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FTIR study and bioadsorption kinetics of bioadsorbent for the analysis of metal pollutants

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Abstract

The elevated concentration of heavy metals in groundwater causes many environmental problems. Present paper reports adsorption behavior of green bioadsorbent (Trichoderma *sp.)* for the removal of heavy metal ions from the industrial effluents. Sampling of the groundwater within a commissioned landfill site showed the contamination of groundwater with some toxic heavy metals. Fungal biomass of Trichoderma *sp.* was tested to treat contaminated groundwater and it was found that dried Trichoderma sp. demonstrated significant ability for the exclusion of Cd^{2+}, Ni^{2+} and Cr^{3+} ions than wet Trichoderma. Fourier Transformation Infrared (FTIR) analysis conducted on wet and dry biomass before and after treatment to observe the changes in functional groups. In addition, Freundlich and Langmuir adsorption isotherms were used to verify the adsorption performance of *Trichoderma sp.*. Langmuir adsorption isotherm was found to be fit better than Freundlich adsorption isotherm. The maximum adsorption capacity of Ni^{2+}, Cd^{2+} and Cr^{3+} ions on *Trichoderma* were found to be 0.1353 (pH4), 0.374 (pH8) and 0.0527 (pH 10), respectively. The results of removal efficiencies of metal on Trichoderma indicates that it can be successfully applied for the removal and recovery of Cd^{2+}, Ni^{2+} and Cr^{3+} ions from the industrial effluents with 100 % recovery.

Keywords: *Heavy metals, bioadsorbent, adsorption, FTIR analysis.*

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1.0 Introduction

Nowadays water pollution by toxic heavy metal ions has become one of the major environmental problems. Three kinds of heavy metals are in major concern, namely toxic metals (e.g. Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (e.g. Pd, Pt, Ag, Au, Ru etc.) and radionuclides (e.g. U, Th, Ra, Am, etc.) [1]. Among the heavy metal ions, nickel, cadmium and chromium play a vital role to contaminate water and are normally found in contaminated water. Nickel is typically used in lead storage batteries and discharged into the environment by various processes such as electroplating, leather tanning, wood preservation, pulp processing, steel manufacturing, mining and metallurgical processes [2]. The exposure of nickel is mainly concerned through the contaminated water and food which can cause a number of gastrointestinal distress and neurological effects [3].

Cadmium is considered as a carcinogenic and mutagenic. It is mostly contributed by electroplating industries and causes significant concentration in metal finishing wastewater. Besides this, other common source of cadmium exposure is galvanized pipes and fixtures if pH of water supply is not controlled [4]. Its toxicity damages body's soft organs (e.g. lung, liver, reproductive system, etc.) and cause a number of diseases (e.g. itai-itai disease, renal damage, emphysema, hypertension, testicular atrophy, etc.) [5, 6]. Chromium is widely used in tanning, electroplating, textile and metal finishing industries which enters the effluent streams and affecting the environment adversely [7]. It becomes an important issue of this era to treat the industrial waste waters for the removal of toxic heavy metal ions. Therefore, a number of methods (such as chemical precipitation and sludge separation, chemical oxidation or reduction, ion exchange, reverse osmosis, adsorption using activated charcoal, electrochemical treatment, inorganic/organic coagulants and evaporative recovery) have been employed to treat wastewater and control the environmental problem [8-16].

For the removal of organic pollutants from water bodies, inorganic coagulants (e.g. Aluminium sulfate (Alum) and polyaluminium chloride (PACl) etc.) are extensively used owing to their high performance [17]. However, there are some drawbacks by using Alum. Firstly maximum use of Alum may induce fatal disease and secondly, it produces a large amount of sludge which significantly becomes a new environmental issue for the treatment, regeneration and reuse [18]. Moreover, organic synthetic flocculants produce polymeric residual which are known to be neurotoxic and carcinogenic to human beings [19]. Besides this, the non-biodegradable flocculating sludge caused environmental problems for sludge treatment process [20]. Thus, use of organic/inorganic compounds in wastewater treatment is directly or indirectly affecting the environment. Therefore, removal of heavy metal ions by the bioadsorption of bacterium, fungi, algae, mosses, macrophytes has become a noteworthy innovative technique [21-23]. It has been found that both living and dead microbial cells can bind with heavy metals either actively or passively [24, 25]. The bioadsorption can be simply achieved by using physical methods without damaging its structural integrity and can accomplish a high efficiency in the treatment of heavy metals from aqueous solution. Thus, to create a pollution free environment, attention has been paid to synthesize novel microorganisms. Removal of toxic heavy metal ions by these microorganisms not only control water pollution, but also develop innovative industrial applications [26-29]. The present paper reports FTIR study and bioadsorption kinetics of bioadsorbent (Trichoderma *sp.*) for the analysis of metal pollutants.

2.0 Materials and method

2.1 Area of study

The area for this study was chosen a decommissioned landfill site. Most of the sites of this area were contained by a natural marine clay liner. Several metal ions (Cd^{2+}, Ni^{2+}, Cr^{3+},

Zn^{2+}, Fe^{3+}, Cu^{2+} and Ca^{2+}) were monitored during the year 2008 and analyzed as given below:

2.2 Sampling of ground water

Each site of a particular area was monitored every time before sampling is conducted. The water in the well was allowed to stabilize to static water level prior to the measurements of water level at least 7 days. The level of water was documented and purging was done for at least 2 or 3 wells volumes. Afterwards, the water level in each well was allowed to recover its pre-purge static water level. Finally, a collection of groundwater sample was carried out within 24h using clean disposable bailer and clean sample container followed by standard method and all the samples were stored at 4ºC when not used.

2.3 Flame Atomic Absorption Spectrophotometer analysis

The concentration of each heavy metal was tested using flame atomic absorption spectrophotometer (FAAS) model Perkin Elmer model A (Analyst 200). The groundwater samples were analyzed for the detection of Cd^{2+}, Cr^{3+} and Ni^{2+} ions by flame atomic absorption spectrometry using an atomic absorption spectrometer. All the measurements were carried out in an air/acetylene flame. Flame atomic absorption spectrophotometer was found to be well suited for the determination of trace metal ions without any interference. The deviation was found to be less than 5 %. Precision was monitored by running triplicates for every sample and mean of the sample were documented in the form of results.

2.4 Preparation of bioadsorbent (Trichoderma sp.)

The biomass of Trichoderma sp. was obtained from Pro-Fil Industrial Resources, Penang, Malaysia. The media of Trichoderma was prepared by mixing vegetable juice (160 mL) with distilled water (90 mL) and agar nutrient (3.0 g) mixed thoroughly at pH 5.0 and heated to boil. The mixture was autoclaved at 121°C for 15min. The broth media were made

up to 30 % by using vegetable juice at pH 5.0 and poured into an Erlenmeyer flask (100 mL) and again autoclaved at 121°C for 15min.

2.5 Treatment of bioadsorbent

Groundwater samples were taken from the five boreholes for the determination of Ni^{2+}, Cd^{2+} and Cr^{3+} ions. In this study two types of Trichoderma biomasses namely vis a vis wet and freeze dried were used. The wet biomasses of Trichoderma were harvested by filtering liquid fungal culture through Whatman paper (with a pore size of 45µm). The biomasses were washed with deionized water several times. After washing, it was weighed to get the wet weight. A portion of wet biomass of Trichoderma sp. was freeze dried using the freeze dryer (model LABCONCO) and ground to 250 µm using grinders (IKA model A11 basic).

Treatment was carried out using 20 g and 2.0 g each of the wet and the dry mass of Trichoderma s*p.* placed in clean plastic bottles together with 150 mL contaminated groundwater sample. The treated bottles were incubated on a rotary shaker by 160 rpm at room temperature (25±2ºC) and the aliquots of filtrate were taken out for analysis at the interval of 24 h, 48 h and 76 h, respectively. The filtrates were analyzed using FAAS analysis for the detection of heavy metal content. At the same time, the fungal culture at the end of each incubation period was filtered and analyzed by FTIR analysis.

2.6 Freundlich and Langmuir isotherm models

The biosorption study includes the plot of the corresponding isotherms, which were established under different experimental conditions by varying the concentrations of metal ions (Ni^{2+}, Cd^{2+} and Cr^{3+} between 0.5 mg/L to 2.0 mg/L). The sorption process was quantified using the corresponding equilibrium parameters derived from the two mathematical models:

Freundlich and Langmuir equations were applied to describe the isotherm:

Freundlich equation:

$$q_e = k_f\,(C_e)^{1/n} \qquad [1]$$

where k_f and n are Freundlich constants and the Freundlich isotherms relationship is exponential. However, it can be linearized by plotting the data in (Log-Log) format to obtain the Freundlich constants and correlation coefficient (r^2). It is given as:

$$\text{Log } q_e = \text{Log } k_f + 1/n \text{ Log } C_e \qquad [2]$$

where C_e is the equilibrium for liquid phase ion concentration (mg/L) and q_e is the equilibrium solid phase ion concentration (mg/L). The Freundlich constant K_f (mg/g) and 'n' represent the adsorption capacity and adsorption intensity of adsorbent, respectively, at a fixed temperature at equilibrium.

Langmuir equation:

$$q = q_{max}\frac{bC_f}{1+C_f}$$

where q_{max} is the maximum sorbate uptake (mg/g) and can be interpreted as the total number of binding sites that are available for biosorption, b and b=1/K is the coefficient related to the affinity between sorbent and sorbate. While C_f is the final equilibrium sorbate concentration.

The Langmuir relationship can be linearized by plotting: $\frac{1}{q}$ vs $\frac{1}{C_f}$ or $\frac{C_f}{q}$ vs C_f .

Calculation for sorbate uptake (q) as follow;

q = V [L] (C_i-C_f)[mg/L] / S [g]

where C_i is the initial sorbate concentration in solution and C_f is the final equilibrium sorbate concentration in solution. V (L) is the volume of the metal-bearing solution contacted with the sorbent and all of these had to be analytically determined (mg/L), while S is the dry amount of (bio) sorbent (g).

2.7 SEM and FTIR analyses

Surface morphology of Trichoderma biomass was studied by scanning electron microscopy (SEM; Carl-Ziess SMT, Oberkochen, Germany). FTIR study was performed to identify the presence of functional groups in the Trichoderma sp. The Infrared (IR) spectra were recorded on a Fourier Transform-IR Spectrometer from Perkin Elmer (1730, USA) using the KBr disc method. The biomass of Trichoderma *sp.* was washed and weighed to get the wet weight. A fix portion (1.0g) of the above wet biomass was freeze dried using freeze dryer model LABCONCO for 5 days and grounded to specific size (250 μm) using a grinder (IKA model A11 basic). The biomass were filtered out before and after adsorption tests on the groundwater, freeze dried and grounded into powder form for the FTIR analysis (model: FTIR-Is 10). FTIR analysis was performed by using the KBr disc method; in which 1.0 mg dried powder of biomass was mixed with 99 mg of potassium bromide (Spectrosol, 100g). The mixture was mixed thoroughly and placed on the KBr die model and compressed using a compressor (model: CARVER) by an applied pressure of 7 Metric tons for about 30s to turn it into pellet forms. The transmittance spectra were obtained in a wavelength range between 500 and 4000 cm^{-1} with a resolution of 4 cm^{-1}.

3.0 Results and discussion

3.1 Morphological characterization

3.1.1 SEM analysis

The morphological characterization and bioadsorption behavior of Trichoderma biomass was studied on five days old fungal culture medium. The fungal culture of Trichoderma shows yellow, greenish appearance for older mycelia in the center while at the peripheral fresh mycelia demonstrates whitish, fluffy morphology (Figure 1). The detailed, interesting morphology of Trichoderma was observed by SEM analysis (Figure 2) which reveals the presence of septate mycelia together with branches of conidiophores, consisting of

a group of 3 or 4 philiades. These philiades have swollen morphology in the middle and narrow morphology at the ends. However, the isolate has not been identified to the species level, therefore it is designated as Trichoderma.

3.1.2 FTIR studies

The identification and the existence of functional groups present in the biomass of Trichoderma before and after treatment with metal ions were studied by FTIR analysis at room temperature (25±2°C). For the comparative study, the spectra of untreated and treated biomass of Trichoderma with heavy metal ions (nickel, cadmium and chromium) were examined. The appended FTIR spectra are shown in Figure 3 (ABCD). In all the spectra, a broadband range of 3200-3550 cm^{-1} corresponds to the presence of hydroxyl groups [30] associated with the N–H bond of amino groups [31]. The relative difference in band intensity to be similar to the difference in the concentration of respective functional groups associated with the bands. It was justified by the presence of broad band in the frequency range of 3200-3550 cm^{-1} corresponds to the existence of the hydroxyl group [32]. Two sharp peaks at 2925 and 2852 $cm^{-1}$are attributed to the -CH asymmetric and symmetric stretching vibrations of methylene hydrogen [33].

Each spectra demonstrates a sharp, intense peak at 1652 cm^{-1} attributed to the aromatic C=C, C=O and conjugated ketones or C=N amide stretching. The methyl asymmetric C-H bends at 1458, 1053 and 1034 cm^{-1} ascribed to C-O stretching of alcohol, sulfoxides, carbohydrates or polysaccharides-like substances. A medium intensity broad band in the range of 1050 cm^{-1} is assigned to υ (C-O-C) asymmetrical stretching. All the observed FTIR frequencies of *Trichoderma* biomass concurred with the study of Csiktusnadi et al., who found the existence of OH, =CH, C=O and $-NH_2$ groups in the *Trichoderma harzianum* [34].

In comparison to the spectra of untreated biomass of Trichoderma with treated heavy metal ions (nickel, cadmium and chromium), a minute shift in peak position was observed (Figure 3 (B, D, C)). These changes were occurred owing to the binding of nickel, cadmium and chromium ions with amino and hydroxyl groups of biomass. Thus, shifting of bands confirms the adsorption of metal ions on the surface of Trichoderma. The most remarkable difference amongst the four spectra is; at an intensity of 3000-3800 cm^{-1} representing hydroxyl (-OH) stretching, and the medium intensity band in the range of 1600-1700 cm^{-1} represents carbonyl (–C=O) stretching. These signify the involvement of hydroxyl groups in the binding of Ni^{2+}, Cd^{2+} and Cr^{3+} ions. A slight change in the frequency of peaks confirms the existence of bioadsorption. In addition, the main functional groups that are responsible for bioadsorption process are the hydroxyl, carbonyl, carboxyl, sulfonate, amide, imidazole, phosphonate and phosphodiester groups as affirmed by Pradhan and Volesky et al. [35, 36]. Some of these groups are present in the biomass of Trichoderma and have good capability to interact heavy metal ions. Besides this, the binding of metal ions with biopolymers were mainly occurred in the peptidoglucan and layer of the cell surface [37].

3.2 Analysis of toxic heavy metal ions

Groundwater sample analysis was carried out to know the concentration of heavy metal ions (Ni^{2+}, Cd^{2+} and Cr^{3+}) from five boreholes. The data in term of concentrations of Ni^{2+}, Cd^{2+} and Cr^{3+} ions are shown in Figure 4. The concentrations of nickel found to be 0.100 mg/L, 0.198 mg/L, 0.321 mg/L, 0.153 mg/L and 0.136 mg/L, respectively. The amount of cadmium was lies from 0.051 to 0.184 mg/L, while the concentration of chromium was obtained from 0.027 to 0.122 mg/L, respectively. Among all samples, borehole 3 shows highest amount of Ni^{2+}, Cd^{2+} and Cr^{3+}ions. It appears that the highest contamination of heavy metals occurred at location borehole 3. Therefore, biomass of Trichoderma was employed to treat groundwater for the bioadsorption of toxic heavy metal ions.

3.3 Bioadsorption behavior of Trichoderma biomass

In order to explore the potentiality of the fungal bioadsorbent for the removal of toxic heavy metal ions from the industrial effluents. Batch experiments were performed at different time interval by placing 20 g and 2.0 g each of the wet and the dry mass of Trichoderma in clean plastic bottles together with 150 ml contaminated groundwater sample (Tables 1-6). The results in Table 1-6 show that initially, no adsorption was observed. However, the absorption of heavy metals was carried out within 24h. The percentage of nickel removal efficiency after 24h from the five samples varied from 77 % to 89.41 %. The variation also occurs between heavy metals from the same source of the groundwater, borehole 1. After 24 h, more than 84 % cadmium in the groundwater of borehole 1 was adsorbed onto the fungal biomass that was higher than chromium (about 81.5 %) and nickel (about 77 %). After 48h all the cadmium and chromium ions from the groundwater borehole 1 removed, leaving 0.002mg/L nickel in the water. Similar observations were made for samples taken from almost all the boreholes. Thus, complete removals of heavy metals by using dried fungal biomass can be achieved in the retention time of 48h.

The removal efficiency of heavy metal ions depends on the source of the contaminated groundwater. However, it appears to be independent of the original concentration in the sample. Among three heavy metals, cadmium demonstrated highest removal affinity towards dried fungal biomass, followed by nickel and chromium, respectively. Given enough contact time, all the heavy metals are being removed from the groundwater onto the fungal biomass.

Tables 4-6 show removal efficiency of heavy metal ions by wet biomass using 20g/150mL groundwater. Both dry and wet bioadsorbents were used for the removal of metal ions. However, wet fungal bioadsorbent found to be less effective than dried biomass. After a time interval of 24h, the removals of the heavy metals from different sources showed the

lower adsorption capacity of dried biomass than dried fungal biomass. At the retention time 76h, almost all the heavy metal ions are being removed from the water which indicates that either wet or dried fungal biomass have the capability to exclude metal ions. The pH of the solution also plays a vital role for the adsorption of metal ions on Trichoderma. The adsorption capacity of metal ions increases with increasing pH, as the enhancement of pH favors the formation of hydroxyl groups. At the same time, the removal of protons from the fungal biomass and the subsequent shifting of equilibrium to the right, which increase interaction between metal ions and negatively charged ligands resulting higher bioadsorption capacity. The affinity sequence for removal of heavy metal ions was found to as follows Cd^{2+} > Cr^{3+} > Ni^{2+}. Thus, fungal biomass of Trichoderma can be fruitfully used for the removal and recovery of nickel, cadmium and chromium ions from contaminated groundwater.

3.4 Adsorption isotherm studies

To study the bioadsorption behavior of Trichoderma biomass for the removal of Cd^{2+}, Ni^{2+} and Cr^{3+} ions from aqueous solutions, the Langmuir and Freundlich adsorption isotherms models were used. By plotting the linear graph (Figures are not shown), the values for K_f, n and r^2 for Freundlich coefficient and q_{max}, b and r^2 for Langmuir coefficient were obtained and results are presented in Table 7. Table 7 shows that both models give fairly good linear fit to the adsorption data, according to the value of r^2. However, the Langmuir equation (r^2) yields a slightly better fit than the Freundlich (r^2). It is well known that the Langmuir equation is intended for homogeneous surface, therefore, a good fit of this equation may reflect monolayer adsorption [38]. Other than homogeneous surfaces, the Freundlich equation is suitable for a highly heterogeneous surface and an adsorption isotherm lacking a plateau which indicates a multilayer adsorption [39]. Besides this, it also frequently gives an adequate description of the adsorption data over a restricted range of concentrations. Hence,

it was found suitable for the experiment in which concentration of metal that covers the range 0.5mg/L to 2.0 mg/L.

In addition, the values of Freundlich constants K and n represent the adsorption capacity as well as the adsorption intensity of the adsorbent, respectively, and the constant b in the Langmuir model reflects the adsorption bond energy (where the constant q_{max} shows the maximum adsorption capacity). Thus, the maximum biosorption capacity of *Trichoderma* sp. for nickel, cadmium and chromium was observed at pH 4 and above this pH. This phenomenon shows that pH dependent *Trichoderma* sp. showed best sorption for nickel at pH 4 and 6 with a capacity of 0.1353 mg/g and 0.0874 mg/g; best cadmium sorption at pH 8 followed by pH 6 and 4 with a maximum cadmium sorption capacity of 0.0374 mg/g, 0.0373 mg/g and 0.0364 mg/g; and best chromium sorption shows the pH trend of pH 10> pH 8> pH6> pH4 with maximum sorption capacity of 0.0527 mg/g, 0.0497 mg/g, 0.0485 mg/g and 0.0421 mg/g, respectively.

A number of effects such as surface charges, valence state, the electronegativity [40], atomic weight and ionic radius [41] favors the biosorption efficiency. In terms of ion uptake capacity, *Trichoderma* sp. prefers adsorption of metals which may be explained on the basis of the property of the biomass, which are electronegativity (Ni, 1.8 Pauling; Cr, 1.6 Pauling and Cd, 1.69 Pauling [42]), molecular weight (Cd, 112.4 u; Ni, 58.7 u; Cr, 51.99 u) and ionic radius (Ni, 69 pm; Cd, 95 pm; Cr, 44pm [43]. The property difference may lie in the number of charges and particle size. Although, adsorption capacity of nickel was found to be high due to its high electronegativity, low atomic weight and lower ionic radius which allows good quality entrapment by cell. However, removal of nickel found to be lower than cadmium and chromium. This is because of the ions which may compete with H^+ ions in solution and the fungi biomass may be more sensitive by the toxic effect of nickel than that of cadmium or chromium. Lower removal percent capacity of chromium than cadmium may be due to the

differences in molecular weights (Cd, 112.4 u; Cr, 51.99 u) which plays an important role in the adsorption process. Chromium has a lower molecular weight than cadmium although their electronegativity (Cd, 1.69 Pauling; Cr, 1.6 Pauling) does not show a discrepancy which allows faster and higher entrapment of metal ions into the biomass cell of *Trichoderma*.

Conclusions

Fungal biomass of Trichoderma has been successfully prepared without any pretreatment and characterized on the basis of SEM and FTIR analyses. Both dry and wet fungal biomass were applied for the bioadsorption of Cd^{2+}, Ni^{2+} and Cr^{3+} ions. However, dry biomass demonstrated maximum bioadsorption capacity than that of wet biomass. The affinity sequence for removal of heavy metal ions was found to be as follows $Cd^{2+} > Cr^{3+} > Ni^{2+}$. The removal of toxic heavy metal ions from ground water samples by Trichoderma biomass via FAAS did not require any pretreatment or pre digestion method. The biomass has been significantly removed heavy metal ions from wastewater with 100 % removal efficiency. Both Freundlich and Langmuir adsorption models are fitted, however Langmuir isotherm is slightly better than Freundlich isotherm, which covers a concentration range of 0.5mg/L to 2.5mg/L for heavy metal ions. The fungal biomass of Trichoderma, thus, exhibits the characteristics of a promising bioadsorbent which can be explored further.

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Table 1 Treatment of nickel in groundwater using 2.0 g dry biomass of Trichoderma at 25±2ºC.

Treatment time	0.0 h	After 24 h		After 48 h		After 76 h	
Groundwater site	Initial concentration (mg/L)	Final concentration (mg/L)	% removal	Final concentration (mg/L)	% removal	Final concentration (mg/L)	% removal
Borehole 1	0.100	0.023	77.00	0.002	98.00	-	100.00
Borehole 2	0.198	0.031	84.34	0.007	96.46	-	100.00
Borehole 3	0.321	0.034	89.41	0.010	96.88	-	100.00
Borehole 4	0.153	0.025	83.66	0.004	97.39	-	100.00
Borehole 5	0.136	0.024	82.35	0.003	97.79	-	100.00

Table 2 Treatment of cadmium in groundwater using 2.0 g dry biomass of Trichoderma at 25±2ºC.

Treatment time	0.0 h	After 24 h		After 48 h		After 76 h	
Groundwater site	Initial concentration (mg/L)	Final concentration (mg/L)	% removal	Final concentration (mg/L)	% removal	Final concentration (mg/L)	% removal
Borehole 1	0.051	0.008	84.31	-	100.00	-	100.00
Borehole 2	0.078	0.011	85.90	-	100.00	-	100.00
Borehole 3	0.184	0.015	91.85	0.001	99.50	-	100.00
Borehole 4	0.149	0.011	92.62	-	100.00	-	100.00
Borehole 5	0.148	0.009	93.92	-	100.00	-	100.00

Table 3: Treatment of chromium in groundwater from PBLS using 2.0 g dry weight of Trichoderma at 25±2ºC.

Treatment time	0.0 h	After 24 h		After 48 h		After 76 h	
Groundwater site	Initial concentration (mg/L)	Final concentration (mg/L)	% removal	Final concentration (mg/L)	% removal	Final concentration (mg/L)	% removal
Borehole 1	0.027	0.005	81.48	-	100.00	-	100.00
Borehole 2	0.066	0.011	83.33	-	100.00	-	100.00
Borehole 3	0.122	0.029	76.23	0.009	92.62	-	100.00
Borehole 4	0.067	0.016	76.12	0.003	95.52	-	100.00
Borehole 5	0.031	0.008	74.19	-	100.00	-	100.00

Table 4 Treatment of nickel in ground water from PBLS using 20 g wet weight of Trichoderma at 25±2ºC.

Treatment time	0.0h	After 24 h		After 48 h		After 76 h	
Groundwater site	Initial concentration (mg/L)	Final concentration (mg/L)	% removal	Final concentration (mg/L)	% removal	Final concentration (mg/L)	% removal
Borehole 1	0.100	0.035	65.00	0.006	94.00	-	100.00
Borehole 2	0.198	0.043	78.28	0.012	93.94	-	100.00
Borehole 3	0.321	0.054	83.18	0.021	93.46	0.002	99.38
Borehole 4	0.153	0.035	77.12	0.010	93.46	-	100.00
Borehole 5	0.136	0.037	72.79	0.009	93.38	-	100.00

Table 5 Treatment of cadmium in ground water from PBLS using 20 g wet weight of Trichoderma at 25±2ºC.

Treatment time	0.0h	After 24 h		After 48 h		After 76 h	
Groundwater site	Initial concentration (mg/L)	Final concentration (mg/L)	% removal	Final concentration, (mg/L)	% removal	Final concentration (mg/L)	% removal
Borehole 1	0.051	0.017	66.67	0.004	92.16	-	100.00
Borehole 2	0.078	0.022	71.79	0.004	94.87	-	100.00
Borehole 3	0.184	0.036	80.43	0.007	96.20	-	100.00
Borehole 4	0.149	0.029	80.54	0.006	95.97	-	100.00
Borehole 5	0.148	0.024	83.78	0.005	96.62	-	100.00

Table 6 Treatment of chromium in groundwater from PBLS using 20 g wet weight of Trichoderma at 25±2ºC.

Treatment time	0.0h	After 24 h		After 48 h		After 76 h	
Groundwater site	Initial concentration (mg/L)	Final concentration (mg/L)	% removal	Average of final concentration, (mg/L)	% removal	Final concentration (mg/L)	% removal
Borehole 1	0.027	0.008	70.37	-	100.00	-	100.00
Borehole 2	0.066	0.020	69.70	0.005	81.48	-	100.00
Borehole 3	0.122	0.031	72.95	0.012	81.81	-	100.00
Borehole 4	0.067	0.022	67.16	0.009	92.62	-	100.00
Borehole 5	0.031	0.011	64.52	0.002	93.55	-	100.00

Table 7 Freundlich and Langmuir coefficient for biosorption of Ni^{2+}, Cd^{2+} and Cr^{3+} ions.

Freundlich coefficient and correlation coefficient (r^2) for biosorption of Nickel by Trichoderma sp. at different pH.				Langmuir coefficient and correlation coefficient (r^2) for biosorption of Nickel by Trichoderma sp. at different pH.			
pH	K_f (mg/g)	n	r^2	pH	q_{max}	b	r^2
2	0.0467	0.6447	0.9293	2	0.0171	0.9257	0.8952
4	0.0338	1.2732	0.9165	4	0.1353	0.4098	0.9414
6	0.0376	1.4457	0.8780	6	0.0874	0.7885	0.9115
8	0.0377	1.6147	0.8759	8	0.0611	1.4740	0.9286
10	0.0386	1.6324	0.8578	10	0.0616	1.5262	0.9165
Freundlich coefficient and correlation coefficient (r^2) for biosorption of cadmium by Trichoderma sp. at different pH.				Langmuir coefficient and correlation coefficient (r^2) for biosorption of cadmium by Trichoderma sp. at different pH.			
pH	K_f (mg/g)	n	r^2	pH	q_{max}	b	r^2
2	0.0327	2.4207	0.8807	2	0.0350	5.4738	0.9586
4	0.0339	2.8703	0.7672	4	0.0364	7.1380	0.8874
6	0.0368	2.8490	0.8245	6	0.0373	8.5730	0.9306
8	0.0383	2.9095	0.8185	8	0.0374	10.0368	0.9326
10	0.0385	3.0479	0.8114	10	0.0370	11.6200	0.9301
Freundlich coefficient and correlation coefficient (r^2) for biosorption of chromium by Trichoderma sp. at different pH.				Langmuir coefficient and correlation coefficient (r^2) for biosorption of chromium by Trichoderma sp. at different pH.			
pH	K_f (mg/g)	n	r^2	pH	q_{max}	b	r^2
2	0.0319	1.8864	0.9677	2	0.0335	3.9841	0.9442
4	0.0723	1.8619	0.9906	4	0.0421	3.6661	0.9973
6	0.0516	1.5803	0.9508	6	0.0485	3.5619	0.9642
8	0.0557	1.5581	0.9635	8	0.0497	3.7774	0.9657
10	0.0601	1.5773	0.9588	10	0.0527	3.9478	0.9724

Figure captions

Figure 1. A snapshot of five days old biomass of Trichoderma s*p*.

Figure 2. SEM image of five-day old culture of Trichoderma sp. (1.00K × magnification).

Figure 3. FTIR spectra of Trichoderma sp. (A). Before treatment, (B). After treatment with Nickel, (C). Chromium and (D). Cadmium ions.

Figure 4. Concentrations of heavy metal ions (Cr^{3+}, Cd^{2+} and Ni^{2+}) in groundwater from five different boreholes.

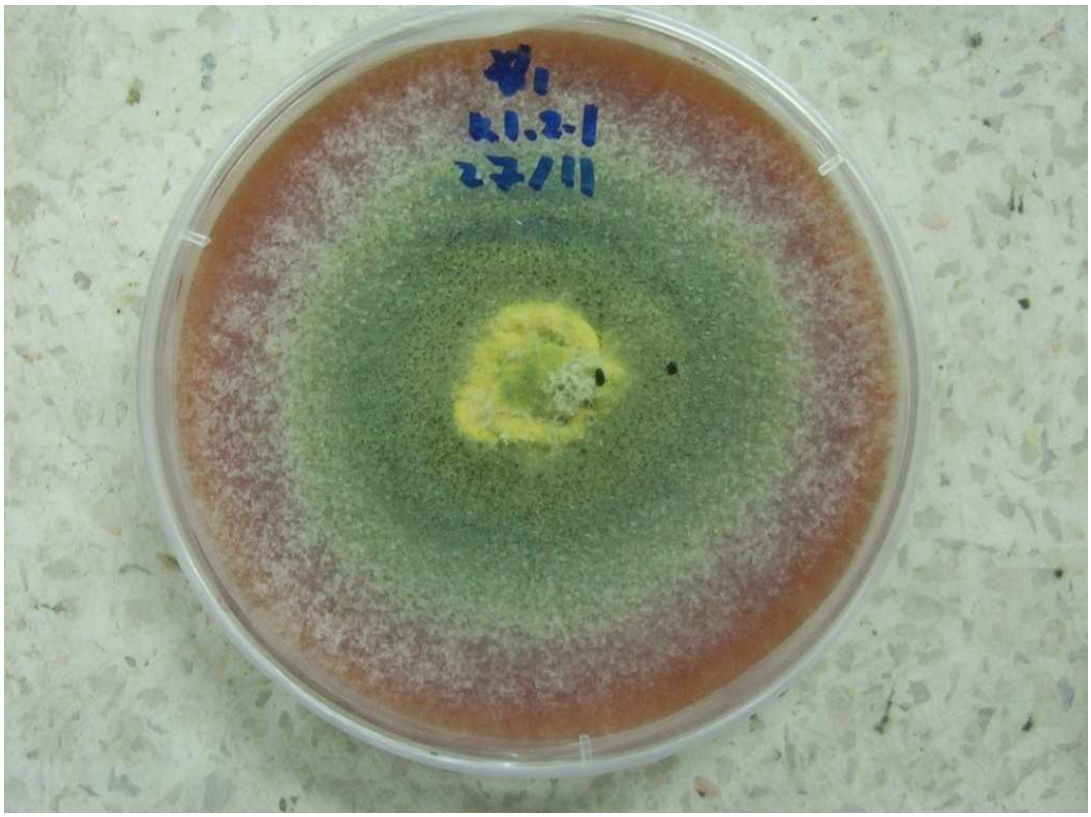

Figure 1

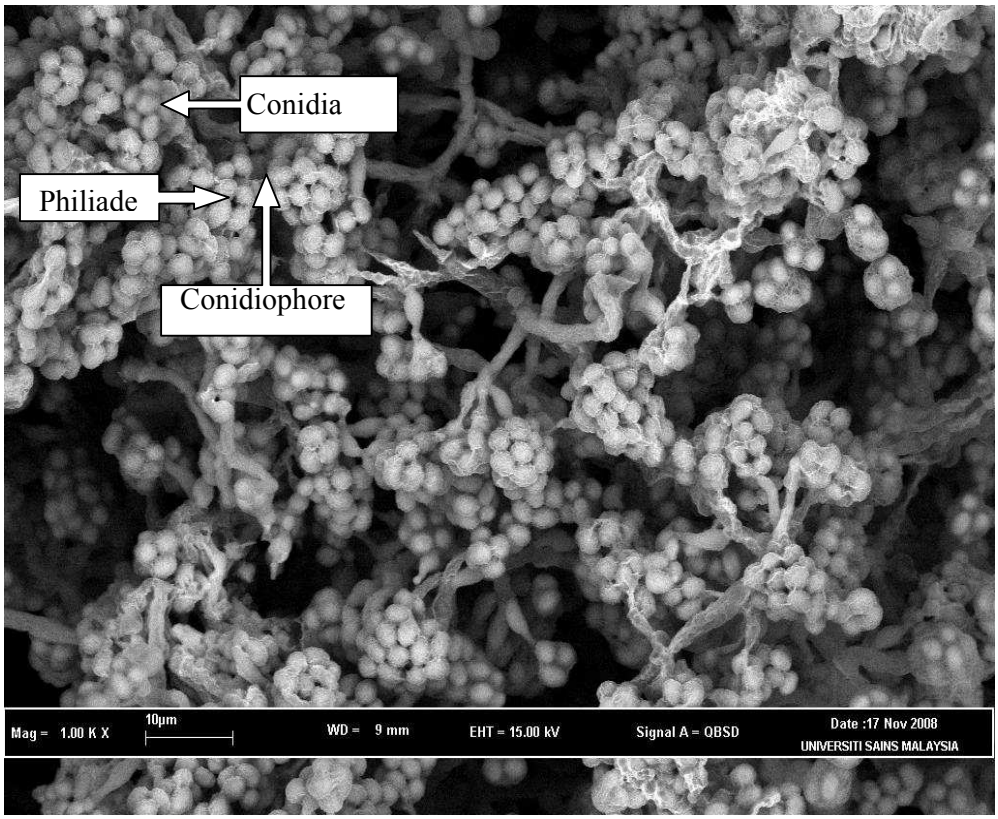
Conidia
Philiade
Conidiophore
Mag = 1.00 K X
10µm
WD = 9 mm
EHT = 15.00 kV
Signal A = QBSD
Date :17 Nov 2008
UNIVERSITI SAINS MALAYSIA

Figure 2

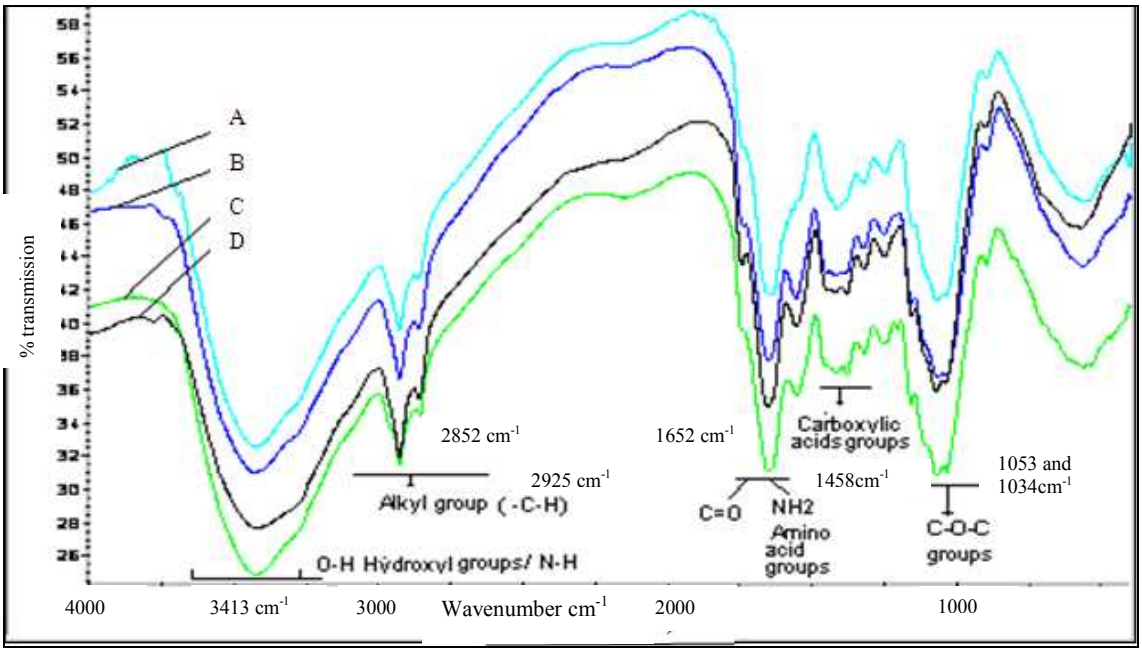
% transmission
A
B
C
D
2852 cm-1
2925 cm-1
Alkyl group (-C-H)
1652 cm-1
Carboxylic acids groups
1458cm-1
C=O
NH2
Amino acid groups
1053 and 1034cm-1
C-O-C groups
O-H Hydroxyl groups/ N-H
4000
3413 cm-1
3000
Wavenumber cm-1
2000
1000

Figure 3

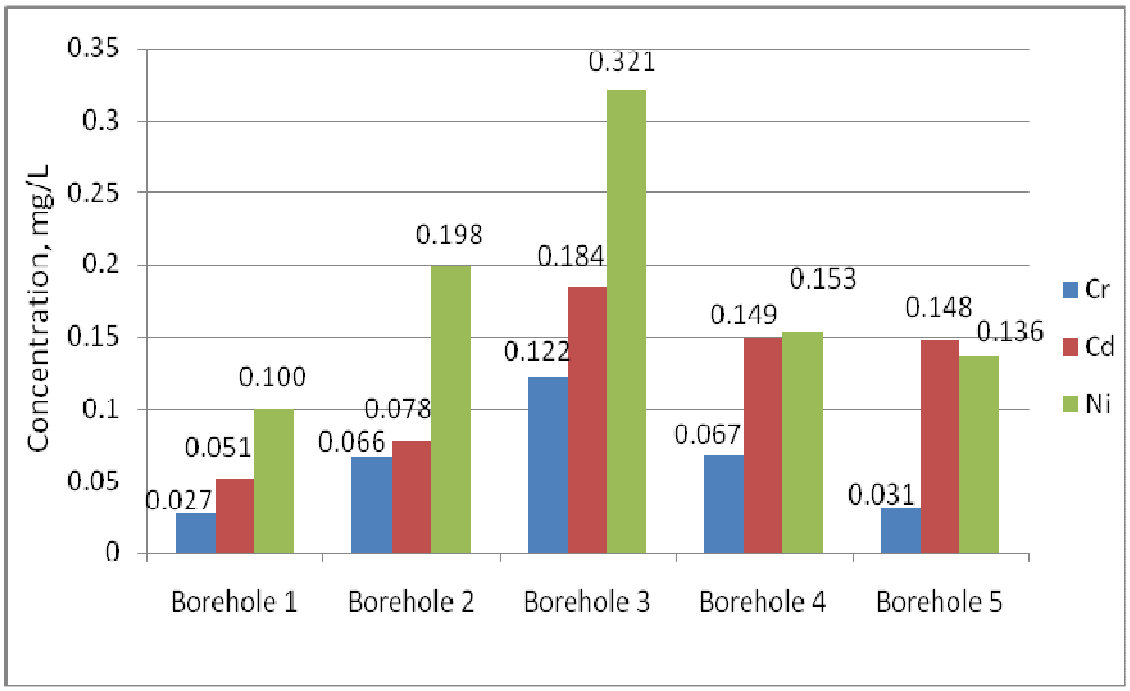
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Concentration, mg/L
0.027
0.051
0.100
0.066
0.078
0.198
0.122
0.184
0.321
0.067
0.149
0.153
0.031
0.148
0.136
Borehole 1
Borehole 2
Borehole 3
Borehole 4
Borehole 5
Cr
Cd
Ni

Figure 4