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Biosorption of zinc(II) on dead and living biomass of *Variovorax paradoxus* and *Arthrobacter viscosus*

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ABSTRACT

There is a great interest in the removal of heavy metals from aqueous media by various biomass. In this study, the potential of using live and dead cells of *Variovorax paradoxus* and *Arthrobacter viscosus* bacteria for the removal of Zn(II) were investigated for the first time. The bacteria were isolated from the ceramic industry sludge. Remaining concentrations of Zn(II) in the metal solutions after biosorption by bacteria were measured by ICP-OES. The removal of Zn(II) from aqueous solution increased with increasing pH and initial ion concentration. The maximum removal efficiency of *V. paradoxus* live and dead cells was calculated as 92.7 and 91.3%, respectively, whereas the maximum removal efficiency of *A. viscosus* live and dead cells was calculated as 89.4 and 90.8%, respectively. The experimental data was found to comply with the Freundlich isotherm model and the biosorption mechanism was expressed with the pseudo-second-order model.

Keywords: *Variovorax paradoxus*; *Arthrobacter viscosus*; Biosorption; Freundlich isotherm; Pseudo-second-order kinetics; Zinc(II)

1. Introduction

Our environment has increasingly been threatened by the heavy metal pollution due to increasing industrialization, population, and lack of environmental awareness in the last few decades. The accumulation of heavy metals in the air, soil, and water, above certain concentrations, may pose severe risks to the ecosystem, from the smallest organisms to the human via the food chain [1].

Zinc is a vital element for all organisms that is also essential for biological processes. Zinc concentration is 0.01–0.2 μg/m^3 in the air, 10–300 mg/kg in the soil, and 0.001–200 μg/L in water depending on its characteristics. However, heavy metal pollution arises resulting from the elevated concentration of such metals in the environment due to the processing of medical products and cosmetics, activities of automotive, ceramic, and construction industries [2,3]. According to environmental protection agency, the recommended or mandatory limit concentration values for zinc must be in the range of 0.03–2.0 mg/L in water depending on

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the hardness of water [4]. Chemical coagulation and filtration, electrochemical processes, chemical oxidation and reduction, ion exchange, evaporation, reverse osmosis, and adsorption are among the techniques applied for the removal of heavy metals, some of which are superior in terms of cost, ease of application, etc. A secondary treatment is required for such methods [5,6]. The removal of heavy metals by microorganisms and other type of biomass may be favorable to overcome the limitations of these methods.

Recently, there has been a significant increase in the number of studies emphasizing the more efficient removal of heavy metals using biomass compared to the conventional techniques. Bacteria [3,7–13], actinomycetes [2,14–16], fungi [17–22], yeast [23,24], lichen [25], macrofungi [26–28], cone [29], algae [30], and various vegetables [31] have been used as biomass for the removal of heavy metals.

The accumulation of yttrium (Y) as a representative of rare earth elements by *Variovorax paradoxus* was studied to evaluate the potential of bioprocesses using micro-organisms for purification and separation [32]. Silva et al. [33] studied the biosorption of Cr(VI) by *Arthrobacter viscosus* bacteria in suspension or supported on NaY zeolites. Cr(VI) has been successfully removed by *A. viscosus* in suspension with an initial concentration of 100 mg/L and biomass concentration of 5 g/L at lower pH (1, 2), and the use of zeolite NaY with bacteria favored the removal of Cr(VI) at higher pH (4). The applicability of *A. viscosus* biofilm supported on zeolites for the removal of Ni(II) from aqueous solutions in continuous mode has been confirmed in another study due to the high removal efficiencies obtained both at laboratory scale (98%) and pilot scale (89%) [34].

In this study, *V. paradoxus* and *A. viscosus* bacteria isolated from the ceramic sludge which presented the highest resistance to zinc (zinc metal tolerance = 15 mM) were studied for the biosorption of Zn(II) from aqueous solutions for the first time in literature. Moreover, the factors acting on the biosorption efficiency, namely pH and initial metal concentration were studied both for the biosorption of Zn(II) on live and dead cells of *V. paradoxus* and *A. viscosus* bacteria. The Langmuir, Freundlich, and Temkin isotherm models were applied to equilibrium data. Kinetic calculations were performed on experimental data in order to explain the biosorption mechanism.

2. Materials and methods

2.1. Isolation and identification of bacteria

The ceramic waste sludge was obtained from a ceramic factory in the vicinity of Eskişehir. Isolation of bacteria was carried out by dissolving 5 g sludge sample in 45 mL 0.1% sterile sodium pyrophosphate solution (pH 7) with stirring at 180 rpm for 30 min. The inoculation of ceramic sludge suspension was performed on petri dishes containing nutrient agar (NA, Merck) after a serial dilution of the sample. Consecutive to the incubation at 35˚C for 24 h, the isolation of bacteria was completed by the inoculation of pure bacterial colonies in terms of morphological peculiarities in nutrient agar. The stock bacteria culture in 20% glycerol was stored at −80˚C in freezer (Thermo scientific, Forma 900 series) for long-term studies.

The automated ribotyping was performed using a robotized instrument (RiboprinterTM Microbial Characterization System, Qualicon, Du Pont, Wilmington, DE, USA) and the RiboprinterTM System Data Analysis Program. The procedure used for processing each sample is described in detail by the manufacturer. Briefly, the isolates were grown overnight at 35˚C, suspended in buffer, heated at 80˚C for 10 min, and lysed. The total DNA was restricted with EcoRI, electrophoretically separated, and transferred to a membrane followed by hybridization.

2.2. Determination of minimum inhibitory concentrations (MIC) of zinc

The minimum inhibitory concentrations (MIC) of the zinc for each isolate were determined by the plate dilution method [35,36]. The metal Zn(II) was used as $ZnSO_4{\cdot}7H_2O$ (zinc sulpfate, Merck) in varying concentrations ranging between 1, 5, 10, 15, and 20 mM. Stock solutions of the metal salt were prepared in sterile Milli-Q water and were added to the nutrient agar in various concentrations, which were then spot inoculated with approximately 10^8 cfu/ml of the test bacterium. The plates were incubated at 35˚C for 24 h, since all the bacteria grow well at this temperature. The lowest concentration of the metal, which inhibits the growth of the micro-organism, was considered as the MIC of the metal against the strain tested.

2.3. Biosorption experiments

In order to obtain live and dead bacteria cells, nutrient broth (NB, Merck) was used. The bacteria from the stock culture growth in the nutrient agar were incubated at 35˚C, 125 rpm until the end of exponential phase. The liquid nutrient media was centrifuged at 4˚C, 10,000 rpm for 15 min by cooled centrifuge (Hettich Universal 320-R). The precipitate was washed three times with distilled water. Dead cells were dried at 80˚C for 12 h, grinded and sieved.

Batch biosorption experiments were carried out by mixing 1 g/L biomass (live and dead cells) with a 50 mg/L-Zn(II) solution at 35˚C temperature and 125 rpm agitation speed. The effect of contact time and solution pH on the Zn(II) biosorption were evaluated. Isotherm experiments were performed as explained above with different concentrations of zinc (5–200 mg/L) at pH 7.0. For kinetic experiments, live cells and dead cells (1 g/L) were mixed with 50 mg/L Zn^{2+}, and the residual Zn^{2+} concentration was measured by collecting samples at intervals starting from 5 min of exposure until 90 min.

The supernatant was analyzed for residual Zn(II) concentration using ICP-OES (inductively coupled plasma-optic emission spectrometer, Varian 720 ES) at a wavelength of 213.857 nm and instrumental detection limits (μg/L) were 0.4 after centrifugation.

The values of Zn(II) adsorbed by the biomass were calculated from the following equation:

$$q_e = \frac{V(C_o - C_e)}{M} \quad (1)$$

$$\text{Removal efficiency } (\%) = \frac{C_o - C_e}{C_o} \times 100 \quad (2)$$

where q_e is the amount of Zn(II) biosorbed on the biomass (mg/g dry), V is the volume of the metal solution (L), M is the mass of bioadsorbent (g), and C_o and C_e are the initial and equilibrium metal concentration (mg/L), respectively [11]. All the experiments were carried out in doublets.

3. Results and discussion

3.1. Isolation and identification of bacteria

A total of 24 bacterial isolates were obtained from the ceramic waste sludge. Their identification was carried out using ribotyping system based on comparative 16S rDNA analysis, and they were defined as *Neisseria meningitidis*, *Neisseria* sp., *Bacillus licheniformis* (two isolates), *Vibrio cholera*, *Pseudomonas pseudoalcaligenes*, *Ocrhrobactrum anthropi*, *Staphylococcus hominis*, *Klebsiella pneumonia*, *Leuconostoc gelidium*, *Citrobacter koseri*, *Haemophilus influenza*, *A. viscosus*, *V. paradoxus* (*four isolates*), *Salinivibrio costicola*, *Enterococcus faecalis*, and unknowns (five isolates). Their identification results based on riboprofiles are given in Fig. 1. Ribotyping is a molecular technique that takes advantage of unique DNA sequences to differentiate bacteria. The genomic DNA is cut at specific sites by doing a restriction digest. This generates pieces of DNA of different lengths. Because different strains of bacteria have the specific "cut-sites" of the restriction enzymes in different places, each strain generates a unique pattern of DNA pieces.

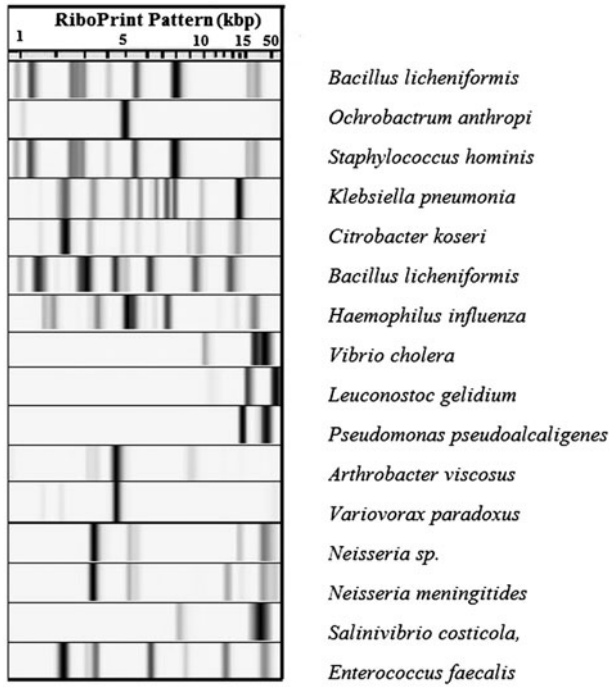

Fig. 1. Riboprinting profiles of bacteria isolated from ceramic waste sludge.

3.2. MIC test of zinc(II)

All the above-mentioned bacterial isolates were tested for their resistance against Zn(II) and only two bacteria isolated from ceramic waste sludge exhibited a maximum MIC value of 15 mM. These bacteria identified as *A. viscosus* and *V. paradoxus* by ribotyping were tested in the following biosorption experiments.

3.3. Effect of contact time

The effect of contact time on the amount adsorbed by both bacteria live and dead cells is shown in Fig. 2. The rapid increase in adsorption was followed by a fluctuation until 90 min. The fluctuation in the amount adsorbed can be attributed to the improper mixing. However, after 60 min the amount of Zn(II) adsorbed didn't change. Therefore, 60 min have been determined as optimum contact time.

3.4. Effect of pH on biosorption

The effect of pH on the biosorption of Zn(II) by both bacteria live and dead cells is shown in Fig. 3.

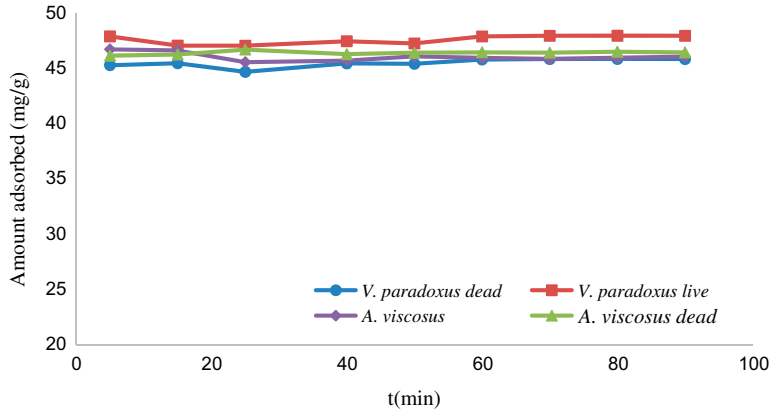

Fig. 2. Effect of contact time on biosorption of Zn(II) by live and dead cells of *V. paradoxus* and *A. viscosus* (biomass dosage 1 g/L, Zn^{2+} 50 mg/L, agitation speed 125 rpm and temperature 35˚C).

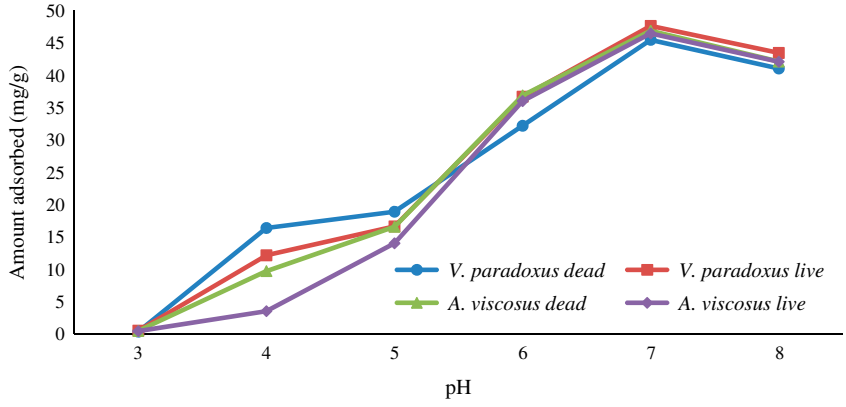

Fig. 3. Effect of pH on biosorption of Zn(II) by live and dead cells of *V. paradoxus* and *A. viscosus* (biomass dosage 1 g/L, Zn^{2+} 50 mg/L, agitation speed 125 rpm, and temperature 35˚C).

Biosorption capacities for all biosorbents increased with increasing pH and began to decline after reaching maximum biosorption at pH 7.0. These results indicated that the adsorption of Zn(II) on bacteria is controlled by ionic attraction. At low pH values, the cell surface becomes positively charged and the attraction between the metal ions and the functional groups of cell wall decreases. When pH increases, retention of metal ions increase due to the more negatively charged cell surface. The retention decreases above pH 7.0 due to the formation of hydroxylated metal complexes that compete with active sites [7]. The maximum biosorption capacities calculated for *V. paradoxus* live and dead cells were 47.7 and 45.5 mg/g, respectively. The maximum biosorption capacities calculated for live and dead cells of *A. viscosus* were 46.5 and 46.9 mg/g, respectively. This is in good agreement with the results obtained by other researchers studying Zn(II) biosorption by different bacterial strains [11].

3.5. Effect of initial Zn(II) ion concentration

The effect of initial metal concentration on the removal efficiency was evaluated under reaction conditions, set at pH 7 and 35˚C equilibrium for 60 min as shown in Fig. 4. Zn(II) removal efficiency slightly increased when the initial Zn^{2+} concentration increased up to 50 mg/L and gradually increased thereafter for live and dead cells of both bacteria. In this study, the maximum removal efficiency of dead cells of *A. viscosus* (90.8%) was higher than live cells of *A. viscosus* (89.4%). This may be due to the passive uptake by the dead cells being independent of energy on the contrary to the active uptake by live cells that requires the metal transfer across the cell wall into the

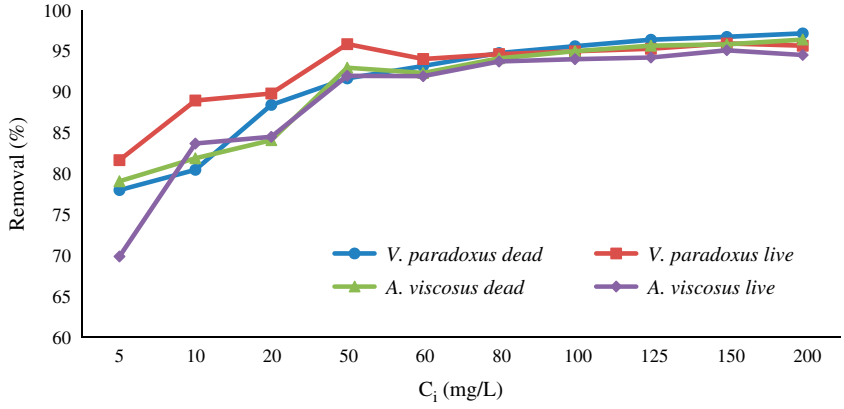

Fig. 4. Effect of initial Zn(II) concentration on removal efficiency (biomass dosage 1 g/L, Zn^{2+} 5–200 mg/L, contact time 60 min, agitation speed 125 rpm, and temperature 35°C).

cytoplasm, and the presence of competition by the H^+ as produced by viable cells. Moreover, drying by heating applied as a pretreatment may expose latent binding sites and improve the uptake of metal ions [8,37]. In contrast, the maximum removal efficiency of dead cells of *V. paradoxus* (91.3%) being lower than live cells of *V. paradoxus* (92.7%) can also be attributed to the heat treatment that decrease the intracellular uptake capacity by destroying the surface-active components.

The recovery values that are higher than those obtained in the previous studies regarding Zn(II) biosorption [11] may be attributed to the different cell structures. The recovery values calculated are higher than those obtained in previous studies about Zn(II) biosorption. This may be attributed to the different cell wall structures.

As can be interpreted from the figure, the amount of Zn(II) adsorbed by the unit mass of bacteria was increased by the increasing initial Zn^{2+} concentration due to the higher probability of interactions between Zn^{2+} and biomass [38]. Furthermore, the increased uptake of Zn(II) may be attributed to the accelerated probable collisions between metal ions and adsorbents resulting from the elimination of mass transfer resistance of Zn(II) at higher initial concentration.

3.6. Biosorption isotherms

Equilibrium data related to the biosorption of zinc ions on *V. paradoxus* and *A. viscosus* dead and live cells were interpreted by the Langmuir, Freundlich, and Temkin isotherm models.

The Langmuir isotherm which implies for the monolayer adsorption is expressed as [21,39]:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \tag{3}$$

where q_m is the maximum biosorption capacity (mg/g) and K_L is the Langmuir equilibrium adsorption constant (L/mg). When the above equation is linearized it becomes:

$$\frac{1}{q_e} = \frac{1}{q_m K_L}\left[\frac{1}{C_e}\right] + \frac{1}{q_m} \tag{4}$$

The values of q_m and K_L can be calculated from the plot of $1/q_e$ vs. $1/C_e$. Correlation factors were obtained as 0.981, 0.866, 0.825, and 0.988 for *V. paradoxus* dead cells, *V. paradoxus* live cells, *A. viscosus* dead cells, *A. viscosus* live cells, respectively, from the Langmuir isotherm plots.

However, negative intercept values were obtained for each plot, which were therefore not included in this paper. The reciprocal of a negative intercept value yields to a negative maximum biosorption capacity, which leads to the conclusion that the experimental data don't fit to the Langmuir model.

Unlike Langmuir isotherm model, Freundlich isotherm model which represents a non-homogenous surface of adsorption is expressed as [40]:

$$q_e = K_F C_e^{1/n} \tag{5}$$

where K_F and n are the Freundlich constant related with adsorption capacity and adsorption intensity. The above equation can be linearized as:

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (6)$$

The values of K_L and n can be calculated from the plot of log q_e vs. log C_e. The Freundlich isotherm plots for the biosorption of zinc ions on *V. paradoxus* and *A. viscosus* live and dead cells are shown in Fig. 5. The calculated values of K_L and n are tabulated in Table 1. As $1/n$ is a measure of heterogeneity, greater heterogeneity is expected at smaller $1/n$ [41]. The value of $1/n$ being greater than unity for all biosorbents used in this study indicates that cooperative sorption which may be interpreted with strong adsorption of solvent, penetration of the solute in the adsorbent, strong intermolecular attraction within the adsorbent layers, and monofunctional nature of the adsorbate [42,43].

Temkin isotherm equation that has been successfully used to describe chemisorption on heterogenous surface was also implied [44]. Temkin isotherm model based on a factor that explicitly taking into account of adsorbent–adsorbate interactions is expressed as [45]:

$$q_e = B_1 \ln(K_T C_e) \quad (7)$$

where K_T is the Temkin isotherm equilibrium binding constant (L/g) and B_1 is the constant related with the heat of sorption (J/mol), which is expressed as:

$$B_1 = \frac{RT}{b_T} \quad (8)$$

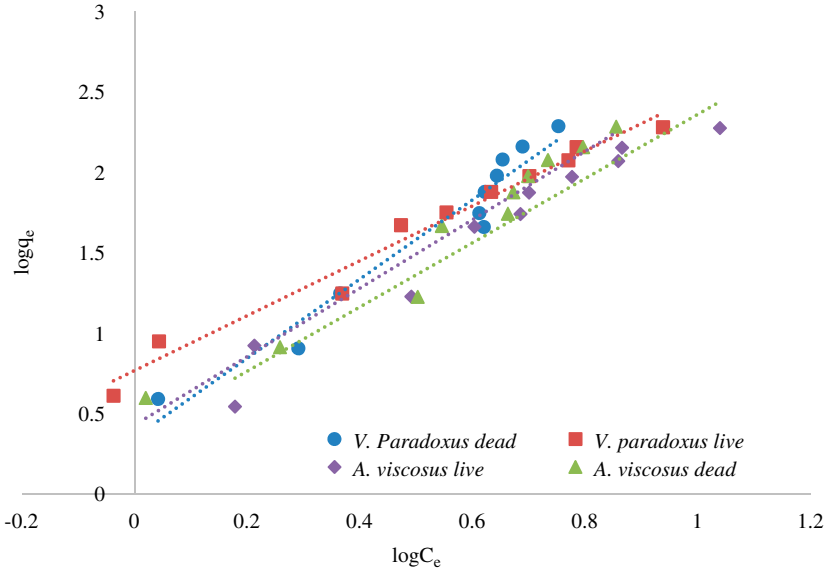

Fig. 5. Freundlich isotherm for the biosorption of Zn(II) on *V. paradoxus* and *A. viscosus* live and dead cells.

Table 1
Freundlich and Temkin isotherm parameters for the biosorption of Zn(II)

Bacteria		*V. paradoxes* (dead)	*V. paradoxes* (live)	*A. viscosus* (dead)	*A. viscosus* (live)
Freundlich	n	0.406	0.585	0.469	0.500
	K_F	2.268	5.869	2.695	2.317
	R^2	0.9507	0.9763	0.9595	0.9595
Temkin	B_1	98.445	76.727	88.164	88.561
	K_T	0.643	0.808	0.630	0.553
	R^2	0.6461	0.8311	0.7042	0.8592

In the above equation, R is the universal gas constant (8.314 J/mol K), T is the temperature (K), and b_T is the Temkin isotherm constant.

Eq. (8) is linearized as:

$$q_e = B_1 \ln K_T + B_1 \ln C_e \quad (9)$$

The values of B_1 and K_T can be calculated from the plot of q_e vs. $\ln C_e$. The Temkin isotherms were plotted for the biosorption of zinc ions on *V. paradoxus* and *A. viscosus* live and dead cells. The calculated values of B_1 and K_T are tabulated in Table 1.

When the correlation factors obtained for Temkin isotherm plots are compared with those of Freundlich isotherm plots, the experimental data are observed to fit best to Freundlich model. Furthermore, Temkin isotherm was suggested to be not appropriate for the prediction of biosorption equilibria due to the more complex nature of adsorption in the liquid phase [46].

3.7. Biosorption kinetics

In order to obtain a better understanding of the biosorption mechanism of zinc ions on *V. paradoxus* and *A. viscosus* live and dead cells, Lagergren's pseudo-first-order and Ho, and McKay's pseudo-second-order models were applied to the experimental data. The pseudo-first-order model derived by Lagergren is expressed as [47,48]:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (10)$$

where q_t is the biosorption capacity at time t (mg/g) and k_1 is the pseudo-first-order rate constant. Integrating at boundary conditions $t = 0$ to $t = t$ and $q_t = 0$ to $q_t = t$, Eq. (11) becomes:

$$\ln(q_e - q_t) = -k_1 t + \ln q_e \quad (11)$$

The pseudo-first-order rate constant, k_1 (L/min), can be calculated from the plot of $\ln(q_e - q_t)$ vs. t. The calculated values of k_1 for the biosorption of zinc ions on *V. paradoxus* and *A. viscosus* live and dead cells are given in Table 2. However, the correlation factors obtained from these plots indicate a poor compliance between the experimental data and the pseudo-first-order model. Therefore, the calculated values of q_e highly differ from the experimental values.

The pseudo-second-order model derived by Ho and McKay [48] and applied to biosorption of metals by others [3,5,49,50] successfully is expressed as:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (12)$$

by rearranging the above equation, the linearized form of Eq. (12) is obtained as:

$$\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_2 q_e^2} \quad (13)$$

The values of k_2 (L/mgmin) can be calculated from the plot of t/q_t vs. t. The pseudo-second-order plots for the biosorption of zinc ions on *V. paradoxus* and *A. viscosus* live and dead cells are shown in Fig. 6. The pseudo-second-order reaction rate constants and the biosorption capacities calculated at equilibrium are given in Table 2. Correlation factors obtained from the plots and tabulated in Table 2 are in the vicinity of or equal to 1. Therefore, experimental data are concluded to fit to the pseudo-second-order model that is an indication of rate-limiting step in biosoption being chemisorption involving valence forces through the sharing and exchange of electrons between the adsorbent and adsorbate as covalent forces and ion exchange [46,48].

Table 2
Pseudo-first-order and pseudo-second-order parameters for the biosorption of Zn(II)

Bacteria		*V. paradoxes* (dead)	*V. paradoxes* (live)	*A. viscous* (dead)	*A. viscous* (live)
Experimental	q_e	45.839	47.934	46.495	45.995
Pseudo-first-order	q_e	0.939	0.617	0.332	0.730
	k_1	0.0101	0.0003	0.0028	0.0015
	R^2	0.0458	0.0001	0.0023	0.0027
Pseudo-second-order	q_e	46.083	47.847	46.512	46.083
	k_2	0.0466	0.1506	4.6225	2.3545
	R^2	0.9999	0.9999	1	0.9998

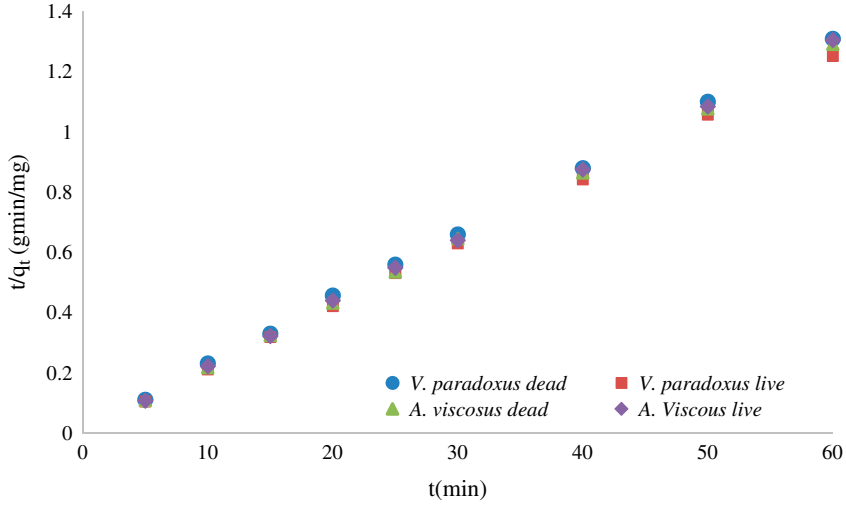

Fig. 6. Pseudo-second-order plot for the biosorption of Zn(II) on *V. paradoxus* and *A. viscosus* live and dead cells.

4. Conclusion

The effects of pH and initial concentration on the biosorption of Zn(II) by the *V. paradoxus* and *A. viscosus* live and dead cells were investigated. The biosorption capacites of both live and dead bacteria were found to have a tendency to increase with increasing pH and initial concentration. The optimum pH was determined as 7 by the experiments carried out for 60 min with 1 g/L of bacteria at 35˚C, 125 rpm. Similar results were obtained for the removal efficiencies presented by live and dead cells of both bacteria (*V. paradoxus* live 92.7%, *V. paradoxus* dead 91.3%, *A. viscosus* live 89.4%, *A. viscosus* dead 90.8%). The experimental data interpreted by various isotherm models were concluded to comply with the Freundlich isotherm model which designates a non-homogenous surface of adsorption. Furthermore, the pseudo-second-order model was found to be favorable to express the biosorption kinetics.

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