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Bioreduction of Iron and Biosorption of Heavy Metals (Ni^{2+}, Co^{2+}, Pb^{2+}) by a Novel Environmental Bacterium, *Tabrizicola aquatica* RCRI19^T

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Abstract: Environmental bacteria have an important role in the removal and improvement of metals from polluted area. Metal–microbe interactions as a form of detoxification of metal have been developed. In this study, we investigated the ability of *Tabrizicola aquatica* RCRI19^T, a novel environmental bacterium isolated from deepwater from Qurugöl Lake nearby Tabriz city, Iran, to Fe(III)-reduction as an electron acceptor in minimal essential elements condition. Subsequently, bioabsorption behaviour of heavy metals (Ni^{2+}, Co^{2+}, pb^{2+}) by strain RCRI19^T has been demonstrated. Our results showed that strain RCRI19^T can reduce 20mM ferric-citrate particularly in anaerobic condition, and a positive correlation was observed between bacterial growth and iron (II) production. Owing to its iron reduction rate, *T. aquatica* RCRI19^T may contribute to iron mineral transformation and element cycling in deepwater of the lake. We further observed that the dead biomass of strain RCRI19^T absorbs of heavy metals (Ni^{2+}, Co^{2+} and Pb^{2+}) from aqueous solution. The optimum conditions of biosorption are in pH = 4 for Ni^{2+}, Pb^{2+} and pH = 5 for Co^{2+}. The equilibrium experimental data fitted both of Freundlich and Langmuir isotherms and displayed monolayer adsorption. Two kinetic models, namely pseudo-first-order and pseudo-second-order were used to describe the kinetics of heavy metal ion biosorption on *T. aquatica*. Pseudo second order was the best of the other kinetic.

Key words: *Tabrizicola aquatica* RCRI19^T, Fe(III)-reduction, bioabsorption of heavy metals.

Introduction

Employment of active bacteria has been included into the perception of biosorption in recent decades (Fomina and Gadd, 2014). Unlike most of the chemical approaches which are expensive and lack the specificity required to treat target metals against a background of competing ions, utilization of biological approaches have a sufficient potential for the highly selective removal of toxic metals (Abdel-Raouf and Abdul-Raheim, 2017; Lloyd and Lovley, 2001). Biosorbents can be considered as the natural ion-exchange substance that fundamentally contains weakly acidic and basic groups (Michalak et al., 2013). Biomaterials containing acidic groups such as hydroxyls and carboxyls are effective in binding metal cations (John et al., 2011).

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However, other biomaterials containing weak basic groups, e.g. amides and amines, have efficiency for adsorbing metal anions (Niu and Volesky, 2003). It has already been indicated that the environmental bacteria with the ability to reduce Fe(III) are significantly useful in the biosorption and reduction of heavy metal (Esther et al., 2015; Zhan et al., 2012). Dissimilatory iron-reducing bacteria (DIRB) have an important role to immobilize heavy metals by reduction to less soluble forms (Lloyd and Macaskie, 2000). For this aim, a lot of microorganisms were studied under different physicochemical conditions, such as pH, temperature, sorbent mass and ionic concentrations (Bleam, 2016). Solubility, adsorption affinity, chemical reactivity, and especially toxicity of metals are related to the valence of the ions (Zhan et al., 2012).

Previously it has been shown that ability of *Tabrizicola aquatic* RCRI19^T a novel genus of environmental bacteria (Tarhriz et al., 2013) in various defined media in order to determine minimal and essential elements for bacterial growth. This also helps us obtain optimized growth condition for use in metal recovery and the capability to Fe(III)-reduction as an electron acceptor (with organic or inorganic electron donors in anoxic and aerobic culture. In the other direction, we investigated the consideration of bioabsorption behaviour of heavy metals (Ni^{2+}, Co^{2+}, Pb^{2+}) by *Tabrizicola aquatic* RCRI19^T, as a novel genus of environmental bacteria.

Materials and Methods

Growth Media and Culture Condition

Six defined marine broth media (DM1-DM6) (Tarhriz et al., 2011) and three defined Pfennig media (DM7-DM9) (Tarhriz et al., 2013) were prepared (Table 1). 200 µl of bacterium with OD: 600 was cultured in every defined medium both in aerobic and anaerobic conditions at 30°C for 72 h. The ability of growth was checked by comparing the measurement of bacterium OD between inoculated samples and the control in order to determine minimal essential elements for bacterial growth.

Fe(III)-reduction by Strain RCRI19^T

Fe(III)-citrate is highly soluble and used as an iron(III) source for the culture of ferric reducing bacteria. A defined medium (DM) as described by Newton et al. (2009) was used for iron(III) reduction. The cultures were incubated in the aerobic condition as well in the anaerobic condition in order to delete O_2 as an electron acceptor on a shaker at 150 rpm at 30°C. Reduction of ferric iron was monitored by measuring Fe(II) using 1,10 phenanthroline method at different times in both test and control cultures. All of the experiments were repeated three times.

Table 1: Compositions of defined modified marine and Pfennig media to determine minimal essential elements for strain RCRI19^T growth

Defined marine medium	*Compositions*
DM_1	Peptone, yeast extract
DM_2	Marine broth medium - [PO_4^{3-}, SO_4^{2-}, NO_3^-]
DM_3	M_2 - [Ferric-citrate]
DM_4	M_2- [Mg^{2+}]
DM_5	M_2-[Ca^{2+}]
DM_6	M_2- [Mg^{2+}, Ca^{2+}]
Defined Pfennig medium	
DM_7	- [SO_4^{3-},PO_4^{3-}, Vitamin B_{12}, Micronutrient solution and NaCl]
DM_8	M_7 - [Ferric-citrate solution]
DM_9	M_8 – [Ca^{2+}]

Also the strain RCRI19^T was cultured with 20 Mm ferric-citrate as an electron acceptor to test whether iron reduction supported growth in autotrophically and heterotrophically conditions at 30°C (Tarhriz et al., 2013). Finally, correlations between biomass yield and ferric iron reduction were assessed in liquid medium at 50, 100, 150, 200, 250, and 300 h after inoculation. The procedure was done three times and the mean values and error bars were reported (Bridge and Johnson, 1998). Preparation of standard iron solution, determination of iron concentration in bacterial samples and the rate of soluble Fe(II) were prepared as described by Kooli et al. (2018).

Biosorption Behaviour in Dead Biomass of RCRI19^T

Solutions of heavy metal consisting of Ni^{2+}, Co^{2+} and Pb^{2+} were prepared from $CoCl_2$, $PbNO_3$ and $NiCl_2$ (Merck) in double distilled water and then sterilized at 121ºC for 20 min. Strain RCRI19^T was cultivated in 100 ml of marine broth medium (Tarhriz et al., 2011). The colonies were centrifuged at 10,000 rpm for 20 min after 72 h, and then the pellets were washed two times with 0.9% NaCl solution and dried at 80°C for 48 hours. Dried dead cells in different concentrations (5, 10, 25, 50, 100, and 200 mg) were investigated to various metal concentrations (10, 25, 50, 100, 200, and

300 mg/l) at the interval time of 15, 30, 45, 60, and 120 min. Each of the samples was centrifuged at 10,000 rpm for 20 min and their supernatant analyzed by means of atomic absorption spectroscopy (AAS – Analytik Jena). The amount of metal's uptake at equilibrium Q_e (mg/g) is calculated using the mass balance equation:

$$Q_e = \frac{(C_0 - C_e) \times V}{m}$$

where C_0 and C_e (mg/l) are the initial and equilibrium metal's concentration, V (L) is the volume of adsorbate (solution) and m (g) is the mass of adsorbent (Anastopoulos and Kyzas, 2015; He and Chen, 2014).

To obtain optimum pH range, 50 mg dried dead cells of RCRI19^T was suspended in 50 mg/l of each metal solutions with various pH values (4, 6, 7). Samples were analyzed by means of atomic absorption spectroscopy (AAS – Analytik Jena) to consider residual metal concentrations.

Modelling of the Biosorption Isotherms

The Langmuir and Freundlich models adsorption isotherms have been tested to fit experimental data and to envision about the interaction type between the affection of *Tabrizicola aquatic* RCRI19^T and heavy metal ions. The Langmuir model assumes monolayer coverage of the adsorbate over a homogeneous adsorbent surface. While the Freundlich model as an empirical equation assumes the adsorbate concentration in solution as increased by the increasing on the adsorbent surface. This model is applied in non-ideal sorption on heterogeneous surfaces and to multilayer sorption (Table 2) (Gadd, 2009; Muñoz et al., 2015; Özdemir et al., 2009; Zouboulis et al., 2004).

Table 2: Biosorption equilibrium parameters of the isotherm models by *T. aquatic* RCRI19^T

Isotherms		*Ni^{2+}*	*Co^{2+}*	*Pb^{2+}*
Langmuir	q_m	31.34±2.1	16.6±1.2	22.12±2.3
$q_e = \frac{q_m b C_e}{1 + bC_e}$	K_b	0.041	0.046	0.021
	r^2	0.9971	0.9949	0.9639
Freundlich	K_n	2.56	2.09	1.42
$q_e = K_F C_{1/n}$	n	2.06	2.57	2.02
	r^2	0.9578	0.9472	0.9854

q_e: Biosorption capacity (mg/g) at equilibrium.
q_m: Maximum biosorption capacity (mg/g).
K_b: Langmuir biosorption equilibrium constant (mg/L).
C_e: Equilibrium concentrations of metal (mg/L).
K_F: Characteristic constant related to the biosorption capacity.
n: Characteristic constant related to the biosorption intensity.

Modelling of Uptake Kinetics

To understand the absorption mechanisms and speed of the process, the experimental data have been adjusted to two kinetic models. The pseudo-first-order equation of Lagergren (solid capacity), and pseudo-second-order equation (solid phase adsorption) (Table 3), to adjust the experimental data to the kinetic models, were used by the IBM SPSS Statistics software (19 version) (Muñoz et al., 2015).

Table 3: Kinetic parameters of the Ni^{2+}, Co^{2+} and Pb^{2+} biosorption by *T. aquatica* RCRI19^T

Pseudo-first order		*Ni^{2+}*	*Co^{2+}*	*Pb^{2+}*
$q = 0$ at $t = 0$ and q	q_e	9.74	11.66	10.38
$= q$ at $t = t$	k_1	0.0703	0.0533	0.01044
$q = q_e\,(1 - e^{-k1t})$	r^2	0.9625	0.9825	0.8671
Pseudo-second order		Ni^{2+}	Co^{2+}	Pb^{2+}
$q = 0$ at $t = 0$ and q	q_e	8.25	12.78	12.09
$= q$ at $t = t$	k_2	0.0056	0.0638	0.0291
$q = t/1/k_2 q_e^2 + t/q_e$	r^2	0.9838	0.9999	0.9999

q_e: Biosorption capacity (mg/g) at equilibrium.
k_1: Pseudo-first-order kinetic rate constant (min^{-1}).
k_2: Pseudo-second-order kinetic rate constant ($g.mg^{-1}.min^{-1}$).

Results

Screening for Bacterium Growth in Defined Marine Broth and Pfennig Media

Tabrizicola aquatica strain RCRI19^T was able to grow in defined marine broth media without PO_4^{3-}, SO_4^{2-}, NO_3^-, ferric-citrate, and Mg^{2+} as well as defined Pfennig media without Vitamin B_{12} micronutrient solution, ferric-citrate solution, NaCl, SO_4^{3-}, PO_4^{3-} in both aerobic and anaerobic conditions.

Reduction of Fe(III) by *Tabrizicola aquatic* RCRI19^T

The results showed that strain RCRI19^T was able to reduce 20mM ferric-citrate under the strictly anaerobic condition in the Peffinig medium (Figure 1). However, it has not observed any iron reduction by strain RCRI19^T in aerobic condition. Probably in aerobic condition oxygen is superior to iron as the terminal electron acceptor. Moreover, we observed the remarkable iron reduction in autotrophically growth with $Na_2S_2O_3$ (5 mM) and Na_2S (5 mM) (Figure 2), while the reduction of iron was lower than heterotrophically growth condition (Figure 1). Furthermore, biomass yields (as

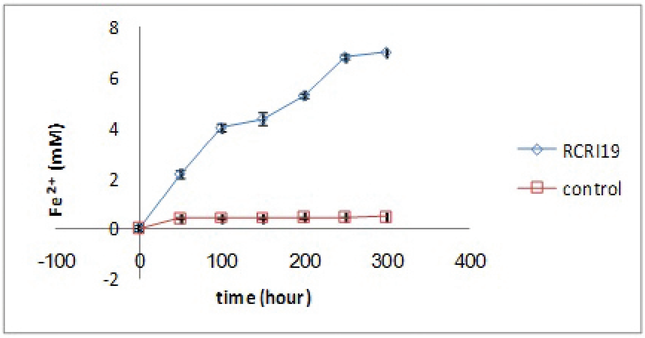

Figure 1: Reduction of ferric iron by RCRI19^T. Strain of bacterium was grown in anaerobic heterotrophically growth containing 20 mM (5.2 mg/mL) ferric citrate and 20 mM pyruvate.

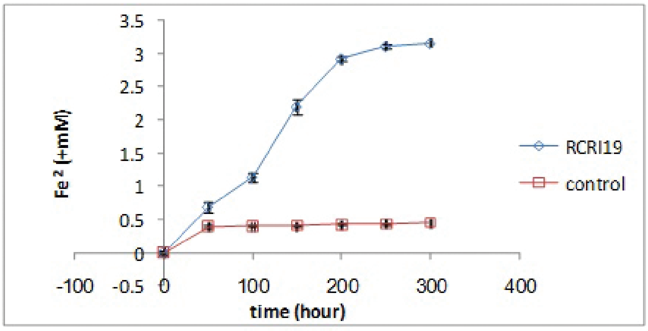

Figure 2: Reduction of ferric iron by RCRI19^T. Strain of bacterium was grown in anaerobic autotrophically growth containing 20 mM ferric citrate, $Na_2S_2O_3$ (5 mM) and Na_2S (5 mM).

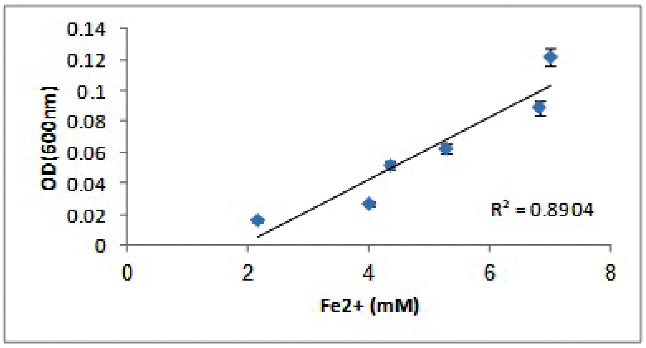

Figure 3: Relationship between ferrous iron production and biomass yields, as determined by optical densities of strain RCRI19^T.

optical densities of bacteria) were correlated with the amount of ferrous ion produced in the defined medium. The result showed that there is a positive correlation between bacterial growth and production of iron(II) by bacterial cells in anaerobic conditions (Figure 3).

Bioabsorption of Metals (Ni^{2+}, Co^{2+}, Pb^{2+}) in Dead Biomass of RCRI19^T

Metal ions are adsorbed to the surface of non-living cells via interactions between metals and functional groups existing on the surface of cells (Jin et al., 2018). Equilibrium data were obtained experimentally using different initial concentrations of heavy metal between 10 and 300 mg/l and a constant biomass concentration (5-200 mg/l), at 30°C and pH 3-7. Our results demonstrated that absorption of metal by RCRI19^T has occurred very rapidly and within several minutes (≤ 60 min) (Figure 4a) as well as the biosorption capacities Q_{eq} of RCRI19^T remarkably increased by enrichment of the metal concentrations. It seems that the uptake rate of the metal ions by RCRI19^T is increased by increasing metal concentration while the amount of bacterial biomass is kept constant (Figure 4b).

Moreover heavy metals adsorptions (Q_{eq}), were reduced by increasing the amount of biomass. It appears when the biomass concentration is getting high, metal ions in the solution would not be adsorbed to the surface of the biomass freely (Figure 4c). The results showed that the pH in the solution is a significant impact on heavy metal uptaking and the maximum biosorption capacity of heavy metal ions are pH = 4 for Ni^{2+}, Pb^{2+} and pH = 5 for Co^{2+} (Figure 4d).

Biosorption Isotherms

Experimental data and plotted isotherms indicated the feasibility of existing one type of binding sites for attaching of metal ions and bioabsorption (Figure 5). The (Q_{eq} against C_{eq}) plot is nearly linear and showed negative slope. It appears that there are separated interactions between the metal ions and the binding sites in the cell surface, and confirms no altering in the affinity of the binding sites for metal ions during the different concentrations (Şahin and Öztürk, 2005). The biosorption isotherms are useful to describe about the interaction adsorbate/biosorbent of any system (Boudechiche et al., 2016). In this study the parameters were obtained from two different Langmuir and Freundlich isotherm models.

Our results indicated that the biosorption process by RCRI19^T followed the Langmuir model (Table 1 and Figure 6), and the regression correlation coefficients which were acquired from Langmuir isotherm were higher than the Freundlich isotherm. However, the results of equilibrium data can be adapted with Freundlich model (Table 1 and Figure 7) and the amount of K_F showed the high capacities of accessible heavy metal ions uptaking. As a result, biosorption equilibrium data fit better to the Langmuir model,

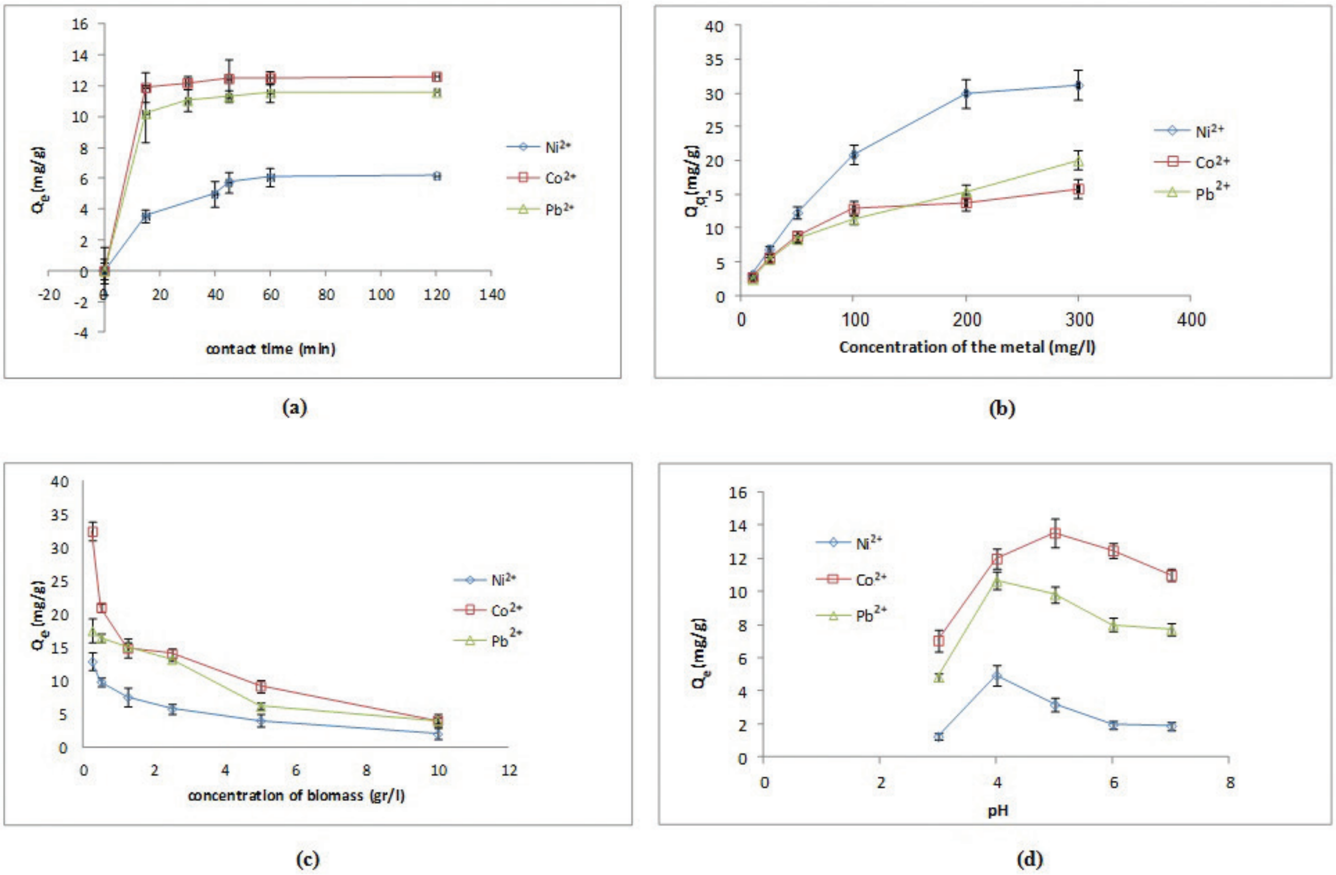

Figure 4: (a) Effect of contact time on equilibrium biosorption capacity of Co^{2+}, $Pb^{2+}$and Ni^{2+} ions. Conditions: pH = 4 for Ni^{2+}, $Pb^{2+}$and = 5 for Co^{2+}, 50 mg of dried powdered cell, 50 mg/l of metal ion concentration. (b) Effect of metal concentration on equilibrium biosorption capacity of ions. Conditions: pH = 4 for Ni^{2+}, $Pb^{2+}$and = 5 for Co^{2+}, 60 min of contact time, 50 mg/l of powdered dried cell. (c) Effect of dried powdered cell concentration on equilibrium. biosorption capacity of Co^{2+}, $Pb^{2+}$and Ni^{2+} ions. Conditions: pH = 4 for Ni^{2+}, Pb^{2+} and = 5 for Co^{2+}, 60 min of contact time, 50 mg/l of metal ion concentration. (d) Effect of pH on equilibrium biosorption capacity of Co^{2+}, $Pb^{2+}$and Ni^{2+} ions conditions: 50 mg/l of metal concentration, 60 min of contact time, and 50 mg of powdered dried cell.

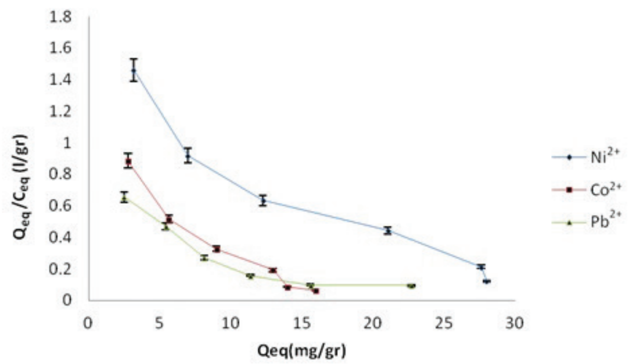

Figure 5: Linear plots for the absorbtion of metal ions on RCRI19^T. Scatchard plot hinted the presence of one type of binding site for metal ions on the biosorbent.

which implies monolayer adsorption and homogeneous surface conditions for Ni^{2+} and Co^{2+}. While Freundlich isotherm, which implies heterogeneous surface condition, is suitable to explain experimental datum for Pb^{2+}.

Biosorption Kinetics

The kinetic parameters for the biosorption of Ni^{2+}, Co^{2+}, Pb^{2+} onto *Tabrizicola aquatica* RCRI19^T were calculated and summarized in Table 2 (Figures 8 and 9). It appears that the biosorption occurs in a very fast initial rate within the first 5 min and this finding suggests that the adsorption of heavy metal by *Tabrizicola aquatic* RCRI19^T may occur via mechanism of cell-surface binding and not intracellular accumulation. The r^2 values of pseudo-second-order equation (k_2: g/mg min, q_e: mg/g) was obtained in the range of 0.9838–0.9999 and appears better than the Pseudo-first-order model equation ($q_{e,exp}$: mg/g, k_1: min^{-1}), to explain the biosorption kinetics.

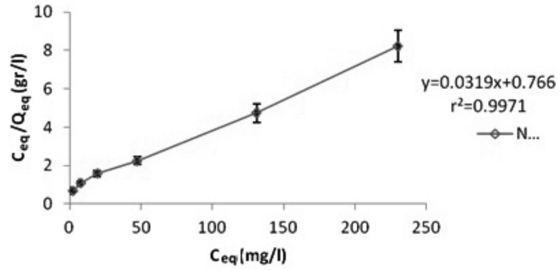

Fig.6a

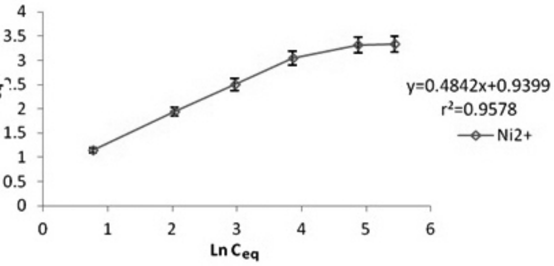

Fig.7a

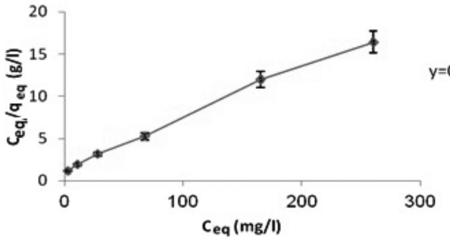

Fig.6b

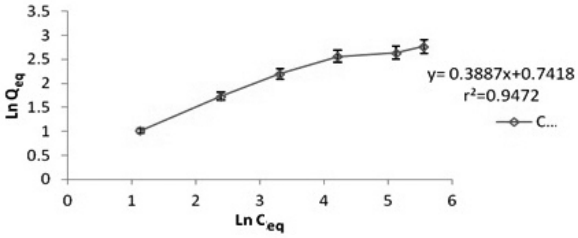

Fig.7b

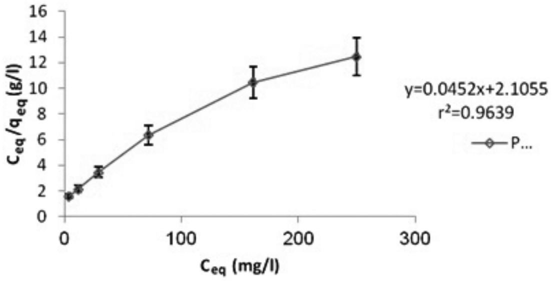

Fig.6c

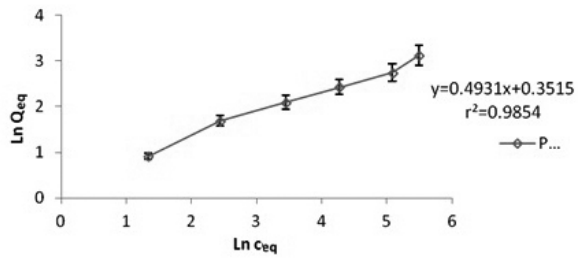

Fig.7c

Figure 6: (a) Langmuir adsorption isotherms of the Ni^{2+} adsorption on $RCRI19^T$. (b) Langmuir adsorption isotherms of the Co $^{2+}$adsorption on $RCRI19^T$. (c) Langmuir adsorption isotherms of the Pb^{2+} adsorption on $RCRI19^T$.

Figure 7: (a) Freundlich adsorption isotherms of Ni^{2+} adsorption on $RCRI19^T$. (b) Freundlich adsorption isotherms of Co^{2+} adsorption on $RCRI19^T$. (c) Freundlich adsorption isotherms of Pb^{2+} adsorption on $RCRI19^T$.

Discussion

The results obtained in this study showed that the new strain, *Tabrizicola aquatica* $RCRI19^T$, was able to reduce 20mM ferric-citrate in strictly anaerobic condition but any reduction was not observed by $RCRI19^T$ in aerobic condition. It appears oxygen has a superior role as the terminal electron acceptor in aerobic condition or probably strain $RCRI19^T$ is similar to the microorganisms with the capacity to catalyze the oxidation of Fe(II) and can reoxidize ferrous iron produced in the presence of O_2 (Lovley et al., 2004). This microorganism has the ability to utilize Fe(III) as a minor electron acceptor and to reduce partially ferric-citrate's in anaerobic conditions. Therefore it appears that *Tabrizicola aquatica* $RCRI19^T$, a new genus of environmental bacteria is used as a bioremediated microorganism. Moreover strain $RCRI19^T$ can grow without consuming anions such as PO_4^{3-}, SO_4^{2-} and NO^{3-} and cations such as Fe^{3+} and Mg^{2+} in both various defined marine and Pfennig media.

The biomass of *Tabrizicola aquatica* $RCRI19^T$ showed a high capacity for the uptake of heavy metals (Co^{2+}, Ni^2 and Pb^{2+} ions). The process of biosorption was regarded to be dependent on experimental conditions such as the initial pH, initial metal ion

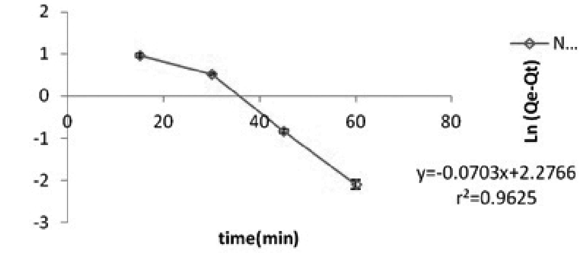

Fig. 8a

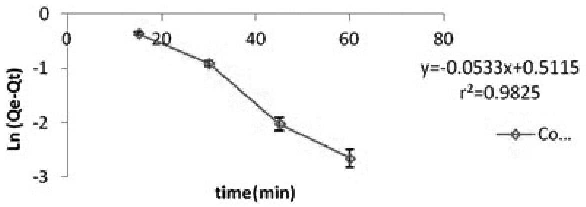

Fig. 8b

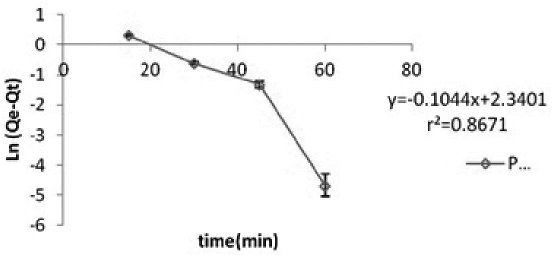

Fig. 8c

Figure 8: (a) Pseudo-first-order kinetic analyzing for Ni^{2+} on RCRI19^T. (b) Pseudo-first-order kinetic analyzing for Co^{2+} on RCRI19^T. (c) Pseudo-first-order kinetic analyzing for Pb^{2+} on RCRI19^T.

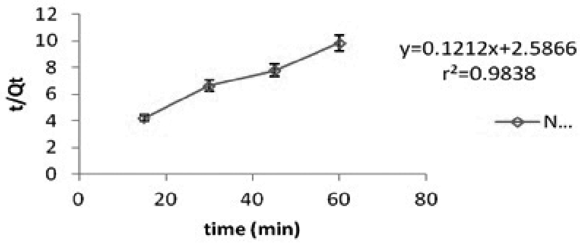

Fig. 9a

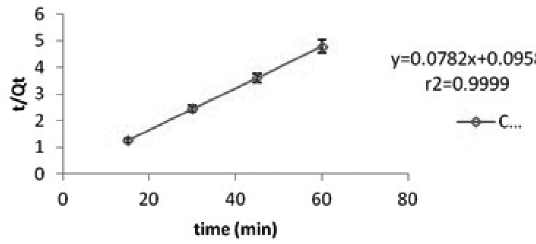

Fig. 9b

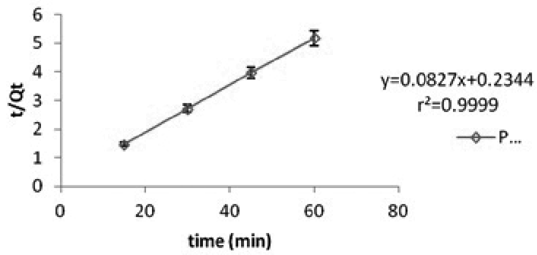

Fig. 9c

Figure 9: (a) Pseudo-second-order kinetic analyzing for Ni^{2+} on RCRI19^T. (b) Pseudo-second-order kinetic analyzing for Co^{2+} on RCRI19^T. (c) Pseudo-second-order kinetic analyzing for Pb^{2+} on RCRI19^T.

concentration and contact time (Figure 4). According to kinetic parameters obtained from pseudo-first-order ($q_{e,exp}$: mg/g, k_1: min^{-1}) and pseudo-second-order (k_2: g/mg min, q_e: mg/g) for biosorption of Ni^{2+}, Co^{2+} and Pb^{2+}, pseudo-second-order provided the best correlation of the experimental data and could be supported as a convenient model for the biosorption of heavy metals by *Tabrizicola aquatica.* The results suggested that strain RCRI19^{T}can be utilized as an effective biosorbent for the removal of Ni(II), Pb(II), and Co(II) ions from aqueous solutions.

Heavy metal pollution of water resources, particularly Pb(II), is a major contribution via human garage activities (Dutta et al., 2010). Recently it has been showed that the range of heavy metal biosorption is from 45 to 104 mg/g for fungi, 22 to 38 mg/g for yeast, and 78 to 90 mg/g for bacteria. Although the most absorption of heavy metals is observed in fungi; however, microbial biomass with low cost and greater ability to establish biofilms are very suitable choice for biosorption process (Muñoz et al., 2015). Garcia et al. (2016) indicated that the dead biomass of *bacillus* sp., can remove heavy metals in aqueous solutions. Our results showed that the increasing of biomass reduced heavy metal absorption (Figure 4c). In fact metal ions cannot be absorbed freely when the biomass concentration is getting high. The results confirmed the study on *Geobacillus* sp., by Babak et al. (2013) showed the absorption capacity increase at the lower concentration of cells.

Indigenous microorganisms like *Tabrizicola aquatica* is more feasible and could be a proper selection for in situ bioremediation because they are well adapted to the climate, physiochemical and nutritional situation of the habitat (Özdemir et al., 2009). Despite the restriction to application of bioremediated microorganisms, due to technological and economic constrains, the future prospect stymies in their application for intents like bioremediation and removing heavy metal from industrial effluents.

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