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Biosorption of Pb and Cu using fixed and suspended bacteria

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ABSTRACT

To compare heavy metal (HM) adsorption capabilities between fixed and suspended morphologies of bacteria, batch adsorption tests of Cu(II) and Pb(II) were performed. Adsorption experiments were performed at 22 °C, neutral pH (7–7.5), and low concentrations of HMs (2.5–25 mg/l). A mixed bed bioreactor (MBBR) and a batch reactor were used to develop the fixed and suspended bacteria, respectively. The dominant bacteria were identified by 16s RNA sequencing as *Enterobacter ludwigii*, *Zoogloea ramigerais* and *Comamonas testosteroni*. The two morphologies exhibited significant differences in percent dry weight and in extracellular polymeric substances (EPS) content. The fixed bacteria displayed 29% EPS content by weight compared to 9.5% EPS found on the suspended bacteria.

The adsorption data fit the Freundlich isotherm for all trials, while data fit the Langmuir isotherm for Cu(II) only. The Langmuir adsorption data for Cu(II) revealed that the q_{max} was greater for fixed bacteria, at 9.80 mg/g compared to 6.52 mg/g for suspended bacteria. Maximum HMs removal was greater by fixed bacteria with 83.7% and 97.5% removal of Pb(II) and Cu(II), respectively, while suspended bacteria removed 72.6% and 83.8% of Pb(II) and Cu(II), respectively.

Introduction

Trace concentrations of HMs are naturally present in the environment, but many industries across the globe such as metal plating facilities, mining operations, fertilizer industries, tanneries, battery manufacturing, pulp and paper mills and pesticide production are contributing to the increase of heavy metal concentrations in our environment [1,2]. The concentration at which a metal becomes toxic will vary between metals, environments and organisms; however, all of the toxic HMs become hazardous at relatively low concentrations. The EPA has stated that the maximum contaminant levels for lead (Pb) and copper (Cu) in drinking water are 15 μg/l and 1.3 mg/l, respectively [3,4].

There are several methods to treat heavy metal contaminated wastewaters such as chemical precipitation, adsorption/biosorption, ion exchange, coagulation/flocculation, flotation, electrochemical, chemical oxidation, and membrane filtration [1,2,5,6]. Biosorption of HMs is a relatively new process that has been reported as a promising method for the removal of HMs from aqueous solutions [1,2]. The major advantages of biosorption are its high effectiveness in reducing heavy metal concentrations and the use of inexpensive biosorbent materials. The biosorption process is particularly suitable to treat dilute heavy metal wastewater.

In aquatic environments, toxic metals are easily absorbed by microorganisms because of their high surface area to volume ratio. This bioaccumulation process may also be used for bioremediation of HM contaminated aqueous solutions. By using bacterial biomass, the same high surface area to volume ratio works in favour of HMs adsorption [7]. Additionally, bacterial biomass displays many ionizable function groups on the cell wall, thus facilitating greater interaction with HM cations [8].

Bacteria are able to transform between suspended and fixed morphologies. The deliberate shift in gene expression required to take planktonic (suspended) bacterial cells to an attached biofilm community is very complex and is initiated by environmental signals detected by a quorum sensing mechanism [9,10]. Biofilm communities are advantageous and allow bacterial cells to adopt specific roles to benefit the biofilm as a whole. One such role is the production of large amounts of extracellular polymeric substances (EPS) [10]. The EPS of the biofilm further increases surface area and are rich in polysaccharides, proteins, nucleic acids and lipids, providing additional ionizable metal binding sites and a net negative charge [11,12].

Many studies have proven bacteria as a capable biosorbent for removal of HMs. The following are some of the recent relevant

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research studies. *S. polyrhiza* biomass was reported to have a maximum adsorption capacity (q_{eq}) of 137 mg Pb/g at optimum pH of 4, initial Pb(II) concentration of 160 mg/l, contact time of 120 min, and temperature of 20 °C [13]. The subtropical estuarine bacteria *Bacillus thioparans* strain U3 was used as a biosorbent for removal of Cu(II) and Pb(II). Equilibrium studies were performed at pH of 4 and 6 for Pb(II) and Cu(II), respectively. The percent removals for Cu(II) and Pb(II) were 46.7% and 94%, respectively. The maximum biosorption capacities were reported to be 27.3 and 90.1 mg/g for Cu(II) and Pb(II), respectively, at 25 °C [14]. *Pseudomonas Putidas* was used as a HM biosorbent at low concentrations of biomass (1 mg/ml) and HMs (0.1–18 mg/l) and neutral pH levels (6–7). The results obtained revealed an 80% reduction of HMs while obtaining much lower q_{eq} values of 6.6 mg Cu/g and 6.9 mg Zn/g [15]. Similarly, immobilized *Pseudomonas Aeruginosa* removed 73% of aqueous Pb(II) while obtaining a max q_{eq} of only 0.74 mg Pb/g [16].

The above studies, along with the majority of available literature, have attempted to optimize conditions to promote biosorption and maximize q_{eq} values. Neutral pH levels and low concentrations of HMs provide a more practical adsorption scenario because target waste streams have a pH of 6–8 and low concentrations of HMs.

The present study takes a novel approach towards Cu(II) and Pb(II) biosorption by developing and comparing naturally forming bacteria of two distinct morphologies i.e. the suspended and fixed bacteria. The study compares the characteristics and HM adsorption performance of these heterogeneous mixes of bacterial species at neutral pH (7–7.5) and low concentrations of HMs (2.5–25 mg/l).

Materials and methods

Bacterial biomass production

It is important to have a consistent source of bacterial biomass to test adsorption capabilities. Two distinct forms of bacteria were produced using two distinct bioreactors, seeded with the same initial bacteria [17]. To produce fixed bacteria, a mixed bed bioreactor (MBBR) was used. The MBBR provides a support material for the bacteria to attach to and grow in the form of a biofilm. To generate unattached (suspended) bacteria, a batch reactor system was used. This system does not provide a support surface for bacteria to grow upon, resulting in a planktonic morphology.

Mixed bed bioreactor (biofilm)

A 5L MBBR, shown in Fig. 1, was used to generate EPS rich bacterial biomass for HMs adsorption tests. The reactor operated under consistent conditions to ensure reproducible biomass for testing. The hydraulic retention time (HRT) of the reactor was maintained at 3 h to ensure low levels of suspended bacteria or low total suspended solids (TSS). The reactor was given a modified synthetic wastewater solution [17,18]. Three individual solutions combine to provide the nutrient demands of the bacteria: Carbon source, Macronutrients, and Micronutrients. Dextrose, Sodium Acetate and Peptone comprise the bulk of the nutrient demands, with a chemical oxygen demand (COD) of 380 mg/l. The reactor was maintained at a constant pH of 7.2. The biofilm was grown on K1 polystyrene media, also shown in Fig. 1. The reactor was completely mixed by air diffusers. The oxygenation rate was high and dissolved oxygen (DO) reached the saturation point of 8.9 mg/l at 22 °C. The bacteria used to seed this system were obtained from a local wastewater treatment plant.

Suspended batch reactor

A 1L completely mixed batch reactor was used to generate unattached bacteria for HMs adsorption tests. This reactor was seeded with the bacteria present in the MBBR in order to compare morphology effects on adsorption between attached and unattached growth. The reactor was operated on a 24 hour growth cycle where 80% of the volume was harvested for biomass and replaced with synthetic wastewater daily. The reactor received all of the daily nutrients: Carbon source, Macronutrients, and Micronutrients [18], in one dose, equal in proportion to the MBBR. The reactor was highly oxygenated, and DO reached a saturation point of 8.9 mg/l at 22 °C.

Microbial characterization

Natural bacterial populations are complex, heterogeneous communities that respond and change with all aspects of their environment [6,19,20]. By providing two reactor environments for the biomass to grow, two distinct forms of bacteria have been produced, and display different characteristics.

Biomass collection

Fixed bacteria were sloughed off of the k1 polystyrene media carriers (Fig. 1) as it was agitated and mixed by the air diffusers. The biomass separated from the carriers exited the reactor with the effluent stream. The fixed biomass was then separated from the

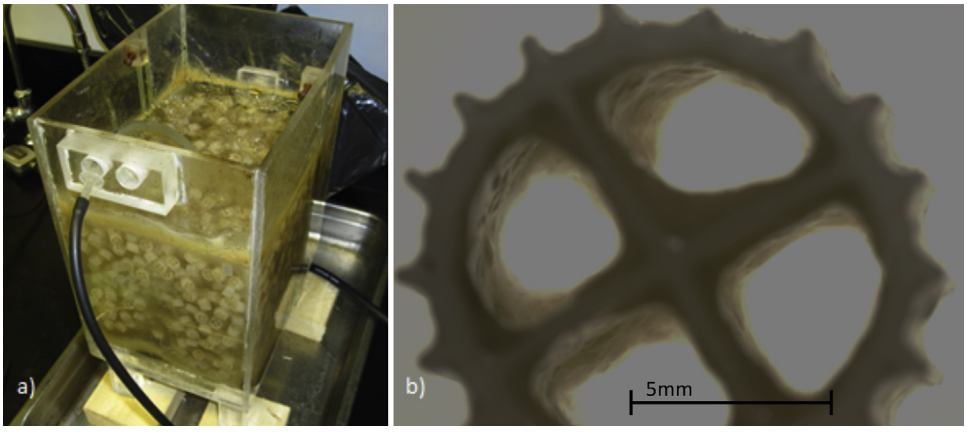

Fig. 1. (a) Image of the bench scale 5 L mixed bed bioreactor used to generate fixed biofilm biomass. (b) Image of a K1 polystyrene media with biofilm growth on the protected inner segments.

effluent by settling. The concentrated solution of biomass was then centrifuged at 8000 rpm for 8 min to pellet the bacteria without lysing the cells [21]. The collection procedure was done at regular intervals such that no biomass to be tested was stored in refrigeration longer than 1 week.

Suspended bacteria were collected daily from the batch reactor when 80% of the volume was replaced with synthetic wastewater. The removed volume was left to settle and the concentrated biomass was then centrifuged and stored in the same manner as the fixed bacteria.

In order to compare different morphologies of bacteria, the dry weight of the collected biomass must be determined. The dry weights of both bacteria were determined by weighing the live bacteria collected before and after desiccation in an oven at 110 °C for 24 h. The change in mass was attributed to the water weight of the bacteria.

EPS analysis

The two distinct morphologies of bacteria used in this study were attached (fixed growth) and unattached (suspended growth). Associated with the distinct morphologies are distinct gene expressions. Attached bacteria up-regulate genes to produce greater quantities of EPS in order to help with attachment and nutrient procurement [22,23]. To determine the amount of EPS present on the two types of bacteria, a modified EPS extraction procedure was used [24]. The suspended and fixed bacteria were washed with a 1 N NaOH solution for 4 h at 4 °C to dissociate the EPS from the bacterial cells. The samples were then centrifuged at 10,000 rpm for 30 min to pellet the bacterial cells and separate the EPS into the supernatant. Because the EPS was not needed for subsequent use, the supernatant was passed through a 0.2 μm pore filter. The filters were then dried and the difference in mass represented the amount of dry EPS captured. The dry mass of EPS can be expressed as a percent of the dry weight bacteria.

RNA testing and identification

The bacteria species used for adsorption experiments were determined by 16s RNA identification. To isolate the bacteria, five biofilm carriers (Fig. 1b) were agitated in 100 ml of sterilized water. The resulting solution was serially diluted at a 1:10 ratio and plated onto nutrient agar plates [25]. The plates were incubated at 37 °C for 36 h. After incubation, three distinct colonies were present on the agar plates. These colonies were analyzed via sanger sequencing technique, by GeneWiz Inc., to determine the 16s rRNA nucleotide sequence [26]. The nucleotide sequence was compared to an online data base via the NCBI nucleotide Basic Local Alignment Search Tool (nBLAST) to determine the best match for bacteria species present in the MBBR. Additionally, the phylogeny of the species was assessed on a tree generated by the nBLAST software [26]. Finally, the observed colony morphologies along with the bacterial cell shape all corroborated to strengthen support for the species identity.

HMs adsorption isotherm tests

Testing conditions

Each trial was completed in triplicate at 22 °C. 85.5 mg of dry weight equivalent (w/w) biomass was added to 50 ml of HMs solution (Pb(II) or Cu(II)) and agitated at 150 rpm for 24 h. Based on preliminary studies and literature 24 h ensured equilibrium had been achieved [14,27]. The HM solution concentrations tested were: 2.5, 5, 7.5, 10, and 25 mg/l. The solutions were prepared by diluting lead(II) and copper(II) standards [28]. pH of the solutions was adjusted to neutral pH (7–7.5) with drop wise addition of NaOH. All measurements were performed gravimetrically and individual results were corrected accordingly. When the interaction of bacteria and HM solution was complete, the biomass was pelleted by centrifugation and the supernatant was collected. The supernatant was stabilized by acidification with HNO_3. The stabilized samples were then analyzed for HM concentration using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Analysis (Varian Vista Pro). Cu(II) and Pb(II) were each monitored at four different wavelengths. Agreement between measured wavelengths showed accuracy and validity of ICP analysis. The difference between the initial concentration of HM and the final concentration after contact with biomass was the amount of HM adsorbed by the bacteria (Eq. (1)).

$$q_{eq} = \frac{(c_i - c_{eq})V}{X} \tag{1}$$

where q_{eq} is the equilibrium HM adsorption capacity in mg/g, c_i is the initial concentration of HM in mg/l, c_{eq} is the equilibrium concentration of HM in mg/l, X is the dry weight equivalent of biomass (g) and V the volume (L) of solution.

Adsorption isotherms

Adsorption is usually described through isotherms, that is, the amount of adsorbate (HM) on the adsorbent (biomass), as a function of concentration at constant temperature. The results obtained in this study were then fitted to Freundlich, Langmuir and Scatchard isotherm models to better define the adsorption process.

Determining the adsorption coefficients of the adsorption isotherm models was done by fitting a trendline in Excel. The solver add-on in Excel was also used to optimize the coefficient values by non-linear regression. The solver method allows the user to test multiple error functions to further optimize the approach. The two methods were then compared based on R^2 values and the one with a higher R^2 value was selected.

Freundlich isotherm. The Freundlich isotherm (Eq. (2)) along with the Langmuir isotherm (Eq. (3)) are the most common isotherm models used when describing the adsorption process. The Freundlich isotherm is a semi-empirical model [14,27,29].

$$q_{eq} = K_f c_{eq}^{1/n} \tag{2}$$

where q_{eq} and c_{eq} represent capacity for HM mass per unit mass of biomass at equilibrium (solid phase concentration) and concentration of HM remaining in solution at equilibrium (liquid phase concentration), respectively. K_f and n are the Freundlich constants which help to characterize the adsorption process.

Langmuir isotherm. The Langmuir isotherm (Eq. (3)) is used to describe mono-layer adsorption. The Langmuir isotherm assumes binding sites on the biomass are homogeneous or have equal affinity for the HM ions. Additionally, the Langmuir isotherm is purely theoretical [29,30].

$$\frac{c_{eq}}{q_{eq}} = \frac{1}{K_L q_{max}} + \frac{c_{eq}}{q_{max}} \tag{3}$$

where q_{eq} and c_{eq} are the same as previously defined. K_L and q_{max} are the Langmuir adsorption binding constant and the maximum binding capacity in mg of HM per g of biomass, respectively.

Scatchard plot. The Scatchard isotherm (Eq. (4)) is a derivation of the Langmuir isotherm and the plot helps to define the interaction between HM and biomass.

$$\frac{q_{eq}}{c_{eq}} = q_{max}K_L - q_{eq}K_L \tag{4}$$

where q_{eq}, c_{eq}, K_L and q_{max} are the same as defined by Langmuir isotherm. The Scatchard plot indicates whether or not the affinity

for HM changes through the adsorption process as HMs use binding sites. This steady adsorption affinity is indicated when there is a high R^2 value and visually, when the trendline of the plot holds a linear, negative slope [14,31].

Separation factor. The separation factor (SF) is a dimensionless constant resulting from the expression of either the Langmuir or Freundlich constant. The SF (Eq. (5)) is inversely related to the strength of binding. Smaller SF values indicate greater favourability of binding [31,32].

$$\mathrm{SF} = \frac{1}{1 + KC_0} \tag{5}$$

where C_0 is the initial concentration of HM in solution and K is either the K_L or K_F coefficient from the Langmuir or Freundlich isotherm, respectively.

Results and discussion

Microbial characterization

Although the fixed growth and the suspended growth were the same species of bacteria, the change in morphology between attached growth and unattached growth alters gene expression [9]. This change in gene expression results in the production of different groups of proteins and ultimately changes the phenotype of the organism [23].

Dry weight

The dry weight analysis revealed another difference between the two morphologies. The suspended growth had a dry weight of 9% while the fixed growth had a dry weight of only 3.5%. This would suggest that the fixed growth bacteria have a higher affinity for water molecules. This could be due to greater surface area, increased favourable binding sites or both.

EPS content

Another difference in morphology was observed in the EPS content. The suspended bacteria contained 9.5% EPS (w/w) of dry weight while the fixed bacteria EPS content was 29% (w/w) of dry weight. This shows that the change in gene expression has resulted in the fixed bacteria producing much higher quantities of EPS. Observations of increased EPS content in biofilms are consistent with literature [18,34,35]. The change in EPS percentage should affect the adsorption characteristics between the morphologies.

16s RNA testing and identification

The nucleotide sequence data of the 16s rRNA was used to identify the bacteria present in the bioreactors. The three dominant species present were identified as *Enterobacter ludwigii*, *Zoogloea ramigerais* and *Comamonas testosterone* (Table 1). *E. ludwigii* is gram-negative rod shaped bacteria [36]. In previous studies, the EPS used from isolated strains of *E. ludwigii* was shown to bind metals [37]. *Z. ramigera* is a gram-negative, aerobic, rod shaped bacteria commonly present and beneficial in sewage treatment systems [37]. *Z. ramigera* is known for producing a zoogleal matrix. The zoogleal matrix is a specific type of EPS known to surround this species and other species in biofilm communities and aids in floc production in waste water treatment [37,38]. *C. testosteroni* is a gram-negative bacterium commonly found in activated sludge and soils [39]. *C. testosteroni* has been classified as a metallophile and has also been implemented in metal remediation studies [40].

Microscopic imaging

Imaging from a compound microscope allowed for closer investigation of the fixed and suspended morphologies. Fig. 2 shows the diverse macro structure present in a natural biofilm as well as independent bacteria cells of the three species identified. Microscope imaging confirmed that all of the species found in the biofilm were rod shaped, which corresponded to literature findings on *E. ludwigii*, *Z. ramigerais* and *C. testosteroni* [36,37,39].

HM adsorption tests

The percentage of HM removed was determined using Eq. (6).

$$\%R = \frac{C_i - C_{\mathrm{eq}}}{C_i} \times 100 \tag{6}$$

where $\%R$ is the percent of HM removed and C_i and C_{eq} are the same as previously defined. Table 2 is a summary of all percent HM removal values for fixed and suspended bacteria at concentrations from (2.5–25 mg/l). Pb(II) removal ranged 62–84% and 65–73% for fixed and suspended biomass, respectively. Cu(II) removal ranged 76–98% and 56–84% for fixed and suspended biomass, respectively.

pH effects

The pH of solution has a great effect on the solubility of HMs. The adsorption tests were performed at a neutral pH (7–7.5) to mimic most natural systems. It is known that HMs precipitate out of solution at higher pH levels [41]. At a neutral pH some of the initial HM concentration is lost to precipitation and must be factored when determining the amount of HM adsorbed by the bacteria. Control tests were performed measuring HM concentration at low pH (2–2.5) and neutral pH. It was determined that 15% of Pb(II) and 18% of Cu(II) was lost to precipitation and cannot be attributed to biosorption.

Effect of concentration

The adsorption tests were performed at five different concentrations: 2.5, 5, 7.5, 10 and 25 mg/l. The q_{eq} (Eq. (1)) increased linearly with increasing initial HM concentration (Fig. 3). The results suggest that the adsorption capacity for both Pb(II) and Cu(II) is greater on the fixed bacteria. This is seen in Fig. 3 with the divergence between the data points of the fixed biomass and the suspended biomass. This divergence results in a greater q_{eq} for the fixed bacteria while adsorbing both Pb(II) and Cu(II) across all concentrations tested.

Isotherm analysis

Adsorption isotherm models are able to describe non-metabolic interactions between either organic or inorganic sorbates onto an

Table 1
Colony morphologies and species characterization of *Enterobacter ludwigii*, *Zoogloea ramigera* and *Comamonas testosteroni* as identified by 16 s RNA sequencing.

Species	Colony morphology			Cell shape	Gram	Metabolism
	Colour	Form	Elevation		(+/−)	
Enterobacter ludwigii	Yellow	Irregular	Raised	Rod	–	Aerobic
Zoogloea ramigerais	Yellow	Circular	Convex	Rod	–	Aerobic
Comamonas testosteroni	Cream	Circular	Convex	Rod	–	Aerobic

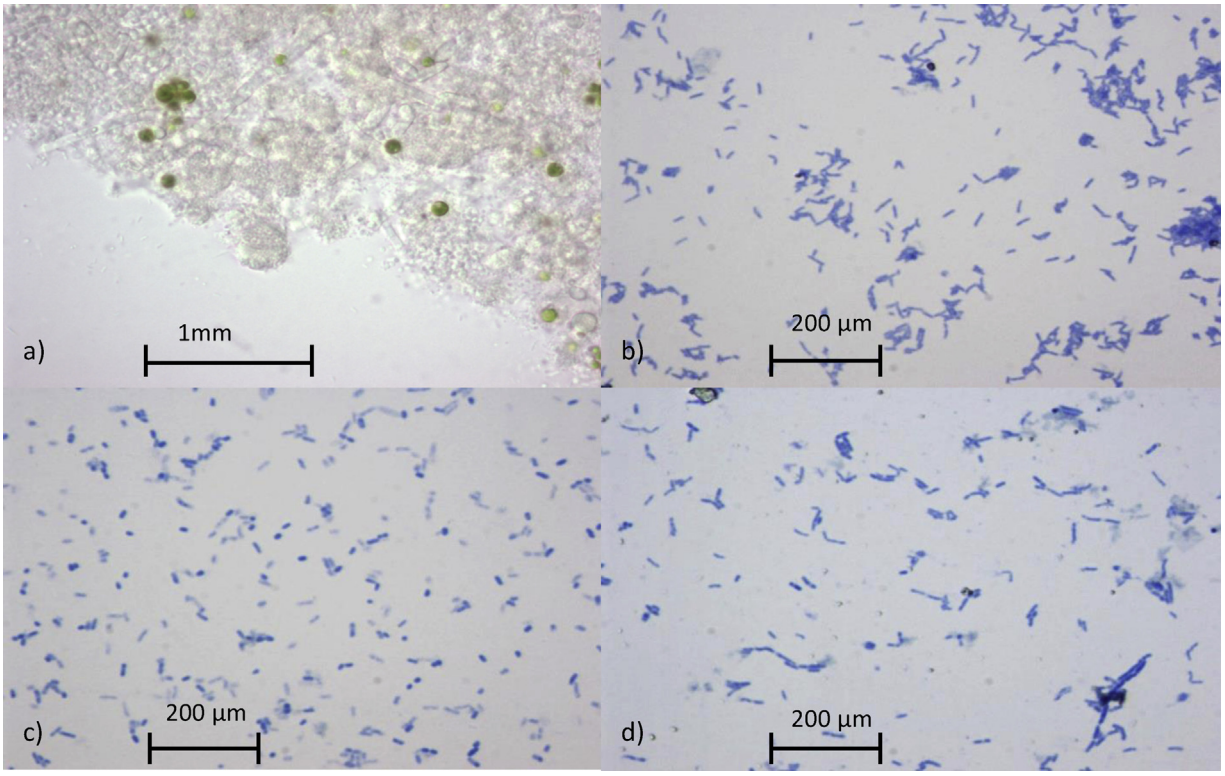

Fig. 2. (a) Image of fixed bacterial biomass community taken at 40× magnification detached from the k1 carrier. (b) Image of *Zoogloea ramigerais* rod-shaped bacterial cells taken at 100× magnification. (c) Image *Enterobacter ludwigii* rod-shaped bacterial cells taken at 100× magnification. (d) Image of *Comamonas testosteroni* rod-shaped bacterial cells taken at 100× magnification. Bacteria colony isolates grown on nutrient agar.

Table 2
Percent of Cu an Pb removed by suspended and fixed bacteria at different initial HM concentrations.

C_i (mg/l)	2.5	5	7.5	10	25
Fixed					
Pb	71	75.9	78.7	83.7	61.7
Cu	96.7	97.3	97.5	88.8	76.4
Suspended					
Pb	71.6	64.2	72.6	72.6	65.2
Cu	83.9	78.4	74.1	74	57.5

adsorbent [42]. The Freundlich, Langmuir, and Scatchard isotherm models were applied to the adsorption data to determine the coefficient values, which describe the adsorption process.

Freundlich isotherm. The Freundlich equation is useful for describing sorbent–sorbate interactions as mono layer binding with heterogeneous binding sites, with different affinities for the sorbate [30,42]. Fig. 4 displays the adsorption data using the Freundlich isotherm. The isotherm constants and the R^2 values are shown in Table 3.

From coefficient values, adsorption characteristics can be determined. The n value is an indication of the strength of interaction between HM and biosorbent, with higher n values indicating higher strength of binding [42,43]. The n values for both Cu(II) and Pb(II) adsorption are greater for the fixed biomass (2.26, 1.84) than the suspended biomass (1.09, 1.14), indicating a stronger interaction and adsorption for fixed biomass (Table 3). The K_f values describe the adsorption capacity of the biomass, with a higher K_f corresponding to a higher capacity for HM adsorption [42,43]. The K_f values were highest for the fixed bacteria when adsorbing both Cu(II) and Pb(II) (4.59, 2.44). Fig. 4 illustrates a close fit for both biomass types and both HMs.

K_f and n values vary with biosorbent and HM, however, the values found in the current study are comparable to the values

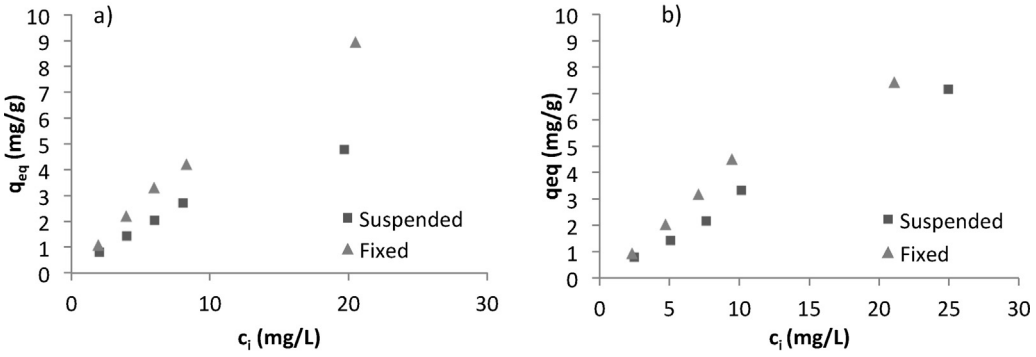

Fig. 3. Comparing the adsorption capacity (q_{eq}), of suspended biomass and fixed biomass at different initial HM concentrations (c_i), a pH of 7 and 22 °C. (a) Cu adsorption and (b) Pb adsorption.

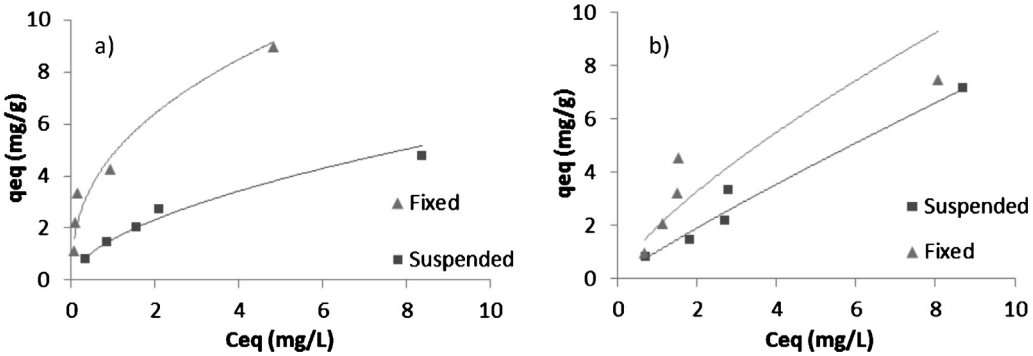

Fig. 4. Freundlich isotherms comparing the adsorption capacity (q_{eq}) of suspended biomass and fixed biomass at different equilibrium HM concentrations (c_{eq}). (a) Cu adsorption and (b) Pb adsorption.

Table 3
Isotherm coefficients and R^2 values for Freundlic, Scatchard and Langmuir Isotherms using fixed and suspended bacteria adsorbing Cu and Pb.

Metal	Biomass	Freundlich isotherm			Scatchard plot			Langmuir isotherm		
		K_F	n	R^2	K_L	q_m	R^2	K_L	q_{max}	R^2
Cu	Fixed	4.59	2.26	0.96	2.4	9.48	0.59	1.89	9.80	0.88
	Suspended	1.67	1.99	0.99	0.42	5.87	0.87	0.33	6.52	0.99
Pb	Fixed	2.44	1.84	0.86	NA	NA	0.03	0.29	10.79	0.91
	Suspended	1.07	1.14	0.97	NA	NA	0.05	NA	NA	0.14

determined by Ozdemir et al. [27]. The close fit to the Freundlich model was also found in much of the literature that detailed HM adsorption onto bacteria [20,33]. Fig. 5 shows the plot of predicted vs. observed values of q_{eq} for Freundlich isotherm, which again shows a good fit and the fact that Freundlich model describes the data well. Corresponding R^2 values (Table 3) are in the range 0.86–0.99.

Langmuir isotherm. The adsorption data from both forms of biomass for Cu(II) fit the Langmuir isotherm well (R^2 = 0.88–0.99), however, Pb(II) binding showed a poor fit to the model. The disparity between the measured and modelled data is clear in Fig. 6. The q_{max} for Cu(II) was higher on the fixed biomass (9.80 mg/g) compared to the suspended bacteria (6.52 mg/g),

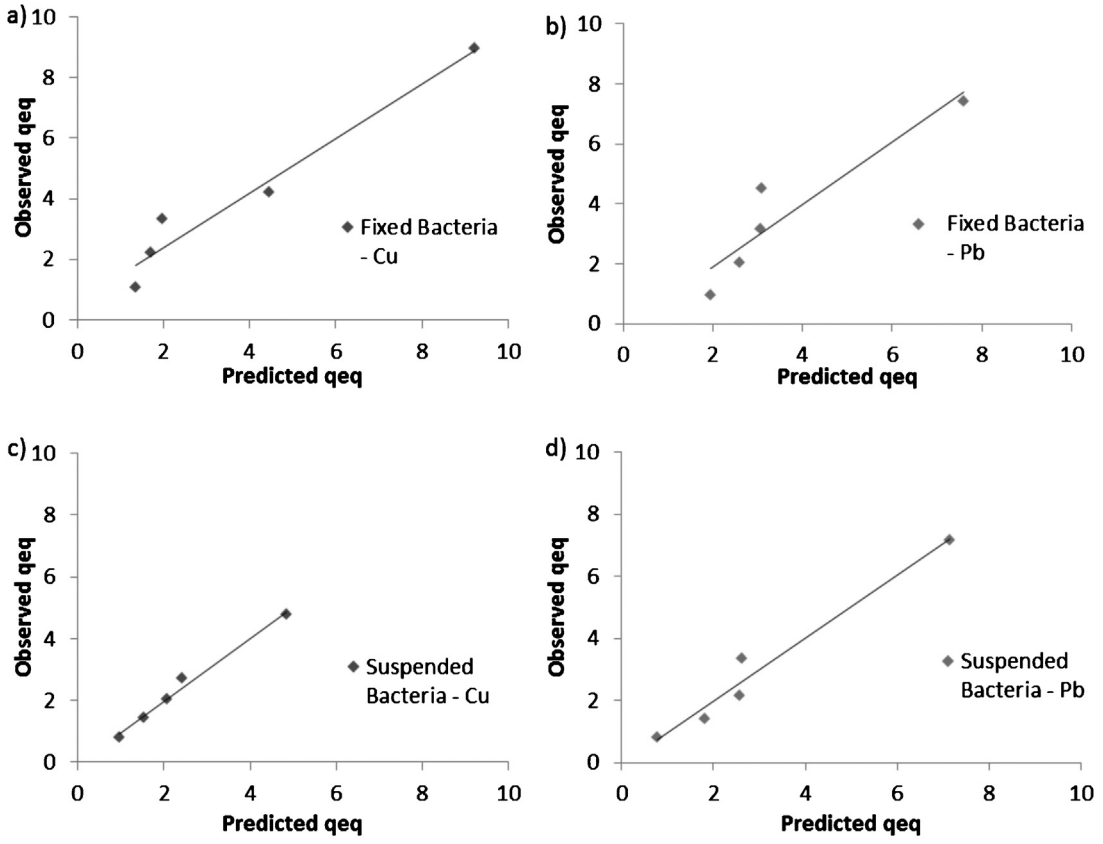

Fig. 5. Plots of measured q_{eq} versus modelled q_{eq} for Freundlich isotherm.

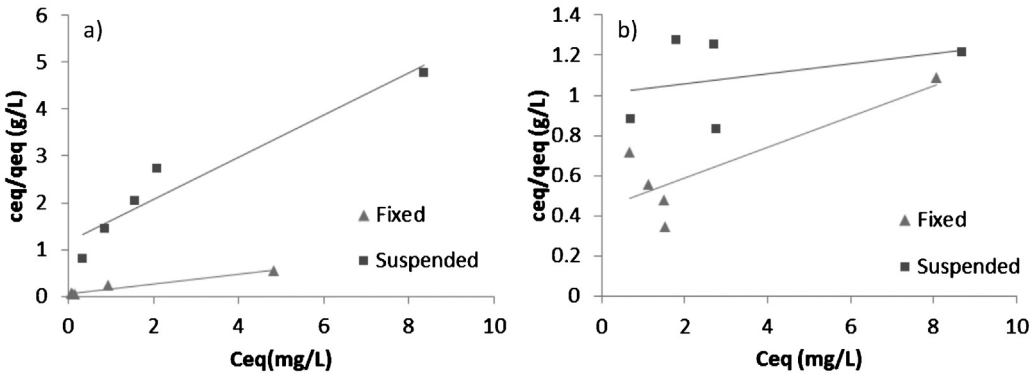

Fig. 6. Langmuir isotherms comparing the c_{eq}/q_{eq} ratio of suspended biomass and fixed biomass at different equilibrium HM concentrations (c_{eq}). (a) Cu adsorption and (b) Pb adsorption.

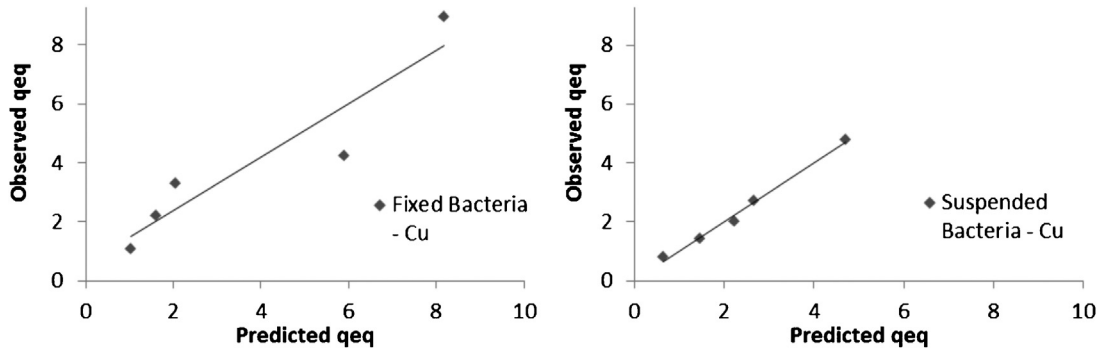

Fig. 7. Plots of measured q_{eq} vs. modelled q_{eq} for Langmuir isotherm.

which is also supported by the K_f values determined from the Freundlich isotherm model.

Fig. 7 shows the measured q_{eq} vs. modelled q_{eq} as determined by the Langmuir isotherm for Cu(II), which shows a good fit and the fact that Langmuir model describes the data well. Corresponding R^2 values (Table 3) are in the range 0.88–0.99. As mentioned above, the Langmuir model was not able to describe the data well in the case of Pb(II).

The q_{max} values from literature are highly variable, depending on what type of bacteria and HM are being used for adsorption. From Ozdemir et al. [27] and Rodriguez-Tirado et al. [14], the q_{max} values determined by the Langmuir isotherm for Cu(II) adsorption were 48.5 mg/g and 27.35 mg/g, respectively. These are higher than 9.80 mg/g and 6.52 mg/g for fixed bacteria and suspended bacteria respectively, as determined by this study (Table 3). The difference in q_{max} is attributed to the low concentrations of adsorbent and HM used in this study. Ozdemir et al. [27], used HM concentrations up to 300 mg/l to obtain such high q_{max} values. At a concentration of 10 mg/l the q_{eq} value obtained by Ozdemir et al. [27], is only 3.1 mg/g. Additionally, much of the literature optimize the pH to levels that are too low for comparison with natural environments.

Scatchard plot. The Scatchard plot of q_{eq}/c_{eq} vs. q_{eq} (Fig. 8) is a modified form of the Langmuir isotherm and shows the interaction between sorbent and sorbate with a changing concentration. A negative and linear slope suggests that affinity for HMs does not change as the concentration changes and supports the Langmuir model [14,27,42]. Fig. 8 reveals poor relationships between trendlines and data or neutral slope for all trials, which supports changing HM affinity with changing HMs concentration. All of the plots showed a low R^2 values (0.03–0.59) except for suspended

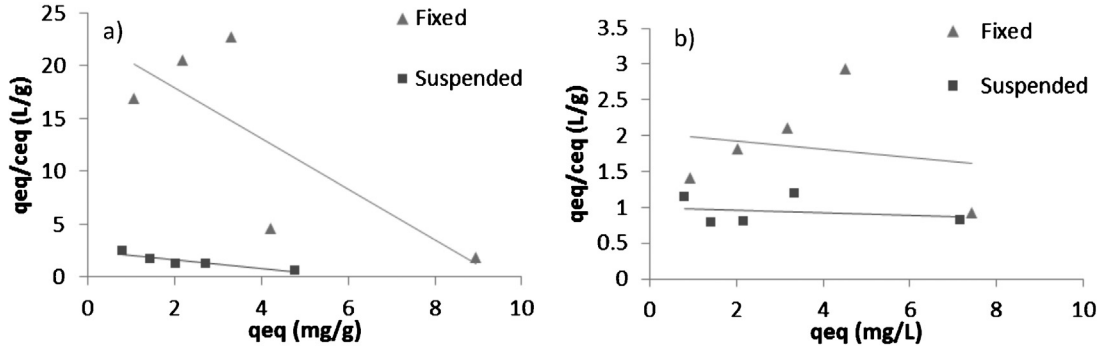

Fig. 8. Scatchard isotherms comparing the q_{eq}/c_{eq} ratio, of suspended biomass and fixed biomass to different equilibrium HM adsorption capacities (q_{eq}). (a) Cu adsorption and (b) Pb adsorption.

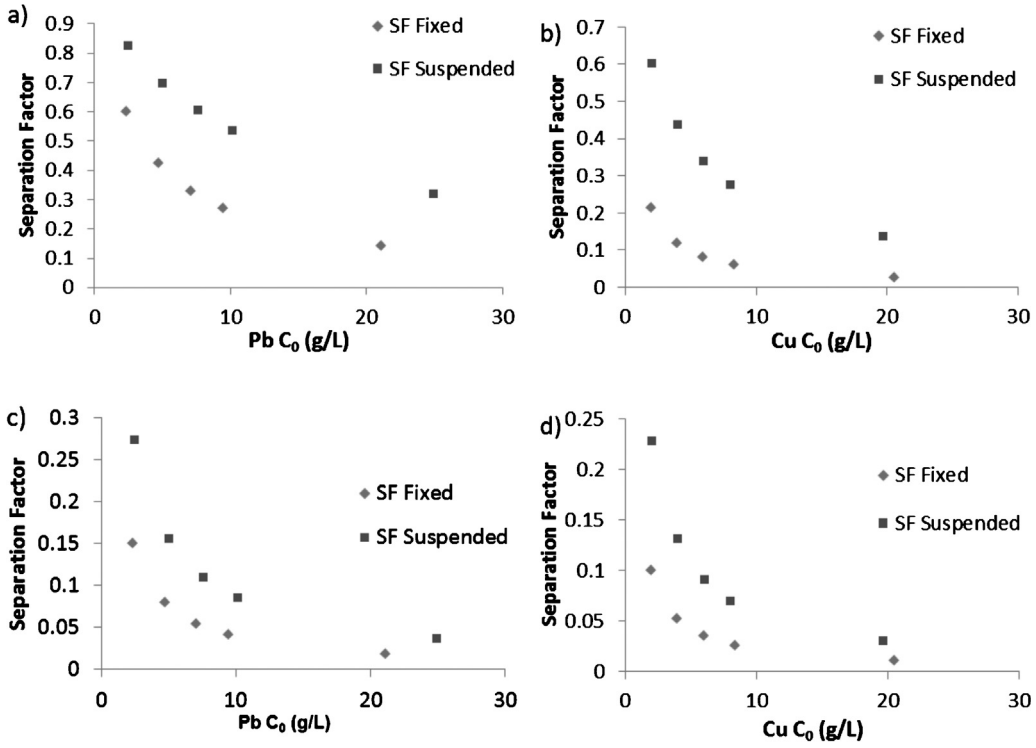

Fig. 9. Separation factors at different concentrations of HM for fixed and suspended bacteria. (a) Separation factor of Pb binding determined using Langmuir coefficient, K_L. (b) Separation factor of Cu binding determined using Langmuir coefficient, K_L. (c) Separation factor of Pb binding determined using Freundlich coefficient, K_F. (d) Separation factor of Cu binding determined using Freundlich coefficient, K_F.

biomass adsorbing Cu(II) (0.87). The poor fit to the Langmuir isotherm suggests that as concentration changes so too does the affinity for the HM ions. This result helps to confirm the close fit suspended biomass binding Cu(II) has to the Langmuir isotherm and the poor fit of Pb(II) adsorption to the Langmuir isotherm.

Separation factor. To better visualize the binding favourability, as dictated from the Freundlich and Langmuir isotherm coefficients, the SF has been plotted with changing initial HM concentration (Fig. 9). Lower SF values indicate stronger sorbate–sorbent interactions.

The charts in Fig. 9 show a clear differentiation between the fixed and suspended bacteria while revealing two things. Firstly, the separation factor decreases with increasing initial HM concentration in all experiments, confirming that higher concentrations of HMs are adsorbed more favourably. Additionally, it is clear that for any concentration and using either isotherm model the SF value is lower for fixed bacteria. This indicates that there is a stronger binding interaction between fixed bacteria and either Pb(II) or Cu(II) than with suspended bacteria.

Conclusion

Utilizing different growth environments, this study developed a naturally occurring, heterogeneous mix of bacteria in suspended and fixed morphologies. Both morphologies of bacteria are capable of adsorption, however, fixed bacteria showed higher capacity and affinity for HMs removal. This could be due to the fact that the fixed bacteria displayed 29% EPS content by weight compared to 9.5% EPS found on the suspended bacteria. The bacteria present in the study were identified by 16s RNA sequencing. The sequences were identified via nBLAST to reveal three bacterial species: *E. ludwigii*, *Z. ramigerais* and *C. testosteroni.* All of these species are gram negative, rod shaped, aerobic and are commonly found in soils and wastewater treatment plants. The Freundlich isotherm model was able to describe the adsorption data well for both fixed and suspended biomass, with R^2 values ranging from 0.86 to 0.99. The Langmuir isotherm model fit the adsorption data well for Cu(II) only, with R^2 values ranging from 0.88 to 0.99. The Freundlich isotherm had higher K_f values for fixed bacteria, suggesting again that the fixed bacteria had stronger interactions with the HMs. The Langmuir isotherm revealed that the q_{max} for Cu(II) adsorption was 9.80 mg/g and 6.52 mg/g for fixed bacteria and suspended bacteria respectively. The poor fit of the Scatchard plot suggests that both morphologies of biomass change their affinity for the HMs as the concentration of HM changes. The SF analysis supported this finding and helped to confirm that the fixed biomass was a superior adsorbent. Finally, the 83.7% and 97.5% removal of Pb(II) and Cu(II) ions from solution shows that at low adsorbent concentrations, fixed biomass would excel at the removal of dilute HMs from waste water streams.

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References

[1] N. Barka, M. Abdennouri, M.E. Makhfouk, S. Qourzal, Biosorption characteristics of cadmium and lead onto eco-friendly dried cactus (*Opuntia ficus* indica) cladodes, J. Environ. Chem. Eng. 1 (2013) 144–149.
[2] F. Fu, Q. Wang, Removal of heavy metal ions from wastewaters: a review, J. Environ. Manage. 92 (2011) 407–418.
[3] US EPA, Lead in Drinking Water, EPA 2013 (2013) 1.
[4] US EPA, Basic Information about Copper in Drinking Water, EPA; 2013 (2012) 1.

[5] T.A. Kurniawan, Y.S. Gilbert, W.L. Chan, B. Sandhya, Physico-chemical treatment techniques for wastewater laden with heavy metals, Chem. Eng. J. 118 (2006) 83–98.
[6] C. Chen, J. Wang, Removal of Pb2+, Ag+, Cs+ and Sr2+ from aqueous solution by brewery's waste biomass, J. Hazard. Mater. 151 (2008) 65–70.
[7] M.D. Mullen, D.C. Wolf, F.G. Ferris, T.J. Beveridge, C.A. Flemming, G.W. Bailey, Bacterial sorption of heavy metals, Appl. Environ. Microbiol. 55 (1989) 3143–3149.
[8] C. Quintelas, B. Fernandes, J. Castro, H. Figueiredo, T. Tavares, Biosorption of Cr(VI) by a *Bacillus coagulans* biofilm supported on granular activated carbon (GAC), Chem. Eng. J. 136 (2008) 195–203.
[9] G. O'Toole, H.B. Kaplan, R. Kolter, Biofilm formation as microbial development, Annu. Rev. Microbiol. 54 (2000) 49–79.
[10] R. Singh, D. Paul, R.K. Jain, Biofilms: implications in bioremediation, Trends Microbiol. 14 (2006) 389–397.
[11] E.D. Van Hullebusch, M.H. Zandvoort, P.N.L. Lens, Metal immobilization by biofilms: mechanisms and analytical tools, Rev. Environ. Sci. Bio/Technol. 2 (2003) 9–33.
[12] C. Pau-Roblot, M. Lequart-Pillon, L. Apanga, S. Pilard, J. Courtois, N. Pawlicki-Jullian, Structural features and bioremediation activity of an exopolysaccharide produced by a strain of *Enterobacter ludwigii* isolated in the Chernobyl exclusion zone, Carbohydrate Polym. 93 (2013) 154–162.
[13] M. Dhanaraj, M.N.V. Prasad, Lead (II) and cadmium (II) biosorption on *Spirodela polyrhiza* (L.) Schleiden biomass, J. Environ. Chem. Eng. 1 (2013) 200–207.
[14] V. Rodríguez-Tirado, C. Green-Ruiz, B. Gómez-Gil, Cu and Pb biosorption on *Bacillus thioparans* strain U3 in aqueous solution: kinetic and equilibrium studies, Chem. Eng. J. 181–182 (2012) 352–359.
[15] R. Pardo, M. Herguedas, E. Barrado, M. Vega, Biosorption of cadmium, copper, lead and zinc by inactive biomass of Pseudomonas Putida, Anal. Bioanal. Chem. 376 (2003) 26–32.
[16] C. Lin, Y. Lai, Adsorption and recovery of lead(II) from aqueous solutions by immobilized *Pseudomonas Aeruginosa* PU21 beads, J. Hazard. Mater. 137 (2006) 99–105.
[17] B. Banihashemi, R.L. Droste, Sorption-desorption and biosorption of Bisphenol A, Triclosan, and 17α-ethinylestradiol to sewage sludge, J. Sci. Total Environ. (2014), http://dx.doi.org/10.1016/j.scitotenv.2013.12.116.
[18] J. Zhao, Y. Li, C. Zhang, Q. Zeng, Q. Zhou, Sorption and degradation of bisphenol A by aerobic activated sludge, J. Hazard. Mater. 155 (2008) 305–311.
[19] C.S. Srinandan, G. D'souza, N. Srivastava, B.B. Nayak, A.S. Nerurkar, Carbon sources influence the nitrate removal activity, community structure and biofilm architecture, Bioresour. Technol. 117 (2012) 292–299.
[20] C.S. Srinandan, V. Jadav, D. Cecilia, A.S. Nerurkar, Nutrients determine the spatial architecture of *Paracoccus* sp. biofilm, Biofouling 26 (2010) 449–459.
[21] J.A. Wyber, J. Andrews, P. Gilbert, Loss of salt-tolerance and transformation efficiency in *Escherichia coli* associated with sub-lethal injury by centrifugation, Lett. Appl. Microbiol. 19 (1994) 312–316.
[22] P. Stoodley, K. Sauer, D.G. Davies, J.W. Costerton, Biofilms as complex differentiated communities, Annu. Rev. Microbiol. 56 (2002) 187–209.
[23] S. Yousaf, M. Afzal, T.G. Reichenauer, C.L. Brady, A. Sessitsch, Hydrocarbon degradation, plant colonization and gene expression of alkane degradation genes by endophytic *Enterobacter ludwigii* strains, Environ. Pollut. 159 (2011) 2675–2683.
[24] H. Liu, H.H.P. Fang, Extraction of extracellular polymeric substances (EPS) of sludges, J. Biotech. 95 (2002) 249–256.
[25] EMD Chemicals, Nutrient Agar. Lot# VM035150, Darmstadt, Germany. 480 S. Democral Rd.
[26] F. Sanger, A.R. Coulson, A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase, J. Mol. Biol. 94 (1975) 441–448.
[27] S. Özdemir, E. Kilinc, A. Poli, B. Nicolaus, K. Güven, Biosorption of Cd, Cu, Ni, Mn and Zn from aqueous solutions by thermophilic bacteria, *Geobacillus toebii* sub. sp. decanicus and *Geobacillus thermoleovorans* sub. sp. stromboliensis: equilibrium, kinetic and thermodynamic studies, Chem. Eng. J. 152 (2009) 195–206.
[28] Fischer Chemicals, Copper and Lead standard reference solutions (FLSC194100, SL21100). 112 Colonnade Road Ottawa, Ontario.
[29] R. Han, H. Li, Y. Li, J. Zhang, H. Xiao, J. Shi, Biosorption of copper and lead ions by waste beer yeast, J. Hazard. Mater. 137 (2006) 1569–1576.
[30] K.A. Shroff, V.K. Vaidya, Kinetics and equilibrium studies on biosorption of nickel from aqueous solution by dead fungal biomass of *Mucor hiemalis*, Chem. Eng. J. 171 (2011) 1234–1245.
[31] D.D. Do, Adsorption Analysis: Equilibria and Kinetics, Imperial College Press, London, 1998.
[32] T.W. Webi, R.K. Chakravort, Pore and solid diffusion models for fixed-bed adsorbers, J. Am. Inst. Chem. Eng. 20 (2) (1974) 228–238.
[33] Y. Sahin, A. Ozturk, Biosorption of chromium(VI) ions from aqueous solution by the bacterium *Bacillus thuringiensis*, Process Biochem. 40 (2005) 1895–1901.
[34] R.D. Monds, G.A. O'Toole, The developmental model of microbial biofilms: ten years of a paradigm up for review, Trends Microbiol. 17 (2009) 73–87.
[35] I.W. Sutherland, The biofilm matrix – an immobilized but dynamic microbial environment, Trends Microbiol. 9 (2001) 222–227.
[36] H. Hoffmann, S. Stindl, A. Stumpf, A. Mehlen, D. Monget, J. Heesemann, K.H. Schleifer, A. Roggenkamp, Description of *Enterobacter ludwigii* sp. nov., a novel Enterobacter species of clinical relevance, Syst. Appl. Microbiol. 28 (2005) 206–212.
[37] R.A. Rosselló-Mora, W. Ludwig, K.H. Schleifer, *Zoogloea ramigera*: a phylogenetically diverse species, FEMS Microbiol. Lett. 114 (1993) 129–133.
[38] T. Montoya, L. Borrás, D. Aguado, J. Ferrer, A. Seco, Detection and prevention of enhanced biological phosphorus removal deterioration caused by Zoogloea overabundance, Environ. Technol. 29 (2008) 35–42.
[39] N. Boon, J. Goris, P. De Vos, W. Verstraete, E.M. Top, Bioaugmentation of activated sludge by an indigenous 3-chloroaniline-degrading *Comamonas testosteroni* Strain, I2*gfp*, Appl. Environ. Microbiol. 66 (2000) 2906–2913.
[40] S. Staniland, M. Coppock, M. Tuffin, L. van Zyl, A.N. Roychoudhury, D. Cowan, Cobalt uptake and resistance to trace metals in *Comamonas testosteroni* isolated from a heavy-metal contaminated site in the Zambian Copperbelt, Geomicrobiol. J. 27 (2010) 656–668.
[41] S.K. Porter, K.G. Scheckel, C.A. Impellitteri, J.A. Ryan, Toxic metals in the environment: thermodynamic considerations for possible immobilization strategies for Pb, Cd, As, and Hg, Crit. Rev. Environ. Sci. Technol. 34 (2004) 495–604.
[42] J. Febrianto, A.N. Kosasih, J. Sunarso, Y. Ju, N. Indraswati, S. Ismadji, Equilibrium and kinetic studies in adsorption of heavy metals using biosorbent: a summary of recent studies, J. Hazard. Mater. 162 (2009) 616–645.
[43] A. Özer, G. Gürbüz, A. Çalimli, B.K. Körbahti, Biosorption of copper(II) ions on *Enteromorpha prolifera*: application of response surface methodology (RSM), Chem. Eng. J. 146 (2009) 377–387.