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Utilization of Pleurotus ostreatus in the Removal of Cr(VI) from Chemical Laboratory Waste

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ABSTRACT : *The presence of Cr(VI) in the chemical waste have the potential to pose significant risks to human health and environments. However, the conventional heavy metal removal have limitations where there are need to introduce alternative treatments. Recently, there have been considerable interests in biosorption of heavy metal using fungus. In the present study, the effectiveness of Cr(VI) removal from chemical waste was evaluated by utilizing living white root fungus (mushroom) viz., Pleurotus ostreatus. Pleurotus ostreatus was best growth at pH 9 and 25°C. The effects of pH, temperature and contact time were evaluated during the treatment. The best operating treatment process was found at pH 5.0 with agitation speed of 150 rpm and temperature of 25°C. Throughout the research, the percentage of removal was found increased with the increasing of contact time between P.ostreatus and liquid laboratory chemical waste. The percentage removal of Cr(VI) at pH 5 is the highest with 20.71% followed by pH 7, 18.89% and pH 9, 18.42%. FTIR analysis proved the involvement of carboxylic (-COOH) and amide (-NH_2) groups on the cell wall of P.ostreatus were known involved in the adsorption process. This validates that Pleurotus ostreatus is a potential biosorbent for laboratory chemical waste treatment.*

Key words - *Biosorption; Chemical Laboratory Waste Treatment; Pleurotus ostreatus; Heavy Metals*

I. INTRODUCTION

The rising interest in research activities has lead to the increasing disposal of chemical waste in the environment. In Malaysia, the Department of Environment (DOE) had notified that that the generation of schedule waste was steeply increased from 1.1 million tonnes to 1.7 million tonnes from 2006 to 2009 [1]. This figure can be expected to be increase with the growing of research activities. Obviously, chemical waste contains heavy metals and classified as hazardous waste which cannot be eliminated by physical and chemical treatment but only stabilized [2]. The difficulty in the treatment of chemical waste was because of their complex compositions as compared to industrial wastewater [3]. The difficulty was contributed by wide variation of chemicals involve, the difference in chemical waste generation and operations which always changing depending on research objectives [4].

The stricter regulation on maximum acceptable concentrations of toxic heavy metals in wastewater discharged into water and drinking waters set up by government had lead researchers to search for suitable treatment methods. Previously, removal of heavy metal from liquid laboratory chemical waste usually achieved through physical chemical process or incineration before discharging into natural body water system [2, 5]. It was including of chemical precipitation [4], electrochemical treatment, reverse osmosis, ion exchange and adsorption on adsorbents [6]. Unfortunately, the available treatments have drawbacks such as generating toxic sludge product from treatment which require another special treatment with great difficulty [7]. Other than that, the removal was ineffectiveness when involving low metals ion concentrations in the range 1-100 mg/L [8] as well as high cost adsorbent.

Therefore, the present study had came out with a novel technologies in developing an alternative treatment process by biological treatment to treat chemical waste in a more environmentally friendly, economical and effective way [9]. Biological treatment utilized natural materials of biological origin such as microbial including bacteria, algae or fungi. Fungi are significantly in reducing the concentration of heavy metals from ppm to ppb level and had emerged as potential treatment methods [8]. Recently, microbial potential have been utilized extensively in wastewater treatment mainly in the removal of metal contaminated effluent [10] and few biomaterials had been recognized as highly potential in chemical waste treatment including coconut coir [11]. *P.ostreatus* is one of the potential biomass for heavy metal removal from synthetics solutions as well as wastewater effluent from electroplating industry [12, 13]. However, *P.ostreatus* is not yet being applied in the removal of heavy metal from liquid laboratory chemical waste. Therefore, the aims of this study

were to evaluate the potential of *P.ostreatus* in the removal of heavy metal from liquid laboratory chemical waste treatment and to study the influences of pH, temperature and contact time on the heavy metals removal.

II. MATERIALS AND METHODS

2.1 Sample and microorganism preparation

Sample of chemical waste was taken from undegraduate student activities in laboratory and was kept in refrigerant at 4°C prior to characterize and treat (APHA, 2005). Microorganism *P.ostreatus* was purchased from C&C Mushroom Cultivated Farm located in southern state of Johor, Malaysia and periodically grows. *P.ostreatus* was cultivate in a Petri dish containing Potato Dextrose Agar (PDA)(Merck) at pH 7 until sporulation for 7 days. Then, the inoculums were prepared by transfering three mycelium agar plugs (8 mm diameter) taken from the edge of white colony PDA into 250 mL Erlenmeyer flask containing 100 mL of malt extract (ME) solution. ME was prepared by adding 20g of ME powder into 1000 mL of distilled water at pH 5. Then, *P.ostreatus* was incubated at 28°C for 15 days and agitated in orbital shaker at 150 rpm. During the course of growing, the activties were done under laminar flow cabinet to prevent external microbial influence. The growth medium and glassware used in the cultivation were sterelized at 121°C and 124kPa for 2 hours in autoclave. Disinfection of the counter space and laboratory surfaces was achieved by wipping with a solution of 70% ethanol to ensure quality of the cultures. Apparatus were exposed under UV light for 15 minutes before every use. After incubation period, *P.ostreatus*'s mycelium was harvested and separated from culture broth by filtration before washed several time with distilled water until it was free from culture broth. The used *P.ostreatus* was sterilized for 30 minutes at 121°C and 124kPa as a post-treatment before sending to respective authorities for disposal.

2.2 Growing study on P.ostreatus

In order to study the effect of pH and temperature on the growth of *P.ostreatus*, the pH of ME broth was adjusted to pH 5, 7 and 9 using 1M HCl and 1M NaOH. The effect of temperature on *P.ostreatus* growth was evaluated between 25, 35 and 45°C. In the growth study of *P.ostreatus*, the observation was done by three phases which are first, fifth and tenth days. The growth study curve was measured by weight of mycelium *P.ostreatus* versus growing day. The growing day was conducted until the growth phase decline.

2.3 Chemical waste characterization

The involvement of functional groups in heavy metal uptake was evaluated by fourier transform infrared spectroscopy (FTIR). Chemical oxygen demand (COD) was measured according to standard method using Hach spectrophotometer DR/2800 in mg/L. Heavy metal concentration in chemical waste was determined using Atomic absorption spectrometer (AAS) (PerkinElmer Analyst 700, USA) with deuterium background corrector. All measurements were carried out in an air/acetylene flame. A 10 cm long slot-burner head, a lamp and an air-acetylene flame were used. Waste sample from Chemistry Laboratory at Faculty of Chemical Engineering was characterized and compared with standard B guideline set up by Department of Environment (DOE) Malaysia (Table 1). The liquid laboratory chemical sample was from the undergraduate student's laboratory experiment known as 'Determination of Chromium'.

Table 1: Characteristics liquid laboratory chemical waste

Test Parameter	Unit	Sample 1	Standard B*
Temperature	°C	28	40
pH		3.03	9
BOD_5 at 20°C	mg/L	20.22	50
COD	mg/L	15, 000	100
Hexavalent Chromium, Cr(VI)	mg/L	2.9	1.00

*Environmental Quality Act, 1974 Environmental Quality Regulations (Sewage and Industrial effluents), 1979

2.4 Batch biosorption study

Heavy metals uptake was performed in 250 mL Erlenmeyer flask containing 100 mL desired concentration of liquid laboratory chemical waste at 25°C and 150 rpm in the incubator shaker. The effect of pH was studies by varying pH 5, 7 and 9 by adding 1M HCl and 1M NaOH which was adjusted at the beginning of experiment and not controlled afterwards. 0.2 g wet weight with average size 4-5 mm of living *P.ostreatus* pallete was added into 100 mL synthetic metal ions solution and shaking at 150 rpm. About 5 mL of sample were collected at definite time intervals (2, 4, 6, 24, 48 and 72 hours) while others parameters (agitation speed: 150 rpm, temperature: 25°C, pH 5) were kept constants. Experiments were repeated with temperatures 25 and

35°C and pH 5, 7 and 9. 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 analyze by using AAS (Model, Varian AA 1275 series). For each of experiment blank, 100 mL of chemical waste without *P.ostreatus* was shaken simultaneously to determine any adsorption of metal onto the wall of flasks and a control of water with 0.2 g mycelium *P.ostreatus* was shaken to determine any leaching of metals from mushrooms.

2.6 Analysis of biosorption efficiency

The amount Cr(VI) ions adsorbed by each gram of biosorbent (q) in mg/g and the efficiency of biosorption (E) in % were calculated using following Equations 1 and 2

$$q = \frac{Ci - Cf}{m} \times V \quad (1)$$

$$E = \left(\frac{Ci - Cf}{Ci}\right) \times 100 \quad (2)$$

Where Ci is the initial concentration (mg/L); Cf is the final concentration (mg/L); m is wet weight of the biosorbent in the aqueous solution (g) and V is volume of aqueous soultion (mL). Experiments were carried out in triplicates and control experiments were also conducted.

III. RESULTS AND DISCUSSION

3.1 Identification of carboxylic and amide group in P.ostreatus

During the studies, the Fourier transform infrared spectroscopy (FTIR) analysis was carried out to identify the involvement of functional groups on *P.ostreatus* cell wall during the removal of Cr(VI) from liquid laboratory chemical waste. Usually, the cell wall of fungal compromise of carboxyl(COOH), phosphate(PO_4), amide(NH_2), thiol(SH) and hydroxide(OH) which are important functional groups during heavy metal ions binding [14]. Figs. 1 and 2 show the FTIR analysis which pointed out the broad adsorption band wavelength of NH (3292.7 to 3337.7) cm^{-1} and COOH (1636.6 to 1630.6) cm^{-1}. Based on the FTIR result obtained, it can be concluded that the NH and COOH groups were involved during Cr(VI) removal. According to Javaid *et al*. (2011), the involvement of –NH group was contributed from chitin and chitosan as the major donor at the *P.ostreatus* cell wall [12].

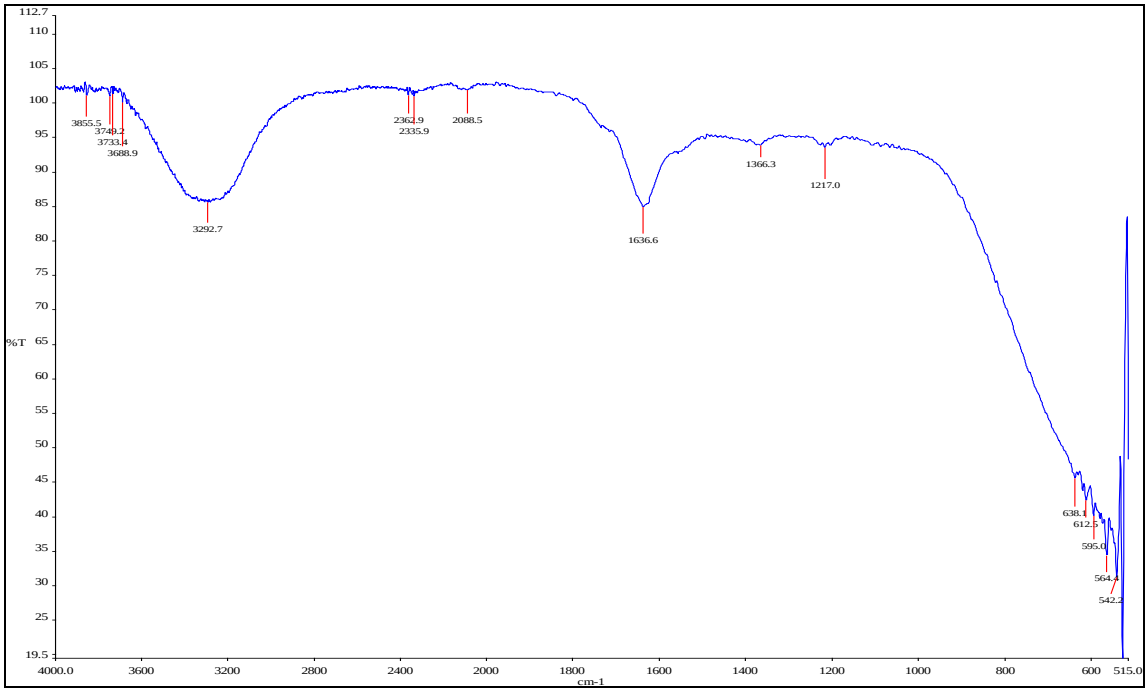


Figure 1: FTIR analysis of Cr(VI) adsorption by *P.ostreatus* before liquid laboratory chemical waste treatment

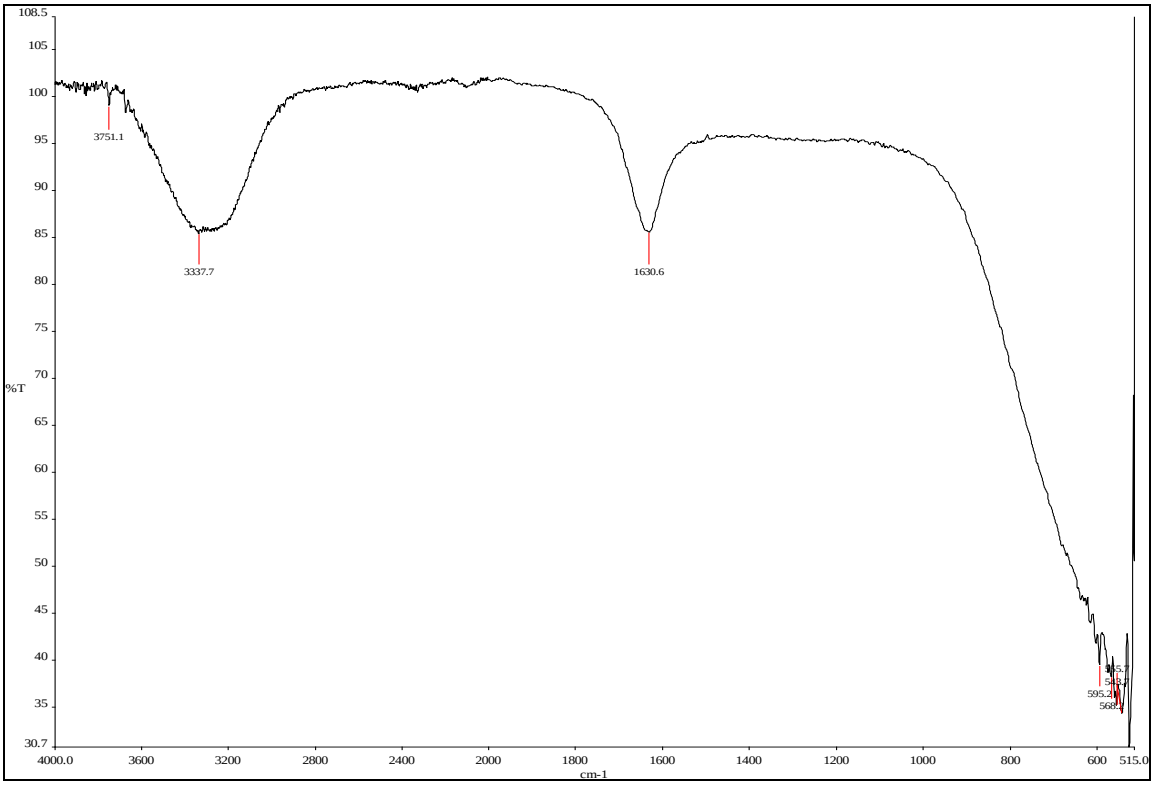
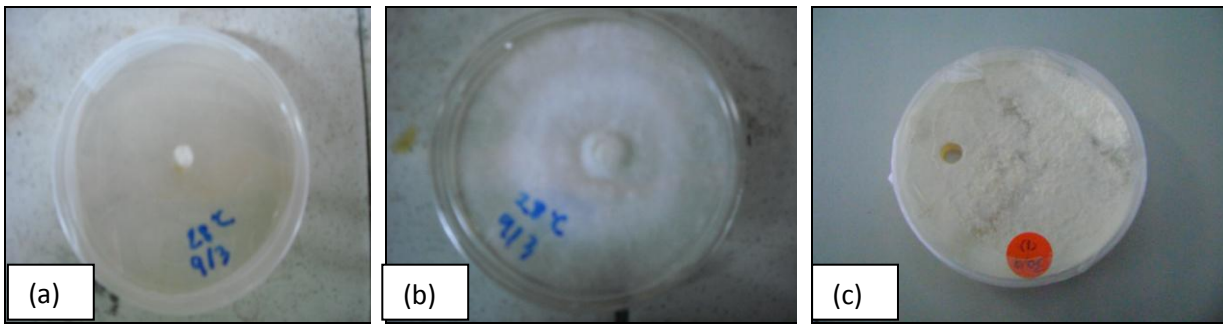


Figure 2: FTIR analysis of Cr(VI) adsorption by *P.ostreatus* after liquid laboratory chemical waste treatment.

3.2 Growth study of P.ostreatus

The pH and temperature plays major roles in the growth of living *P.ostreatus.* A visual examination of the growth culture of *P.ostreatus* on the PDA was shown in Figs. 3. On the first day, *P.ostreatus* was cultivated on PDA at 28°C Fig. 3(a) and after fifth days, white hype *P.ostreatus* was quarterly covered on the PDA Petri dish and is ready to be transferred into 250 mL Erlenmeyer flask for inoculation as shown in the Fig. 3(c).



Figures 3: The cultivation of *P.ostreatus* on PDA agar slant. (a) day-1, (b) day-5, (c) day-10

After 10 days, three mycelium agar plugs from PDA containing *P.ostreatus* culture were transferred into Erlenmeyer flask containing ME medium and was growth for 13 days in the incubator shaker at 150rpm and 25°C (Fig. 4 (a)). *P.ostreatus* was grown in the form of spherical pellets of dull white color (Fig. 4(b)).

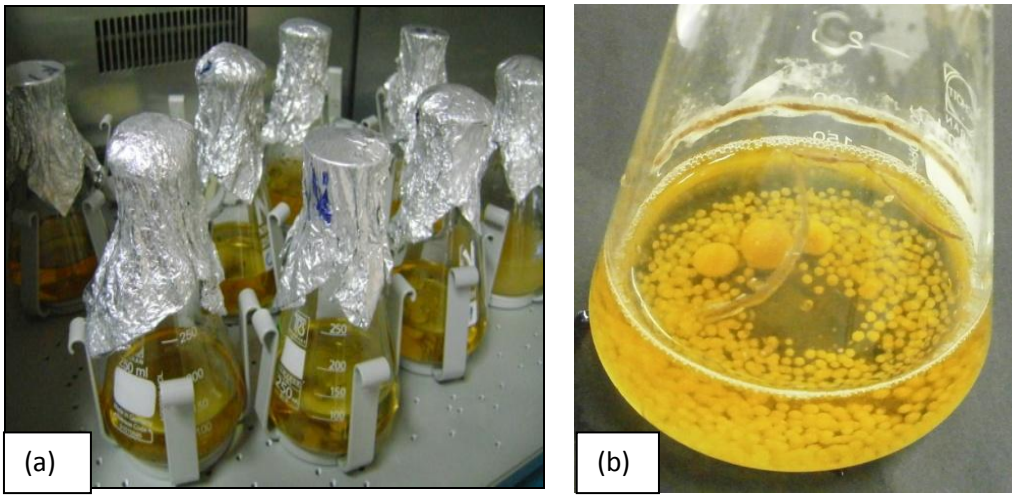


Figure 4: The inoculation of *P.ostreatus* (a) ME broth on rotator shaker (b) *P.ostreatus* in ME

The growth study of *P.ostreatus* was measured by weight of mycelium growth and it is shown from Fig. 5 that *P.ostreatus* exhibits a typical S-shaped curve. The growth curve of *P.ostreatus* is similar to Wu et al. (2003) on *Pleurotus tuber-regium* [15]. Typically, the growing of fungi is rapidly in the beginning and followed with an exponential growth phase and plateau or known as stationary phase. However, after that the growing was declined. The *P.ostreatus* mycelium experienced lagged phase growth for period four days where the rate of growth or cell division was slower. However, the growth increased rapidly until seven days during exponential growth phase until fixed nutrient was enough for growth. During that time, no noticeable change in *P.osteatus* mycelium concentration in the flask or the growing was stationary. Finally, the growth was declined due to the limited nutrient when the oxygen becomes depleted or metabolic by-products accumulate to toxic level which inhibited the *P.ostreatus* growing.

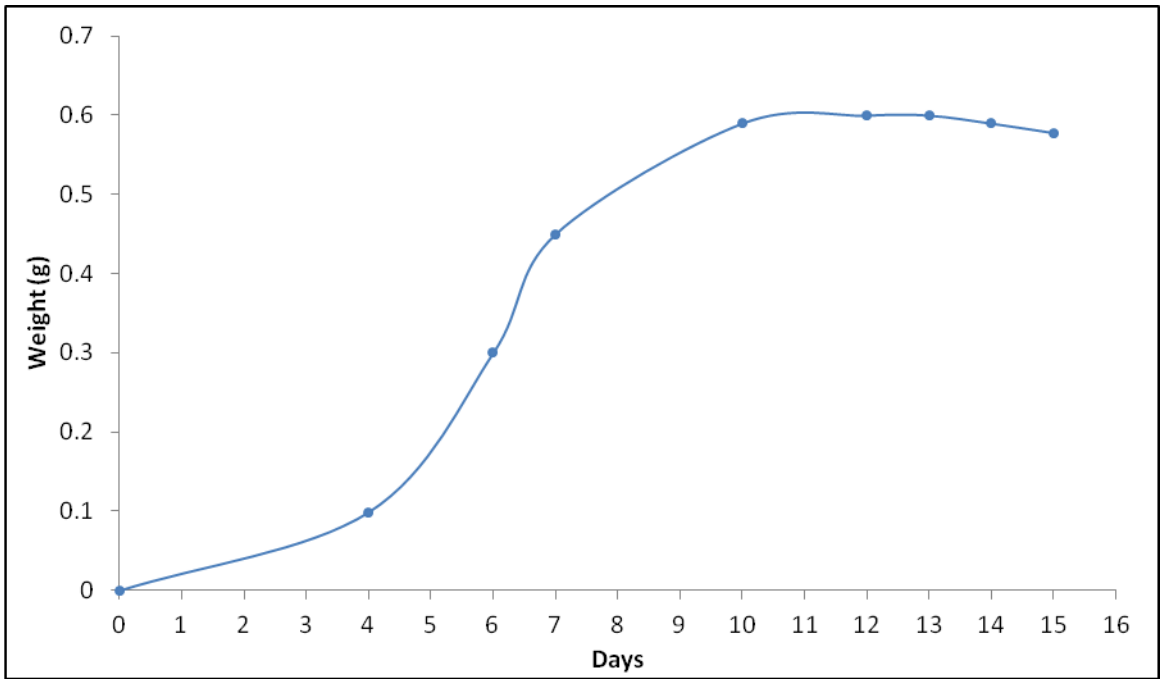


Figure 5: The growth phase study on *P.ostreatus*

Cell age of biosorbent will influence on metal biosorption when involving living microorganism [16]. It was believed that the cell at lag phase or early stage growth phase has a higher biosorptive capacity for metal ion compare to the stationary phase [17]. The inoculation of *P.ostreatus* at different temperature and pH were found to produce different palletize size of *P.ostreatus*. The growing of *P.ostreatus* as effect of pH between 5, 7 and 9 were shown in Fig. 6 at constant temperature but different pH. The inoculated *P.ostreatus* resulted in

different mean size diameter of 3.8 mm, 4.2 mm and 5.2 mm for pH 5, 7 and 9, respectively. It can be observed that the *P.ostreatus* growth size was smaller at lower pH compared to higher pH. The largest palletize *P.ostreatus* size was found at pH 9 which indicated that the *P.ostreatus* highly growth in basic condition. Figs. 6(a, b, c) show the diameter size of inoculated fungi at different pH and constant temperature of 25°C.

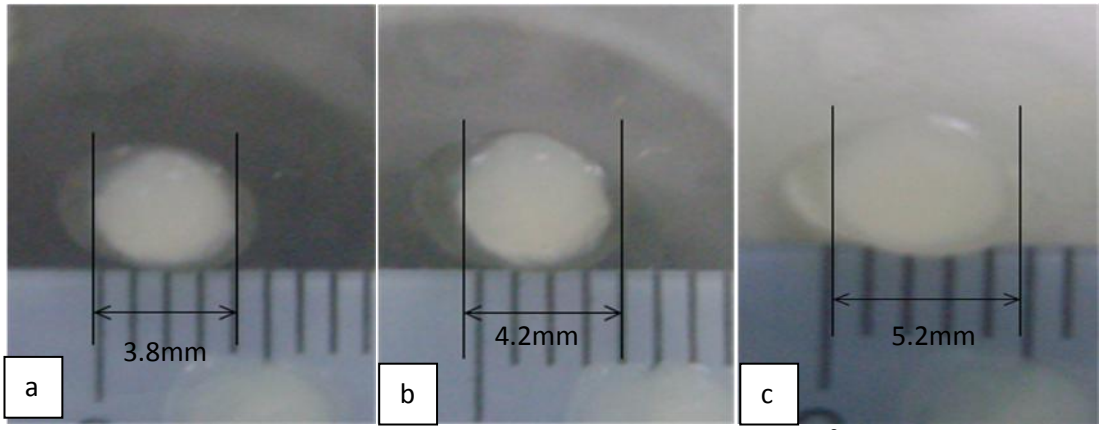


Figure 6: Diameter size of *P.ostreatus* inoculated at constant temperature 25°C and different pH a) pH5 b) pH7 c) pH9

The study on the effect of pH 5, 7 and 9 at constant temperature of 35°C on the inoculation of *P.ostreatus* was shown in Fig. 7. The inoculation of *P.ostreatus* produced different mean size diameter of 3.4, 3.6 and 4.0 mm for pH 5, 7 and 9. The effect of pH towards the inoculation at 35°C shows the same behavioral pattern with the inoculation at 25°C where the largest growth was found at pH 9.

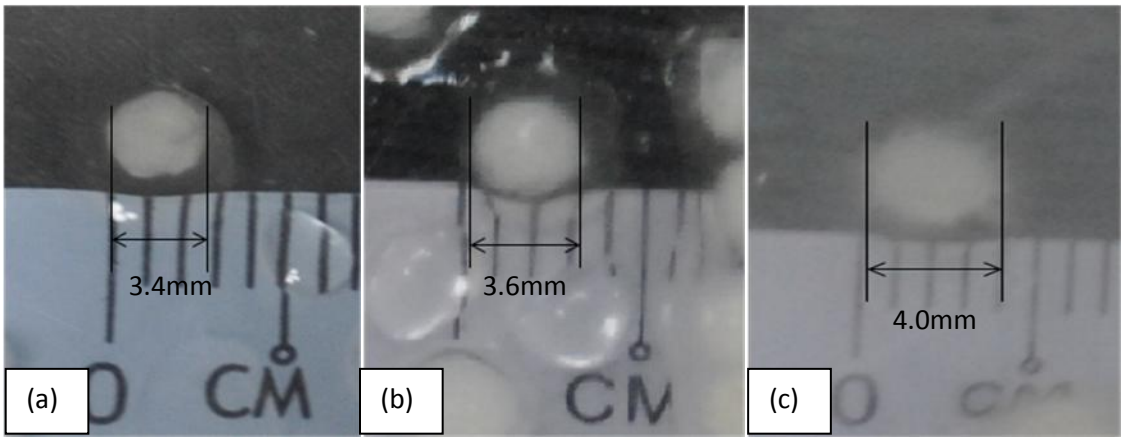


Figure 7: Diameter size of *P.ostreatus* inoculated at constant temperature 35°C and different pH a) pH 5 b) pH 7 c) pH 9

To study the effect of temperature on the inoculation of *P.ostreatus*, the growth temperature was set up at higher temperature than 35°C which is at 45°C. It can be seen in Figure 8 that there is no existence of *P.ostreatus* growth was found at 45°C and at varying pH 5, 7 and 9, respectively.

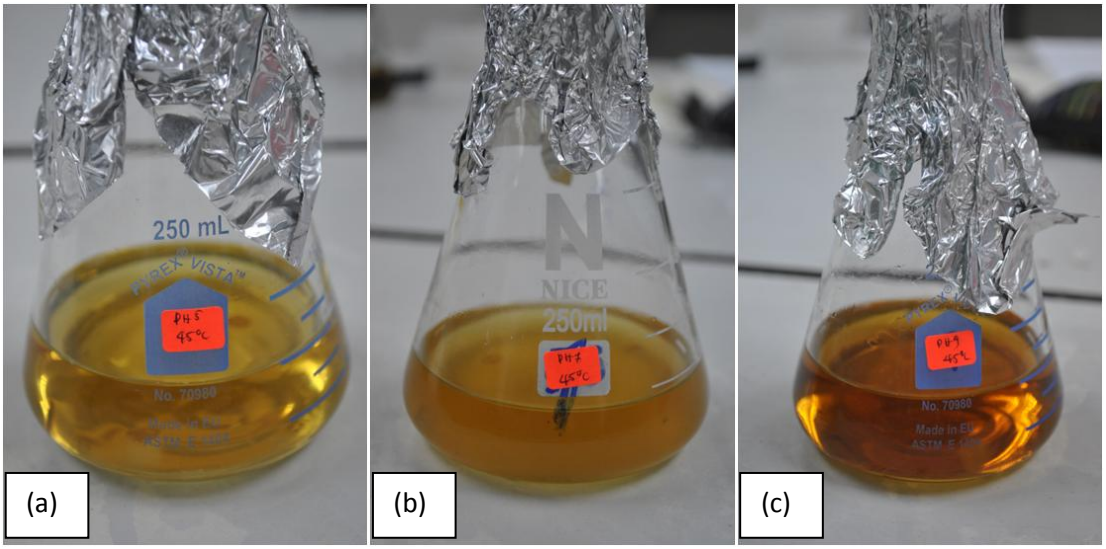


Figure 8: Inoculation of *P.ostreatus* at constant temperature 45^{o}C and different pH a) pH 5 b) pH 7 c) pH 9

Therefore, batch biosorption studies were not conducted at a temperature higher than 45^{o}C. This can be concluded that the suitable inoculation condition for *P.ostreatus* was at 25^{o}C and pH 9. The best growth temperature for *P.ostreatus* was similar with Nwokoye et al. (2006) who observed the best growing temperature for *P.ostreatus* was found at 28^{o}C and pH 9 [18].

3.3 Effect of pH in the treatment of liquid laboratory chemical waste

It was found that as the pH increased from 5 to 9 as the biosorption capacity decreased. The highest removal was observed when initial pH of liquid laboratory chemical waste was at 5 with 13.36% removal compared to pH 7 (9.46%) and pH 9 (8.90%) at an early 2 hours contact time (Fig. 9). The maximum removal of Cr(VI) was observed at pH 5 which approached 17.02% comparing to pH 7 (15.10%) and pH 9 (12.59%). The fact of pH 5 is the best biosorption of metal ions approved by the study of Huang *et al*. (1988) who found that metal ions removal was increased with pH greater than 4. At pH 5, the removal of Cr(VI) is faster because the proton competition between Cr(VI) and H_3O^+ are lower thus increase the potential of Cr(VI) bind to *P.ostreatus* active sites. At this time the affinity of the *P.ostreatus* surface for Cr(VI) also increase. It was proved from the studied of Romera *et al*. (2007)[19]. It was observed at pH 9, the percentage removal of Cr(VI) is the lowest which maximum uptake at 48 and 72 hours contact time was only 12.59% from 8.90% (Fig. 9). Less biosorption of Cr(VI) was observed at higher pH of 7 and 9 because of the high competition between metal ions and hydrogen ions for biosorption available sites [20]. During this pH, the lower removal was caused by the metal hydrolysis process where formation of hydroxylated complexes of Cr(VI) [21, 22]. During this time, the competition between H_3O^+ is lower thus adsorption increase as ionic competition for active sites increased [20]. Nemr *et al*. (2011) stated that at pH higher than 3 the biosorbent posses more functional group carrying a net negative charge, which tends to repulse the anions from aqueous solution [9]. It can be seen that pH is one of the important parameter need to be considered in the biosorption process of heavy metal in liquid laboratory chemical waste. The selection of pH must be suitable, wherein it is not toxic to *P.ostreatus*. Based on the result obtained, the optimum pH in biosorption process was found to be 5.

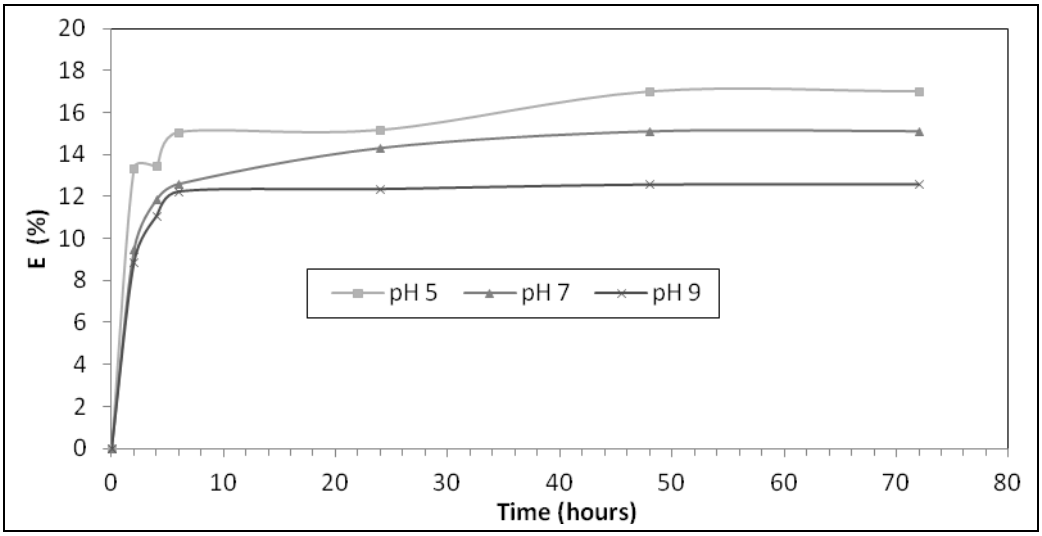


Figure 9: Efficiency removal of Cr(VI) from liquid laboratory chemical waste at different pH 5,7 and 9 by *P.ostreatus* at 25^{0}C and 150 rpm

3.4 Effect of contact time between P.ostreatus and liquid laboratory chemical waste

It was observed that the rapid uptake of Cr(VI) from liquid laboratory chemical waste at temperature 25^{0}C and pH 5 during the first 2 hours with 190 mg/g biosorption capacity (13.36% removal efficiency) (Fig. 10). Almost similar percentage removal reduction (13.36 % and 13.46%) was observed from 2 hours to 4 hours because of slower diffusion of metal ions into the interior of the *P.ostreatus* cell wall [23]. However, the increasing biosorption capacity and efficiency was observed at contact time 6 hours to 48 hours. It was because the available sites of biosorption increase as Cr(VI) had adsorb into intracellular of *P.ostreatus* [24]. Insignificant uptake of Cr(VI) concentration was observed at 48 hours and remained nearly constant until 72 hours where it suggested that the equilibrium condition was achieved. After this period, the amount of Cr(VI) adsorbed did not significantly change with time.

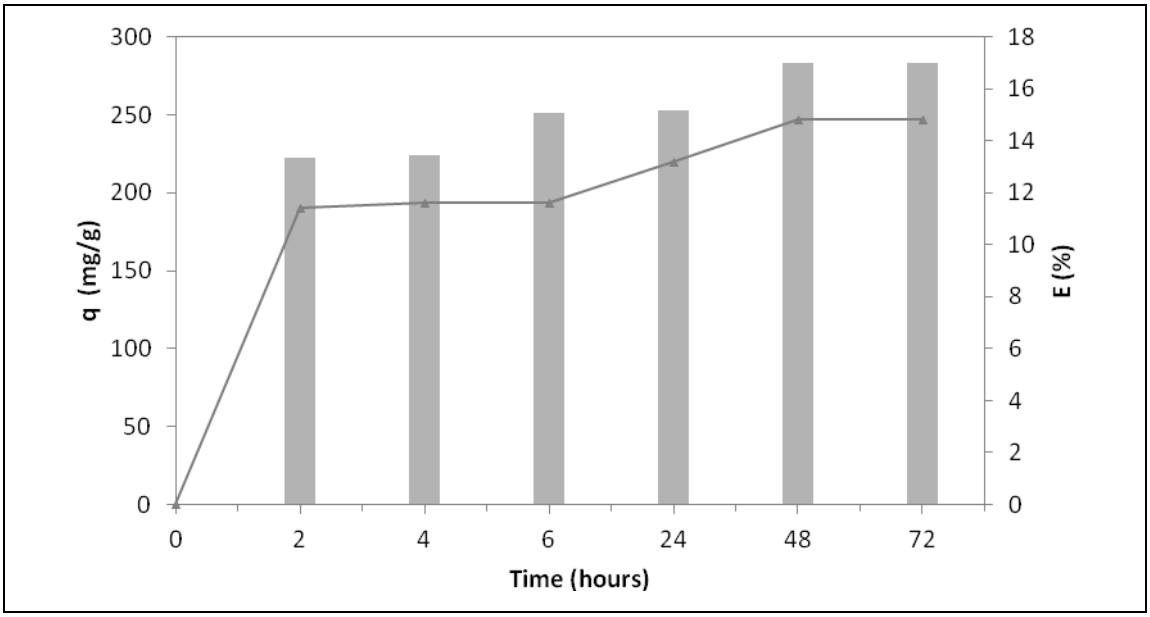


Figure 10: Biosorption capacity (mg/g) Cr(VI) concentration removal by *P.ostreatus* at temperatue at 25^{0}C and agitation speed at 150 rpm

3.5 Effect of temperature on biosorption by P.ostreatus

It was observed that the biosorption of metal ions decreased with increasing temperature from 25 to 35 °C (Figs. 11, 12, 13). The similar trend on biosorption decreased with increasing temperature which also

observed by Huiping *et al*. (2007). It was because of exothermic nature of biosorption process [17]. The pH 5 was observed the highest biosorption capacity at temperature 25°C (189.95 mg/g) as compared to 35°C (104.26 mg/g) at early 2 hours contact (Fig. 11). It was found that during 2 hours contact time, at pH 7 the biosorption difference was 33.6 mg/g (Fig. 12) and pH 9 was 124.09 mg/g (Fig. 13). The highly difference biosorption capacity was observed at pH 9 where at 25°C the Cr(VI) biosorption capacity at 129.08 mg/g and only 4.98 mg/g observed at 35°C at early 2 hours (Fig. 13). The decrease in biosorption capacity is due to damage of active binding sites at higher temperature [25]. The pH 9 and temperature 35^{0}C showed lowest biosorption capacity of Cr(VI) (Fig. 13). High pH showed lowest biosorption but too high temperature decreased metal sorption due to distortion of some sites of the cell surface for Cr(VI) biosorption [26]. Based on the result, biosorption of Cr(VI) is exothermic process. As a whole, Cr(VI) were effectively adsorbed by *P.ostreatus* in the temperature range of 25 to 35°C and maximum absorption was observed at 25°C at all pH (Figs. 11,12,13).

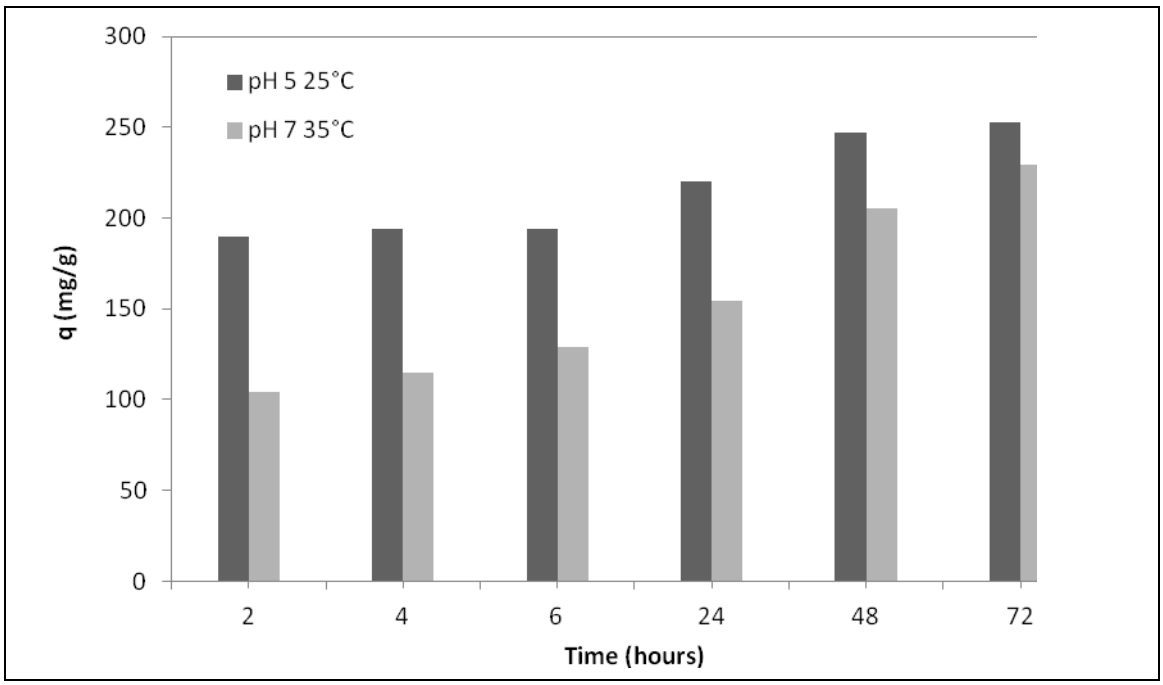


Figure 11: Graph bar of final concentration of Cr(VI) versus contact time at temperature 25 and 35°C; agitation speed 150 rpm; pH 5

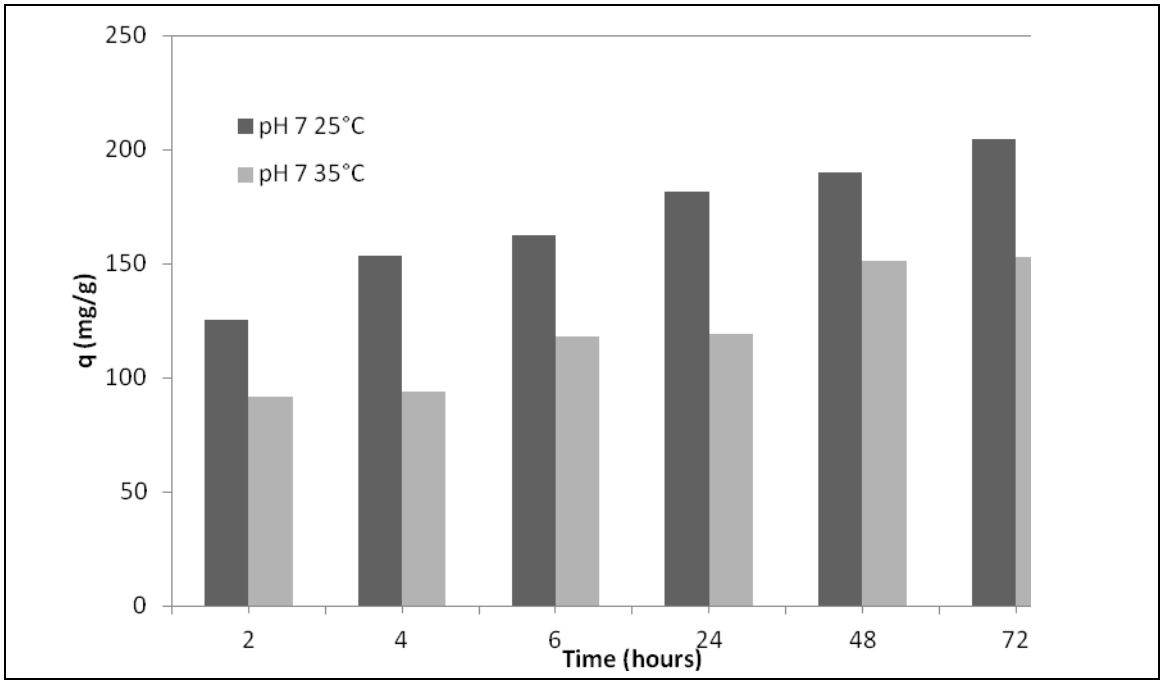


Figure 12: Graph bar of final concentration of Cr(VI) versus contact time at temperature 25 and 35°C; agitation speed 150 rpm; pH 7

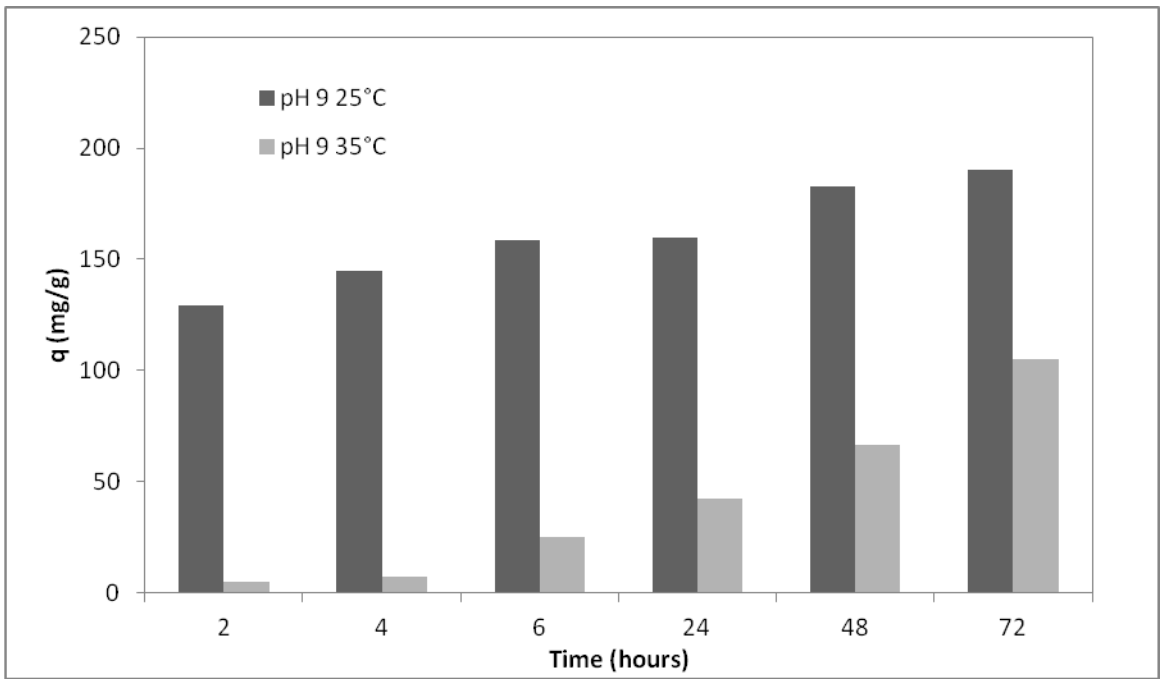


Figure 13: Graph bar of final concentration of Cr(VI) versus contact time at temperature 25 and 35°C; agitation speed 150 rpm; pH 9

IV. CONCLUSION

On the basis of data presented above, *P.ostreatus* employed in this study is a potential fungus to be applied for removal of toxic heavy metals from liquid laboratory chemical waste. It was observed that there are involvement of two important functional groups, COOH and NH on *P.ostreatus* in Cr(VI) adsorption. As pH increased from 5 to 9, the biosorption efficiency decreased. The highest biosorption efficiency was observed at pH 5 with 17.02% and the lowest was at pH 9 with 12.60% and only 15.11% for pH 7. The best pH condition for the treatment was at 5 and temperature 25°C. These treatment condition were significantly proven towards treatment of liquid laboratory chemical waste particularly Cr(VI). Therefore, the research is significant in

helping to reduce heavy metals content in liquid laboratory chemical waste. However, research is also need to be exploring the possibilities *P.ostreatus* of recovery and regeneration of precious metal ions. If it can happen, it can protect the environment and hence, contribute to the valuable treatment. Lastly, the research work should be extended to longer biosorption day for liquid laboratory chemical waste to remove heavy metals at the lowest concentration.

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