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Biosorption of Lead(II) using *Trichoderma viride* in the Aqueous Solution

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ABSTRACT

Lead is considered as one of water pollutant that is toxic, corrosive, and irritant. One method that can be applied for reducing Pb(II) in the environment is by using microorganisms. In this work, the study of biosorption of lead in the water samples was conducted using *Trichoderma viride*. The research was focused on the determination of optimum conditions of biosorption including initial pH, biosorption time, and initial concentration of lead. Profiles of functional groups contained in the *T. viride* have been monitored using FT-IR spectrophotometry. Results showed that the maximum biosorption of Pb(II) was achieved at initial pH 4.5, with equilibrium of contact time at 20 h, and optimum concentration of 50 mg/L, and adsorption capacity of 85 mg/1x10^6 *T. viride* colonies. The FTIR results indicated that biosorption process affected the functional groups in the *T. viride*. These have shown in the absorption bands at ~3200 cm^{-1}, ~2850 cm^{-1}, ~2260 cm^{-1}, ~1650 cm^{-1}, ~1450 cm^{-1}, 1180 cm^{-1}, and in the finger printing regions. The biosorption mechanism was proposed through the adsorption process between positively charged metal ions and the negative charge on the functional groups, such as $–COO^–$, $–OPO_3^{2–}$, and $–NH_2^–$, on the cell surface.

Key words: biosorption, heavy metal, FTIR, pH, lead, *Trichordema viride*

INTRODUCTION

Heavy metal pollution caused by both natural processes and human activities is a serious problem in the environment. At high concentrations, heavy metal ions poison aquatic life in the waters. These metal ions are non-degradable and are persistent in the environment [1]. One of the toxic metal ion is Lead(II). When absorbed in the body, Lead(II) can inhibit the activities of the enzymes, since this ion can form covalent bond with –SH group from cysteine or methionine residues [2]. In addition, Pb(II) tends to accumulate in the bones, due to similar ionic radii with Ca^{2+}. Therefore, the removal of Pb(II), in particular in the water, is essential to the protection of environment.

Conventional methods such as chemical precipitation, coagulation, solvent extraction, membrane filtration, osmosis, are being used for the removal of heavy metal ions from aqueous waste [3]. These methods have certain disadvantages, including incomplete metal removal, high reagents and energy requirements, often ineffective when heavy metals concentrations are higher than permissible concentration [4].

In recent years, bio-removal of metal ions has emerged as an option due developing economic and eco-friendly waste water treatment process [4]. Biosorption is a promising method for removing toxic metal ions with living and dead microbial cells from aqueous

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solutions [5]. It can be used efficiently for the treatment of large volumes of waste with low concentrations of pollutants. Thus, the process does not depend on the viability of biomass [6]. The application of microorganisms as biosorbents has several advantages due to their small size and the ability to adsorption, binding the concentrations of heavy metals from aqueous solutions including very dilute concentrations by certain types of inactive, dead and living microbes [7]. The same microorganisms with other microbial groups can accumulate metals from their external environment by of physics-chemistry and biological mechanisms [8]. In this process, the uptake of heavy metals occurs as a result of physico-chemical interactions of metal ions with the cellular compounds of biological species.

In the current work, the biosorption process of Pb(II) in aqueous solution are conducted using *T. viride*. The capacity of *T. viride* to adsorp metal ions has been studied before [8], and results showed that *T. viride* effectively adsorbed Ni^{2+} from the aqueous solution. Another study has been conducted [9], and the results indicated that *T. viride* had the capacity to adsorp Cr(VI), the adsorption model followed Langmuir model and Freudlich isotherm.

In the adsorption process, there are some factors that contribute to the adsorption capacity, including pH, contact time, the number of adsorbent and or/ adsorbate. Therefore, in this study, those factors are studied. The changes in the *T. viride* functional groups before and after biosorption process are followed by using FT-IR spectrometer. The FT-IR is particularly to monitor changes in the functional groups in *T. viride* affected by biosorption process.

EXPERIMENT

Chemicals and instrumentation

Culture of *T. viride* was obtained from the microbial stock collection of Food and Nutrition Laboratory, Gadjah Mada University. All reagents of analytical or higher purity grade were purchased from Merck or Sigma-Aldrich and were used as received: HCl (37% aqueous solution), HNO_3 (trace pure, 65% w/w in H_2O), NaOH (99.9%), ethanol (96%), n-hexane (≥95%, HPLC grade), $CoCl_2$, $CaC1_2$, $(NH_4)_2S0_4$, $MgSO_4.7H_2O$, KH_2PO_4, Na_2HPO_4, $(PbNO_3)_2$, NaCl, $NaKC_4O_6H_4$ (Na, K-tartrat), KCl, NH_4Cl dan K_2HPO_4, urea, phenol, CH_3COOH (100%; 1.05g/mL), CH_3COONa, and urea ($CO(NH_2)_2$). The growth media for *T. viride* was potatoes dextrose agar (PDA).

The determination of Pb(II) concentrations before and after biosorption process was conducted using Atomic Absorption Spectroscopy (AA-6200/Shimadzu). The functional groups changes in the *T. viride* before and after biosorption process was determined using FTIR spectrophotometer (8400S/Shimadzu).

Methods

Cultivation of T. viride

The solid growth medium for sub-culturing *T. viride* was PDA (potatoes dextrose agar). A loop full of *T. viride* was grown in the sterile PDA for 6 days, at 37 °C. Next, spores grown in the PDA was dissolved in 1 mL of sterile bi-distilled water. These were cultivated in 100 mL liquid growth medium in 250-mL Erlenmeyer flasks on a rotary shaker at room temperature, for 36 h at 150 rpm. The suspension was the *T. viride* inoculums.

Biosorption of Pb(II) using T. viride

The solution of Pb(II) was prepared from $Pb(NO_3)_2$ to achieve final concentration of Pb(II) 50 mg/mL. An aliquot the solution (25 mL) was transferred to 250-mL Erlenmeyer flask, and 10 mL of *T. viride* inoculums that contain 1.37 x 10^6 CFU and 25 mL of liquid growth medium were added to the solution. The biosorption process of Pb(II) was conducted

in batch system, at room temperature with different initial pHs at: 4; 4.5; 5; 5.5; 6; 6.5 and 7, for 24 h. The initial pH was adjusted with the addition of 0.1 M HCl or 0.1M NaOH. The process was repeated under the influence of different contact times at: 5; 10; 15; 20; 30; 40; and 50 h. The initial pH that contributed to the optimum amount of Pb(II) absorbed was then used, whereas other conditions were the same, such as using 10 mL inoculums and at room temperature. The effects of the initial concentrations of Pb(II) were determined using different concentrations of: 30; 40; 50;60; and 70 mg/mL. The conditions for this experiment were at room temperature, 10 mL of inoculum, at optimum pH, and at optimum contact time. At the end of each biosorption process, the filtrate was separated and the final Pb(II) concentrations in the solution were determined using AAS, and Pb(II) adsorbed (%) was calculated.

The precipitates were collected and were analyzed using FTIR spectrophotometer. The adsorption capacity was calculated in the optimum condition of pH, contact time, and concentration. The adsorption capacity (q) was calculated as the mole of adsorbed, measured at equilibrium, per number of *T. viride* colonies.

RESULT AND DISCUSSION

Influence of pH to the Biosorption of Pb^{2+} by *T. viride*

Figure 1 shows the relationship between pH variations and the percentage of Pb(II) adsorbed. The conditions used were at room temperature, biosorption time of 24 h, initial concentration of Pb(II) was 50 mg/mL, and the amount of inoculum used was 10 mL that contained 1.37 x 10^6 colonies of *T. viride.* The biosorption capacity of *T. viride* for Pb(II) was affected by the initial pH of aqueous metal solution. It was found that at initial pH 4, biosorption of Pb(II) by *T. viride* was 63.8%. The amount of Pb(II) adsorbed increased dramatically to 95% at pH 4.5. A further increase in pH adversely affected the biosorption capacity. At pHs of 5; 5.5; 6; 6.5; and 7, the amount of Pb(II) adsorbed decreased to 87.2%, 76.2%, 71.4%, 64.8%, and 60.8%, respectively. Therefore, the optimum pH condition obtained at pH 4.5.

The pH of the medium affects both the solubility of metal ions and ionization of the functional groups such as $-COO^-$, $-OPO_3^{2-}$, dan $–NH^-$ of the cell wall of *T. viride*; which are acidic and carry negative charges rendering cell walls to be potent scavenger of cations [10]. At very low pH, the functional groups remain in protonated form and create less conducive binding charges condition for the biosorption due to the reduction in negatively charged surface. However, with the increase in pH, the negative charge density on the cell surface increases due to deprotonation of the metal-bindings sites, thus increasing the attraction of metallic ions with positive charge and allowing the biosorption on the cell surface [11]. Several researchers have investigated the effect of pH on biosorption of heavy metal ions by using different kinds of microorganism and have reported the similar results [2, 12].

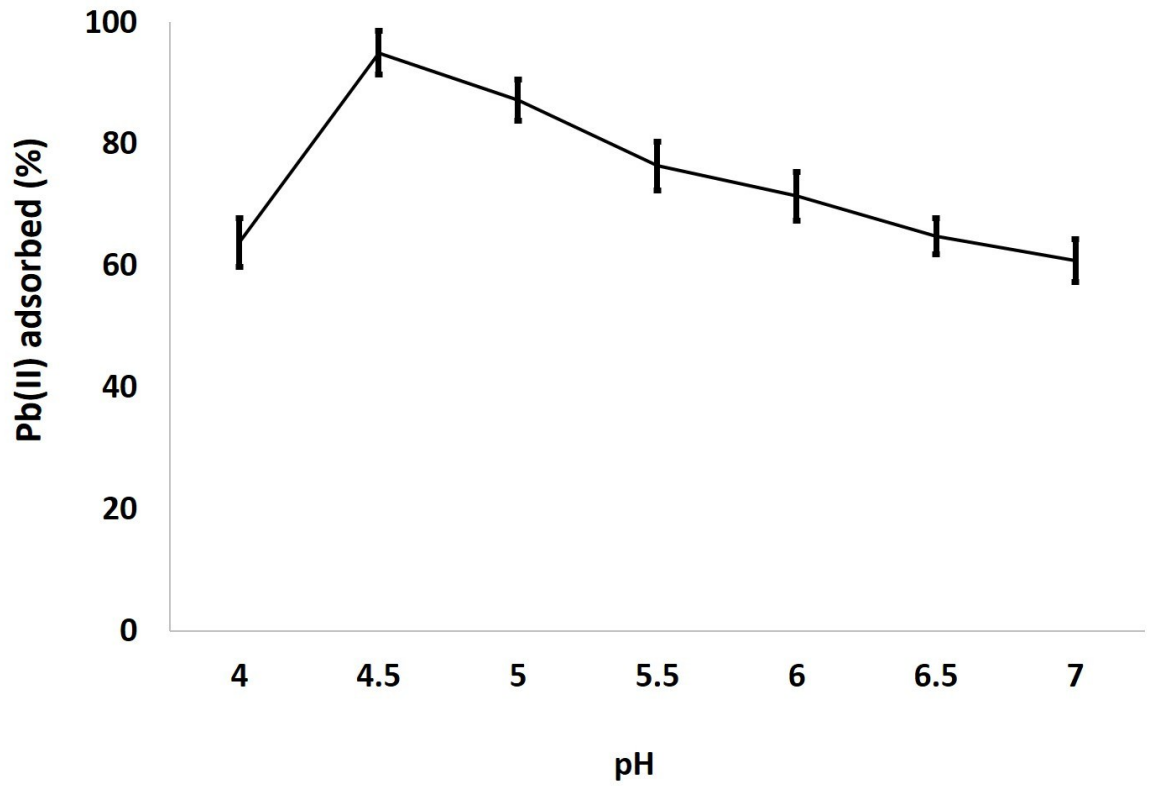

Figure 1. Result of biosorption of Pb(II) using *T. viride* under the influence of pH. Values are means and standard deviation of three replicates experiment

Influence of Contact Time to the Biosorption of Pb(II) by *T. viride*

The effect of contact time to the biosorption process of Pb(II) by *T. viride* is presented in Figure 2. The conditions applied were at room temperature, initial concentration of Pb(II) was 50 mg/mL, at pH 4.5, and the amount of inoculum used was 10 mL that contained 1.37 x 10^6 colonies of *T. viride.* In the initial 5 h of contact time, the amount of Pb(II) adsorbed was 40.3%. Increasing contact time led to increase in the amount of Pb(II) adsorbed to 67.8%, 77.6%, and 90.2% at 10, 15, and 20 h, respectively. Saturation levels were gradually attained after 20 h. Thus, Pb(II) biosorption was found to be in a two-stages process. This consist of an initial rapid passive binding of metals to negatively charged sites on the cell walls, followed by a slower active uptake of metal ions in the cells. These results are in agreement with biosorption studies of various metals studied with groups of microorganisms, where higher rates of metal binding have been reported before [3]. The biosorption kinetics observed in biosorption process is proposed by involving no energy-mediated reactions and metal removal from solution is due to purely physico-chemical interactions between microorganism and metal solution [13].

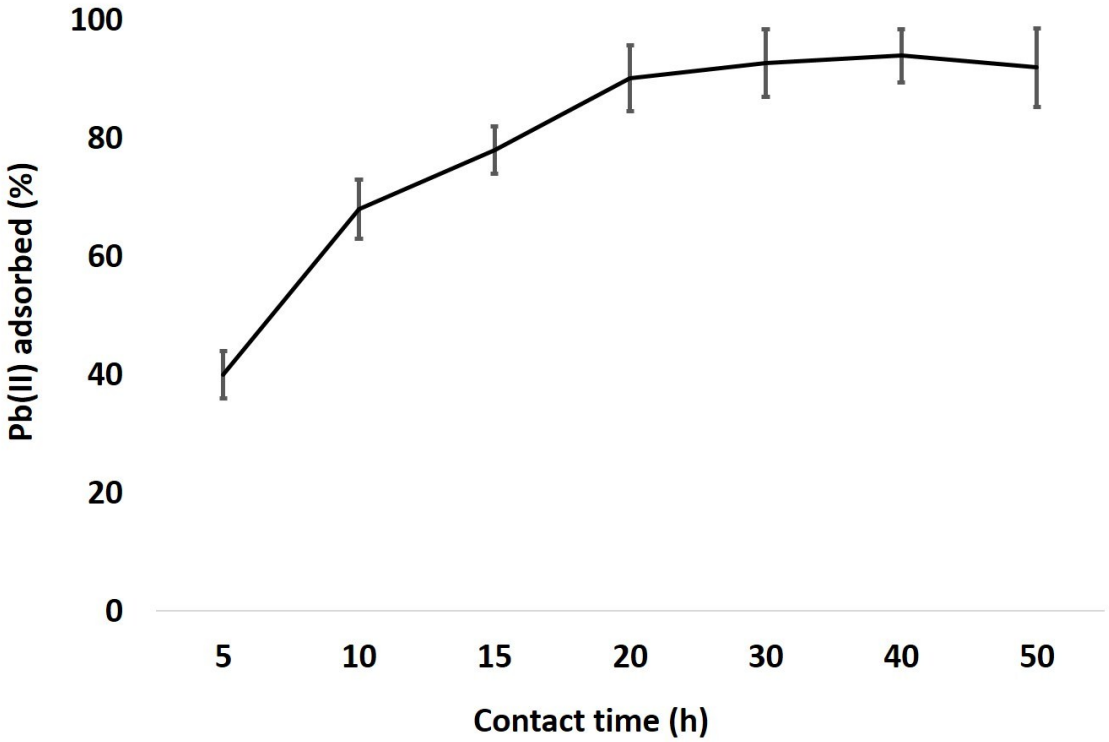

Figure 2. Result of biosorption of Pb(II) using *T. viride* under the influence of contact time. Values are means and standard deviation of three replicates experiment.

Influence of Pb(II) Concentration to Its Biosorption by *T. viride*

Effect of initial Pb(II) ions concentration on its biosorption by *T. viride* was investigated by incubating *T. viride* with previous optimum conditions that obtained before (pH 4.5, contact time 20 h), with 100 mL of Pb(II) solutions of concentration ranging from 30 to 70 mg/mL. It was found that the amount of Pb(II) taken up by the cells increased with an augmentation of Pb(II) concentration from 30 to 50 mg/mL rapidly, in the percentages of 51.5%, 67.2%, and 77.4%, respectively. Relatively slow increase was observed at concentration greater than 50 mg/mL. In addition, at concentration of 60 and 70 mg/mL, the amount of Pb(II) adsorbed did not differ significantly than the amount of Pb(II) adsorbed at concentration of 50 mg/mL, at 79.8% and 79.5%, respectively. Therefore, the 50 mg/mL concentration is the concentration of adsorption equilibrium.

The influence of concentration in the biosorption can be explained that at the beginning of the process, active sites at the cell walls of *T. viride* adsorbed metal ions fast. This may be due the unoccupied of the negatively active sites on the surface of cell walls, or may be due to the higher collision between the metal ions and negatively functional groups [14]. At high equilibrium concentration, uptake of Pb(II) ions owing to surface binding was negligible due to saturation of biosorbent-binding sites. The slow increase in biosorption capacity at high concentrations could be related to the different concentration gradient between the solution and the inside of the microbial cells. At very high solute levels, solid-liquid equilibrium becomes limited by diffusion of metal ions into the cell [15]. The limiting factor for the availability of active sites to bind metal ions indicates that adsorption can achieve equilibrium and saturation. In these conditions, pH 4.5, contact time 20 h, and concentration of Pb^{2+} 50 mg/mL, the adsorption capacity was calculated, the adsorption capacity of *T. viride* toward Pb(II) was 8.5 x 10^{-5} mg/colony, or 85 mg/1 x 10^{6} colonies of *T. viride*.

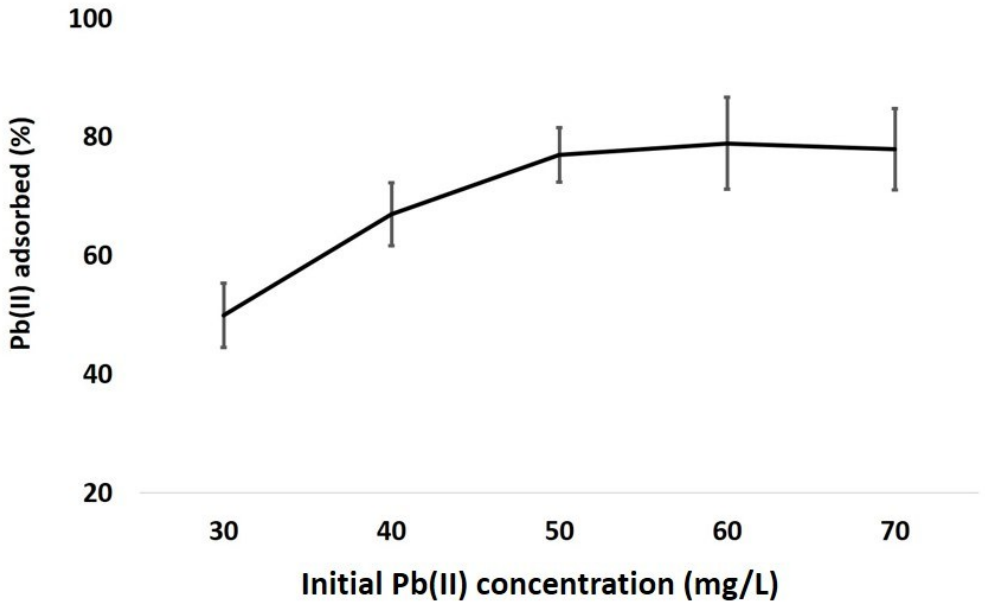

Figure 3. Result of biosorption of Pb(II) using *T. viride* under the influence of initial Pb(II) concentration. Values are means and standard deviation of three replicates experiment.

Changes in the Functional Groups of *T. viride* in the Biosorption Process of Pb(II)

In this study, solid samples from biosorption process separated from their filtrates have been characterized their functional groups before and after Pb(II) biosorption. Figure 4 shows the FTIR results from *T. viride* (before biosorption), and after biosorption in the pH 4.5, contact time 20 h, and concentration of 50 mg/mL. The conditions chosen were optimum conditions.

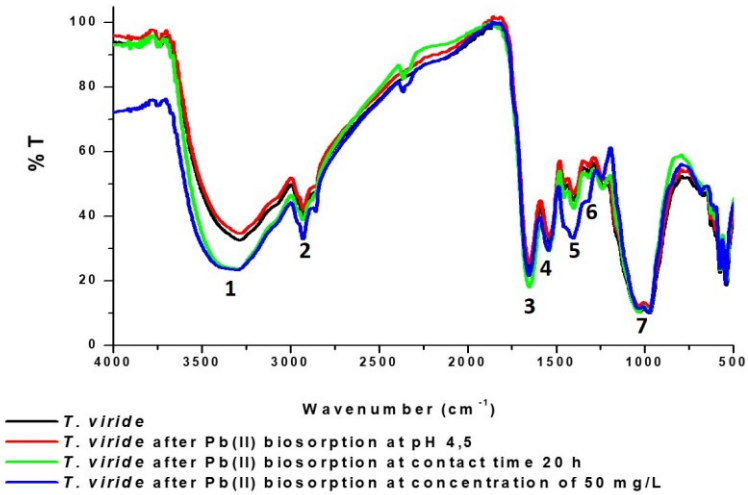

Figure 4 The FTIR spectra from *T. viride* and after biosorption process of Pb(II) by *T. viride* under the influence of optimum conditions of pH 4.5, contact time of 20 h, and initial concentration of 50 mg/mL. The number labelled with 1-7 indicated functional groups affected by biosorption process.

As can be seen in Figure 4, there were seven significant areas changed due to biosorption process of Pb(II). Those areas were 3450-3100 cm^{-1}, 2850-2950 cm^{-1}, 2260-2120 cm^{-1}, 1690-1630 cm^{-1}, 1180-1160 cm^{-1}, and 1000-990 cm^{-1}. The band assignment for those are N–H bending from amine group, C–H stretching from alkane, C≡N stretching from amide, C=O bending from amide, C–H bending from alkane, C–N bending from amine group, and C–O bending from ester and or/ ether [16, 17]. In addition, in the finger printing regions (1000-500 cm^{-1}) also showed some changes, these regions are generally phosphate groups and alkanes bands from nucleic acids (DNA) [18]. The changes and relative intensities in the FTIR bands are tabulated in Table 1. Under the influences of pH and contact time, all bands showed increases in intensity, whereas concentration did not lead to the same changes. Under the influence of concentration, most band intensities decreased, only band at 2260-2120 cm^{-1} showed increase intensity, while band at 1000-900 cm^{-1} showed relatively similar intensity, compared to *T. viride* bands.

From Figure 4 and Table 1, it is suggested that IR peaks that changed during biosorption process was mainly from proteins and lipids. Moreover, carbohydrates and also nucleic acids also showed little changes after biosorption process. This is possible since *T. viride* as biosorbent is also microorganisms that contains biomolecules, including proteins, lipids, carbohydrates, and nucleic acids.

Taken together, data from the biosorption and FTIR suggest that mechanism of biosorption of metal ions by *T. viride* in this study is proposed through the adsorption on the surface of the cells. This is possible, as in extreme conditions, for instance in environment with high metal ion concentrations, such as designed in this study, the *T. viride* will undergo a detoxification mechanism [18]. The mechanism of detoxification of heavy metal ions is carried out by extracellular formation of polymers that preventing the entry of metal ions into the cells [19, 20]. These were achieved by binding metal ions to the cell surface, depositing metal ions around cell walls and extracellular medium, or transforming metal ions into non-toxic forms [20]. The binding of Pb(II) by *T. viride* involves interactions of positive metal ions on the negatively charged cell walls of the extracellular polymer of *T. viride*, such as -COO^-, -OPO_3^{2-}, dan $-NH^-$.

Table 1. FTIR changes in intensity of bands of interests relative to *T. viride* bands under the influence of optimum conditions of pH, contact time, and concentration

No	Wavenumber (cm^{-1})	Assignment	pH*	Contact Time*	Concentration*
1	3450-3100	N–H amine	↑	↑	↓
2	2850-2950	C–H alkane	↑	↑	↓
3	2260-2120	C≡N	↑	↑	↑
4	1690-1630	C=O amide	↑	↑	↓
5	1350-1470	C–H alkane	↑	↑	↓
6	1180-1160	C–N amine	↑	↑	↓
7	1000-990	C–O ester or ether	↑	↑	↔

* Relative changes to FITR bands of *T. viride* before biosorption of Pb(II). The up-arrow (↑) indicates increase in intensity, down-arrow (↓) indicates decrease in intensity, and flat-arrow (⟷) indicates no changes.

CONCLUSION

This work has described biosorption process of Pb(II) using *T. viride*, which can effectively adsorb Pb(II) from aqueous solution under the conditions of initial pH, contact time, and initial Pb(II) concentration. The optimum conditions obtained were pH 4.5, contact time of 20 h, and at concentration of 50 mg/mL, with the adsorption capacity of 85 mg/1x10^6 *T. viride* colonies. The proposed mechanism for biosorption is through adsorption of Pb(II) ions in the cell walls of *T. viride*, involving negatively charged functional groups in *T. viride* such as COO^-, $-OPO_3^{2-}$, dan $-NH^-$, and positive charged from metal ions. The FTIR spectra revealed that the groups that are affected during the biosorption process are mainly from proteins and lipids, and also slightly from carbohydrates and nucleic acids. The biosorption process of Pb(II) using *T. viride* can be applied as one of green alternative solutions for heavy metal removal in the environment.

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