*Polish Journal of Chemical Technology, 17, **1,** 79 — 87, 10.1515/pjct-2015-0012*

Biosorption of lead(II), zinc(II) and nickel(II) from industrial wastewater by *Stenotrophomonas maltophilia* and *Bacillus subtilis*

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The biosorption of Pb(II), Zn(II) and Ni(II) from industrial wastewater using *Stenotrophomonas maltophilia* and *Bacillus subtilis* was investigated under various experimental conditions regarding pH, metal concentration and contact time. The optimum pH values for the biosorption of the three metals were in the range 5.0–6.0, while the optimal contact time for the two bacterial species was 30 min. Experimental data was analyzed using Langmuir and Freundlich isotherms; the former had a better fit for the biosorption of Pb(II), Zn(II) and Ni(II). The maximum adsorption uptakes (q_{max}) of the three metals calculated from the Langmuir biosorption equation for *S. maltophilia* were 133.3, 47.8 and 54.3 for Pb(II), Zn(II) and Ni(II), respectively, and for *B. subtilis* were 166.7, 49.7 and 57.8 mg/g, respectively. *B. subtilis* biomass was more favorable for the biosorption of Pb (II) and Ni (II), while *S. maltophilia* was more useful for the biosorption of Zn (II).

Keywords: heavy metals, biosorption, *Bacillus subtilis*, *Stenotrophomonas maltophilia*.

INTRODUCTION

Pollution of the environment with toxic heavy metals is spreading throughout the world alongside industrial expansion; the resultant contamination of soils, groundwater, sediments, surface water and the air poses significant problems for both human health and the environment[1]. Despite serious environmental concerns due to their toxicity even at low concentrations[1–5], various industrial processes, such as electroplating, metal finishing, metallurgical work, tanning, chemical manufacturing, mining and battery manufacturing, result in the continuous introduction of heavy metal polluted wastewater to the environment (mainly Pb, Hg, Cu, Cd, Zn, Ni and Cr).

With regard to their impact on human health, each heavy metal imparts different effects and symptoms. For instance, in the case of minor Zn exposure, common symptoms are irritability, muscular stiffness, loss of appetite and nausea[6, 7]. Pb is extremely toxic and can inflict damage to the nervous system, kidneys and reproductive system, particularly in children[4, 7]. The presence of Ni above a critical level might bring about serious lung and kidney problems, aside from gastrointestinal distress, pulmonary fibrosis and skin dermatitis[8].

Conventional methods used to remove dissolved heavy metal ions from wastewaters include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, solvent extraction, reverse osmosis, membrane technologies and evaporation recovery. These processes may be ineffective or extremely expensive, especially when the metals in solution are in the range 1–100 mg/l[8, 9]. Another major disadvantage of conventional treatment methods is the production of toxic chemical sludge and its subsequent disposal/treatment being costly and not eco-friendly. Therefore, it is so significant to find a cost effective and environment-friendly method of removing toxic heavy metals down to a level considered environmentally safe[4, 10].

One of the most promising technologies involved in the removal of toxic metals from industrial waste streams is biosorption, based on the ability of many algae, fungi, yeasts and bacteria to concentrate heavy metals from aquatic environments[8, 11, 12]. It offers the advantages of low operating costs, the possibility of metal recovery, regeneration of the biosorbent, minimization of the volume of chemical and/or biological sludge to be disposed of, and high efficiency in detoxifying very dilute effluents[13–15]. This complex process depends on the properties of metal ions, the cell wall composition of microorganisms, cell physiology, as well as physicochemical factors such as pH, temperature, contact time, ionic strength, and metal concentration[15]. It occurs through complexation, coordination, physical adsorption, chelation, ion exchange, inorganic precipitation or some combination of these processes[11, 16]. These processes involve the active participation of several anionic ligands present in the biomass, such as phosphoryl, carboxyl, carbonyl, sulfydryl and hydroxyl groups, to immobilize metal ions[17].

The main objective of this work was to study differences in the adsorption of Pb(II), Zn (II) and Ni(II) between *Stenotrophomonas maltophilia* and *Bacillus subtilis* cells isolated from a wastewater treatment plant. Factors affecting biosorption (i.e. pH, reaction duration, metal concentration) were also studied. Biosorption isotherms and kinetics were determined from biosorption measurements.

EXPERIMENTAL

Microorganisms

Lead, zinc and nickel resistant bacterial strains were isolated from a wastewater treatment plant located in Głubczyce (Poland). Samples were diluted 10–10.000 fold in sterile distilled water and plated on Nutrient Agar (Merck). To isolate resistant strains, the media were amended with 100 mg/l Pb(II), 50 mg/l Zn(II) and 50 mg/l Ni(II) and a standard spread plate method was performed. The inoculated plates were incubated for 48 h at 30°C after which larger identical colonies from each plate were isolated. The most effective bacterial strains for the biosorption of Pb(II) and Ni(II) were identified according to Bergey's Manual of Systematic Bacteriology[18]. Morphological, physiological and biochemical characteristics of the isolated bacterial species (*S. maltophilia* and *B. subtilis*) are given in Table 1.

Table 1. Biochemical characterization of the isolate strains

	Result	Properties	Result
Gram reaction	+	Gram reaction	–
Cell shape	Rod	Cell shape	Rod
Cell diameter >1.0 μm	–	Motility	+
Spores round	+	Catalase test	+
Catalase	+	Citrate test	+
Oxygen requirements	Aerobic	Oxidase test	–
Voges-Proskauer test	+	Methyl red test	–
Glucose	+	Voges-Proskauer test	–
Xylose	+	Gelatin test	+
Mannitol	+	Starch test	–
Hydrolysis of casein	+	Arabinose	–
Hydrolysis of gelatin	+	Dextrose	+
Hydrolysis of starch	+	Fructose	–
Utilization of citrate	+	Galactose	–
Nitrate reduced to nitrite	+	Inositol	–
Formation of indole	–	Lactose	–
Growth at pH 6.8, nutrient broth	+	Maltose	+
Growth at pH 5.7, nutrient broth	+	Mannitol	–
Growth in NaCl 2%	+	Mannose	–
Growth in NaCl 5%	ND	Sorbitol	–
Growth in NaCl 7%	–	Sucrose	+
Growth in NaCl 10%	ND	Trehalose	+
Growth at 5°C	+	Xylose	–
Growth at 10°C	+	Nitrate reduction	+
Growth at 30°C	ND	Type strain	–
Growth at 40°C	–		*Stentrophomonas maltophilia*
Growth at 50°C	–		
Growth at 55°C	–		
Growth at 65°C	–		
Type strain	*Bacillus subtilis*		

+: 90% or more strain positive, –: 90% or more strain negative, ND: not detected.

Preparation of biomass

Bacterial strains of *S. maltophilia* and *B. subtilis* were cultivated aerobically at 30°C in Nutrient Broth (Merck) constantly agitated at 150 rpm in glass flasks. After inoculation, cells were harvested by means of centrifugation for 20 min at 3000 rpm. The cell pellet was rinsed three times with sterile deionized water, then freeze dried using a lyophilizer (Alpha 1-2 LD plus, Christ, Germany). For the purpose of the biosorption experiments, 1 g portions of bacterial cell mass (separately for *S. maltophilia* and *B. subtilis*) were suspended in 1 l of deionized water.

Wastewater sample

Wastewater was obtained from a chemical manufacturing plant in Głubczyce (Poland). The composition of the wastewater is given in Table 2. The concentrations of the other metal ions present in the wastewater were so minimal that they would not effect the removal of lead, zinc and nickel ions from the wastewater. If necessary, the effluent was diluted with deionized water to an appropriate concentration of heavy metals.

Table 2. Characteristics of wastewater used

Characteristics	Value
pH	2.5–5.3
Lead [mg/l]	326.4
Zinc [mg/l]	178.2
Nickel [mg/l]	249.3
Cadmium [mg/l]	0.1
Cobalt [mg/l]	0.1
Copper [mg/l]	0.2

Heavy metal assay

The concentrations of Pb(II), Zn(II) and Ni(II) in the biosorption experiments were determined spectrophotometrically (Photolab Spectral, WTW, Germany). Before measuring the samples were passed through a Whatman filter (pore size 0.45 μm) and then diluted with deionized water. The initial and the final concentrations of heavy metals used in batch mode studies were estimated spectrophotometrically. The removal efficiency of the microorganisms was calculated from the difference between initial and final concentrations.

Biosorption experiments

Parameters of Pb(II), Zn(II) and Ni(II) sorption by *S. maltophilia* and *B. subtilis* are presented in Table 3.

Experimental tests were conducted in a BIOSTAT A-plus bioreactor containing 1.0 l of wastewater at constant level of biomass (1.0 g/l) at 30°C and agitation of 200 rpm. Biosorption experiments were carried out to investigate the effects of pH, contact duration and initial metal concentration. The pH values were adjusted between 2.0–7.0 by adding 0.1 M NaOH or 0.1 M HNO_3. The contact durations ranged from 0–60 min. The initial Pb(II), Zn(II) and Ni(II) concentrations varied from 15.9 to 325.3 mg/l, 11.2 to 172.3 mg/l and 14.3 to 245.8 mg/l, respectively. The metal uptake (mg metal/g dry biomass) was calculated according to:

$$q_e = \frac{(C_0 - C_e)V}{M} \tag{1}$$

Where C_0 and C_e are the respective initial and equilibrium metal concentrations in the solution (mg/l), V is the volume of the solution (l), and M is the dry weight of the biomass (g). The metal sorption ability of the biomass was determined by the above-mentioned procedure in all the following experiments unless stated otherwise.

Adsorption isotherms

Heavy metals biosorption isotherms were obtained at constant pH and ionic strength. To test the fit of data, Langmuir and Freundlich isotherm models were applied

Table 3. Parameters characterizing biosorption experiments

Biosorption experiments	Biosorbent dose [mg/l]	Temperature [°C]	Agitation speed [rpm]	pH	Time [minute]	Heavy metal concentration		
						Pb(II) [mg/l]	Zn(II) [mg/l]	Ni(II) [mg/l]
Effect of pH	1.0	30	200	2	30	98.4	39.6	45.6
	1.0	30	200	3	30	98.4	39.6	45.6
	1.0	30	200	4	30	98.4	39.6	45.6
	1.0	30	200	5	30	98.4	39.6	45.6
	1.0	30	200	6	30	98.4	39.6	45.6
	1.0	30	200	7	30	98.4	39.6	45.6
Effect of contact time	1.0	30	200	5.5	5	98.4	39.6	45.6
	1.0	30	200	5.5	10	98.4	39.6	45.6
	1.0	30	200	5.5	15	98.4	39.6	45.6
	1.0	30	200	5.5	20	98.4	39.6	45.6
	1.0	30	200	5.5	25	98.4	39.6	45.6
	1.0	30	200	5.5	30	98.4	39.6	45.6
	1.0	30	200	5.5	35	98.4	39.6	45.6
	1.0	30	200	5.5	40	98.4	39.6	45.6
	1.0	30	200	5.5	45	98.4	39.6	45.6
	1.0	30	200	5.5	50	98.4	39.6	45.6
	1.0	30	200	5.5	55	98.4	39.6	45.6
	1.0	30	200	5.5	60	98.4	39.6	45.6
Effect of heavy metal concentration	1.0	30	200	5.5	30	15.9	11.2	14.3
	1.0	30	200	5.5	30	39.6	20.6	31.5
	1.0	30	200	5.5	30	81.5	42.3	60.2
	1.0	30	200	5.5	30	125.4	62.4	90.2
	1.0	30	200	5.5	30	147.8	86.0	123.6
	1.0	30	200	5.5	30	162.7	104.3	159.4
	1.0	30	200	5.5	30	201.3	132.2	188.6
	1.0	30	200	5.5	30	250.6	149.9	221.4
	1.0	30	200	5.5	30	325.3	172.3	245.8

to this study. The Langmuir isotherm model is valid for monolayer sorption onto a surface and a finite number of identical sites, and is given by:

$$q_{eq} = \frac{q_{max}\ bC_{eq}}{1+bC_{eq}} \quad (2)$$

or presented in linear form as follows:

$$\frac{1}{q_{eq}} = \frac{1}{q_{max}} + \frac{1}{bq_{max}\ C_{eq}} \quad (3)$$

Where q_{max} is the maximum amount of the metal ion per unit weight of the cell to form a complete monolayer on the surface bound at a high C_{eq} (mg/l) and b, a constant related to the affinity of the binding sites, q_{max} represents a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in the comparison of adsorption performance, particularly in cases where the sorbent did not reach full saturation in experiments. Another essential factor of the Langmuir isotherm is R_L, which can be calculated according to the following equation:

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

Where C_0 is the highest metal concentration (mg/l).

The empirical Freundlich isotherm model based on a heterogeneous surface is given by:

$$q_{eq} = K_f \cdot C_{eq}^{1/n} \quad (5)$$

Where K_f and n are Freundlich constants characteristic of the system, K_f and n are indicators of adsorption capacity and intensity, respectively. Freundlich parameters can be determined from the linear form of the eq. (5) by plotting the lnq_{eq} versus lnC_{eq} the slope is the value of $1/n$ and the intercept is equal to lnK_f. The Freundlich isotherm is also more widely used and provides information on the monolayer adsorption capacity, in contrast to the Langmuir model[16]. All data shown are the mean values of three replicate experiments, and error bars are indicated wherever necessary.

RESULTS AND DISCUSSION

Characteristics of biosorbents

In this study the bacterial strains of *Stenotrophomonas maltophilia* and *Bacillus subtilis* were identified according to Bergey's Manual of Systematic Bacteriology[18]. Their biochemical and microscopic characteristics are given in Table 1. *B. subtilis* is a gram-positive aerobic rod-shaped bacterium ubiquitous in soils and waters, with a well-known parietal structure[19]. *S. maltophilia* is common in water and soil environments; many reports have indicated its potential wide application in biotechnology, including the biological control of plant pathogens, bioremediation and biosorption[20].

Effect of pH

The effect of hydrogen ion concentrations on the biosorption of heavy metals has been the subject of many studies, which shows the importance of this parameter on the solubility of the metal ions as well as on the ionization of the fixing sites[5, 17, 21]. In this work, pH was varied in order to determine its optimum value for maximum biosorption of lead, zinc and nickel ions. It can be seen from Figure 1 and Figure 2 that the biosorptive capacity of Pb(II), Zn(II) and Ni(II) by the two bacterial strains was very low at a low pH value and it increased with pH until reaching an optimum between 5.0 and 6.0. At levels higher than 6.0 the heavy metals begin to precipitate. *S. maltophilia* demonstrated a maximum capacity (q_e) of 71.4 mg/g for Pb(II) and 29.8 mg/g for Zn(II) at pH 5.0 while *B. subtilis* demonstrated 78.8 and 30.0 mg/g respectively. The maximum biosorption of Ni(II) by *S. maltophilia* was 39.8 mg/g at pH 6.0, and 40.1 mg/g for *B.*

subtilis. At low pH values, cell wall ligands were closely associated with hydronium ions H_3O^+ and so restricted the biosorption of Pb(II), Zn(II) and Ni(II) as a result of the competition between H_3O^+ and the heavy metals with the bacterial biosorbent cell wall ligands[22].

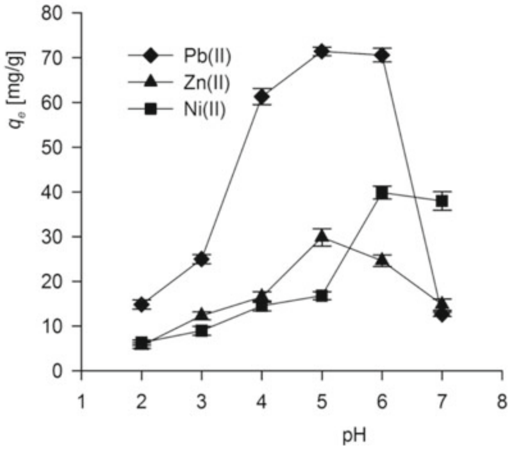

Figure 1. Effect of pH on the biosorption of Pb(II), Zn(II) and Ni(II) by *Stenotrophomonas maltophilia*. Metal concentration: Pb(II) 98.4 mg/l, Zn(II) 39.6 mg/l, Ni(II) 45.6 mg/l, contact time 30 min

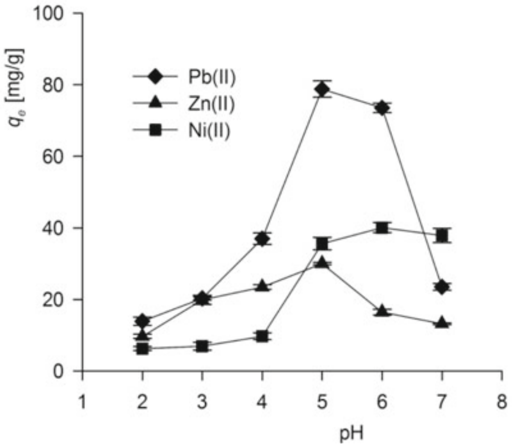

Figure 2. Effect of pH on the biosorption of Pb(II), Zn(II) and Ni(II) by *Bacillus subtilis*. Metal concentration: Pb(II) 98.4 mg/l, Zn(II) 39.6 mg/l, Ni(II) 45.6 mg/l, contact time 30 min

Differences between the analyzed bacterial strains in their ability to bind metal ions are small. The higher metal binding capacity of *B. subtilis* (gram-positive) compared to *S. maltophilia* (gram-negative) can be traced back to the cell wall makeup. The gram-positive cell wall features a layer about 20–30 nm thick of peptidoglycan (PG) into which teichoic acids (TA) and teichuronic acids (TUA) are embedded. Peptidoglycan is a linear polymer of alternating glucosamine and muramic acid with peptide side chains. These side chains bear one carboxyl group at the terminal amino acid, and additional functional groups on certain intermediate amino acids like asparagine, lysine, cysteine, or aspartic acid. Teichoic acid contains phosphodiesters. Teichuronic acids feature carboxyl groups both of which contribute to the negative charge of the biomass and enable ion exchange. Gram-negative bacteria have a much thinner (only 1–3 molecules thick) PG layer which makes up about 10% of the weight of the total cell wall, which can be 30–80 nm thick. The PG layer of gram-negative bacteria does not contain TA or TUA, therefore they offer less negatively charged carboxyl groups, which is a reason for their lower biosorptive capacity[23]. On the other hand, a characteristic of these bacteria is an outer membrane which contains lipopolysaccharides (LPS) and phospholipids. Their phosphonate groups create a negative surface charge conducive to cation binding. As the pH increases, the competing effect of H_3O^+ ions decreases. More functional groups such as carboxylic, phosphate and amino acid groups carrying negative charges are exposed[17, 24]. The degree of ionization of these negative groups also increases; leading to electrostatic attractions between the positively charged cations such as Pb(II), Zn(II) and Ni(II) and the negatively charged binding sites, thereby promoting the binding of heavy metals[9, 25]. This suggested that the biosorption of metals from wastewater is based on ion exchange. This finding also indicated that Pb(II), Zn(II) and Ni(II) removed was mainly bound to the cell walls and external surfaces of the biomass. Lead biosorption was maximal at pH 5.0, a value in agreement with results obtained by Veglió et al.[26] who found the maximal pH for lead by *Arthrobacter* sp. was 5.0. Çabuk et al.[27] also reported the was pH = 5.0 to be optimal for the biosorption of Pb(II) by *Bacillus* sp. ATS-2. Similar results were observed by Sassi et al.[5], Bahadir et al.[4] and Ho[28], while Çolak et al.[29] concluded that the optimum pH for Pb(II) biosorption was 6.0 for *Bacillus* strains, and Rodríguez-Tirado et al.[7] observed an optimum pH of 4.0 for *Bacillus thioparans* U3.

Maximal pH for zinc biosorption for the presented two bacterial species was 5.0. This is in agreement with Li et al.[30] and Chen et al.[16], who also reported the optimum pH for Zn(II) removal by *Pseudomonas putida* CZ1 was 5.0. Other studies like Joo et al.[17] and Aston et al.[31] reported that maximal pH for zinc biosorption for *Pseudomonas aeruginosa* and *Acidithiobacillus caldus* BC13 were 6.0 and 4.0, respectively.

The maximal pH for Ni(II) biosorption for the presented bacterial biomass was 6.0. Pahlavanzadeh et al.[10] and Liu et al.[22] reported maximal removal of nickel in the pH range 5.0–6.0. In contrast, Gabr et al.[15] and Lopez et al.[32] found the maximal pH for nickel biosorption for *Pseudomonas aeruginosa* ASU 6a and *P. fluorescens* 4F39 were 7.0 and 8.0, respectively. A decrease in the removal of Ni(II) at a pH above 6.0 is due to the formation of $Ni(OH)_2$. Substantial precipitation of nickel and nickel hydroxide occurs at high pH values. The formation of hydroxide precipitate reduces the amount of free nickel ions[13].

Effect of contact duration

The kinetics of metal ion sorption is an important parameter for designing sorption systems and is required for selecting the optimum operating conditions for full-scale batch metal removal process[22]. The effect of contact duration on the extent of biosorption of Pb(II), Zn(II) and Ni(II) by bacterial biomass is shown in Figure 3 and

Figure 4. The rate of Pb(II) biosorption by *S. maltophilia* and *B. subtilis* was very rapid, reaching almost 72.5% and 74.3% of the maximum adsorption capacity within 5 min of contact, respectively. However, it took longer for Zn(II) and Ni(II) to be adsorbed by *S. maltophilia* and *B. subtilis*, which reached approximate 67.4–73.5% and 63.9–78.9% of the maximum biosorption capacity within 20 min, respectively. The initial fast uptake was likely due to the high initial Pb(II), Zn(II) and Ni(II) concentration and empty metal binding sites on the microbes. The slower subsequent phase was likely due to the saturation of metal binding sites. Therefore, one can conclude that the appropriate equilibrium time for measurements was reached at 30 min. This represents the equilibrium time at which an equilibrium metal ion concentration is presumed to have been attained. This short time required for biosorption is in accordance with the results given by other authors[10, 15–17, 21, 30]. Gabr et al.[15], Joo et al.[17] and Pahlavanzadeh et al.[10] showed that the maximum biosorption of lead, zinc and nickel was reached after 30–40 min. A rapid metal sorption is also highly desirable for successful deployment of biosorbents for practical applications[16].

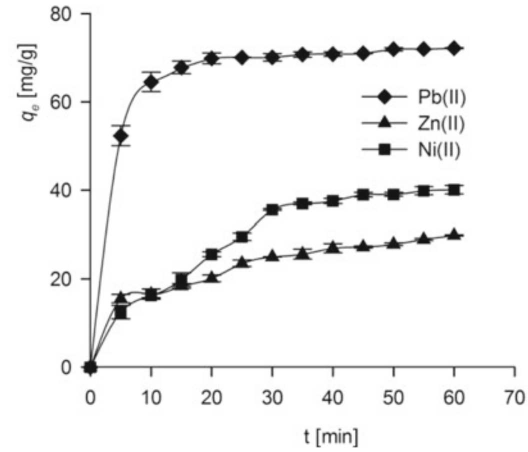

Figure 3. Effect of contact time on the biosorption of Pb(II), Zn(II) and Ni(II) by *Stenotrophomonas maltophilia*. Metal concentration: Pb(II) 98,4 mg/l, Zn(II) 39,6 mg/l, Ni(II) 45,6 mg/l, pH 5.5

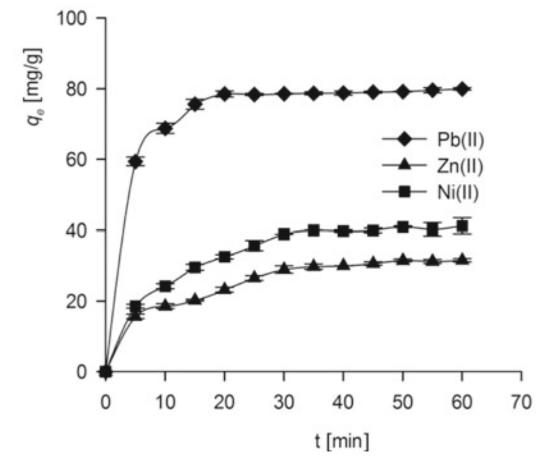

Figure 4. Effect of contact time on the biosorption of Pb(II), Zn(II) and Ni(II) by *Bacillus subtilis*. Metal concentration: Pb(II) 98,4 mg/l, Zn(II) 39,6 mg/l, Ni(II) 45,6 mg/l, pH 5.5

Biosorption isotherm

The biosorption isotherm curve represents the equilibrium distribution of metal ions between the aqueous and solid phases. The equilibrium distribution is important in determining the maximum biosorption capacity. Several isotherm models are available to describe this equilibrium distribution. Langmuir and Freundlich models are widely applied in equilibrium analysis to understand sorption mechanisms[9, 15–17, 21]. The Langmuir model considers sorption by monolayer type and supposes that all the active sites on the sorbent surface have the same affinity for heavy metal ions[33]. The Freundlich isotherm is an empirical equation which assumes a heterogeneous biosorption system with different active sites[30]. The linearized Langmuir adsorption isotherms of each metal for *S. maltophilia* and *B. subtilis* are shown in Figures 5 and 6.

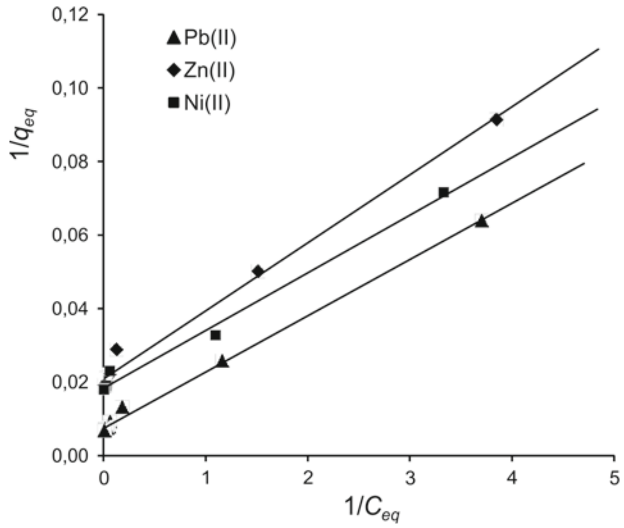

Figure 5. The linear from of Langmiur adsorption isotherm of Pb(II), Zn(II) and Ni(II) by *Stenotrophomonas maltophilia*. Metal concentration: Pb(II) 15.9–325.3 mg/l, Zn(II) 11.2–172.3 mg/l, Ni(II) 14.3–245.8 mg/l, contact time 30 min., pH 5.5

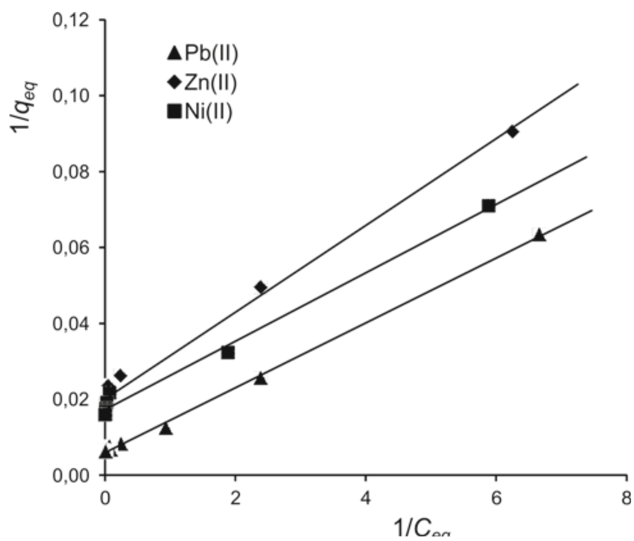

Figure 6. The linear from of Langmiur adsorption isotherm of Pb(II), Zn(II) and Ni(II) by *Bacillus subtilis*. Metal concentration: Pb(II) 15.9–325.3 mg/l, Zn(II) 11.2–172.3 mg/l, Ni(II) 14.3–245.8 mg/l, contact time 30 min., pH 5.5

The values of q_{max}, b and R^2 are given in Table 4. High coefficients of determination (R^2 = 0.990–0.998) for the Langmuir isotherm were obtained for all heavy metal biosorption with the bacterial isolates. A good fit of the Langmuir model indicates that the biosorption of Pb(II), Zn(II) and Ni(II) could be characterized by a monolayer formation of metal ions on the surface of the biomass and belongs to a single type phenomenon with no interactions between sorbed metals. This result was consistent with a number of earlier studies focusing on the adsorption of lead, zinc and nickel ions[15, 16, 27, 34]. The Langmuir q_{max} represents the saturation level of sorbed metal ions at high solution concentrations[30]. In the experiment of Pb(II) and Zn(II) biosorption, the q_{max} values for *S. maltophilia* were respectively 133.3 mg/g and 47.8 mg/g, compared to 166.7 mg/g and 49.7 mg/g for *B. subtilis*. It was also found that the value of Ni(II) biosorption by *B. subtilis* was higher (57.8 mg/g) than that of *S. maltophilia* (54.3 mg/g). The data show that at a high concentration of metal ions in wastewater the *B. subtilis* biomass showed a higher level of saturation with Pb (II), Zn (II) and Ni (II) than *S. maltophilia*.

It is known that b is the constant related to the affinity of the binding sites, which allows us to make a comparison of the affinity of the biomass for metal ions. As shown in Table 4 the affinity of *B. subtilis* to Pb(II) and Ni(II) (0.019 and 0.047 l/mg, respectively) was higher than that of *S. maltophilia* (0.016 and 0.036 l/mg, respectively). However, the values obtained for Zn(II) indicate that *S. maltophilia* possesses a higher adsorption affinity for Zn(II) as compared to *B. subtilis*. As reported by Li et al.[30], the parameter indicates the shape of the isotherm and nature of the biosorption proces ($R_L > 1$ unfavorable; $R_L = 1$ linear; $0 < R_L < 1$ favorable; $R_L = 0$ irreversible). The values shown in Table 4 indicate that the use of *B. subtilis* biomass was more favorable for the biosorption of Pb (II) and Ni (II), while *S. maltophilia* was more useful for the biosorption of Zn (II).

The Freundlich isotherm equation was originally empirical in nature, but it was later interpreted to be used in the case of sorption on heterogeneous surfaces or surfaces supporting sites of different affinities. The linear plots of lnq_{eq} versus for the two isolates are displayed in Figures 7 and 8.

The values of K_f, n and R^2 are shown in Table 4. The Freundlich isotherm represents the amount of metals sorbed when the solution concentration in the equilibrium is unity[9, 15, 30]. In Table 4, the magnitude of K_f shows a higher uptake of Pb(II), Zn(II) and Ni(II) using *B. subtilis* compared to *S. maltophilia*. The values of K_f were found to be 52.5, 21.3 and 26.0 l/mg biomass *B. subtilis* for Pb(II), Zn(II) and Ni(II) sorption and 36.4, 19.0, 23.6 l/mg for

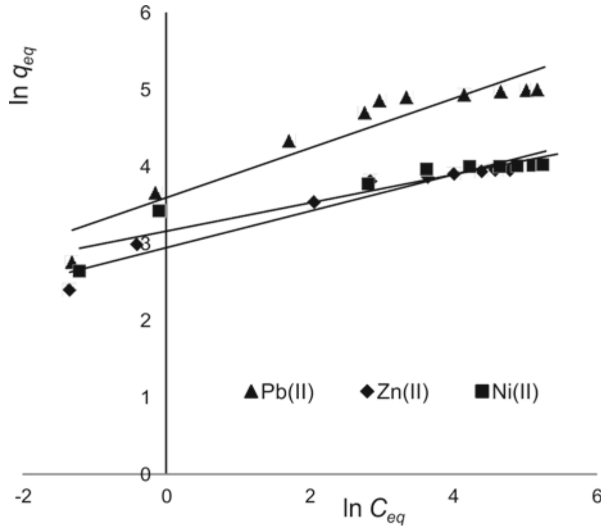

Figure 7. The linear from of the Freudlich adsorption isotherm of Pb(II), Zn(II) and Ni(II) by *Stenotrophomonas maltophilia*. Metal concentration: Pb(II) 15.9–325.3 mg/l, Zn(II) 11.2–172.3 mg/l, Ni(II) 14.3–245.8 mg/l, contact time 30 min, pH 5.5

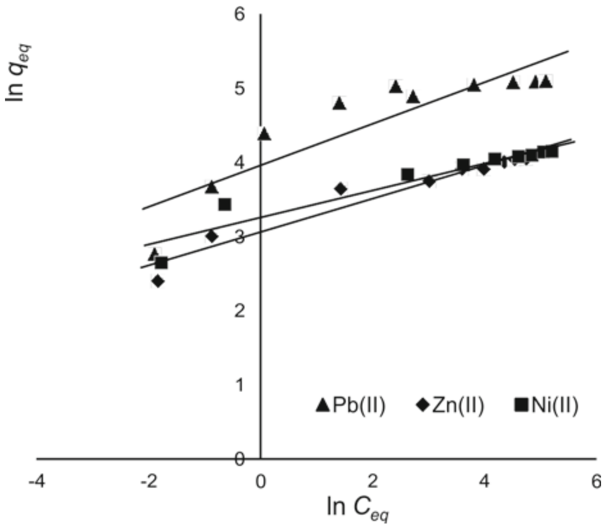

Figure 8. The linear from of the Freudlich adsorption isotherm of Pb(II), Zn(II) and Ni(II) by *Bacillus subtilis*. Metal concentration: Pb(II) 15.9 –325.3 mg/l, Zn(II) 11.2–172.3 mg/l, Ni(II) 14.3–245.8 mg/l, contact time 30 min., pH 5

S. maltophilia, respectively. The results suggest that *B. subtilis* cells were characterized by higher biosorption than *S. maltophili,* even at low concentrations of metal ions in the wastewater. Çolak et al.[29] showed that the value of obtained for lead sorption by *B. cereus* and *B. pumilus* were 30.78 and 39.40 l/mg biomass. Likewise, lead and nickel ions sorption by *P. aerugionas* ASU 6a gave 40.04 and 24.14 l/mg biomass, respectively[15]. A value of

Table 4. Langmuir and Freundlich parameters fo the biosorption of Pb(II), NI(II) and Zn(II) by *S. maltophilia* and *B. subtilis*

Metals	Biosorbent	Langmuir isotherm				Freundlich isotherm		
		q_{max} [mg/g]	b [l/mg]	R^2	R_L	K_f [l/mg]	n	R^2
Pb(II)	*Stenotrophomonas maltophilia*	133.3	0.016	0.996	0.16	36.4	3.91	0.891
	Bacillus subtilis	166.7	0.019	0.998	0.14	52.5	4.81	0,789
Zn(II)	*Stenotrophomonas maltophilia*	47.8	0.057	0.990	0.09	19.0	4.06	0.937
	Bacillus subtilis	49.7	0.044	0.990	0.11	21.3	3.61	0.938
Ni(II)	*Stenotrophomonas maltophilia*	54.3	0.036	0.990	0.10	23.6	5.38	0.873
	Bacillus subtilis	57.8	0.047	0.990	0.08	26,0	5.71	0.903

n which is related to the distribution of bonded ions on the sorbent surface, represents beneficial adsorption[35]. Larger values of *n* imply stronger interactions between the biosorbent and the heavy metals[30]. In this study, the *n* values for *B. subtilis* were 3.61–5.71 while those for *S. maltophilia* were 3.91–5.38, from which it could be derived that the effect of lead and nickel ions on *B. subtilis* was stronger than that on *S. maltophilia* biomass. Values of the correlation coefficient (R^2 = 0.789–0.938) are lower than the Langmuir model in the studied concentration range. Generally it can be stated that the sorption of lead, zinc and nickel by the analyzed bacterial strains depended on the initial concentration of the metal ions in wastewater. *B. subtilis* had high K_f and q_{max} values, indicating high sorption capacity, especially with regard to Pb(II), over the entire range of heavy metal ion concentrations in wastewater. The values obtained for *S. maltophilia* suggest its potential usefulness for the removal of Pb(II), Zn(II) and Ni(II) from wastewater containing low concentrations of these metals.

Figure 9 shows the efficiency of *S. maltophilia* and *B. subtilis* in removing of Pb(II), Zn(II) and Ni(II) from industrial wastewater (wastewater composition is shown in Table 2). Regardless of the type of used bacteria biomass, the efficiency of Pb(II), Zn(II) and Ni(II) removal was over 96% when the concentration in the wastewater did not exceed, 39.6, 20.6 and 31.5 mg/l, respectively.

Efficiency decreased with the increase of metal concentration in the wastewater, especially with regard to zinc and nickel ions. Only for lead ions did it remain above 80%, despite high concentrations of metals in the wastewater; 162.7, 104.3 and 159.4 mg/l, respectively for Pb(II), Zn(II) and Ni (II). In raw wastewater, removal efficiency was less than 49% of lead ions, and less than 33% zinc and 25% nickel ions. The differences in the results obtained after the application of *S. maltophilia* and *B. subtilis* were around 10–12%. The high affinity of living cells of genera *Pseudomonas* and *Bacillus* for Pb(II) is also confirmed in other studies by this author[36].

The descending order of selectivity of metal ions by the biomasses was Pb>Zn>Ni. This preferential type of adsorption may be ascribed to the difference in ionic radii and the electro-negativity of the metal ions. The ionic radius of Pb(II) is 1.20 A^o, while that of Zn(II) and Ni(II) is 0.9 A^o. The smaller the ionic radius, the greater its tendency to be hydrolyzed, leading to reduced biosorption. The electro-negativity of Pb(II) (2.33 Pauling) is greater than that of Ni(II) (1.91 Pauling) and Zn(II) (1.60 Pauling). Both the aforementioned factors contributed to the bacterial biomass had a greater affinity for lead than for nickel or zinc[36]. A comparison between the results of this work for *S. maltophilia* and *B. subtilis* and other studies found in literature is presented in Table 5. Thus, the comparison of adsorption capacities shows that the studies species of bacteria were efficient biosorbents of Pb(II), Ni(II) and Zn(II).

CONCLUSIONS

In this study, the live bacterial biomasses of *S. maltophilia* and *B. subtilis* were used as effective biosorbents of Pb(II), Zn(II) and Ni(II) from wastewaters. The biosorption performances were strongly affected by parameters such as pH, contact duration and heavy metal concentration. The optimum pH for the biosorption of Pb(II), Zn(II) and Ni(II) by the two bacterial species was achieved at pH 5.0–6.0 for 30 min. The uptake of metals was very fast. Adsorption equilibrium was reached

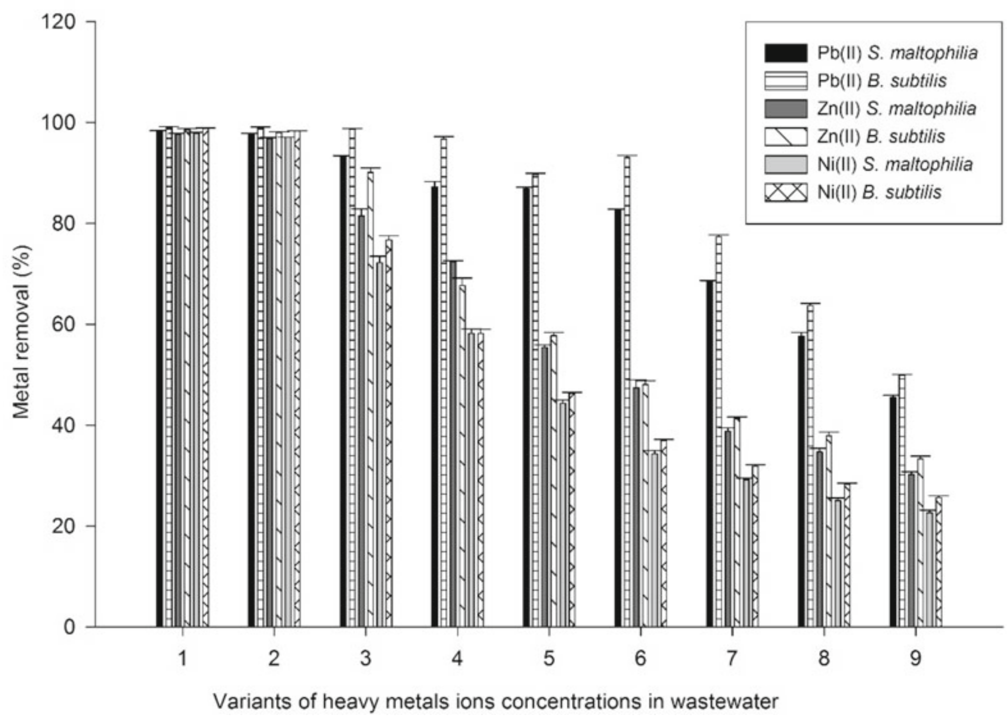

Figure 9. Comparison of metals removal from wastewater by *S. maltophilia* and *B. subtilis*. Variants concentrations of heavy metals ions in wastewater (mg/l): 1: Pb(II)–15.9, Zn(II)–11.2, Ni(II)–14.3; 2: Pb(II)–39.6, Zn(II)–20.6, Ni(II)–31.5; 3: Pb(II)–81.5, Zn(II)–42.3, Ni(II)–60.2; 4: Pb(II)–125.4, Zn(II)–62.4, Ni(II)–90.2; 5: Pb(II)–147.8, Zn(II)–86.0, Ni(II)–123.6; 6: Pb(II)–162.7, Zn(II)–104.3, Ni(II)–159.4; 7: Pb(II)–201.3, Zn(II)–132.2, Ni(II)–188.6; 8: Pb(II)–250.6, Zn(II)–149.9, Ni(II)–221.4; 9: Pb(II)–325.3, Zn(II)–172.3, Ni(II)–245.8

Table 5. Comparison of other biosorbents from selected literature with the present work

Metal ion	Biororbent	pH	q_{max} [mg/g]	Reference
Lead	*B. subtilis*	5.0	166.7	This work
	S. maltophilia	5.0	133.3	This work
	P. aeruginosa ASU 6a	6.0	123.0	Gabr et al.[15]
	Rhodococcus oparus	5.0	95.2	Bueno et al.[37]
	B. cereus	6.0	23.2	Çolak et al.[29]
	B. pumilus	6.0	29.6	Çolak et al.[29]
	Dairy sludge	5.0	148.6	Sassi et al.[5]
	Granular sludge	5,0	286.0	Hawari et al.[33]
Zinc	*B. subtilis*	5.0	49.7	This work
	S. maltophilia	5.0	47.8	This work
	Bacillus jeotgali U3	7.0	128.2	Green-Ruiz et al.[35]
	Pseudomonas putida CZ1	5.0	27.4	Chen et al.[16]
	P. aeruginosa ASU 6a	6.0	83.3	Joo et al.[17]
	B. cereus AUMC B52	6.0	66.6	Joo et al.[17]
Nickel	*B. subtilis*	6.0	58.7	This work
	S. maltophilia	6.0	54.3	This work
	Bacillus sp.	7.0	244.0	Zaidi and Musarrat[34]
	Yarrowia lipolytica NCIM 3589	7.5	138.9	Shinde et al.[38]
	P. aeruginosa ASU 6a	7.0	113.6	Gabr et al.[15]
	Granular sludge	5,0	25.0	Hawari et al.[33]

within 30 min of biomass addition. The applied biomass had a high affinity for Pb(II), and then Zn(II) and Ni(II).

The batch experimental results fitted well to the Langmuir isotherm model. The maximum adsorption uptake (q_{max}) of respectively Pb(II), Zn(II) and Ni(II) calculated from the Langmuir equation for biosorption by *S. maltophilia* and *B. subtilis* were 133.3, 47.8 and 54.3 and 166.7, 49.7 and 57.8 mg/g. Efficiency of removing metal ions from wastewater decreased with their increasing concentration. Only in the case of Pb(II) did it not decrease below 80%, even when the concentration of Pb(II), Zn(II) and Ni(II) were 162.7, 104.3 and 159.4 mg/l respectively. The results of the experiments show the possibility of using *B. subtilis* and *S. maltophilia* as sorbents of Pb(II), Zn(II) and Ni(II) from wastewater.

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