Jurnal Teknologi

Full paper

Textile Effluent Discoloration by Immobilized *Phanerochaete Chrysosporium* into PVA-Alginate-Sulfate Beads

Nor Atikah Husna Ahmad Nasir[b], Nor Fadhilatul Shilla Mohd Asri[b], Nor Azimah Mohd Zain[a*], Mohd Suardi Suhaimi[b], Ani Idris[b]

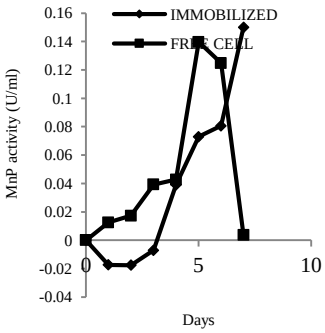
[a]*Department of Industrial Biotechnology, Faculty of Biosciences and Bioengineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*
[b]*Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

*Corresponding author: azimah@fbb.utm.my

Article history

Received :19 November 2012
Received in revised form :
8 January 2013
Accepted :18 January 2013

Graphical abstract



Abstract

This paper presents preliminary research on immobilized *Phanerochaete chrysosporium* in PVA-alginate-sulfate beads to discolor textile effluents. It is an alternative technique from the current physico-chemicals. The main focus of this study was to determine the colour removal, Chemical oxygen deman (COD) removal and manganese peroxidase activity of the immobilized *P.crysosporium*. Immobilized *P.crysosporium* also confers advantages such as reusability and improved cell performance. Scanning electron microscope (SEM) was also performed to characterize the immobilization matrix. The immobilized results were compared with that of free cells. Immobilized cells were able to discolor 47.14% compared to free cells which recorded 10.78% colour removal. The COD removal of immobilized cell is more than 60% as compared to that of free cells, which could only reduced 30% of COD. Finally, the manganase peroxidase activities showed a slight difference between the immobilized and free cell at 0.15U/L and 0.13U/L respectively.

Keywords: PVA-alginate-sulfate beads; *Phanerochaete chrysosporium*; COD; MnP; SEM

Abstrak

Kertas kerja ini membentangkan kajian awal *Phanerochaete chrysosporium* yang di sekat gerak ke dalam manik PVA-alginat-sulfat untuk melunturkan warna sisa tekstil. Ia adalah teknik alternatif terhadap teknik fizik dan kimia yang sedia ada. Tujuan utama kajian ini adalah untuk menentukan tahap penyingkiran warna, penyingkiran COD dan aktiviti enzim manganase peroxsidase oleh *P.chrysosporium.* yand di sekat gerak. *P.chrysosporium* yang disekat gerak mempunyai kelebihan kerana boleh diguna pakai berulang kali serta meningkatkan prestasi sel. Selain itu, ujian SEM juga dijalankan bagi tujuan mengenal pasti ciri matrik PVA-alginate-sulfate. Hasil kajian akhirnya dibandingkan dengan sel bebas bagi memastikan keberkesananya. Sel yang disekat gerak berjaya melunturkan warna sehingga 47.14% manakala hanya 10.78% dilunturkan oleh sel bebas. Tambahan itu, nilai COD yang berjaya disingkirkan oleh sel sekat gerak melebihi 60 % sedangkan sel bebas hanya mampu mengurangkan COD kurang daripada 30%. Manakala, perbezaan aktiviti enzim manganase peroxidase yang sedikit antara sel yang disekat gerak dan sel bebas iaitu 0.15U/L dan 0.13U/L masing-masing.

Kata kunci: PVA-alginate-sulfate beads; *Phanerochaete chrysosporium*; COD; MnP; SEM

1.0 INTRODUCTION

In textile industries and bleaching purposes, dyes stuffs are most appropriate and widely used as they are cheaper and easy to use. Almost 10,000 different dyes and pigment are produced annually worldwide with a volume of more than 7×10^5 ton.[1-2] Unfortunately, data show that 10% of dye effluents are found in water.[3] Worst, some of the dye effluents removal could seep through the aquifer and finally pollute the underground water. Improper discharge to water without prior treatment would negatively affects sunlight penetration, retards the photosynthesis process and interferes gas solubility in water.[4] This could affect the balance of aquatic ecosystems. Thus, there are many dye treatment processes suggested such as chlorination, ozone treatment, flocculation, precipitation and ion-pair extraction .[5] However, these techniques produce large amounts of sludge and most of them are expensive.[6-7] In order to overcome these limitations, biodegradation by using microorganism is gaining popularity.[5] This is because treatments by using microorganisms is cheaper and

environmentally friendly compared to existing treatment methods.

The aim of this experiment is to develop a simple technique to discolor dye effluents by using immobilized *Phanerochaete chrysosporium* into PVA –alginate-sulfate beads. *P. chrysosporium*,is one of the best microorganisms that is widely studied in textile effluent discoloration. This white rot fungus is able to produce enzymes with a low substrate specificity that increases their capability to degrade different groups of textile dyes.[8- 9] In addition, *P. chrysosporium* is known to degrade wide range of recalcitrant xenobiotic compounds, including azo dyes that are commonly used in textile industry.[10-13]

Immobilization gives better operational stability and higher efficiency.[14-15] Recently, cell immobilization by using polyvinyl alcohol (PVA) has sparked interest of many researchers.[16] PVA offers unique properties such as high stability, non-toxic to organism and can be produced cheaply at industrial scale.[17] Thus, the idea of immobilizing the potent *P.chrysosporium* in PVA-alginate-sulfate beads would be an interesting supposition.

■2.0 EXPERIMENTAL

2.1 Materials

Polyvinyl alcohol (PVA) 60,000 MW and boric acid were purchased from Merck Schuchardt OHG, Darmstadt, Germany. Sodium alginate was obtained FlukaChemie GmbH, Buchs, sodium sulfate from GCE Laboratory Chemicals and calcium chloride from R&M Marketing, Essex, UK. *Phaneorachate chrysosporium* ATCC24725 suspension was obtained from Faculty of Chemical Engineering, Universiti Teknologi Malaysia. The commercial textile used in this experiment consists of different dyes which are Yellow FG, Turqoise Blue, Red 3BS, Blue RSP and Black B, courtesy of Razali Batik Kota Bharu, Kelantan. All dyes were mixed together to 30 ppm of final concentration.

2.2 Inoculum Preparation

The fungi were inoculated on Potato Dextrose Agar (PDA) (OXOID Ltd. England) and incubated at 37ºC until the intensive mycelia grow occurred. Fungal biomass was harvested by using 1% (v/v) Tween 80 solution after 7 days of incubation. Later on, spores counts were carried out to determine spore concentration in the suspension by using haemocytometer-light microscope.

2.3 Immobilization of *P. Chrysosporium*

Spore suspension (10ml) was mixed with 90 ml of PVA (12% w/v) and sodium alginate (1% w/v) solution. The mixed solution was then dropped into a mixed solution of boric acid (5% v/v) and calcium chloride (2% w/v) by using syringe in order to form beads and stirred for 30-50 minutes. Later on, the beads were store for 24 h at 4ºC in sterilize distilled water. After the incubation period, the beads were stirred in 10% v/v boric acid solution for 30 minutes and then with 0.5 M sodium sulfate solution for another 30 minutes. The beads were kept at 4ºC until further used.[18]

2.4 Germination of Immobilized Fungal Spores for Discolorisation Study

Growth medium containing 2% w/v malt extract was used to germinate the immobilized fungal spores to be used as the starting culture. The immobilized spores were incubated at 37ºC until the growing biomass in the alginate entered idiophasic growth.[19] The germinated spores were drained and used later to discolor the samples.

After four days of incubation, the growth medium was replaced aseptically with the dye solution and nitrogen limited medium that contains 1.5% w/v glucose, 0.04% w/v malt extract, 174 µM $MnSO_4$ H_2O, 0.0004% w/v $MgSO_4$ $7H_2O$, 20mM 3, 3-dimethylglutrate.[19-20]. Samples were incubated at 38ºC and 100 rpm and sampling was performed every 24 hours to access the discoloration percentage, COD and manganase peroxidase activity. The experiment was carried out in three replications to ensure its reproducibility.

Additionally, a flask (without *P.chrysosporium*) was prepared as a control.

2.4 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was carried out to determine the presence of *P.chrysosporium* inside the PVA-alginate-sulfate beads. PVA-alginate-sulfate beads were dried by using a filter paper and cut by using a scalpel to obtain the cross section of the beads. Then, the bead was placed on a stand and the cross section image was obtained by using SEM (Model JSM-6390).[18]Then, sample was coated for four times by using Platinum coating with Auto-Fine Coater JFC-1600(Joel, USA Inc, USA).

2.5 Percentage of Dyes Discoloration

Percentage of dye was measure by using HACH DR/4000U spectrophotometer .Then the rate of discoloration was calculated using equation 1[5]:

$$\text{Percentage of discoloration} = \frac{(A_o - A_t)}{A_o} \times 100 \quad (1)$$

where A_0 = initial absorbance
A_t = absorbance at time '*t*'

2.6 COD Removal

Chemical Oxygen Demand of each dye was observed by monitoring changes in its spectrum (nm) by using HACH DR/4000U spectrophotometer. The rates of COD removal were calculated using equation 2:

$$\text{Percentage of COD removal} = \frac{(A_o - A_t)}{A_o} \times 100 \quad (2)$$

where
A_0 = initial absorbance
A_t = absorbance at time '*t*'

2.7 Manganese Peroxidase Activity Assay

Based on Bermegyer 1974,[21] 0.53 g of KH_2PO_4 and 1.06 g K_2HPO_4 was dissolved in 100 ml of distilled water and the pH was determined to be 7.0. Then, 22.3mg of guaiacol was dissolved in 10 ml of distilled water. It was stored in ice and prepared fresh daily. 0.1 ml of 30% hydrogen peroxide will be prepared fresh daily by diluting with distilled water to 120 ml. Then, diluents were kept in ice at 4ºC.

Reaction volume containing 0.1M Potassium Phosphate Buffer (2.8 ml), 0.018 M guaiacol (0.05 ml), 30% hydrogen peroxide (0.05 ml) and sample (0.1 ml) were pipetted into a 1 cm cuvette. The rates of increase in absorbance at 436 nm wavelength were recorded after the initial lag phase. The rate of enzyme activity will be calculated based on equation 3.

$$(U/ml) = \frac{DA_{436/min} \times 4 \times V_t \times \text{dilution factor}}{A_o(e \times V_s)} \quad (3)$$

Volume activity (U/ml):

V_t =final volume of reaction mixture (ml) = 3.00

V_s =sample volume (ml) = 0.1

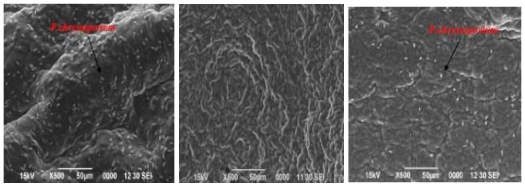
e =micromolar extinction co-afficient of tetraguaicol (cm^2/micromol) =25.5

4 =derives from unit definition and principle

3.0 RESULTS AND DISCUSSION

3.1 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) analysis was carried out in this experiment in order to observe the entrapment of spores in the PVA-alginate-sulfate matrix. This is important to confirm the spores are in the immobilization matrix. The SEM micrographs of immobilized *P. chrysosporium* are presented in Figure 1(a) and Figure 1(b). Figure 1(a) shows the surface or external part of the PVA-alginate-sulfate with immobilized *P. chrysosporium.* Figure 1(b) shows the cross section or internal part of PVA-alginate-sulfate with immobilized *P. chrysosporium*. Figure 1(c) shows the control beads without fungus. These figures conclude that the beads were successfully immobilized with the fungus. The SEM figures reveal that that the fungus appear more on the surface compared to the inside of the beads. This may be due to the restriction of space inside the beads compared to the beads surface.



(a) (b) (c)

Figure 1 SEM image of PVA-alginate-sulfate matrix. a) external image b) cross section image c) control beads

3.2 Percentage of Discoloration

Table 1 represents the characterization of 30mg/L of synthetic dyes before and after treatment by using immobilized and free cells. Three parameters were used to determine the effectiveness of the method; American Dyes Manufacturing Index (ADMI) removal, COD removal and MnP activity. The ADMI and COD values obtained from the untreated dyes are 600 and 3955 mg/L, respectively. After treatment using the immobilized fungus, the ADMI removal and COD removal are 47.04% and 63.49%, respectively. Meanwhile, the MnP activity for the immobilized fungus is 0.15 U/mL. In contrast with immobilized cells, the dye treated with free cells showed a low ADMI removal, COD removal and MnP activity, of 10.78%, 29.25% and 0.1397 U/mL, respectively. The results showed that the immobilized cells exhibited higher ADMI and COD removal compared to free cells. In addition, the immobilization system also increased the MnP activity for the fungus.

Table 1 Characteristic of dye after treatment by immobilized and free cells

Parameter	Treated by Immobilized cells	Treated by Free cells
%ADMI removal	47.04	10.78
%COD removal	63.49	29.25
MnP activity (U/mL)	0.15	0.1397

The discoloration pattern of mixed dye within seven days of incubation is shown in Figure 2. It is observed that the immobilized cells are able to discolor more efficiently compared to free cells. The highest rate of discoloration is 47.04% which was observed on the seventh day. This shows that the discoloration percentage by immobilized cells is approximately 36% higher than the free cells. On the sixth day of the experiment, the ADMI removal for immobilized cells is increased steadily compared to that of free cells. This may be due to the sudden drop of MnP activity in free cells on fifth day of the experiment (Figure 3).

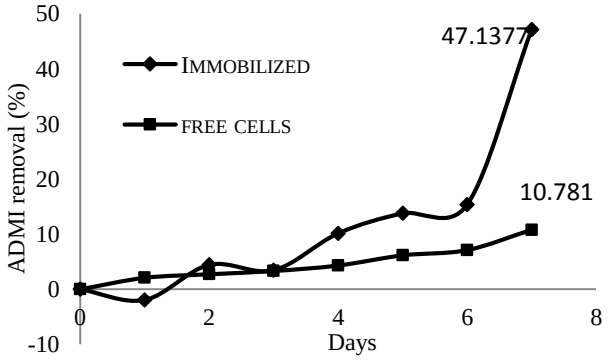
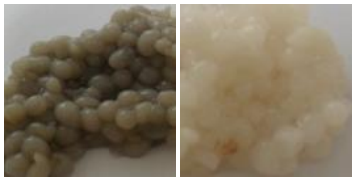


Figure 2 Percentage of dye discoloration by immobilized beads

Free cells discoloration might occur through biodegradation process. This is due to the cell extracellular enzymes such as manganase peroxidase, lignin peroxidase and laccase secreted by fungus.[22] In addition, discoloration by using PVA-alginate-sulfate beads might occur through bio-absorption and bio-degradation process. This is explained in Figure 2 since the rate of discoloration by immobilized cell is higher than free cell. In this state, PVA-alginate-sulfate beads acts both as a support and absorbent for the dye adsorption and this is proven by the increasing adsorption of immobilized beads.[23]

Results in Figure 3 show that the beads are colored at first and became colorless on the 14th day of experiment. Based on the observation, the beads became colored after two hours of immersion in the dye solution while the MnP activity was detected after the 1st day of the experiment (Figure 4). This observation concluded that the immobilized beads absorb the dyes and degrade it later. Comparing this result with the control experiment (beads without fungus), apparently biodegradation is the dominant mechanism in dye discoloration. The predominance of biodegradation in dye discoloration was also recorded by Mielgo and co-workers[24] where 98% of discoloration was attributed to biodegradation.



(a) (b)

Figure 3 (a) Beads were colored during treatment (b) beads become colorless at the end of treatment

3.3 COD Removal Determination

Table 2 represents COD removal by immobilized and free cells in 30mg/L mixed dyes. COD signifies the quantity of oxygen required to oxidize the organic pollutants present in liquid sample especially waste water. The quantity of oxidant consumed is expressed in terms of its oxygen equivalent.[25-26] The reduction in COD value indicates a corresponding reduction in the concentration of pollutants.[27] Immobilized cells successfully removed 63.49% of COD, which is 34.24% higher better than free cells. Wijetunga *et al*. 2008,[27] mentioned that azo dyes or organic substituents produced from their degradation are elements that could contribute to high COD reading. Thus, immobilized beads, can be useful tool to remove COD value.

Table 2 COD removal by free cell and immobilized beads

Dye color	Cell form	% of COD removed
Mixed	Immobilized	63.49
	Free cell	29.25

3.4 Manganese Peroxidase Assay

Figure 4 represents the manganese peroxidase (MnP) activity of immobilized and free cells. By comparing the enzyme activity, results show MnP activity for immobilized cell is 0.15 U/mL while in free cell is 0.14 U/mL. Although the difference is marginal, results proved that the immobilized cells are able to give better enzyme activity compared to free cells. PVA – alginate-sulfate beads might protect the mycelia from any potential inhibitors and mechanical stress thus contributes to a slightly higher enzyme activity. Figure 4 also reveals that the MnP activity for the free cells fluctuates compared to that of the immobilized cells steadily increases until the end of the experiment. This may be due to the protected environment in the immobilization system that stabilized the MnP activity.[28]

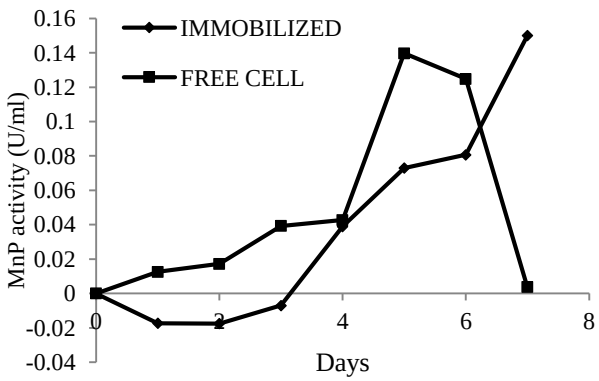


Figure 4 Manganase peroxidase activity within seven days

This observation suggests an involvement of MnP in the dye discoloration via biodegradation. Furthermore, no MnP could be detected in the control sample. MnP has long been associated with the ability of *P.chrysosporium* to degrade several organic pollutants .[20] Urek and Pazarlioglu (2007),[20] have studied in vitro discoloration of several dyes using the purified MnP from *P.chrysosporium* with a promising outcome. It was postulated that the biodegradation of such compounds occurs via Mn-mediated oxidation that generate Mn^{3+} chelated with organic acids produced by fungal cells.[29-31] The resulting Mn^{3+}organic acids complex later oxidize organic compounds which in this case are the dye molecules.

4.0 CONCLUSION

The findings of the study conclude that *P.chrysosporium* can be successfully immobilized into PVA-alginate-sulfate beads. Apparently, immobilization has to some extent improved the cells ability in the discoloration of textile effluents. This is because immobilization cells are able to discolor 47.04% compared to free cells which can achieve only 10.78%. It is believed that MnP is secreted by *P. chrysosporium* which is responsible for dye discoloration since it showed positive results. Decreasing rate of COD represents the ability of immobilized cells to degrade and discolor dyes. Finally, SEM analysis shows that *P. chrysosporium* spores has been entrapped within the immobilization systems in order to make it functional.

Acknowledgement

We would like to thank the Ministry of Higher Education for the financial support of this research through FRGS funding vote no.78678.

References

[1] Meyer, U. 1981. Biodegradation of Synthetic Organic Colorants. Microbial Degradation of Xenobiotics and Recalcitrant Compound, FEMS symposium 12, ed. T. Leisinger, A. M. Cook, R. Hutter & J. Nuesch. London: Academic Press. 371–285.

[2] Zollinger H. 1987. Properties and Application of Organic Dyes and Pigments. *Colour Chemistry*. New York: VCH Publisher. 92–100.

[3] Deveci, T., Unyayar, A. and Mazmanci, M. A. 2004. Production of Remazol Brilliant Blue R Decolourising Oxygenase from the Culture Filtrate of *Funalia Trogii* ATCC 200800. *Journal of Molecular Catalysis B: Enzymatic.* 30(1)**:** 25–32.

[4] Banat, F. A. and Simandl, J. 1996. Removal of Benzene Traces from Contaminated Water by Vacuum Membrane Distillation. *Chemical Engineering Science.* 51(8)**:** 1257–1265.

[5] Sani, R. K., Azmi, W., Banerjee, U. C. 1998. Comparison of Static and Shake Culture in the Decolorization of Textile Dyes and Dyes Effluents by *Phanerochaete Chrysosporium. Folia Microbial.* 43(1): 85–88.

[6] Cooper, P. 1993. Removing Color from Dye House Wastewater. *Journal of the Society of Dyers and Colourists*. 109(3): 97–100.

[7] Stephen, J. A. 1995. Electro-oxidation of Dyestuffs in Waste Water. *J.Chem. Technol. Biotechnol.* 62(2): 111–117.

[8] Cripps, C., Bumpus, J. A., Aust ,S. D. 1990. Biodegradation of Azo and Heterocyclic Dyes by *Phanerochaete Chrysosporium. Appl Environment. Microb*. 56(4): 1114–1118.

[9] Ollikka, P., Alhanmaki, K. Leppanen, V-M, Glumoff, T., Raijola, T. Souminen, I. 1993. Decolorization of Azo, Triphenyl Methane, Heterocyclic and Polymeric Dyes by Lignin Peroxidase Isoenzymes from *Phanerochaete Chrysosporium. Appl. Environ. Microbial.* 59(12): 4010–4016.

[10] Tien, M. and Kirk, T. K. 1983. Lignin-degrading Enzyme from the Hymemocyete *Phanerochaete Chrysosporium*. *Science.* 221(4611): 661–663.
[11] Vasdev, K., Kuhad, R. C., Saxena, R. I. 1995. Decolorization of Triphenylmethane Dyes by the Bird's Nest Fungus *Cyathus Bulleri*. *Current Microbiol.* 30(5): 269–272.
[12] Haemmerli, S. D., Leisola, M. S. A., Sanglard, D., Fietcher, A. 1986. Oxidation of Benzo(*a*) Pyrene by Extracellular Ligninases of *Phanerochaete Chrysosporium. J. Biol. Chem*. 261(15): 6900–6903.
[13] Cameron, T. P., Hughes, T. I., Kirby, P. E, Fung, V. A., Dunkei, V. C. 1987. Mutagenic Activity of 27 Dyes and Related Chemicals in the *Salmonella* Microsome and Mouse Lymphoma T K Assay. *Mutant. Res*. 189(3): 223–261.
[14] Linko, S., Haapala, R. 1996. Progress in Biotechnology. In: Wijffels RH, Buitellar RM, Bucke C, Tramper J, editors. Immobilized cells: Basic and Applications, Amsterdam, The Netherlands: Elsevier. 40-53
[15] Ramakrishnan, S. V., Jamuna, R., Emery, A. N. 1992. Production of Ethanol by Immobilized Yeast Cells. *Appl Biochem Biotechnol*. 37: 275–282.
[16] Wang Jianlong, Hou Wenhua, Qian Yi. 1995. Immobilization of Microbial Cells Using Polyvinyl Alcohol (PVA)-Polyacrylamide Gels. *Biotechnology Techniques*. 9(3) :203–208.
[17] Szczesna-Antczak, M. and Galas, E. 2001. *Bacillus Subtilis* Cells Immobilized in PVA-cryogels. *Biomolecular Engineering*. 17(2): 55–63.
[18] Idris, A., Mohd Zain, N. A., Suhaimi, M. S. 2008. Immobilization of Baker's Yeast Invertase in PVA-alginate Matrix Using Innovative Immobilization Technique. *Process Biochemistry*. 43. 331–338.
[19] Knapp, J. S., Newby, P. S. and Reece, L. P. 1995. Decolorization of Dyes by Wood-Rotting Basidiomycete Fungi. *Enzyme and Microbial Technology*. 17: 664–668.
[20] Urek, R. O. and Pazarlioglu, N. K. 2007. Enhanced Production of Manganese Peroxidase by *Phanerochaete chrysosporium*. *Brazilian Archives of Biology and Technology*. 50: 913–920.
[21] Bergmeyer, H. U. 1974. Methods of Enzymatic Analysis. 2nd ed. New York: Academic Press. 457–458.
[22] Shah, V. and Nerud, F. 2002. Lignin Degradation System of White-Rot Fungi and Its Exploitation for Dye Decolorization. *Can. J. Microbiol*. 48(10): 857–870.
[23] Wang Bao-e and H. U. Yong-you. 2007. Comparison of Four Support for Adsorption of Reactive Dyes by Immobilized *Aspergilus fumigates* Beads. *Journal of Environmental Sciences*. 19(4): 451–457.
[24] Mielgo, I., Moreira, M. T., Feijoo, G., Lema, J. M. 2001. A Packed-Bed Fungal Bioreactor for the Continuous Decolourisation of Azo-dyes (Orange II). *Journal of Biotechnology*. 89: 99–106.
[25] Rump, H. H. 1999. Laboratory Manual for the Examination of Water, Waste Water and Soil. New York: Wiley. 800.
[26] Hofrichter, M. 2002. Review: Lignin Conversion by Manganase peroxidase (MnP). Enzyme and Microbial Technology. 30(4): 454–466.
[27] Wijetunga Somasiri, Li Xiufen, Wenquan Ruan and Jian Chen. 2008. Colour and COD Removal, Reactor Performance, and Stability in Textile Waste Treatment by Up Flow Anaerobic Sludge Blanket Reactor At Mesophilic Temperature. *Electronic Journal of Environment, Agriculture and Food Chemistry*. 7(10): 3461–3475.
[28] H'ela, Z., Marc, L., Sami, S. 2002. Degradation of 4-chlorophenol by the White Rot Fungus *Phanerochaete chrysosporium* in Free and Immobilized Cultures. *Bioresour Technol*. 84: 145–150.
[29] Glenn, J. K., Akileswaran, L., Gold, M. H. Mn (II) Oxidation is the Principal Function of the Extracellular Mn-peroxidase from *Phanerochaete chrysosporium*. *Archieves of Biochemistry and Biophysics.* 198: 251(2): 688–696.
[30] Gold, M. H. and Wariishi, H. 1989. Lignin Peroxidase Compound III Formation, Inactivation, and Conversion to the Native Enzyme. 243(2): 165–168.
[31] Wariishi, H., Vallis, K., and Michael, H. Gold. 1992. Manganese (I1) Oxidation by Manganese Peroxidase from the Basidiomycete *Phanerochaete Chrysosporium* Kinetic Mechanism and Role of Chelators. *The Journal of Biological Chemistry*. 267(33): 23688–23695.