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Bioaccumulation of Fe^{3+} by bacteria isolated from soil and fermented foods for use in bioremediation processes

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It is known that in areas where mining activity exist, a great ecological imbalance with accumulation of iron particles in the soil can occur, which in high concentrations damages cellular structures of plants and microorganisms. Bacteria resistant to high concentrations of iron with the ability to reduce Fe^{3+} can make it bioavailable as an electron acceptor. The aim of this work was to select bacteria resistant to Fe^{3+} and to evaluate the accumulation of this element in the bacterial biomass of selected strains. Of 183 isolates tested using three different iron concentrations (0.005, 0.5 and 1 g/L) in culture medium chemically defined, 12 bacterial strains showed better growth in 1 g/L of iron. The three best isolates (*Bacillus simplex* UFLA CESB127, *B. subtilis* UFLA SCF590 and *Acetobacter tropicalis* UFLA DR6.2) were selected for further experiments. The selection of physical factors was performed using the Plackett-Burman design in order to assess the effect of the parameters on the accumulation of iron, followed by a central composite rotational design. A pH valuev of 3.5 and iron concentration of 0.750 g/L presented the best conditions for the accumulation of iron by bacterial isolates. After the optimization step, the bioaccumulation of Fe^{3+} was 99.22%. The validation confirmed the model of the experimental results. These results indicate the potential use of these isolates in the removal of iron, which in turn may be a promising alternative to conventional methods of treatment of contaminated soils.

Key words: Bioaccumulation, ferric iron, *Bacillus subtillis*, response surface methodology.

INTRODUCTION

Contamination of soils and industrial effluents with trace elements such as cadmium, lead, iron, manganese, mercury, chromium, copper, nickel, zinc has become a serious problem worldwide due to their high toxicity and bioaccumulative potential (Lodeiro et al., 2006). In undisturbed environments these elements are found in concentrations below 5 g/mL, but in contaminated areas they are often found above the permissible concentrations (Volesky, 2001; Lodeiro et al., 2006; Li et al., 2011).

The persistence of trace elements in the environment compromises the quality of soil, water and air with direct consequences on macro and microorganisms. Some

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species of bacteria are naturally resistant to higher concentrations of these elements making them the focus of approach to use non-pathogenic microbial biomass as an innovative and promising alternative to clean up contaminated areas. Some studies on bacteria of the phyla Firmicutes, Actinobacteria and Proteobacteria showed that they were capable of resisting and reducing mercury (Hg^{2+} to Hg^{0}) in the soil with high concentrations of this metal (Chakravarty et al., 2007). Strains of *Bacillus thuringiensis*, *Bacillus* sp. and *Paenibacillus polymyxa* are capable of absorbing cadmium (Cd^{2+}), arsenic (As) and copper (Cu^{2+}), respectively, due to the high affinity that these microorganisms demonstrated to these metals. The configuration of their surface polymers have been explored due to the ability to link to these metals (El-Helow et al., 2000; Nakamura et al., 2000; Acosta et al., 2005; Zhang et al., 2008; Kumar et al., 2010).

Iron is the fourth most abundant element found naturally in the earth's crust potentially making it the largest acceptor of electrons present in this environment (Stucki and Kostka, 2006; Dong et al., 2009; Marschner et al., 2011). However, mining activities mean that iron is the main polluter found in soil in particulate form and can compromise the integrity of molecules such as DNA, proteins and lipids (Naidoo and Chirkoot, 2004; Kuki et al., 2009). Many microorganisms have the ability to grow in high concentrations of trace elements. In general, this ability may be the result of intrinsic or induced mechanisms and may also be influenced by cell concentration, concentration of metal, composition of the culture medium, ionic strength and the presence of other ions in solution (Ledin, 2000). Among these factors, the pH value and stage of development of biomass are the factors that most influence the accumulation of elements (Guan et al., 1993).

Bacteria belonging to the genus *Bacillus* have the ability to reduce Fe^{3+}, when it is in excess in soils. These bacteria are widespread in natural systems such as soils, waters and marine sediments, and some of these microorganisms in these environments are potentially amenable to reducing Fe^{3+} (Lovley, 2000; Scheid et al., 2004; Liu et al., 2011). *Bacillus subtilis*, is a bacterium that is easily manipulated and has no or only a low level of pathogenicity. It has been studied for biotechnological potential of bioaccumulation (Diderichsen and de Boer, 1991). *B. subtilis* and *Bacillus licheniformis* show the capacity to adsorb various heavy metals, such as Cd^{2+}, Pb^{2+} and Cu^{2+}, and even compete for these metals (Daughney et al., 1998; Fein et al., 2005). Yang et al. (2012) reported the ability of *B. subtilis* to adsorb As^{5+} when complexed with Fe^{3+}, that is, Fe-bac performed well in the adsorption and reduction of As^{5+}.

Thus, the aim of this work was to select bacteria tolerant to Fe^{3+} and evaluate the nutritional and physical parameters that increase metal accumulation in bacteria with potential for use in bioremediation processes *in situ*.

MATERIALS AND METHODS

Microorganisms

The bacterial isolates tested belong to the Collection of Microorganisms from the Laboratory of Physiology and Genetics of Microorganisms, Department of Biology, Federal University of Lavras (UFLA, Brazil). These bacteria were isolated from Cerrado soils of Minas Gerais, from coffee fruit and cocoa fermentation (Silva et al., 2008; Coba et al., 2012; Pereira et al., 2012).

Cultivation of isolates

One hundred and eighty-three isolates were reactivated in a nutrient broth: 3 g/l of beef extract, 5 g/l peptone) and after 24 h, plated on nutrient agar (nutrient broth plus 15 g/l agar), incubated at 28°C for 24 h to check for purity by microscopic observation.

Selection of bacterial isolates under different conditions of iron

The evaluation of the growth of the isolates was performed using chemically defined culture medium (CD) (Pas et al., 2004) supplemented with different concentrations of ferric chloride ($FeCl_3$) (0.005, 0.5 and 1 g/L). The medium CD consisted of: 10 g/L of glucose, 0.5 g/L $(NH_4)_2SO_4$, 0.1 g/L KCl, 0.1 g/L NaCl, 0.5 g/L $MgSO_4.7H_2O$, 0.1 g/L $CaCl_2.2H_2O$, 1.0 g/L KH_2PO_4, 0.005 g/L H_3BO_3, 0.002 g/L $CuSO_4.5H_2O$, 0.01 g/L $ZnSO_4.7H_2O$, 0.002 g/L $Na_2MoO_4.2H_2O$, 0.001 g/L KI, 15 g/L Agar (pH 5.0 and no added vitamins). The bacterial inoculum was standardized according to the Mc Farland No. 5 scale (1.5×10^9 CFU/ml) and 10 µl of bacterial suspension added to each concentration tested in triplicate. The CD medium without the addition of iron was used as a positive control for each isolate. The colony diameters of the different isolates tested was performed after 48 h of incubation at 28°C, and the isolates with greater than or equal to 0.2 mm diameter were selected for the experimental design.

Experimental design

Identification of variables with significant effect on the growth of isolates: Delineation Plackett-Burman

Isolates selected under different conditions of iron were subjected to experimental design of Plackett and Burman (1946) for assessment of growth and the concentration of Fe^{3+} on microbial biomass (dependent variables) to fifteen different physicochemical and nutritional conditions generated from the 8 independent variables X1= pH (3.5 , 4.5 and 5.5), X2= concentration of glucose (5.0, 10.0 and 15.0 g/L), X3= iron concentration (0.005, 0.5 and 0.995 g/l), X4= concentration of the inoculum (10^6, 10^7 and 10^8 CFU/ml), X5 = nitrogen concentration (0.0 , 1.0 and 2.0 g/L), X6 = concentration of potassium (0.0 , 1.0 and 2.0 g/L), X7 = concentration of phosphate (0.0, 4.0 and 8.0 g/L), and X8 = ecoenzyme (enzyme complex whose function is the effect of buffer solution) (0.0, 5.0 and 10.0 g/L). The CD culture medium was used as base culture medium for all the different conditions tested. All cultures were maintained at 28°C for up to 7 days.

Determination of microbial biomass and content of Fe^{3+}

The production of biomass was measured in dry weight at 60°C

Table 1. Condition variables at different levels using CCRD for evaluation of the absorption of iron ferric isolate selected.

Variable	Code	Levels				
		-1.41	-1	0	+1	+1.41
pH	X1	3.295	3.5	4.5	5.5	5.705
Iron ferric (mg/L)	X2	147	250	500	750	852

after 7 days of growth for each test performed. The content of Fe^{3+} residual was assessed by an atomic absorption spectrophotometer (Varian SpectrAA 10 Plus) at 320 and 0.2 nm slit (Ewing, 1989; AOAC, 1997). The sample was obtained from the culture medium in the different conditions tested by rotary shaker at 150 rpm at intervals of 24 h and 7 days of and growth. The concentration of bioaccumulation by bacterial biomass was calculated by subtracting the initial concentration in each assay and the values of residual Fe^{3+}obtained from culture media by analysis in a spectrophotometer.

After identifying the independent variables that had a significant effect on the dependent variables, a central composite rotational design (CCRD) was applied for the purpose of optimizing the process of bioaccumulation by the isolates.

Central composite rotational design (CCRD)

Eight CCRD experiments were performed, grown in the culture medium CD (Pas et al., 2004) by varying the pH and the concentration of Fe^{3+} ($FeCl_3$ solution) (Table 1). The concentration of Fe^{3+} in the biomass was measured as described in determination of microbial biomass and content of Fe^{3+}.

The validation of the methodology used in the optimization process was performed by testing the best conditions obtained in the optimization and comparing them with the values predicted by the model. The experiment was done in triplicate, based on five points under conditions of interest within the surface and applying the same experimental procedures used to build the models. Statistical analyzes and graphs were performed using Design Expert® version 8.0 software (Stat-Ease Inc., Minneapolis, MN, USA).

Scanning electron microscopy with energy dispersive spectroscopy X-ray (SEM -EDS)

The samples of biomass previously centrifuged at 6000 g, from the validation stage were lyophilized. The samples were deposited on 1.0 mm diameter glass slides and immersed in a modified Karnovisky fixative solution (2.5% glutaraldehyde, 2.5% paraformaldehyde in 0.05 M cacodylate buffer pH 7.2, 0.001 M $CaCl_2$)/1 h. Subsequently, the slides were washed in 0.005 M cacodylate buffer to remove the glutaraldehyde residue of the Karnovisky solution and 5 drops of osmium tetroxide solution was added for fixation. After 4 h, the samples were washed in distilled water and dehydrated in an acetone series (25, 50, 75, 90 and 100%) and then placed in a desiccator to dry for 1 h. After drying, the samples were mounted on aluminum supports (stubs) and metalized by vacuum precipitation to a micrometric film of conductive material (carbon) on the surface of the material analyzed. Samples were observed in a scanning electron microscope (LEO EVO 40XVP) coupled with an energy dispersive system (X-ray microanalysis-EDS) and analyses of results were performed using the Genesis software under conditions: 30.000 kV, spot ranging from 5 to 7 and high vacuum (Alves, 2004).

RESULTS AND DISCUSSION

Selection of bacterial isolates under different conditions of iron

Of the 183 strains tested in different concentrations of Fe^{3+}, 12 showed higher tolerance to iron than the control, that is, colony diameter >0.4 mm in the CD medium with 0.5 g/L Fe^{3+} (data not shown). Of the 12 isolates with higher growth, three grew similarly with the control in the absence of iron (0.6 mm), and with a diameter of 0.3-0.5 mm when grown on CD medium with 1 g/L of Fe^{3+} (Table 2). The selection of bacteria capable of growing in higher concentrations of trace elements has the potential for bioremediation processes. This biotechnology may involve stimulation of the microbial community, the native population (biostimulation), introduction of a viable population (bioaugmentation), bioaccumulation (performed by living cells) or the use of biomass from dead cells (biosorption) (Abou-Shanab, 2011). In this work, bacterial strains from different origins were able to grow in high ferric iron concentrations; especially the species *B. subtilis*. Hsieh et al. (2009) studied the tolerance and accumulation of Hg^{2+} by *Bacillus megaterium* (and the successful transfer of the gene responsible for to *Arabdopsis thaliana* plant) *B. subtilis* previously grown in 8 mM solution of Fe^{3+} was capable of absorbing arsenic (Yang et al., 2012). This suggests that bacteria may be tolerant and/or resistant to multi-metals and thus can be used in soil contaminated by these elements. Because *B. subtilis* is tolerant to metals, this makes it a promising organism for the process of bioremediation of soil, since, along with *Corynebacterium* and other Firmicutes, dominate soils contaminated with metals (Ellis et al., 2003).

Variables having a significant effect on the accumulation of Fe^{3+}

Bacillus simplex UFLA CESB127, *B. subtilis* UFLA SCF590 and *Acetobacter tropicalis* UFLA DR6 were the three isolates that showed higher growth (Table 2). Twelve isolates from soil of the Cerrado of Minas Gerais state, from fermenting coffee fruit and from the fermentation of cocoa underwent 15 trials, with 8 independent variables analyzed using the Plackett and Burman design (1946) (Table 3). The results of the trial are given in Table 4.

Fe^{3+} is an element that can be chemically reduced or used as an electron acceptor, in an anaerobic response. Iron can be classified as essential, but is toxic to organisms in general when in high concentrations (Valls and Lorenzo, 2002). Operating costs in the bioremediation

Table 2. Evaluation of growth (mm) of 12 strains of bacteria isolated from different sources, QD cultured in medium at concentration of 1 g/L. Maximum diameter for each species are in bold.

Strain	Source	Species/genera	Media	
			QD (Without iron)	QD [1] g/L
			mm	
UFLA CESB127	Cerrado Soil	***Bacillus simplex***	**0.6**	**0.5**
UFLA CESB135	Cerrado Soil	*Bacillus simplex*	0.5	0.3
UFLA SCF590	Fermentation Coffee	***Bacillus subtilis***	**0.6**	**0.4**
UFLA SCF747	Fermentation Coffee	*Bacillus subtilis*	0.6	---
UFLA BR2.11	Fermentation Cocoa	*Acetobacter ghanensis*	0.5	0.3
UFLA ER5.15	Fermentation Cocoa	*Acetobacter* sp.	0.5	0.3
UFLA DR2.31	Fermentation Cocoa	*Acetobacter syzygii*	0.4	---
UFLA DR3.11	Fermentation Cocoa	*Gluconobacter* sp.	0.6	0.3
UFLA DR4.13	Fermentation Cocoa	*Acetobacter* sp.	---	---
UFLA DR6.12	Fermentation Cocoa	***Acetobacter tropicalis***	**0.6**	**0.4**
UFLA DR13.1	Fermentation Cocoa	*Acetobacter* sp.	---	---
UFLA DR3.21	Fermentation Cocoa	*Acetobacter* sp.	---	---

QD [1g/L] = QD media added, 1 g/L of $FeCl_3$ solution.

process should be considered, and thus the optimal conditions for bioaccumulation should be previously established in the laboratory in order to reduce these costs. Therefore, applying the methodology of an experimental design such as Plackett-Burman and CCRD help to establish the physical and chemical factors that increase the efficiency of bioaccumulation. Of the eight factors used to perform the Plackett-Burman, pH and concentration of Fe^{3+} were those that had the most significant effect on the accumulation of iron. Previous studies for adsorption of Cr^{6+} in a consortium of denitrifying bacteria determined that the pH and the amount of active (living) biomass were the determining factors for increased accumulation of this metal (Guan et al., 1993).

Among the independent variables tested only the pH and concentrations of Fe^{3+} showed significant negative and positive effects, respectively on bioaccumulation by bacterial biomass. Other factors showed no significant effects, but the magnitude of the effect of inoculum concentration (-24.25) was interesting showing that the lowest concentration of cells may be used in subsequent studies (Table 4).

The statistical significance of the model was checked by F-test. The analysis of variance (ANOVA) for bioaccumulation showed that the regression model was significant and the lack of fit was not significant (Table 4). The statistical model relate to biomass lack of fit and therefore was not significant, not allowing the optimization of this response. The cell biomass density is also a factor that previously was shown to influences the bioaccumulation. Inoculums with high cell density accumulated less metal due to the electrostatic interactions of the functional groups of the cell surface (Ledin, 2000). In our study, the cell concentration presented negative effect on the bioaccumulation of Fe^{3+}, indicating that the lower cell density should be used in the process (Table 4). In some cases, both organic and inorganic electron donor compounds may be used for the reduction of iron, for example when the availability of the metal for the microorganism occurs when it is in soluble form (Weber et al., 2006).

Central composite rotational design (CCRD)

B. subtilis UFLA SCF590 showed the best accumulation capacity of Fe^{3+} (highest accumulation of 0.828 g/l) and was selected for the optimization process using the CCRD. The experimental design was 2^2, with 11 trials being conducted, including four axial tests and another three central points. It was observed that the best conditions occurred in test 3 (pH= 3.5 and concentration of Fe^{3+} of 0.750 g/L) showing maximum accumulation of Fe^{3+} of 0.771 g/L (Table 5). Other tests also showed a high degree of accumulation of Fe^{3+} by biomass, but these conditions involved raising the pH and/or lowering the concentration of Fe^{3+}.

The model fit was assessed by the coefficient of determination R^2. The regression equation obtained indicated R^2 of 0.9922, with their predicted and fitted values of 0.9480 and 0.9845, respectively, suggesting an adequate fit of the model to quadratic experimental data and indicating that the model can explain 99.22% of the variability in response. The experimental results were modeled with a polynomial equation of second order to explain the dependence of the growth of the microorganism on the two analyzed factors

Table 3. Ferric iron accumulation (g/L) determined in Plackett-Burman design experiments after incubation of 3 bacterial isolates for 7 days. Absorption maxima are in bold and the percentage of biomass accumulation are in parenthesis.

Assay	pH	Glucose (g/L)	$FeCl_3$ (g/L)	Inoculum (UFC/mL)	NH_4NO_3 (g/L)	K_2HPO_4 (g/L)	Na_2PO_4 (g/L)	Ecoenzyme (g/L)	Iron ferric accumulation (g/L)		
									***B. simplex* UFLA CESB127**	***B. subtilis* UFLA SCF590**	***A. tropicalis* UFLA DR6.12**
B1	7.5	15	0.005	10^8	2	2	0	0	NA (0%)	NA (0%)	NA (0%)
2	3.5	15	0.995	10^6	2	2	8	0	**795 (79.5%)**	**828 (82.8%)**	**588 (58.8%)**
3	7.5	5	0.995	10^8	0	2	8	10	441 (44.1%)	527 (52.7%)	577 (57.7%)
4	3.5	15	0.005	10^8	2	0	8	10	NA (0%)	NA (0%)	NA (0%)
5	3.5	5	995	10^6	2	2	0	10	585 (585%)	818 (81.8%)	775 (77.5%)
6	3.5	5	0.005	10^8	0	0	8	0	NA (0%)	NA (0%)	NA (0%)
7	7.5	5	0.005	10^6	0	2	8	10	NA (0%)	NA (0%)	NA (0%)
8	7.5	15	0.005	10^6	2	2	0	10	NA (0%)	NA (0%)	NA (0%)
9	7.5	15	0.995	10^6	0	0	8	0	529 (52.9%)	0.1 (53.1%)	479 (47.9%)
10	3.5	15	0.995	10^8	0	0	0	10	566 (56.6%)	757 (75.7%)	742 (74.2%)
11	7.5	5	0.995	10^8	2	0	0	0	534 (53.4%)	602 (60.2%)	559 (55.9%)
12	3.5	5	0.005	10^6	0	0	0	0	NA (0%)	NA (0%)	NA (0%)
13	5.5	10	0.5	10^7	1	1	4	5	533 (53.3%)	557 (55.7%)	554 (55.4%)
14	5.5	10	0.5	10^7	1	1	4	5	526 (52.6%)	572 (57.2%)	558 (55.8%)
15	5.5	10	0.5	10^7	1	1	4	5	603 (60.3%)	569 (56.9%)	559 (55.9%)

NA – Not accumulated.

(pH and concentration of Fe^{3+}).

$$Y = 548.33 - 32.39X_1 + 230.28X_2 - 17.00X_1X_2 - 5.79X_1^2 - 58.29X_2^2,$$

where Y is the estimated accumulation of Fe^{3+} and X1 and X2 are the coded values, respectively, for pH and concentration of Fe^{3+} making it possible to confirm that the accumulation of iron can be estimated on the basis of quadratic effect on both factors .

The statistical significance of the model was checked by F-test (Table 6). The analysis of variance (ANOVA) for the accumulation of iron showed that the regression model was significant and lack of fit is not-significant, which can then describe the mathematical model based on the significant variables. The mathematical model is capable of describing the accumulation of iron based on the significant variables.

Regression analysis between X1 and X2 (Table 6), evaluated after 7 days of growth was significant for the confidence interval of 95 % ($p<0.05$). As noted, X1 and X2 factors showed values of -32.39 and 230.28, that is, negative and positive effects, respectively. This means that the lower the pH value in conjunction with the higher concentration of Fe^{3+} induced a greater accumulation of this metal by the bacteria. It was also noted that the quadratic effect of X2 was significant but other values for the interaction and the quadratic for X1 were not significant. This analysis of variance result showed the existence of significant differences between the effects caused by each factor analyzed.

The prediction of the optimal operating conditions for the accumulation of Fe^{3+} was determined experimentally using response surface methodology (RSM). The interaction effect of the parameters that significantly affect the accumulation of Fe^{3+} by *B. subtilis* UFLA SCF590 is shown in Figure 1. The curve in the

Table 4. ANOVA analysis showing each variable described for iron ferric absorption on 7 days of culture in Plackett-Burman experiments design.

Factors	Maineffect	Value F	Value P Prob> F	Significance
Model	384.07	74.93	<0.0001	***
pH	-61.92	18.86	0.0074	**
Glucose	14.08	0.98	0.3686	NS
Iron ferric	338.58	563.97	<0.0001	***
Inocullum	-24.25	2.89	0.1497	NS
NH4NO3	36.08	6.41	0.0525	NS
K2HPO4	23.58	2.74	0.1590	NS
Na2PO4	-24.25	2.89	0.1497	NS
Ecoenzime	11.75	0.68	0.4474	NS
Lackoffit		6.86	0.1155	NS

(***): Significant at >99.9% (for 0.0001 <P value <0.001); (**) Significant levels between 99 and 99.9% (for 0.001 <P <0.01); NS: not significant (NS P <0.05 was considered to be non-significant).

Table 5. Two variable central composite rotational design (CCRD) and their responses to the iron ferric accumulated from isolated *Bacillus subtilis* UFLASCF590.

Assays	pH	Iron ferric (g/L)	RESPONSE	
			Iron ferric accumulation (g/L)	Accumulation percentage
1	3.5	0.25	0.255	100
2	5.5	0.25	0.204	81.6
3	3.5	0.75	0.771	100
4	5.5	0.75	0.652	86.9
5	3.3	0.50	0.582	100
6	5.7	0.50	0.519	100
7	4.5	0.1475	0.135	91.5
8	4.5	0.852	0.756	88.7
9	4.5	0.50	0.545	100
10	4.5	0.50	0.562	100
11	4.5	0.50	0.538	100

Table 6. ANOVA analysis of DCCR for the experimental results of the isolated *Bacillus subtilis* UFLASCF590 of culture for 7 days.

Factor		Effect	ValueF	Value P Prob> F	Significance
Regressionmodel		548.33	127.64	<0.0001	***
X_1	pH	-32.39	11.80	0.0185	***
X_2	Ferro	230.28	596.66	<0.0001	***
X_1X_2		-17.00	1.63	0.2583	NS
X_1^2		-5.79	0.27	0.6278	NS
X_2^2		-58.29	26.99	0.0035	***
Lackoffit			7.11	0.1258	NS

(***) Significant at >99.9% (for 0.0001 <P value <0.001); NS: not significant (NS P <0.05 was considered to be non-significant).

response surface was plotted against two independent variables (pH and concentration of Fe^{3+}) for the predicted response Y (accumulation of Fe^{3+}).

A RSM showed the model proposed for the

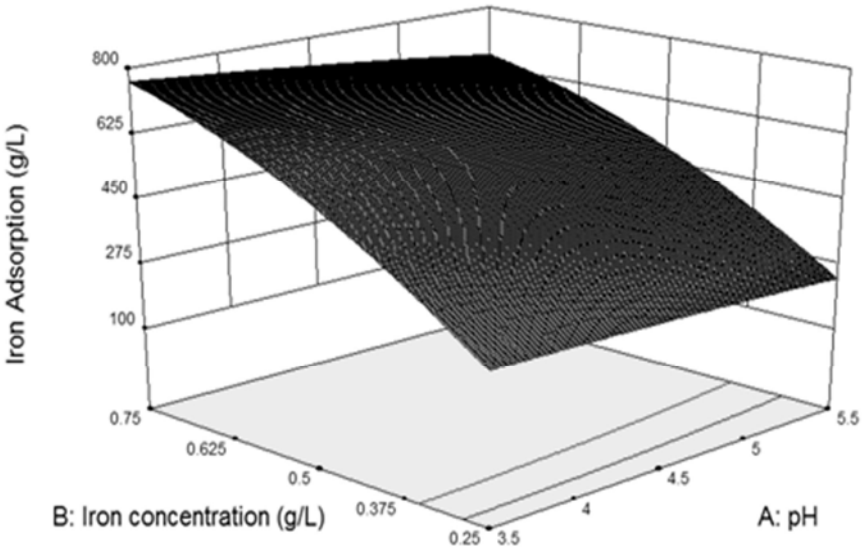


Figure 1. Effect of pH and ferric iron concentration on absorption by *Bacillus subtilis*. Response surface for the ferric iron absorption during the central composite rotational design–CCRD.

accumulation of Fe^{3+} after 7 days of testing. Table 5, indicates that the maximum accumulation of Fe^{3+}occurs with lower pH values (A) and with maximum concentration of Fe^{3+} tested (B) (Figure 1). By analyzing the response surface, we obtained a pH of 3.5 and concentration of Fe^{3+} of 0.750 g/l in optimal conditions resulting in a cumulative total biomass of 0.771 g/L. This validation was conducted with new experiments carried out under the optimum conditions shown in the CCRD, with the aim of confirming the proposed model. The comparison between the experimental results and those predicted by the model, confirmed the effect of the factors evaluated and the close agreement of the model with experimental results. Fe^{3+} solubility increases with a decrease of pH (Weber et al., 2006), which explains the higher bioaccumulation with lower pH values. The degree of bioaccumulation in this work by *B. subtilis* (UFLA SCF590) is reliable since there was no precipitate in the culture medium ensuring that the decrease in metal concentration in the medium corresponded to the accumulation of microbial biomass.

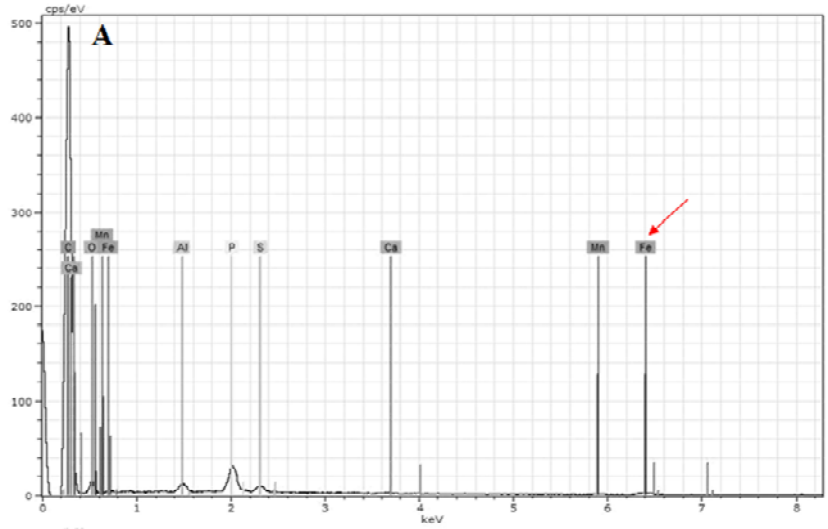
The transport of Fe to the interior of cells involves mobilization strategies (in this case reduction) in addition to a transport of high affinity (Miethke et al., 2013). The presence of other metals such as Cu^{2+}, Ni^{2+} and Cr^{2+} can stimulate the production of siderophores (is an organic compound that acts on iron uptake by organisms, p.e., bacteria) even in the presence of high concentrations of iron, acting as a system of extracellular resistance mechanism (Schalk et al., 2011) to toxic metals. Recently, it was observed that Fe^{3+} can be transported to the interior of the bacterial cell by specific transporters even without suffering prior reduction (Miethke et al., 2013).

Scanning electron microscopy with energy dispersive spectroscopy X-ray (SEM-EDS)

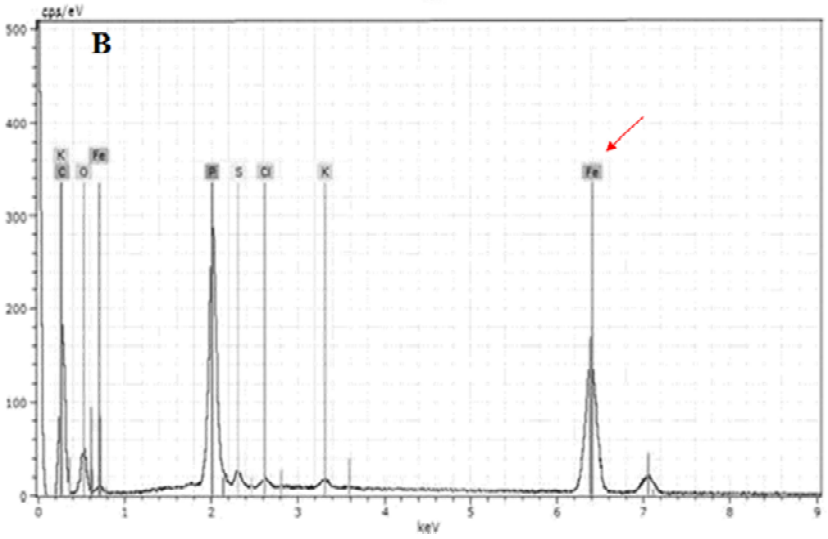
The energy dispersive X-ray (EDS) was used to qualitatively determine the Fe^{3+} produced from biomass in the control culture (no addition of iron) and in the culture after validating the optimal culture condition (Figure 2). Spectra of chemical composition by energy dispersive X-ray, are presented in Figure 2. The peaks for Ca and Mn disappeared and there was a decrease in peak S after the biomass was exposed to Fe^{3+} at a concentration of 0.750 g/L. Conversely, there was an increase in Cl and K peaks and no change in the Al and P peaks. These results indicated that the accumulation of Fe^{3+} by *B. subtilis* UFLASCF590 also includes ion exchange mechanisms. It was observed that the presence of Fe^{3+} in the biomass (peak of Fe^{3+} at 6.25 Kev) was approximately 5 times higher than in the control, that is, 34.3% concentration of atomic mass as compared to the control which showed 7.04%, confirming the accumulation of Fe^{3+} by *B. subtilis* (Figure 2). Spectroscopy energy dispersive X-rays (EDS) are thus a useful tool to evaluate the chemical characteristics of the biomass. From the analysis by EDS, we could see that the transport of Fe^{3+} involved the displacement of other intra and extracellular ions such as Ca and Mn, Cl and K, respectively. The exchange in the ion concentration was also observed after the absorption of Cu and Pb by *Bacillus* sp. (Tunali et al., 2006).

Conclusion

These results demonstrated that the isolate *B. subtilis*



ELEMENT	ATOMIC NUMBER	[Atomic Mass] standard %
Carbon (C)	6	0
Oxygen (O)	8	40,78007
Aluminium (Al)	13	7,486932
Phosphorus (P)	15	22,09808
Sulfur (S)	16	1,257599
Chlorine (Cl)	17	0,59126
Potassium (K)	19	0,970376
Calcium (Ca)	20	0
Manganese (Mn)	25	0
Iron (Fe)	26	34,30262
Copper (Cu)	29	0
Total	---	100



ELEMENT	ATOMIC NUMBER	[Atomic Mass] standard %
Carbon (C)	6	0
Oxygen (O)	8	49,05615
Aluminium (Al)	13	7,486932
Phosphorus (P)	15	22,34158
Sulfur (S)	16	7,015405
Chlorine (Cl)	17	0
Potassium (K)	19	0
Calcium (Ca)	20	0,816485
Manganese (Mn)	25	2,40733
Iron (Fe)	26	7,042312
Copper (Cu)	29	3,833808
Total	---	100

Figure 2. Analyzes micrograph and EDS taken by SEM and EDS of the *Bacillus subtilis* with the presence of Fe III (a) and control (b).

UFLA SCF590 is potentially useful in bioremediation processes in environments contaminated with Fe^{3+}. The process of removal of Fe^{3+} by this isolate was confirmed to be an active process of bioaccumulation involving the displacement of other ions. The maximum accumulation was obtained at pH 3.5 and a concentration of Fe^{3+} of 0.770 g/l with 100% efficiency.

Further studies are being developed using experimental microorganisms in the best conditions in order to evaluate the characteristics of bioaccumulation in soils previously contaminated with Fe^{3+}.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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