THE APPLICATION OF FUNGUS, P.OSTREATUS IN THE REMOVAL OF DANGEROUS SUBSTANCES

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ABSTRACT: Fungus, *Pleurotus ostreatus* was used in this present study to remove dangerous substance including heavy metals and naphthalene which commonly exist in both water and soil. These chemicals should be removed due to their toxicity characteristics. However the conventional removal methods have some limitations where there is a need to introduce alternative method biologically. The removal of naphthalene from soil and heavy metal ions from chemical waste had proved the capabilities of *Pleurotus ostreatus* in the removal of dangerous chemical at higher efficiency. Furthermore, almost Fe(II) 17.02%, Cu(II) 46.6%, Pb(II) 76% and Zn(II) 17.88% of Zn(II) are successfully being removed from chemical waste under suitable treatment conditions. In the removal of naphthalene from the soil, 84% of it being degraded and further enhanced with additional of oil palm fibre (OPF) as nutrient for *Pleurotus ostreatus* to 98% within 22 days, respectively. This validates that a *Pleurotus ostreatus* is a good biosorbent agent in the removal of dangerous chemicals.

Keywords: *Pleurotus ostreatus*, biodegradation, biosorption, naphthalene, heavy metal ions

I. INTRODUCTION

Due to the increasing industrial activities to meets the demand of human need; contamination of dangerous chemicals either in water or land is common problem faces by human. The water and soil contamination by the dangerous chemicals such as naphthalene and heavy metal ions should be tailored by researcher because it can cause harms to the environment and human health. In an extreme case, it can bring hazards to human health. Therefore, these dangerous chemicals should be removed at all extent [1].

However, the available technologies in the removal of these chemicals usually involve unreasonable cost and there are some limitations including ineffective removal involving lower concentration of contaminants and lower removal efficiency. The introduction of biological method utilizing fungus in the removal of these chemicals had attracted many researchers due to their several advantages [2]. Previous studies proved that the biosorption technique is have more advantageous compared to chemical and physical processes due to its lower operating cost, higher efficiency removal even in dilute effluent, steady performance and the potential recovery of valuable metals and harmless secondary product [3].

Now, the researchers are interested to go towards an application of largely available quantity of microorganism named fungus in the removal of these dangerous chemicals. Among them, *Pleurotus ostreatus (P.ostreatus)* is the most suitable choices because of its low cost and high availability [4]. Previous studies have shown the capabilities of *P.ostreatus* in removal of various chemicals from different sources of waste either from electroplating or tannery effluents [5]; [6]; [7] and [8]. It was believed that the addition of oil palm fibre (OPF) enhanced biosorption capacity of fungus. The aim of the present study is to investigate the capabilities of *P.ostreatus* in the removal of dangerous substances from soil and chemical waste solution by varying their operating conditions.

II. MATERIAL AND METHODS

a) Characteristics study of *P.ostreatus*

P.ostreotus was cultured in Potato Dextrose Agar (PDA) before being transferred in 100 mL malt extract

and agitated in an oscillary shaker (MAX-Q 200; Barnstead Lab-Line, USA) at 125 rpm and 12 for 13 days to establish stationary growth phase. The resulting samples were filtered and dried in oven at approximately 10 for 24 hours and was used to determine the rate of mycelium growth as opposed to the bioremoval rate.

b) Sample Preparation

Sample were taken at 2-day intervals, whereby a 0.5 g soil sample was placed in microwave digester and combined with 17.5 mL of n-hexene (Merck, Malaysia) and 7.5 mL of acetone (Merck, Malaysia). The extraction of naphthalene from soil samples was performed at 120ºC for 20 minutes using a pressurized microwave extraction system (Multiwave 3000, Anton Paar, Austria).

c) Naphthalene Concentration Determination

The relative concentration of naphthalene present in the soil samples was determined using a high performance liquid chromatography (HPLC) Perkin Elmer Series 200(USA) with a 150×3.2-mm Brown-lee 4µm PAH reversed column(USA) combined with an ultraviolet-visible spectrometer scanning at hmax = 254 nm, which is the optimum wavelength for naphthalene detection. The mobile phase was 60:40 (v/v) acetonitrile/water (HPLC grade; Merck, Malaysia) flowing at 0.5 mL/min.

d) Bioremoval Study of Naphthalene from Soils

Soil purchased from nursery in Shah Alam, Malaysia was sieved to obtain a particle size of less than 2 mm and sterilized using an autoclave at 121ºC for 20 minutes prior to being spiked with naphthalene in accordance with the method described by Bishnoi, Kumar and Bishnoi (2008)[9]. The soil pH prior to spiking was determined to be 7.36. Soil sample (20 g) was individually spiked with naphthalene to a concentration of 2000 mg/kg using acetone as carrier solvent in 250 mL Erlenmeyer flasks. The soil water content of the samples prior to bioremoval was determined to be 60% (w/w) at the beginning of the experiments accordance with the work of Antizardislao, Lopez-Real and Beck (2009) and maintained as such throughout the duration of the bioremoval through the addition of deionized water as and when required [10]. The flask was agitated at 125 rpm and 25ºC for a period of 3 days to ensure the complete vaporization of the volatile acetone. Triplicate batches of the synthesized contaminated soil samples were considered in the degradation studies, namely, (i) control-spiked soil; (ii) spiked soil with fungus; (iii) spiked soil with both fungus and OPF, thereby enabling evaluation of the effect of fungus and OPF addition on the biodegradation kinetics. With respect to the third set of triplicates, 0.1 g of OPF, obtained from a palm oil mill located in Labu-Nilai, Malaysia which had been finely powdered using a blander to promote homogenous mixing, was added and mixed with the soil. For characterization purpose, the OPF underwent elemental analysis using a Flash EA 1112 ThermoFinnigan Elemental Analyzer (USA) and both *P.ostreatus* was subjected to thermogravimetric analysis (TGA) using TA Instrument Q-500(USA).

e) Bioremoval Studies of Heavy Metals

First batch experiments were conducted using synthetic heavy metals solutions where the effects of pH, temperature and stirring speed were evaluated. The heavy metals removal uptake was performed in 250 mL Erlenmeyer flask containing 100 mL desired concentration of heavy metals solution at 25°C in the incubator shaker at 150 rpm. The effect of pH was studied by varying pH between 2 to 6 using 1 M HCl and 1 M NaOH. The pH was adjusted at the beginning of experiment and not being controlled throughout the study. About 6 g of living *P. ostreatus* (approximately 100 pellets) with average size between 4 to 5 mm were added into 100 mL synthetic metal ions solution.

The effect of contact time between *P.ostreatus* and metal ion solution were studied by collecting about 5 mL of sample after 1 hour while other parameters were kept constant. The experiments were repeated at different stirring speeds (50, 100, 150, 200 rpm) and temperatures (20, 25, 30 and 35°C). Each experiment was followed by centrifugation and filtration through Whatman filter paper No.1. The residual filtrates were placed in incubator at 20°C before being analyzed using AAS. After suitable operating treatment conditions (pH, temperature and agitation speed) were identified from previous batch studies, the bioremoval study was repeated using liquid laboratory chemical waste. The amount Cu (II), Fe (II), Zn (II) and Pb (II) ions absorbed by each gram of *P.ostreatus* and the efficiency of biosorption (E) were calculated using following equation:

$$\text{Biosorption efficiency, E} = \frac{c_i - c_f}{c_i} \times 100\% \quad \text{(I.1)}$$

Where *Ci* is the initial concentration (mg/L); *Cf* is the final concentration (mg/L).

III. RESULTS AND DISCUSSION

a) Characteristics studies of *Pleurotus ostreatus*

Fig. III. 1 presents the growth of *P.ostreatus* mycelium which exhibits a typical S-shaped growth curved [11]. In this study, the mycelium exhibits minimal growth for the first 4 days, followed by 6 days of exponential growth after day 10, growth asymptotes and no further growth is observed. Mycelium harvesting is thus performed at day 10 once maximum mass is attained. The maximum growth rates for the first 4 days and the exponential phase are 0.18 and 0.58 g/L.day, respectively, and are of importance with respect to bulk fungus production and identifying when the mycelium mass is maximized and its suitable for cultivation and

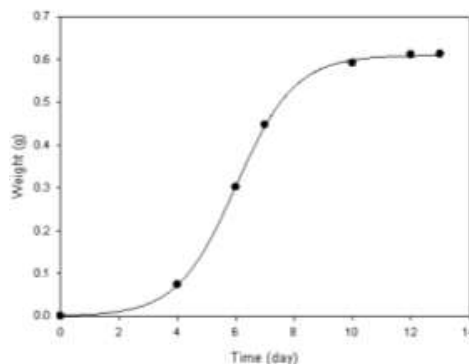consequent use in the biodegradation of naphthalene-contaminated soil.

Figure.III.1: Growing phase of P.ostreatus

Elemental analysis of *P.osteatus* shows 41.3% carbon, 0.7% hydrogen and 5.1% nitrogen as shown in Table III.1 which is significant in that carbon is a micronutrient typically found in large portions in organism cells [10].

Elements	C	H	N	S
% of dry weight	40.96	7.37	4.00	0.97

Table III.1: Compositions of P.ostreatus on CHN-S Elemental Analyzer

Dried mycelium (20 mg) and OPF (16 mg) samples were heated under nitrogen from 30ºC to 750ºC at a rate of 10ºC/min. the TGA curves for *P.ostreatus* and OPF are presented in Fig. III.2, according to which approximately 10% of the OPF sample's weight is lost as the temperature increases from 75-100ºC, which corresponds to the evaporation of adsorbed of water. Significant structural (cell wall) weight loss occurs at around 110ºC. The fungus initially undergoes a more rapid weight loss with respect to the increasing temperature than the OPF, which may be attributed to direct weight loss. Over the range 100-350 ºC, both of the fungi and OPF lose approximately 60% of their initial mass, which would correspond to the decomposition of carbohydrates.
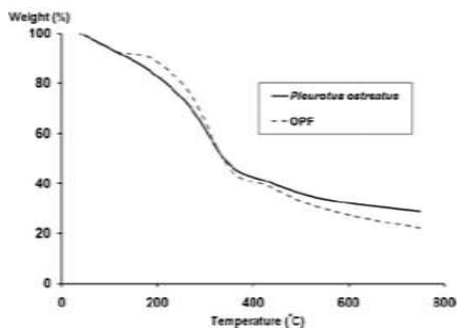
Figure III.2: TGA curves for Pleurotus and OPF.

b) Bioremoval study from soil

Fig. III. 3 shows the naphthalane biodegrades in the absence of the fungus and nutrient (control sample); such biodegradation is termed ―natural attenuation‖ and its most likely due to the influence of chemical, physical and biological process unrelated to the fungus or nutrient such as external biodegradation caused by microbes present in the added deionized water used to maintain the soil moisture content at 60% (w/w) or as a consequences of the natural volatility of naphthalene. Irrespective of this apparent unprompted decreased in naphthalene concentration, the batched inoculated with both *P.ostreatus* and OPF evidently exhibits higher naphthalene biodegradation rates than the control samples. The samples treated with only *P.ostreatus* exhibits initial plateau for about 10 days, after which rapid degradation appears to occur, achieving relative naphthalene percentages of approximately 10% (w/w).
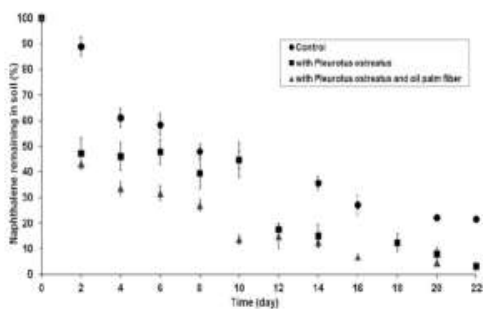

Figure III.3: Bioremoval of naphthalene in soil.

c) Biodegradation kinetics of naphtalene

Assuming the degradation is first order with respect to the naphthalene concentration in accordance with LaGrage, Buckingham and Evans (2001) [13]:

$$\underline{S} = S_0 e^{-kt} \qquad (III.1)$$

Where S is the relative naphthalene percentage, is the initial relative naphthalene percentage, k is the biodegradation rate constant (day^{-1}) and t is the time (day). Plotting the natural logarithm of the relative naphthalene percentage with time yields a straight line of gradient k (Fig. III.4). It is evident that the smallest rate constant value corresponds to that for the control sample, which in comparison with the other determined rate constants indicates promotion of naphthalene biodegradation in the presence of the fungus and OPF.
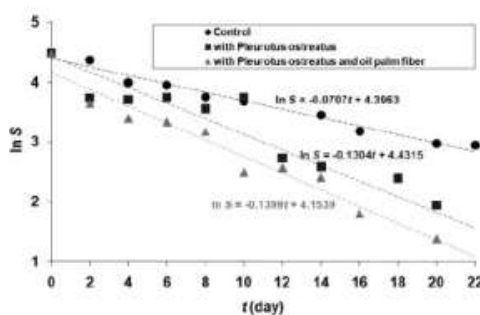

Figure III.4: First order biodegradation kinetics of naphthalene in soil

Regression analysis of the experimental data indicates that there is a greater scatter in the fungus-only samples with respect to the R^2 values but in general the experimental data are well described with respect to first order biodegradation kinetics.

d) Bioremoval study from chemical waste

Biosorption of heavy metals from aqueous solution is affected by many factors. Other than cell surface, the conditions of pH, temperature, stirring speed and *P.ostreatus* dosage can influence biosorption performance. Hence, finding the best operating

conditions is important to achieve maximum biosorption efficiency.

In terms of contact time, Figure III.5 shows the removal of Fe (II), Cu (II), Pb (II) and Zn (II) at different contact time interval. The removal efficiency started from 5 minutes contact time and until 72 hours (600 minutes). It is proved that the biosorption efficiency increased with increasing contact time and larger amount of heavy metal ions was removed within the first 10 minutes. It is apparent that there are two phases of metal ions biosorption which is clearly exhibited. The first phase is the rapid accumulation into the high availability of *P.ostreatus* binding sites which finally occupy within certain contact time and the following phase is much slower when equilibrium achieved.
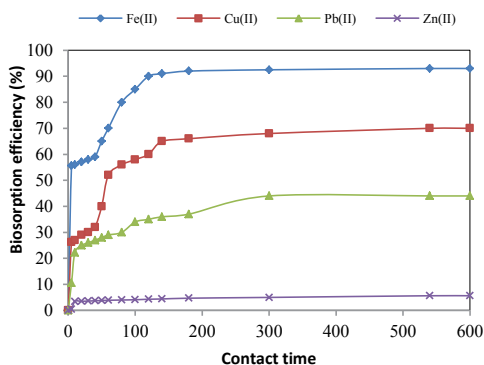

Figure III.5: Effect of contact time

Biosorption of heavy metal ions are also depending on the operating pH which is gradually increased with increasing solution pH. It is due to the fact that pH of contact solution increase the negative charge density on the cell surface due to deprotonation of the cell surface where is there are metal binding sites (functional groups) [7].
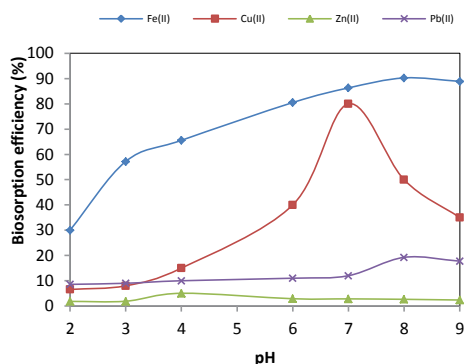

Figure III.6: Effect of pH

To assess the biosorption efficiency of *P.ostreatus* of Fe (II), Cu (II), Pb (II) and Zn (II) as effect of temperature between 20 to 40°C. The relationship of temperature with biosorption efficiency is correlated to the thermodynamics parameters of the biosorption process whether the process depending of temperature is either exothermic or endothermic. Usually, when temperature is changing, thermodynamic parameters also change [18]. Temperature is related to the energy (physical force) hence, the increase temperature can affect the physical forces responsible for sorption. Bhainsa and D'Souza (2008) stated that many reactions including biosorption are to be temperature dependent [6].

Figure III.7 presents the biosorption efficiency of Fe (II) as effect of temperature from 25°C to 40°C. The results show that the biosorption efficiency increased from 20 to 25°C but decreased afterward. It was found that the best operating temperature for biosorption of Fe (II) is at 25°C where the biosorption efficiency is the highest (84.85%). However, the lowest biosorption efficiency is when the temperature is 40°C with 61.89% removal. The boundary layer which surrounds the *P.ostreatus* contains important active site for biosorption process. This layer became an external mass transfer between two phases of biosorbent and heavy metal ions solution.

In order to increase the biosorption rate, appropriate

stirring speed should be introduced to minimize mass transfer resistance. It is shown that the biosorption is increased with increasing agitation speed until suitable speed was achieved which is 150 rpm. About 84.85%, 44.5%, 9.05% and 1.05% of Fe (II), Cu (II), Pb (II) and Zn (II) were removed at 150 rpm. Lowest absorption was found at 50 rpm.
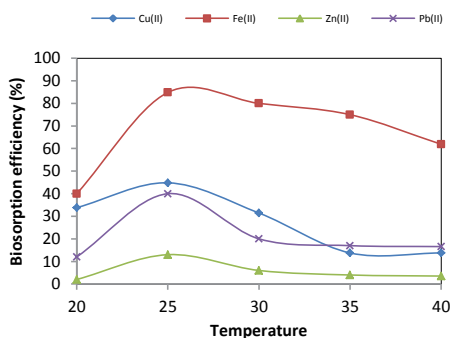

Figure III.7: Effect of temperature

As shown in Fig. III.8, the best stirring speed was at 150 rpm. It was believed that all active binding sites on the biosorbent surface was sufficient enough for metal ions biosorption thus provide more binding. At highest speed, a lowest biosorption was identified which contributed by the vortex phenomena which hard for heavy metal ions to bind on the surface of *P.ostreatus*. In addition, too lower speed the lower dispersion of *P.ostreatus* in aqueous solution reduces the capability between surface and heavy metal ions to contact each other. Not fully dispersed of *P.ostreatus* in the chemical waste led towards agglomeration of particles and it takes more time to reach equilibrium.
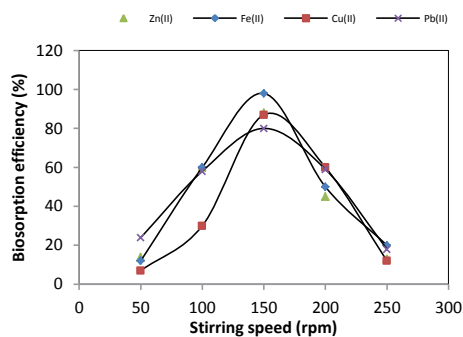

Figure III.8: Effect of Stirring Speed

Figure III.9 shows that Fe (II) had dominated of the competitive binding with 55.35% of removal. It showed the highest biosorption in the multi metal solution in the order of Fe (II)> Cu (II)> Pb (II)> Zn (II). There are no significant changes in comparison with the order of highest biosorption efficiency from previous single system. This can be attributed to the fact that strong driving force of the dominant metal ion which is Fe (II) in the multi metal mixture where competitive uptake occurs. It was proved by the study of Kapoor *et al*., (1999) who had found that the removal of heavy metal ions when present together in solution by fungi *A.niger* were lower compared to present independently [13] and [14].

The good performance of *P.ostreatus* on the biosorption of heavy metal ions solution from single, multi-metal solution indicated the capability of *P.ostreatus*. It is then tested with the chemical waste samples. However, there is slightly difference in the absorption of heavy metal ions from chemical waste but the removal is still within the allowable limits.

It is due to the inherent characteristics of the chemical waste sample. It is expected that the chemical waste also contains contaminants, impurities. It is including light metal ions (e.g. Na, K, Mg (II) and Ca (II) and anions. These impurities will compete with the desired heavy metal ions to be removed. It is believed that the process of the removal of heavy metals is ion exchange where the deprotonated negatively charged groups in *P.ostreatus* biosorbent, monovalent ion (e.g. Na and K) compete with divalent ions (e.g. Cu (II), Fe (II), Pb

(II), Zn (II) to electrostatically attracted during biosorption.

However, it is also depending on the ions where the divalent ions have stronger influences than monovalent ions [15] and [16]. In many instances, the presence of anions such as nitrate, chloride and sulfate decreased metal sorption [16]. The greater the total metal concentration in the chemical waste, the most effective was the competitive metal uptake [17]. It was because of the strong driving force and large difference in concentration between biosorbent surface and metal solution [17]. It is apparent that the highest biosorption of Fe (II) was achieved at 69.60%. The other metal ions show lower biosorption efficiency with 46.46% Cu (II), 36.81% Pb (II) and 17.38% Zn (II).
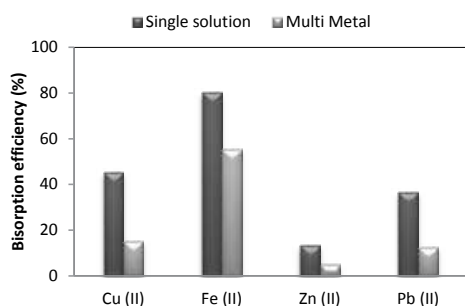

Figure III.9: Comparison single and multiple ions

Parameter	Initial (mg/L)	Final (mg/L)	Percentage removal (%)
pH	0.06	-	-
BOD5	20.22	-	-
COD	15,000	-	-
Fe(II)	282.94	233.76	17
Zn(II)	2.06	1.10	46.6
Zn(II)	2.33	0.56	76
Cu(II)	1.79	1.47	17.88

Table III.2: Final concentration and percentage removal

IV. CONCLUSION

The present study presents the potential of *P.ostreatus* for the biological removal in a batch of dangerous chemicals; naphthalene and heavy metalions. The observation leads to the conclusion that The *P.ostreatus* had successfully removed heavy metal ions from chemical waste especially Fe (II), Cu (II), Pb (II) and Zn (II) by biosorption under specific conditions, even it shows the removal in a lower concentration. However, the *P.ostreatus* show selectively removal and the obvious findings show that the tolerance of heavy metal ions removal is in the order of Fe(II) >Pb(II)> Cu(II)>Zn(II).

In the removal of naphthalene from soil, in the present or absence of OPF enhanced the rate of naphthalene biodegradation in contaminated soils from 80% in the control to 84% and 98% with respect to the degradation rate, respectively. A linear relationship between OPF mass and the biodegradation rate constant: k=0.0249 x m + 0.1291, demonstrated its utility in fungal degradation of naphthalene. The biodegradation kinetics determined in this study may be of interest with respect to scaling up from laboratory scale and also in the tailoring of in and ex situ contaminated soil remendation system.

One of the significant findings to emerge from this study is, *P.ostreatus* show the ability to remove heavy metal ions even at very low concentrations. The pH can affect the metal ions solution behaviour whereas the selection of pH should be the best wherein it is not toxic to *P.ostreatus* which can influence the biosorption process. It was found that the suitable pH for Cu (II) and Fe (II) is pH 6; Pb (II), Zn (II), pH 5 and pH 4 to achieve the maximum biosorption by living *P.ostreatus*. The desired temperature value for biosorption was found to be at 25°C. The increasing temperature from increased the biosorption efficiency and decreased afterwards. The present study opens new vitas management of dangerous chemical contaminated industrial effluent using low cost and environmental friendly biological methods.

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