Desalination and Water Treatment
www.deswater.com

doi:10.5004/dwt.2017.20330

68 (2017) 267–273
March

Metal removal from multi-metal solutions by metal-tolerant *Stenotrophomonas maltophilia* isolated from river sediment

Carrie Siew Fang Sim, Adeline Su Yien Ting*

School of Science, Monash University Malaysia, Jalan Lagoon Selatan, 46150 Bandar Sunway, Selangor, Malaysia, Tel. 603-5514 6000, Ext. 62012, Fax 603-55146184, email: carriesim@hotmail.com (C.S.F. Sim), Tel. +60 3-5514 6105, Fax +60 3-55146184, email: adeline.ting@monash.edu, adelsuyien@yahoo.com (A.S.Y. Ting)

Received 11 August 2016; Accepted 31 October 2016

ABSTRACT

The efficacy of *S. maltophilia* in removing Pb^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} from multi-metal solutions was determined by comparing against single-metal solutions. Results showed that the removal of the cations was higher in single than multi-metal solutions with mean uptake of 70 mg g^{-1} of metals removed compared to less than 10 mg g^{-1} metals, respectively. Biosorption of Pb^{2+} was the highest in both single (70.89 mg g^{-1}) and multi-metal solutions (8.05 mg g^{-1}). Metal removal by *S. maltophilia* in multi-metal solutions was influenced by different pH and adsorbent dosages used, in which pH 7 and low adsorbent dosages (0.10–0.50 g) led to higher amount of metals removed (pH 7: 3.26 –12.28 mg g^{-1}; 0.10–0.50 g: 2.30–8.71 mg g^{-1}). ATR-FTIR analysis revealed that metal-binding sites for *S. maltophilia* were attributed to functional groups such as hydroxyl (–OH), amine ($–NH_2$) and carboxyl (–COOH). Biosorption by *S. maltophilia* was found to comply with pseudo-second order, suggesting that the biosorption process is chemically rate-limited. This study showed that the metal-tolerant *S. maltophilia* has good potential as biosorbent for removal of metals, with recommended dosage and pH at 0.10–0.50 g and pH 7, respectively.

Keywords: Adsorbent dosages; Biosorption; Multi-metal solutions; pH; Single-metal solutions; *Stenotrophomonas maltophilia*

1. Introduction

Toxic metals such as lead (Pb^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}) and cadmium (Cd^{2+}) are commonly found in the environment. Cu^{2+} and Zn^{2+} are essential metals with physiological and biological functions while Cd^{2+} and Pb^{2+} are non-essential metals with no known functions [1]. Irrespective of their roles, excess of metals could have serious health implications. Cd^{2+} has devastating effects on kidney; Cu^{2+} hampers brain and liver functions; and Pb^{2+} interferes with memory and nervous system [2,3]. Traditionally, physico-chemical methods such as electrochemical precipitation, ion exchange, and reverse osmosis were used to remove toxic metals. However, these methods have several limitations which include cost-ineffective and the by-production of toxic sludges. As an alternative, microorganisms such as bacteria and fungi are sought, and this approach has been found to be relatively cheap and environmentally sustainable [4]. In this study, metal removal by bacteria is explored. Bacteria rely on the peptidoglycan layer found in their cell wall for metal removal. Peptidoglycan, which is made up of N-acetylglucosamine (NAG) and N-acetylemuramic acid (NAM), has structures such as glycoproteins, teichoic acids and teichuronic acids in Gram-positive bacteria [5,6] and are rich in the following functional groups: amine ($–NH_2$), carbonyl (C=O), carboxyl (–COOH) and hydroxyl (–OH) groups. Gram-negative bacteria have outer membranes consisting of lipopolysaccharide (LPS), phospholipids and

*Corresponding author.

proteins [7] which are also rich with the various functional groups. These functional groups aid in the binding of positively-charged metal cations to bacteria surface, enabling the removal of metal cations from the environment [8]. Bacterial species known to be efficient in metal removal include *Bacillus licheniformis*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Streptomyces rimosus* and *Staphylococcus xylosus* [9–14].

In this study, *Stenotrophomonas maltophilia,* a Gram negative, obligate aerobic bacterium was investigated for its metal removal potential in multi-metal solutions. As compared to other organisms, this bacterium is less conspicuous for metal removal activities. This isolate is ubiquitous in the environment and was isolated from a river sediment sample in Penchala River, Malaysia. *S. maltophilia* has been found to tolerate and remove Cu^{2+}, Pb^{2+} and Cd^{2+} in single metal solutions [15,16] therefore in this study, the efficacy of *S. maltophilia* in removing metals from multi-metal solutions was further investigated. This approach is more reliable as multi-metals are often present in natural environments instead of single-metal. Furthermore, multi-metal solutions in this study mimic wastewaters and provide an insight on the biosorption efficacy of *S. maltophilia* in the presence of various metals. The influence of pH and adsorbent dosages were also determined to optimize metal removal in multi-metal solutions. To ascertain the possible kinetic mechanism adopted by *S. maltophilia* in metal removal, kinetic modelling was also performed. The functional groups present on *S. maltophilia* were also identified via Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy analysis.

2. Materials and methods

2.1. Culture establishment and preparation of single and multi-metal solutions

The bacterium *S. maltophilia* was maintained on Nutrient Agar (NA, Merck) at 25 ± 2°C. To generate cell biomass, *S. maltophilia* was inoculated into 250 mL of Nutrient Broth (NB, Merck) and incubated (25 ± 2°C, for 3–5 d). The culture was then centrifuged (9000 rpm, 7 min), rinsed thrice with sterile distilled water and autoclaved (121°C, 20 min). The non-viable cells were dried overnight in an oven (80°C ± 2°C) and powdered using pestle and mortar. The powdered *S. maltophilia* was sieved using a fine sieve (mesh size of 0.08 cm) and was kept in sterile bottles (25 ± 2°C) until further use.

Stock solutions of metal salts (analytical grade of $Pb(NO_3)_2$ (R&M Chemicals), $Cu(NO_3)_2{\cdot}3H_2O$ (Sigma-Aldrich), $Zn(NO_3)_2{\cdot}6H_2O$ (R&M Chemicals) and $Cd(NO_3)_2{\cdot}4H_2O$ (Sigma-Alrich)) were prepared separately by dissolving salts in Milli-Q water to obtain stock solutions with concentrations of 1000 mg L^{-1}. Multi-metal solutions were prepared by mixing each of the individual metal solutions together to give rise to mixed-solution with a final concentration of 100 mg L^{-1}. The initial pH of metal solutions was adjusted to pH 5 using 0.1 M HCl and 0.1 M NaOH.

2.2. Metal biosorption in multi-metal solutions

The non-viable *S. maltophilia* was weighed to 0.1 g and added into 15 mL of 100 mg L^{-1} multi-metal solutions as described in section 2.1. Controls were prepared using single metal solutions of 100 mg L^{-1}. The mixture was incubated (30 ± 2°C, 150 rpm) and sampling was performed at 15, 30, 60, 120, 240, 360 and 480 min [17]. At each sampling time, the solutions were filtered and the collected filtrates were analysed using Atomic Absorption Spectroscopy (AAS) [Agilent Technologies 240 Series AA] with an air-acetylene flame. The current was pre-set at 15mA with wavelengths set as follows: 217.0 nm, 324.8 nm, 228.8 nm and 213.9 nm for Pb^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+}, respectively. Calibration of AAS was performed using standard solutions for every set of analyses. Amount of metal removed per g of biosorbent (mg g^{-1}) (Q) was calculated as follows:

$$Q = [(C_i - C_f) \cdot V] / m$$

where C_i: initial metal concentration (mg L^{-1}); C_f: final metal concentration (mg L^{-1}); V: total volume of solution (mL); M: mass of dried biosorbents (g).

2.3. Kinetic modeling in single and multi-metal solutions

The biosorption mechanism by *S. maltophilia* was predicted using the common pseudo-first and pseudo-second kinetic orders. Kinetic modelling predicts the rate-controlling mechanisms by understanding the adsorption pathway. The kinetic models are as follows:

Pseudo-first order kinetics:

$$\log(q_e - q_t) = \log q_e - (k_1 / 2.303)t \quad (1)$$

where q_e: metal adsorbed at equilibrium determined experimentally (mg g^{-1}); q_t: metal adsorbed at particular time (mg g^{-1}); k_1: rate constant derived from slope; t: time;

Pseudo-second order kinetics:

$$\left(\frac{t}{q_t} - \frac{1}{k_2 q_e^2}\right) + \left(\frac{1}{q_t}\right) t \quad (2)$$

where t/q_t: contact time divided with adsorption at particular time; k_2: rate constant from y-intercept; q_e: metal adsorbed at equilibrium determined experimentally from equation slope (mg g^{-1}); q_t: metal adsorbed at particular time (mg g^{-1}) .

2.4. Influence of pH on metal biosorption in multi-metal solutions

The influence of pH was tested in multi-metal solutions to establish the effect on the metal removal efficacy by *S. maltophilia*. The biomass (0.10 g) of *S. maltophilia* was added into 15 mL of 100 mg L^{-1} multi-metal solutions and the initial pH values were adjusted to pH 3, 4, 5, 6 and 7 using 0.1 M HCl and 0.1 M NaOH. The mixture was then incubated (30 ± 2°C, 150 rpm). Sampling was performed for 480 min, in which the metal solutions were collected and analysed as described in section 2.2.

2.5. Influence of adsorbent dosages on metal biosorption in multi-metal solutions

The biomass of *S. maltophilia* was prepared according to the desired biosorbent dosages of 0.10, 0.50, 1.00 and 1.50 g. The biosorbent was subsequently added into 15 mL of 100 mg L^{-1} multi-metal solutions and incubated (30 ± 2°C, 150 rpm) [18]. Sampling was performed for 480 min, in which the metal solutions were collected and analysed as described in section 2.2.

2.6. ATR-FTIR analysis

S. maltophilia was first oven-dried at 50°C over night. The samples were then ground to powder and analyzed with FTIR (Thermo Scientific Nicolet 1810) under ambient condition. The single reflection ATR (Attenuated Total Reflection, diamond crystal) spectra (within 4000–400 cm^{-1}) were acquired using the FTIR. A total of 16 scans were collected, averaged and the resolution determined (4 cm^{-1}). Data were collected within the range of 4000–700 cm^{-1} to eliminate interference. The functional groups indicated by the peaks in the spectra were then identified.

2.7. Statistical analysis

All assessments were performed in triplicates and repeated once. Data collected were analysed with ANOVA and means compared using Tukey's Standardized Range Test at $p < 0.05$. Analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0.

3. Results and Ddiscussion

3.1. Metal biosorption in multi-metal solutions

The efficacy of metal biosorption by *S. maltophilia* was lower in multi-metal solutions compared to single-metal solutions. In multi-metal solutions, 8.05, 5.60, 1.61, 1.27 mg g^{-1} was removed for Pb^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+}, respectively (Fig. 1). These were significantly lower than the amount removed in single-metal solutions, which were 70.89, 27.04, 17.01 and 7.85 mg g^{-1} for Pb^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+}, respectively (Fig. 1). In both the single- and multi-metal solutions, the most preferred metal uptake was Pb^{2+}, followed by Cu^{2+}. There was however, no clear preference for Cd^{2+} or Zn^{2+} in multi-metal solutions, despite the higher uptake of Cd^{2+} than Zn^{2+} in single-metal solutions.

The biosorption efficacy by *S. maltophilia* in multi-metal solutions was clearly inferior to biosorption in single-metal solutions. This may be due to the interference and competition among the metal cations to bind to *S. maltophilia*. Poorer removals of metals in multi-metal solutions have also been reported with other biosorbents such as fungus, algae as well as perennial plants [19–21]. Although the biosorption in multi-metal solutions was inferior to single-metal solutions, the metal uptake trend was rather similar with $Pb^{2+} > Cu^{2+}$ and followed by either Cd^{2+} or Zn^{2+}. Other biosorbents such as seeds, algae, cyanobacteria and seeds also showed such trend of preferred cationic removal [22,23]. This is attributed to the inherent nature of the cations. Ionic radius,

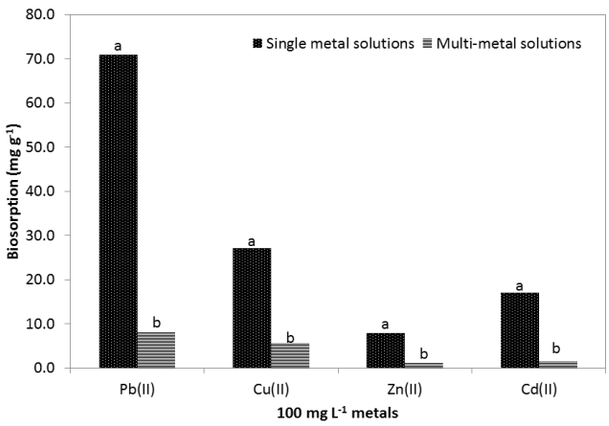

Fig. 1. Means of metal removed by *S. maltophilia* in single and multi-metal solutions. Means with the same letters within the same type of metal are not significantly different ($p < 0.05$).

one of the cationic properties, affects the ease of cations to bind to functional groups [24]. Pb^{2+} which was the most easily adsorbed (and in large amount) ion in this study has larger size (1.19Å) than Cu^{2+} and Cd^{2+} (0.73Å and 0.97Å, respectively). This suggests a correlation between ionic size and the tendency of adsorption. In addition, adsorption also depends on the mass of cations and their electronegativity [25]. Smaller cations are easily displaced by larger ions due to their lighter weight [26,27]. In this study, the highest removal in single- and multi-metal solutions was for Pb^{2+} which is the heaviest, with 207.20 atomic mass per unit (a.m.u.). This was followed by Cu^{2+} and Cd^{2+} with 63.55 and 112.41 a.m.u., respectively. The inherent nature of the cations therefore contributes to the biosorption trend, with higher uptake of Pb^{2+} and Cu^{2+} than Zn^{2+} and Cd^{2+}.

Sorption equilibrium was achieved for most metals after 360 min (Fig. 2). Increase in metal adsorbed thereafter (at 480 min) was not significantly different, as observed for Pb^{2+} and Cu^{2+} in multi-metal solutions, and Pb^{2+} and Zn^{2+} in single-metal solutions. On the contrary, the removal of Zn^{2+} continued to increase in multi-metal solutions, as well as the biosorption of Cu^{2+} in single-metal solutions. Biosorption of Cd^{2+} continued to increase and did not achieve equilibrium in both single and multi-metal solutions (at 480 min) (Fig. 2).

3.2. Kinetic modelling in single and multi-metal solutions

Kinetic modelling was performed to assess the biosorption mechanism by *S. maltophilia* for metals which achieved equilibrium: Pb^{2+} and Cu^{2+} in multi-metal solutions, and Pb^{2+} and Zn^{2+} in single metal solutions. Comparing the R^2 of pseudo-first and pseudo-second orders, pseudo-first orders generated lower values (0.83–0.97) than pseudo-second orders (0.89–1.00) (Table 1) hence revealing that biosorption performed by *S. maltophilia* complied with pseudo-second order. This was consistently calculated in both metal solutions with $R^2 = 0.98$ for both biosorption of Pb^{2+} and Cu^{2+} in multi-metal solutions. In single-metal solutions, the R^2

value was higher for the removal of Pb^{2+} (R^2 =1.00) compared to Zn^{2+} (R^2 = 0.89) (Table 1). The compliance to pseudo-second order was further validated when the amount of metal cations removed experimentally (q_{eqex}) was similar to the amount estimated from pseudo-second order kinetic (q_{eq}) (Table 1). On the contrary, the amount of metal estimated from pseudo-first order varies greatly from the experimental readings. A difference of 6-fold was obtained using pseudo-first order hence the non-suitability of this kinetic model for the biosorption of *S. maltophilia*. Comparatively, only 1-fold difference between the q_{eqex} and q_{eq} was obtained using pseudo-second order. These further confirmed the biosorption compliant to pseudo-second order model. In terms of speed, the adsorption process was rapid with the calculated rate constant, *k*, of pseudo-second order kinetic model ranging from 0.03–13.12 g mg^{-1} min^{-1} (Table 1). The adsorption process was however affected in multi-metal solution as observed for Pb^{2+}, with the lower rate constant of 0.05 g mg^{-1} min^{-1} in multi-metal solution than the 13.12 g mg^{-1} min^{-1} in single metal solution.

Compliance to pseudo-second order kinetic by *S. maltophilia* in Cu^{2+} and Pb^{2+} has also been reported by [16] and [28]. This suggests that *S. maltophilia* adsorbed metal cations via chemisorptions. In the rate-limiting process, sharing and exchanging of electrons occurs [17,29]. On the contrary, pseudo-first order kinetic suggests a resisting external force occurring in the beginning of biosorption [29]. Comparing the two common pseudo-first and pseudo-second order, compliance to pseudo-second order is more typical for microbial biosorbents hence the expected compliance of *S. maltophilia* to pseudo-second order in single- and multi-metal solutions.

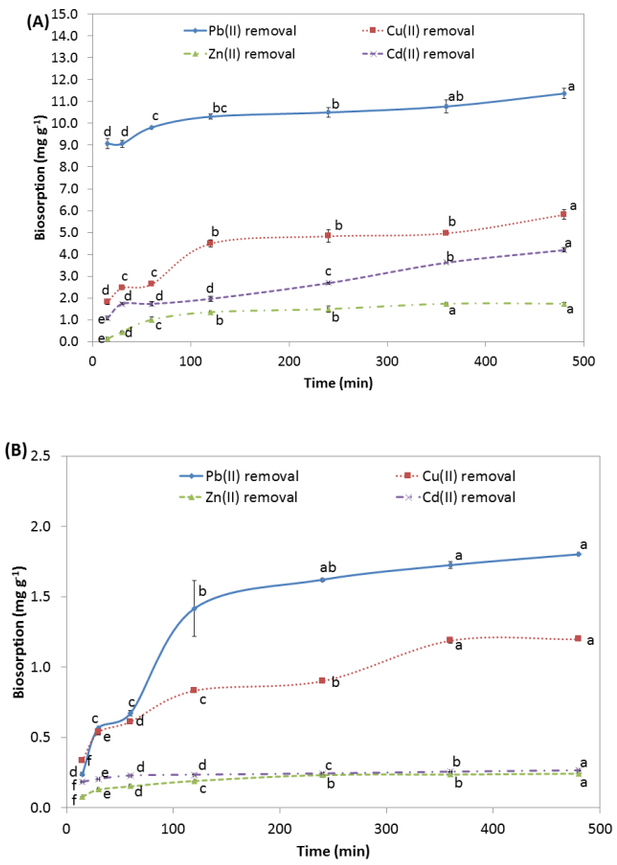

Fig. 2. Metal biosorption by *S. maltophilia* in (A) single and (B) multi-metal solutions. Means with the same letters are not significantly different ($p < 0.05$).

3.3. Influence of pH on metal biosorption in multi-metal solutions

The influence of pH on metal biosorption in multi-metal solutions was not as expected, as the curve obtained was not a typical normal distribution "bell-curve" plot. Under the influence of acidic conditions (pH 3–6), biosorption activities were weak compared to pH 7 (Fig. 3). In fact, in solutions with the neutral pH 7, a significant increase in biosorption for all metals was observed, ranging from 3.26 mg g^{-1} to 12.28 mg g^{-1} (Fig. 3). It is concluded that pH 3 negatively influences the biosorption activities by *S. maltophilia* as the removal of Pb^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+} was the lowest with 5.68, 3.74, 1.07 and 0.77 mg g^{-1}, respectively (Fig. 3). At pH 4, significant improvements in metal biosorption were observed (Fig. 3). The different pH conditions however did not influence metal preference as the trend of metal uptake preference remains the same with $Pb^{2+} > Cu^{2+} > Cd^{2+} = Zn^{2+}$.

The optimum pH for *S. maltophilia* to perform biosorption was at pH 7, suitable for most biosorbents especially bacteria and fungi [30,31]. pH plays an important role in biosorption as it determines the amount of hydrogen ions present, which influences metal solubility and surface charges of biosorbent [32]. Hydrogen ions are naturally bound to

Table 1
Pseudo-first and pseudo-second order kinetics for the removal of Pb^{2+}, Zn^{2+} and Cu^{2+} by *S. maltophilia*, for metals with biosorption processes which reached equilibrium in single and multi-metal solutions (after 480 min)

	Metal cations	Experimental q_{eqex} (mg g^{-1})	Pseudo-first order			Pseudo-second order		
			k_1 (min^{-1})	q_{eq} (mg g^{-1})	R^2	k_2 (g mg^{-1} min^{-1})	q_{eq} (mg g^{-1})	R^2
Single metal solutions	Pb^{2+}	11.37	0.01	6.02	0.92	13.12	11.33	1.00
	Zn^{2+}	1.73	0.04	10.20	0.86	0.04	2.39	0.89
Multi-metal solutions	Pb^{2+}	1.80	0.02	2.93	0.97	0.05	2.24	0.98
	Cu^{2+}	1.20	0.03	1.62	0.83	0.03	1.32	0.98

functional groups but become displaced and replaced by metal cations in the presence of those metals. pH also affects the solubility of metals in terms of metal speciation. With the increase in pH, the solubility of metals decreases resulting in the precipitation of hydroxides [33]. Despite the observed precipitation of metals in this study, particularly at pH 7, it was negligible. This suggests the effect of the complex environment towards metals in real wastewater. Additionally, pH also has an effect on the ionization state of functional groups. With an increase in pH, deprotonation occurs, resulting in more negatively-charged surface of *S. maltophilia*. This further encourages the binding of metal cations. Carboxyl (–COOH), for example, becomes deprotonated at pH higher than 4, heightening cationic attraction while simultaneously repelling negatively-charged anions [34].

3.4. Influence of adsorbent dosages on metal biosorption in multi-metal solutions

Adsorbent dosages appeared to have no specific influence on the biosorption efficacy of *S. maltophilia* towards the various metals tested, although the biosorption trend suggested slightly higher metal removal when lower adsorbent dosages (0.10–0.50 g) were used. Pb^{2+} (8.71 mg g^{-1} by 0.10 g biosorbent), Cu^{2+} (5.09 mg g^{-1} by 0.10 g biosorbent) and Zn^{2+} (2.30 mg g^{-1} by 0.50 g biosorbent) removal was effectively achieved with the use of 0.10–0.50 g biosorbent (Table 2). On the contrary, Cd^{2+} removal (8.04 mg g^{-1}) was the most efficient with 1.50 g of biosorbent. The various adsorbent dosages used also resulted in various trends of metal uptake. With 0.10 g biosorbent, the metal preference uptake trend was $Pb^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+}$. Usage of *S. maltophilia* at 0.50 and 1.50 g showed differing metal preference uptake trend with $Cd^{2+} > Pb^{2+} > Zn^{2+} > Cu^{2+}$; and with 1.00 g *S. maltophilia* used, the trend was $Cd^{2+} > Zn^{2+} > Pb^{2+} > Cu^{2+}$ (Table 2).

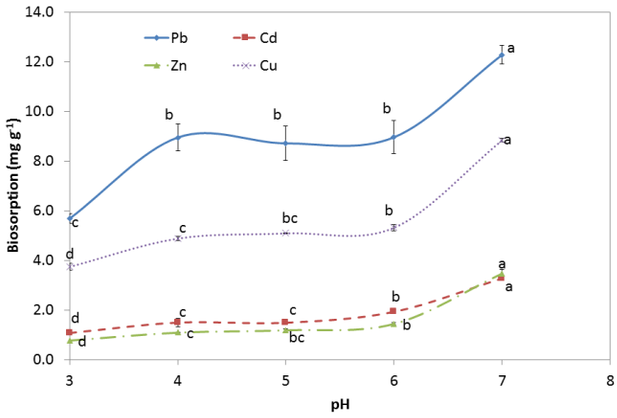

Fig. 3. Biosorption capacities of *S. maltophilia* in removing Pb^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} in multi-metal solutions at different initial pH conditions. Values are means of triplicates. Error bars indicate standard deviations of means. Means with the same letters are not significantly different ($p < 0.05$).

The non-specific influence towards biosorption in terms of adsorbent dosages could be attributed to the aggregation of biosorbents observed, similarly reported by [35] and [36]. Aggregation or "lump-formation" largely relies on the size of the biosorbents [37]. The finer the biosorbents (powder form in this study), the higher the possibility of aggregation. The aggregation caused the surface area of *S. maltophilia* to become either more adequate or limited for the binding of a particular metal in the multi-metal solutions. This indirectly affects the diffusional path length [37]. Taking 0.10 g of *S. maltophilia* for example (uptake trend of $Pb^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+}$), it was possible that more Pb^{2+} binding sites were exposed while the binding sites for Cd^{2+} were covered. The diffusional path length decreased for Pb^{2+} while increased for Cd^{2+} hence the higher removal of Pb^{2+} than Cd^{2+}. However, the uptake preference reversed when 0.50 and 1.50 g of *S. maltophilia* were used instead (uptake trend of $Cd^{2+} > Pb^{2+} > Zn^{2+} > Cu^{2+}$). This was attributed to possibly more binding sites for Cd^{2+} which subsequently decreased the diffusional path length. The non-specific removal trend revealed in this study had proven that multi-metal solutions are more complex than single-metal solutions in terms of adsorbent dosages, depending on the metal cations and their interaction with the type of biosorbents used.

3.5. ATR-FTIR analysis

ATR-FTIR spectra showed eight prominent peaks at wavenumbers 3265.88, 2924.87, 1633.36, 1519.94, 1455.20, 1393.04, 1223.66 and 1065.54 cm^{-1} (Table 3, Fig. 4). This affirms the heterogeniety of the surface of *S. maltophilia*. The presence of hydroxyl (–OH) and amine ($–NH_2$) functional groups were concluded based on the detection of peaks at 3265.88 cm^{-1}. Hydroxyl (–OH) and amine ($–NH_2$) groups are commonly associated with alcohols, phenols and proteins,

Table 2
Influence of adsorbent dosages of *S. maltophilia* on metal removal in multi-metal solutions. Values are means of triplicates (±standard deviations). Means with the same letters for each within the tested metal are not significantly different (probability values set at <0.05)

Adsorbent dosages (g)	Biosorption (mg g^{-1})			
	Pb	Cu	Cd	Zn
0.10	8.712 ± 0.701[a]	5.086 ± 0.011[a]	1.486 ± 0.002[c]	1.182 ± 0.045[c]
0.50	2.328 ± 0.027[c]	2.016 ± 0.052[b]	2.507 ± 0.015[b]	2.301 ± 0.060[a]
1.00	1.111 ±0.018[d]	0.702 ± 0.000[c]	1.246 ± 0.007[d]	1.175 ± 0.021[c]
1.50	6.492 ± 0.130[b]	1.883 ± 0.093[b]	8.040 ± 0.000[a]	2.146± 0.000[b]

Table 3
Types of functional groups identified on *S. maltophilia* using ATR-FTIR

Wavenumbers (cm^{-1})	Types of functional groups	Types of compounds
3265.88	Hydroxyl, –OH	Alcohols / Phenols
	Amine, NH_2	Proteins
2924.87	Methyl, $–CH_3$	Aliphatic acids
1633.36	Alkene, –C=C–/ Carbonyl, C=O	Aldehydes / Ketones
1519.94	Aromatic carbon C=C/ Nitro, NO_2	
1455.20, 1393.04	Methyl, $–CH_3$	Aliphatic acids
1223.66 1065.54	Carboxyl, –COOH	Alcohols / Ethers/ Carboxylic acids/ Esters

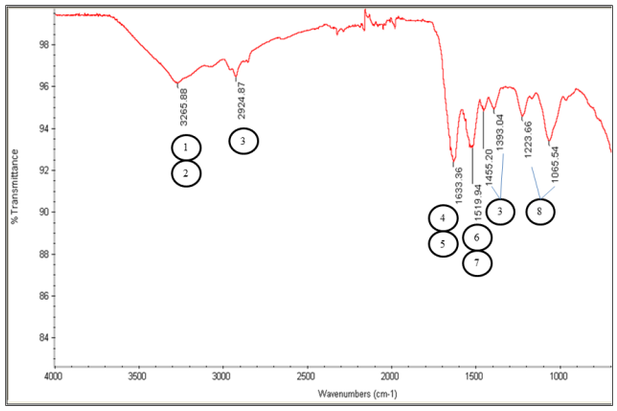

Fig. 4. ATR-FTIR spectra for *S. maltophilia*. Identified functional groups are indicated by 1 denoting hydroxyl (–OH), 2 denoting amine ($–NH_2$), 3 denoting methyl ($–CH_3$), 4 denoting alkene (–C=C–), 5 denoting carbonyl (C=O), 6 denoting aromatic carbon C=C, 7 denoting nitro (NO_2) and 8 denoting carboxyl (–COOH).

which are abundant in the cell wall structure of a typical bacterium. Alkene (–C=C–) and carbonyl (C=O) groups, attributed to aldehydes or ketones, respectively in the bacterium, were also from visible peaks at 1633.36 cm^{-1}. In addition, detection of peaks at 2924.87, 1393.04 and 1455.20 cm^{-1} suggested presence of methyl ($–CH_3$) groups from aliphatic acids, while peaks at 1519.94 cm^{-1} revealed the existence of aromatic carbon (C=C) and nitro (NO_2). Another key functional group, carboxyl (–COOH), was also found in *S. maltophilia* with peaks detected at 1223.66 and 1065.54 cm^{-1}, which may be contributed by alcohols, ethers, carboxylic acids and esters in the bacterium (Table 3, Fig. 4).

The identification of functional groups present on *S. maltophilia* sheds light on their involvement in binding of metals to cell surface. Most functional groups identified are oxygen rich radicals such as hydroxyl (–OH) and carbonyl (C=O). Apart from oxygen, nitrogen-rich functional groups were also identified. These functional groups are usually regarded as the primary metal binding sites for metal cations including Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}. These functional groups may have regulated the sorption mechanism, complying with pseudo-second order kinetic compared to pseudo-first order.

4. Conclusion

This study demonstrated that firstly, the bacterium *S. maltophilia* as a biosorbent, was able to remove metals from both single- and multi-metal solutions. The biosorption of metal cations by *S. maltophilia* in single and multi-metal solutions was promising despite its lower efficacy in multi-metal solutions. In both the single and multi-metal solutions, the trend of metal removed was Pb^{2+} > Cu^{2+} followed by either Cd^{2+} or Zn^{2+}. In terms of pH and adsorbent dosages, the optimization of these factors is important. This study, vastly differing from single-metal studies, have shown that pH 7 with no specific trend of adsorbent dosages (recommended dosage at 0.10–0.50 g) were the optimum conditions for biosorption in multi-metal solutions.

Acknowledgement

This project was supported by the Ministry of Higher Education (MOHE) under the FRGS scheme (FRGS/2/2013/STWN01/MUSM/02/2). The authors are also grateful to Monash University Malaysia for the facilities to conduct the study.

Disclosure Statement

No conflict of interest was reported between the authors.

References

[1] G.M. Gadd, L. Ramsay, J.W. Crawford, K. Ritz, Nutritional influence on fungal colony growth and biomass distribution in response to toxic metals, FEMS Microbiol. Lett., 204 (2001) 311–316.
[2] G.A. Drasch, An increase of cadmium body burden for this century- an investigation on human tissues, Sci. Total Environ., 26 (1993) 111–119.
[3] T.A Davis, B. Volesky, A. Mucci, A review of the biochemistry of heavy metal biosorption by brown algae, Water Res., 3 (2000) 4311–4330.
[4] M. Gavrilescu, Removal of heavy metals from the environment by biosorption, Eng. Life Sci., 4 (2004) 219–232.
[5] A.G. Moat, J.W. Foster, M.P. Spector, Microbial Physiology, New York, Wiley-Liss. 2002.
[6] L.M. Prescott, J.P. Harley, D.A. Klein, Microbiology, United Kingdom, McGraw-Hill, 2002.
[7] N. Kuyucak, B. Volesky, Biosorbents for recovery of metals from industrial solutions, Biotechnol. Lett., 10 (1988) 137–142.
[8] X. Deng, P. Wang, Isolation of marine bacteria highly resistant to mercury and their bioaccumulation process, Biores. Technol., 121 (2012) 342–347.

[9] 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.
[10] C.C. Lin, Y.T. Lai, Adsorption and recovery of lead (II) from aqueous solutions by immobilized *Pseudomonas aeruginosa* PU21 beads, J. Hazard. Mater., 137 (2006) 99–105.
[11] S. Tunali, A. Cabuk, T. Akar, Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil, Chem. Eng. J., 115 (2006) 203–211.
[12] G. Uslu, M. Tanyol, Equilibrium and thermodynamic parameters of single and binary mixture biosorption of lead (II) and copper (II) ions onto *Pseudomonas putida*: effect of temperature, J. Hazard. Mater., 135 (2006) 87–93.
[13] M. Zhou, Y.G. Liu, G.M. Zeng, X. Li, W.H. Xu, T. Fan, Kinetic and equilibrium studies of Cr (VI) biosorption by dead *Bacillus licheniformis*biomass, World J. Microbiol. Biotechnol., 23 (2007) 43–48.
[14] M. Ziagova, G. Dimitriadis, D. Aslanidou, X. Papaioannou, E.L. Tzannetaki, M. Liakopoulou-Kyriakides, Comparative study of Cd (II) and Cr (VI) biosorption on *Staphylococcus xylosus* and *Pseudomonas* spp in single and binary mixtures, Biores. Technol., 98 (2007) 2859–2865.
[15] D. Pages, J. Rose, S. Conrod, S. Cuine, P. Carrier, T. Heulin, W. Achouak, Heavy metal tolerance in *Stenotrophomonasmaltophilia*, PLoS ONE, 3 (2008) 1539–1542.
[16] A.S.Y. Ting, C.C. Choong, Utilization of non-viable cells compared to viable cells of *Stenotrophomonasmaltophilia* for copper (Cu(ii)) removal from aqueous solutions, Adv. Environ. Biol., 3 (2009) 204–209.
[17] W.S. Tan, A.S.Y. Ting, Efficacy and reusability of alginate-immobilized live and heat-inactivated *Trichodermaasperellum* cells for Cu(II) removal from aqueous solution, Biores. Technol., 123 (2012) 290–295.
[18] C.S.F. Sim, W.S. Tan, A.S.Y. Ting, Endophytes from *Phragmites*-for metal removal: evaluating their metal tolerance, adaptive behaviour and biosorption efficacy, Desal. Water Treat., 57 (2016) 6959–6966.
[19] I. Ahmad, M.I. Ansari, F. Aqil, Biosorption of Ni, Cr and Cd by metal tolerant *Aspergillusniger* and *Penicillium* sp. using single and multi-metal solution, Indian J. Exp. Biol., 44 (2006) 73–76.
[20] R. Ramsenthil, R.M. Meyyappan, Single and multi-component biosorption of copper and zinc ion using microalgal resin, Int. J. Environ. Sci. Dev., 1 (2010) 298–301.
[21] K.M. Al-Qahtani, Biosorption of binary mixtures of heavy metals by *Medicago sativa*, World Appl. Sci. J., 16 (2012) 465–473.
[22] S. Singh, S. Pradhan, L.C. Rai, Metal removal from single and multimetallic systems by different biosorbent materials as evaluated by differential pulse anodic stripping voltametry, Process Biochem., 36 (2000) 175–182.
[23] V. Obuseng, F. Nareetsile, H.M. Kwaambwa, A study of the removal of heavy metals from aqueous solutions by *Moringaoleifera* seeds and amine-based ligand 1,4-bis[N,N-bis(2-picoyl)amino]butane, Anal. Chim. Acta., 730 (2012) 87–92.
[24] S.K. Chandra, C.T. Kamala, N.S. Chary, A.R.K. Sastry, R.T. Nageswara, M. Vairamani, Removal of lead from aqueous solutions using an immobilized biomaterial derived from a plant biomass, J. Hazard. Mater., B108 (2004) 111–117.
[25] S. Sannasi, J. Kader, B.S. Ismail, S. Salmijah, Sorption of Cr(VI), Cu(II), Pb(II) by growing and non-growing cells of a bacterial consortium, Biores. Technol., 97 (2006) 740–747.
[26] J.S. Chang, J.C. Huang, Selective adsorption/ recovery of Pb, Cu and Cd with multiple fixed beds containing immobilized bacterial biomass, Biotechnol. Prog., 14 (1998) 735–741.
[27] 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.
[28] S.K. Sen, S. Raut, T.K. Dora, P.K.D. Mohapatra, Contribution of hot spring bacterial consortium in cadmium and lead bioremediation through quadratic programming model, J. Hazard. Mater., 265 (2014) 47–60.
[29] J. Febrianto, A.N. Kosasih, J. Sunarso, Y.H. Ju, N. Indraswati, S. Ismadji, Equilbrium and kinetic studies in adsorption of heavy metals using biosorbent: a summary of recent studies, J. Hazard. Mater., 162 (2009) 616–645.
[30] J.L. Wang, C. Chen, Biosorption of heavy metals by *Saccharomyces cerevisiae*- a review, Biotechnol. Adv., 24 (2006) 427–451.
[31] S. Congeevaram, S. Dhanarani, J. Park, M. Dexilin, K. Thamaraiselvi, Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates, J. Hazard. Mater., 146 (2007) 270–277.
[32] N. Lokeshwari, K. Joshi, Biosorption of heavy metal (chromium) using biomass, Glob. J. Environ. Res., 3 (2009) 29–35.
[33] A. Saeed, M. Iqbal, M.W. Akhtar, Removal and recovery of lead (II) from single and multimetal (Cd, Cu, Ni, Zn) solutions by crop milling waste (black gram husk), J. Hazard. Mater., 117 (2005) 65–73.
[34] K.C. Sekhar, S. Subramaniam, J.M. Modak, K.A. Natarajan, Removal of metal ions using an industrial biomass with reference to environmental control, Int. J. Miner. Process., 53 (1998) 107–120.
[35] S. Karthikeyan, R. Balasubramaniam, C.S.P. Iyer, Evaluation of the marine algae *Ulvafasciata* and *Sargassum* sp. for the biosorption of Cu(II) from aqueous solutions, Biores. Technol., 98 (2007) 452–455.
[36] R. Boota, H.N. Bhatti, M.A. Hanif, Removal of Cu(II) and Zn(II) using lignocellulosic fiber derived from *Citrus reticulate* (Kinnow) waste biomass, Sep. Sci. Technol., 44 (2009) 4000–4022.
[37] H.N. Bhatti, S. Hamid, Removal of uranium (VI) from aqueous solutions using *Eucalyptus citriodora* distillation sludge, Int. J. Environ. Sci. Technol., 11 (2014) 813–822.