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Abstract

This study investigated the reduction of Pb(II) by bioaccumulation and biosorption in an artificial biosorbent. The biosorbent was prepared by mixing grounded manure and fine-grain sand at a ratio of 1:1 (w/w) with the media packed into a laboratory scale column (diameter of 4.7 cm). The sweet soy sauce (food grade) solution with pH ranged of 5.0-5.5 and the concentration of 15 g COD L⁻¹ was fed at a rate of 500 mL d⁻¹ to the column surface for a period of 101 d. The active biofilm was then acclimatised for another 79 d, by adding 0.2 mg L⁻¹ of Pb(NO₃)₂ into an acidic substrate solution. After 180 d, biofilm was matured as the removal efficiencies of COD and Pb(II) were constant at 30 and 60%, respectively. The rate of bioaccumulation was evaluated using the modified Gompertz model. The living microbes can gradually consume Pb(II) and the bioaccumulation efficiency is 14.0 mg Pb g⁻¹ organic matter (OM). The Pb(II) ions act as the trace element, which can enhance the growth of microbes in the biosorbent. The biosorption can be described using Freundlich model. Under the acidic Pb(II) solution (pH 4.0), the highest distribution coefficients of biosorption is 6.3 mg Pb g⁻¹ OM, and the sorbed Pb is biochemically fixed onto OM. The acidic Pb(II) solution can prevent the deposition of Pb(II) prior to biosorption. Pb(II) can be stored in accordance with the microbial cell activities by 56% of total Pb(II) removal. Approximately, 44% of Pb(II) is adhered on OM. At the active zone of biosorbent, the free Pb(II) species in the forms of soluble and exchangeable Pb(II) are well sorbed. The biosorbent could present the high benefit to bound the toxic Pb(II) even the contaminated water is highly acidic (pH = 4.0).

Keywords

biomathematical, model, application, bioaccumulation, pb, biosorption, ii

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Application of biomathematical model for Pb(II) biosorption and bioaccumulation

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ABSTRACT

Key Words: Adsorption isotherm, bioaccumulation, biomathematical model, biosorption, lead, modified Gompertz model

This study investigated the reduction of Pb(II) by bioaccumulation and biosorption in an artificial biosorbent. The biosorbent was prepared by mixing grounded manure and fine-grain sand at a ratio of 1:1 (w/w) with the media packed into a laboratory scale column (diameter of 4.7 cm). The sweet soy sauce (food grade) solution with pH ranged of 5.0-5.5 and the concentration of 15 g COD L⁻¹ was fed at a rate of 500 mL d⁻¹ to the column surface for a period of 101 d. The active biofilm was then acclimatised for another 79 d, by adding 0.2 mg L⁻¹ of Pb(NO₃)₂ into an acidic substrate solution. After 180 d, biofilm was matured as the removal efficiencies of COD and Pb(II) were constant at 30 and 60%, respectively. The rate of bioaccumulation was evaluated using the modified Gompertz model. The living microbes can gradually consume Pb(II) and the bioaccumulation efficiency is 14.0 mg Pb g⁻¹ organic matter (OM). The Pb(II) ions act as the trace element, which can enhance the growth of microbes in the biosorbent. The biosorption can be described using Freundlich model. Under the acidic Pb(II) solution (pH 4.0), the highest distribution coefficients of biosorption is 6.3 mg Pb g⁻¹ OM, and the sorbed Pb is biochemically fixed onto OM. The acidic Pb(II) solution can prevent the deposition of Pb(II) prior to biosorption. Pb(II) can be stored in accordance with the microbial cell activities by 56% of total Pb(II) removal. Approximately, 44% of Pb(II) is adhered on OM. At the active zone of biosorbent, the free Pb(II) species in the forms of soluble and exchangeable Pb(II) are well sorbed. The biosorbent could present the high benefit to bound the toxic Pb(II) even the contaminated water is highly acidic (pH = 4.0).

INTRODUCTION

The problem of lead contamination in groundwater is seriously concerned, particularly in the western region of Thailand, as a result of mining activities. The slag or residue contains high concentration of lead. The Pb contaminated wastes can be flushed to surface and groundwater resource. The extremely high concentration of lead is detected in water and crops, which

causes the lead poison in animals and human. Naturally, lead is mostly found in the form of inorganic lead and it is classified as the toxic heavy metal, which can pose the risk on human health and destroy the ecological system [1]. In order to prevent the movement of lead at source, the in-situ remediation technique might be employed. The permeable reactive barrier (PRB) is a novel technology, which can separate Pb(II) from aqueous solution by biosorption and bioaccumulation

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[2]. The active media with highly adsorptive surface is normally used for biomedica construction and the media are good habitat for the active microbes. The biofilm microbes play a significant role in controlling the propagation of Pb(II) in surface and subsurface systems. However, the growth of biofilm and the formation of PRB are little known [3-5]. A significant effort is put into this work to develop the predictive tool, which can describe the growth of biofilm forming microbes and their activities involved biosorption and bioaccumulation of Pb(II) on the synthesised PRB. This work outlines the development of biomathematical model involving the key factor for Pb remediation technologies and chemical or biological immobilisation of metals.

The predictive tools include the modified [6], the Monod kinetics [7] and adsorption isotherm [8]. The growth and rate of substrate consumption of the bio-system can be defined by the modified Gompertz model. The Monod kinetic equation is applied to examine the specific microbial growth rate, which is influenced by the substrate utilisation and the substrate inhibition. Mostly the mechanism of lead remediation in solid phase is an adsorption process, which can be detected by adsorption isotherm. The biomathematical models are employed to define the interactions among Pb(II), dead and living biomass, which can contribute to Pb(II) retention in biosorbent. Both Pb(II) bounded to organic or ionic constituents are assessed through FTIR (Fourier Transform Infrared Spectroscopy) and sequential extraction to illustrate the stability of sorbed Pb(II) and its interactions with biosorbent.

MATERIALS AND METHODS

1. Preparation of Biosorbent

Physical and chemical properties of biomaterial (manure) and sand are examined including bulk density, permeability, cation exchange capacity, pH, nutrients and metal compounds. The test procedures are conducted in accordance with the ASTM [9] and APHA [10] standards. The manure (soil 2:1 cow dung) with a diameter less than 2 mm and medium grain sizes sand between sieve size No. 50 (0.3 mm) and No. 40 (0.425 mm) is mixed together at the ratio of 1:1 (w/w). The manure is applied as a source of natural microbe and fine sand is used as the high permeability porous media. The mixture is packed into column, which has a diameter of 4.7 cm and effective height of 16 cm, without compaction. The bulk density of biomaterial is at 0.529 g cm^{-3} . Even though manure contains the organic carbon that can be supplied as a carbon source to microbes, it may not be enough to serve the active microbes. The sweet soy sauce (food grade) is then added to increase the levels of available substrates in the biosystem. The characteristics of sweet soy sauce are quantified in accordance with the

APHA [10] Standard Methods. The sweet soy sauce solution (15 g COD L^{-1} with an adjusting the pH at a range of 5.0-5.5) [11] is fed to inoculate the microbes. The substrate feeding rate to the column surface is 500 mL d^{-1} and it is controlled by the peristaltic pump. The quality and quantity of influent and effluent samples are daily determined. The constant COD removal efficiency confirms that the biofilm is matured within 101 d. The biofilm is then acclimatised by adding 0.2 mg L^{-1} of $\text{Pb}(\text{NO}_3)_2$ into an acidic sweet soy sauce solution with the mixed substrate solution COD 15 g L^{-1} . The mixed solution is fed at the same flow rate as at the incubation stage. The characteristics of influent and effluent samples are analysed daily. The biofilm is acclimatised for another 79 d until the removal efficiencies of COD and Pb(II) are constant. The experimental setup and equipments are illustrated in Fig. 1.

2. Experimental Procedure

After 180 d, the matured biosorbent is separately collected for every 3.5 cm as the presence of microbial community may vary layers by layers. The functional groups of biopolymer of every layers of biosorbent are examined by FTIR spectrometer [12]. The sequential extraction technique is employed to examine the various forms of Pb(II) adhered onto living microbes due to bioaccumulation [13]. The biomedica are separated into 2 parts which are non-autoclaved (A1) and autoclaved biosorbent (A2). Firstly, the bioaccumulation test is conducted using the samples of A1-biosorbent. The kinetic rate constants are analysed using biomathematical models, particularly modified Gompertz equation and the Monod equation [6,7]. The biosorbent samples are weighed at 1 g. These samples are mixed with solutions of $\text{Pb}(\text{NO}_3)_2$ at 2.5 and 4 mg L^{-1} at different pH levels (4, 7 and 9). The mixtures are shaken at 250 rpm at constant 25°C , until achieving the equilibrium time. The effluent samples from every tests are filtered and analysed for Pb(II) ion concentration by an atomic adsorption spectroscopy (AAS)

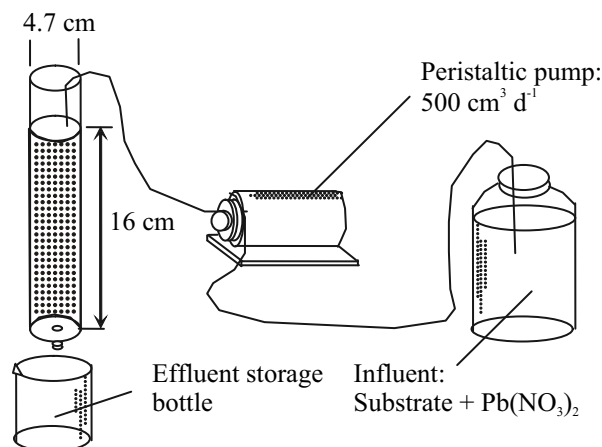


Fig. 1. Equipment setup.

according to NIOSH guidelines [14]. Secondly, biosorption test is conducted by modifying the isotherm test with A2-biosorbent. The different biosorbent samples (1-20 g) are mixed with Pb(II) solutions at 2.5 mg Pb L⁻¹ at pH 4. The mixtures are shaken at 250 rpm with temperature 25 °C, until achieving the equilibrium time. The filtered effluent samples were analysed for Pb(II) concentration by AAS according to NIOSH guidelines. The forms of Pb(II) adhered onto the biomass is determined via the sequential extraction method. The partitions of particulate Pb are categorised into five fractions, which are either bound-to-organic, bound-to-inorganic compounds or soluble Pb(II), by extracting with the various solutions under certain conditions as proposed by Tessier et al. [15].

3. Biomathematical Model Applications

The modified Gompertz model is employed to fit the observations of time versus Ln(C/C₀). The term C/C₀ refers to the remaining of substrate and Pb(II). The equations are presented as follows [6].

$$\ln\left(\frac{C}{C_0}\right) = A \exp\left[-\exp\left(\frac{\mu_m e}{A}(\lambda - t) + 1\right)\right] \quad (1)$$

where μ_m is the specific substrate consumption rate, λ is the delay reaction time (lag time), t is the observation time, and A is asymptote [$L_n(C/C_0)$].

The Monod kinetics equation is applied to express the growth-linked substrate utilisation of the living biomass in the batch Pb(II) biosorption experiment. The Pb(II) may involve the inhibition of substrate utilisation. The equations are given as follows [7].

$$\mu = \frac{\mu_{\max} S}{K_s + S} I_{pb} \quad (2)$$

where S is the limiting COD substrate concentration (mg L⁻¹), K_s is the half saturate concentration constant (mg COD L⁻¹), μ_{\max} is maximum specific growth rate (h⁻¹) and I_{pb} is the empirical term for Pb(II) inhibition.

The biosorption experiment for the dead biomass is fitted by the isotherm models. The Langmuir and Freundlich models are employed. The Langmuir model is suited to the uniform layer formation of adsorbate, which can provide the homogeneous active site. The linearised Langmuir isotherm is given as follows [16].

$$\frac{C_e}{q_e} = \frac{1}{bQ_{\max}} + \frac{C_e}{Q_{\max}} \quad (3)$$

where q_e is the amount of Pb(II) adsorbed per unit weight of biosorbent (mg Pb g⁻¹), C_e is the Pb(II) concentration in aqueous solution at equilibrium (mg L⁻¹), Q_{\max} is maximum adsorptive capacity (mg Pb g⁻¹) and b is the adsorptive intensity (L mg⁻¹ Pb).

To determine the degree of favourable adsorption, the dimensionless constant, R_L is defined. The equation can be written as follows [16].

$$R_L = \frac{1}{1 + bC_0} \quad (4)$$

where C_0 is the initial Pb(II) concentration (mg Pb L⁻¹). The favourable adsorption is presented when $0 < R_L < 1$. The $R_L > 1$ and $R_L = 1$ are the unfavourable and linear adsorption, respectively. The $R_L = 0$ is the irreversible adsorption.

The Freundlich isotherm model is served to evaluate the adsorption with the non-uniform distribution. This adsorption pattern indicates that the active adsorbent has the heterogeneous surface. The linearised Freundlich is expressed as follows [16].

$$\text{Log} q_e = \text{Log} K_F + \frac{\text{Log} C_e}{n} \quad (5)$$

where K_F and n are the Freundlich constants. The constant n refers to the level of favourable adsorption and K_F presents the adsorption capacity of adsorbent. If the value of $(1/n)$ is close to 1, it indicates a normal Langmuir isotherm.

RESULTS AND DISCUSSION

1. Properties of Manure and Substrate Solution

The average values of triplicate sampling are presented in Table 1. The laboratory experiment has shown that the manure itself contained the organic substances and nutrients, which can be supplied to the microbes. The pH of manure is slightly alkaline. The manure can be the habitat of natural living microbes [17]. However, the ratio of COD:TKN:soluble P of manure is 295:6.5:1. The ratio of these substrates is higher than the recommended ratio for biological treatment of C:N:P ratio 250:5:1 [18]. However, the manure may partially contain the non-available C and N substrates. To be ensured that the microbes can obtain enough consumable substances C and N, the sweet soy sauce was fed to inoculate the living microbes. The ratio of COD:TKN:soluble P of sweet soy sauce is 160:2:1. Nevertheless the C:N:P of sweet soy sauce is lower than the recommended ratio [18], the fractions of available C, N and P of sweet soy sauce is highly and promptly to be consumed by microbes. Therefore, the solution of sweet soy sauce is employed to the system in order to enhance the assimilation process of microbes.

2. Biofilm Formation

Under natural condition, the mixed microbial species at contaminated site can tolerate with the Pb(II) contaminants. The physical appearance of biofilm is shown in Fig. 2. The biofilm producing seeds were from manure or cow dung. The natural microbial seeds were incubated with sweet soy sauce to increase the biomass of Pb(II) consuming microbe prior to

Table 1. Properties of manure and sweet soy sauce

Parameters	Manure	Sweet soy sauce
COD (g kg ⁻¹)	678	606
TKN (g kg ⁻¹)	14.9	7.9
Soluble P (g kg ⁻¹)	2.3	3.9
pH*	8.00	4.33
Organic Matter, OM (%)	39.3	N/A

*pH (1:5) was applied to manure sample, but the conventional pH measurement was used to sweet soy sauce solution. N/A is non-available.

acclimatisation with Pb(NO₃)₂. A thick biofilm was observed within 111 d after feeding the synthetic acidic substrate with Pb(II) solution at a hydraulic loading rate of 0.291 m³ m⁻² d⁻¹ (500 mL d⁻¹). However, a thick layer of dark coloured biofilm is presented on the top layer of 0-1 cm only. The clogging and ponding had been observed since the loading rate was reduced to 0.174 m³ m⁻² d⁻¹ (300 mL d⁻¹). The removal efficiencies of COD and Pb(II) were constant at 30 and 60%, respectively. The biomedias were then removed from the column to determine their properties.

The FTIR spectra can reflect the functional groups of biopolymer observed in the thick film layer on biosorbent, the results are illustrated in Fig. 3. The possible functional group at every assigned wave number are summarised in Table 2 [19-23]. Table 3 provides the summary of the presence of functional groups of the biopolymer in each layer along the biosorbent. The functional groups of alkyl, hydroxyl or amino substituent and C=O stretching in carboxyl and amide group are observed in the entire column as same as the raw biomaterial. These functional groups present in the background of the raw biomaterial are hydrophobic biosorbent containing the long chain of exchangeable

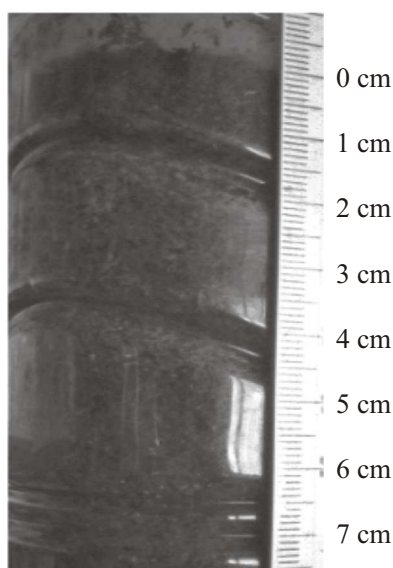


Fig. 2. Physical appearance of biofilm on the top layer biosorbent (0-7cm).

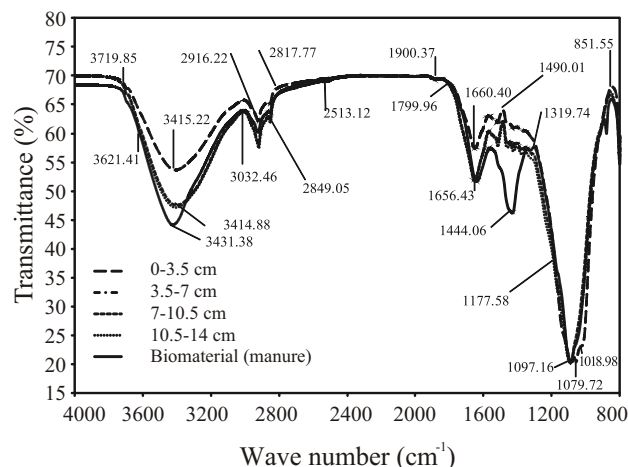


Fig. 3. FT-IR spectra of biosorbent and biomaterial.

anionic species. The functional group C=O stretching in carboxyl and amide group are developed after biofilm forming. These functional groups indicate that the biofilm can either bind or sorb the Pb(II) onto the peptide linkages of biofilm. The functional group C=O stretching in carboxyl and amide group is peaked at the top layer (0-3.5 cm), indicating the high numbers of peptide linkages that are originated from the biomass.

3. Bioaccumulation Test

The accumulation of sorbed Pb(II) in the non-autoclaved sample (A1-biosorbent) is determined at the top layers (0-3.5 cm) of biosorbent as the dense community of biofilm is obtained. The data are fitted with the modified Gompertz model (Fig. 4a). The pH of Pb(II) solution could inhibit the activities of living cell, therefore the substrate with Pb(II) solutions at pH of 4.0, 7.0 and 9.0 were studied. The bioaccumulation of Pb(II) onto A1-biosorbent occurred quickly, as the lag time is very short. Under pH levels of 4.0, 7.0 and 9.0, the rate constants of the reaction are 0.046, 0.050, 0.052 mg sorbed Pb(II) h⁻¹, respectively. The maximum accumulated Pb(II) on biosorbent under pH at 4.0, 7.0 and 9.0, are 14.0, 16.0 and 16.6 mg Pb g⁻¹ organic matter (OM), respectively. This suggests that under the alkaline condition (pH = 9.0), the biofilm can well accumulate the Pb(II). On the other hand the acidic condition (pH = 4.0) can inhibit the accumulation of Pb(II) into the biofilm. This effect is due to the fact that pH may influence bioactivity or stimulate Pb(II) precipitation. Since the forms of Pb(II) species may change with pH and some species can be deposited. The forms of Pb(II) species are evaluated using the diagram for concentrations of Pb(II) species at various pH level [24] as shown in Fig. 4b. Among these pH levels, the Pb(II) species are almost in the ionic form under pH of 4. The ionic Pb(II) species are completely dissolved in the substrate solution, resulting in the low amount of accumulated Pb(II) in the biofilm at this

Table 2. Possible functional group at assigned wave number [19-23]

Possible assignments	Wavenumber (cm ⁻¹)
H-bonded OH groups (polysaccharides) and NH ₂ stretching (proteins)	3600-3200
Aliphatic C-H stretching (fatty acids)	2960-2850
C=O carbonyl stretching, -CN stretching (amide I) group of protein peptide bond	1660-1650
C=N stretching (amide II)	1542
Phenolic -OH and C=O carboxylate stretching	1455-1423
C-H bending, CH ₃ stretching, COO symmetric stretching (amino acid side chains, fatty acids)	1396-1389
C-O-C and -OH stretching of polysaccharides	1207
P-O-C, P-O-H stretching (phospholipids, ribose phosphate chain pyrophosphate)	970-1022

Table 3. Functional group of biopolymer appeared in artificial biosorbent

Sample (depth below the surface)	Functional group of biopolymer			
	Hydroxyl or amino compound (3500-3200)*	Alkyl Group (C-H stretching) (3000-2850)*	C=O Stretching in carboxyl and amide group (1650-1550)*	Alkyl group-Hydroxyl or possibly amino substituent (1375-1300)*
Biosorbent 0-3.5 cm (inlet)	Yes	Yes	Yes	Yes
3.5-7 cm	Yes	Yes	Yes	Yes
7-10.5 cm	Yes	Yes	Yes	Yes
10.5-14 cm (outlet)	Yes	Yes	Yes	Yes
Biomaterial (manure)	Yes	Yes	No	Yes

*the wavenumbers (cm⁻¹) of FTIR spectra [22].

acidic pH. In the biosystem with the neutral condition (pH = 7.0), Pb(II) species are presented in the forms of $\text{Pb}(\text{HCO}_3)^+$. The cationic Pb(II) may be less accumulated in regard with the biopolymer containing the anionic exchangeable ions. On the other hand, the Pb(II) is mostly insoluble at pH of 9.0. Hence, it could not be denied that the Pb(II) species may be removed by the precipitation rather than bioaccumulation. Regarding the sequential extraction, Pb(II) can be mostly bounded with carbonate group and OM, forming the particulates of $\text{Pb}(\text{CO}_3)$ and $\text{Pb}(\text{II})$ -OM complexes. These results indicate that the Pb(II) bioaccumulation without precipitation occurs under acidic condition only. For the system with pH at 4.0, the accumulated Pb(II) species are predominantly in the form of Pb(II) bound to OM or microbial cell [15,25].

In the case of acclimatised microbial seed, the inhibition of Pb(II) on bioactivity may influence the biosystem. The bioaccumulation process of Pb(II) under the acidic pH is further studied in the macroscopic scale. The Monod model is employed to determine the linkage between substrate utilisation rate and microbial growth. The graphical plot of Monod model is illustrated in Fig. 5. The constants for Monod model are summarised in Table 4. Since the Pb(II) was accounted as the co-substrate or trace element, which could be biologically utilised by the acclimatised microbes, as indicated higher μ_{max} value of the biosystem fed by the pure substrate solution. The pure

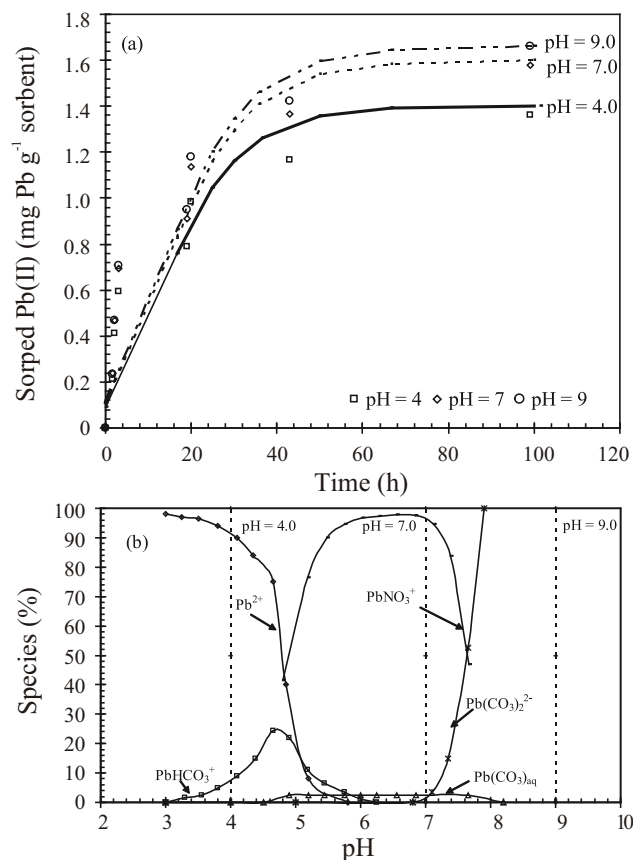


Fig. 4. (a) Biosorbent is fitted with modified Gompertz model and (b) Species of Pb(II) at various pH level.

solution with $[S_0] \ll K_s$, refers that the plenty amount of substrates are served to microbes. There is no substrate competitive condition in the biosystem when it is supplied with Pb(II) mixed substrate solution. In other words, the Pb(II) ions could stimulate the substrate utilisation of biofilm as they are trace element.

4. Biosorption Test

The pH of $Pb(NO_3)_2$ synthetic wastewater is controlled at 4, with only ionic Pb(II) species present. The reaction between Pb(II) and biosorbent can reach the equilibrium time within 100 min. The data of isotherm test are well fitted with Freundlich isotherm model. The Freundlich model constants are summarised in Table 5. The final pH of bioslurry samples is 7.6 which indicates that the manure had the pH buffering system, which could self-adjust and maintain the pH of biosystem at the neutral range. In accordance with the diagram for Pb(II) species at various pH levels, the Pb(II) species are stored in the manure by forming the precipitants of $Pb(CO_3)$ [26]. The manure has a low affinity and capacity for Pb(II) adsorption as the low values of $1/n$ and K_F are yielded. The adsorption isotherm results confirm that the Pb(II) can be adsorbed onto A2-biosorbent. The higher values of $1/n$ and K_F point out that the biosorbent can provide the higher affinity and capacity for Pb(II) biosorption. Since the value of $1/n$ is close to 1, the A2-biosorbent could sorb Pb(II) and form a mono layer of sorbed Pb(II) onto the surface. The A2-biosorbent represents the homogeneity surface, which can effectively biosorb Pb(II) species. The maximum biosorption capacity is $6.2 \text{ mg Pb g}^{-1} \text{ OM}$ at the top portion of biosorbent.

The sorbed form of Pb(II) on the biosorbent is determined by the sequential extraction process. The results are given in Fig. 6. After leaching with $Pb(NO_3)_2$ solution, a low amount of Pb remains in the biomaterial and the Pb species are mostly trapped onto OM. In the matured biosorbent, a higher amount of sorbed Pb is kept in that biosorbent than the initial biomaterial. The Pb is predominately fixed on the OM at the top layer, expecting that biofilm plays a significant role in Pb(II) sorption [27]. The high amounts of residual Pb are present in the entire column and the levels of residual Pb are constant in every layer. The residual Pb(II) refers to the inert Pb(II), which is sorbed by the organic constituents containing in the substrate solution. The amounts of Pb bound-to-Fe & Mn are stagnant along the column, assuming Pb(II) species are sorbed onto oxides of Fe and Mn originated from substrate solution too. Little amount of Pb bound to carbonate is present in the biosorbent. The Pb bound to carbonate cannot tolerate in the acidic pH condition, so this sorbed Pb may dissolve and liberate from the biosystem, or form the complex of $Pb(CO_3)$ -OM. Consequently, the amounts of total sorbed Pb are decreased along the depth of biosorbent, indicating the

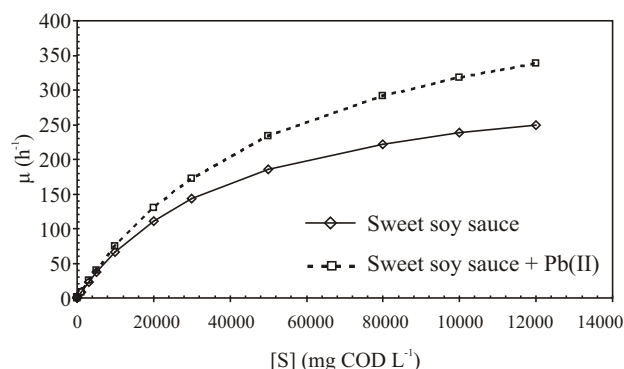


Fig. 5. Specific substrate utilisation rate fitted by Monod model.

Table 4. Constants for Monod equation

Substrate	K_s (g COD L ⁻¹)	μ_{max} (h ⁻¹)	R^2
Sweet soy sauce	4010	33	0.949
Sweet soy sauce + Pb(II)	57	500	0.855

Table 5. Parameters of adsorption isotherm on manure and biosorbent

Sample	$1/n$	K_F	R^2
A2-Biosorbent (autoclaved)	0.10	0.09	0.921
Biomaterial (manure)	1.04	0.62	0.917

absence of active living biomass. The soluble and exchangeable Pb(II) species are the major concern, as they can easily escape from the thin biofilm. The lower amounts of free Pb(II) could be observed at the lower portion of biosorbent. The finding suggests that the dead biomass can slightly retard the migrations of soluble and exchangeable Pb(II). This confirms that even the dead biomass, the harmful Pb(II) species in the forms of exchangeable and soluble can be stored as same as the living biomass in the fresh biosorbent.

The major mechanisms for Pb(II) restoration in this active biosorbent are bioaccumulation and biosorption. A 56% of Pb(II) is kept on living cell by bioaccumulation and another 44% of Pb(II) is sorbed onto dead cell by biosorption. In the active zone of biosorbent, the biopolymers play an important role in both bioaccumulation and biosorption. Besides, the synthetic biosorbent was acidic resistant, it can be applied to restore free Pb(II) from the acidic Pb contaminated wastewater.

CONCLUSIONS

Acclimatised biofilm from manure is prepared by mixing this natural culture with sweet soy sauce, and the biofilm is then acclimatised by feeding substrate mixed with Pb(II) solution. Until the culture can tolerate Pb(II), the biofilm is ready to work as biosorbent. The biosorbent contains the active microbe,

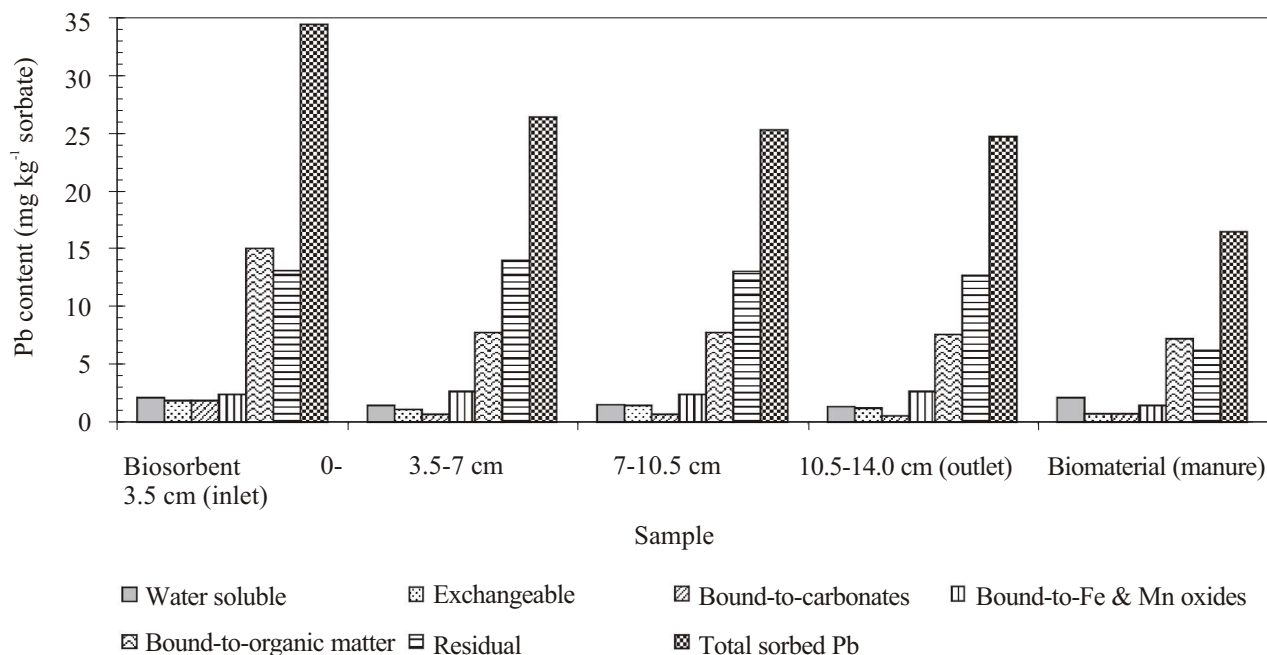


Fig. 6. Sequential extraction of biosorbent.

which can generate the biopolymers and they can adsorb and bind the Pb(II) via the biosorption and bioaccumulation mechanisms. The autoclaved specimens are employed to test the biosorption of Pb(II) onto dead biomass and the natural specimens are examined the biosorption and bioaccumulation of Pb(II) in the living biomass. At this stage, the effect of type of Pb(II) species on the activities of biofilm forming microbe can be described and the findings are useful for media and culture selection to fabricate the laboratory scale biobarrier. The bioaccumulation reaction by living biomass can keep 14 mg Pb g⁻¹ OM. In addition, the dead biomass can retard Pb(II) by sorption. The biosorption capacity is maximised at the top portion of biosorbent with 6.2 mg Pb g⁻¹ OM. Hence, the living microbes can restore Pb(II) approximately 56% by bioaccumulation and 44% by dead biomass sorption. The benefits of this artificial biosorbent include enrichment of actively acclimatised microbial communities, high pH buffering capacity and acidic pH resistance. The bioaccumulation and biosorption can be maintained, if the microbes are well functioned. In this study, the biosorbent can actively restore Pb(II) in a medium period (> 3 months).

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