 (2003) 57–68.
[3] T. Robinson, G. McMullan, P. Nigam, Remediation of dyes in textile effluent: a critical review on current treatment technologies with a propose alternative, Bioresour. Technol., 77 (2001) 247–255.
[4] H. Zollinger, Colour Chemistry: Syntheses, Properties and Applications of Organic Dyes and Pigments, 3rd ed., Wiley VCH, New York, 2003.
[5] T. Leisinger, A.M. Cook, R. Hutter, J. Nuesh, Microbial Degradation of Xenobiotic and Recalcitrant Compounds, 12th FEMS Symposium, London, 1981, pp. 371–385.
[6] E. Forgacs, T. Cserháti, G. Oros, Removal of synthetic dyes from wastewater: a review, Environ. Int., 30 (2004) 953–971.
[7] A.A. Pourbabaee, F. Malekzadeh, M.N. Sarbolouki, F. Najafi, Aerobic decolorization and detoxification of a disperse dye in textile effluent by a new isolate of *Bacillus* sp., Biotechnol. Bioeng., 93 (2006) 631–635.
[8] U. Shedbalkar, R. Dhanve, J. Jadhav, Biodegradation of triphenylmethane dye cotton blue by *Penicillium ochrochloron* MTCC 517, J. Hazard. Mater., 157 (2008) 472–479.
[9] P. Xu, G.M. Zeng, D.L. Huang, C. Lai, M.H. Zhao, Z. Wei, N.J. Li, C. Huang, G.X. Xie, Adsorption of Pb(II) by iron oxide nanoparticles immobilized *Phanerochaete chrysosporium*: equilibrium, kinetic and mechanisms analysis, Chem. Eng. J., 203 (2012) 423–431.
[10] G. Mcmullan, C. Meehan, A. Conneely, N. Kirby, T. Robinson, P. Nigam, Mini review: microbial decolourisation and degradation of textile dyes, Appl. Microbiol. Biotechnol., 56 (2001) 81–87.
[11] M.S.M Annuar, S. Adnan, S. Vikineswary, Y. Chisti, Kinetics and energetics of azo dye decolorization by *Pycnoporus sanguineus*, Water Air Soil Pollut., 202 (2009) 179–188.
[12] A. Idris, M.S. Suhaimi, N.A.M. Zain, R. Rashid, N. Othman, Discoloration of aqueous textile dyes solution by *Phanerochaete chrysosporium* immobilized in modified PVA matrix, Desal. Wat. Treat., 52 (2014) 6694–6702.
[13] D.K. Sahoo, R.C. Gupta, Evaluation of ligninolytic microorganism for efficient decolourization of a small pulp and paper mill effluent, Process Biochem., 40 (2005) 1573–1578.
[14] P. Remmersand, K.D. Vorlop, Production of Highly Elastic Polyvinyl Alcohol Biocatalyst Beads and Their Use for Continuous Denitrification, Proc. DECHEMA Biotechnology Conference 5, VCH, Weinheim, 1992, pp. 939–942.
[15] K.M. Khoo, Y.P. Ting, Biosorption of gold by immobilized fungal biomass, Biochem. Eng. J., 8 (2001) 51–59.
[16] N.A.M. Zain, M.S. Suhaimi, A. Idris, Hydrolysis of liquid pineapple waste by invertase immobilized in PVA–alginate matrix, Biochem. Eng. J., 50 (2010) 83–89.
[17] D.S. Arora, P.K. Gill, Comparison of two assay procedures for lignin peroxidase, Enzyme Microb. Technol., 28 (2001) 602–605.
[18] [18] A. Paszczyński, V.-B. Huynh, R. Crawford, Enzymatic activities of an extracellular, manganese-dependent peroxidase from Phanerochaete chrysosporium, FEMS Microbiol. Lett., 29 (1985) 37–41.
[19] H. Wariishi, K. Vallis, M.H. Gold, Manganese(II) oxidation by manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium*. Kinetic mechanism and role of chelators, J. Biol. Chem., 267 (1992) 23688–23695.
[20] G. Palmieri, P. Giardina, C. Bianco, A. Scaloni, A. Capasso, G. Sannia, A novel white laccase from *Pleurotus ostreatus*, J. Biol. Chem., 272 (1997) 31301–31307.
[21] N.A.M. Zain, M.S. Suhaimi, A. Idris, Development and modification of PVA–alginate as a suitable immobilization matrix, Process Biochem., 46 (2011) 2122–2129.
[22] X. Xiong, X. Wen, Y. Bai, Y. Qian, Effects of culture conditions on ligninolytic enzymes and protease production by *Phanerochaete chrysosporium* in air, J. Environ. Sci., 20 (2008) 94–100.
[23] A. Kunamneni, S. Camarero, C. García-Burgos, F.J. Plou, A Ballesteros, M. Alcalde, Engineering and application of fungal laccase for organic synthesis, Microb. Cell. Fact., 7 (2008) 32.
[24] D.W. Gao, X.H. Wen, Y. Qian, Effect of nitrogen concentration in culture mediums on growth production of *Phanerochaete chrysosporium*, J. Environ. Sci., 17 (2005) 190–193.
[25] A. Idris, E. Misran, N. Hassan, A.J. Aishah, E.S. Chan, Modified PVA-alginate encapsulated photocatalyst ferro photo gels for Cr(VI) reduction, J. Hazard. Mater., 227–228 (2012) 309–316.
[26] A. Idris, N.A.M. Zain, M.S. Suhaimi, Immobilization of Baker's yeast invertase in PVA–alginate matrix using innovative immobilization technique, Process Biochem., 43 (2008) 331–338.