Chemosphere 74 (2009) 239–244

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Soil acidity determines the effectiveness of an organic amendment and a native bacterium for increasing soil stabilisation in semiarid mine tailings

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ARTICLE INFO

Article history:
Received 21 April 2008
Received in revised form 12 September 2008
Accepted 12 September 2008
Available online 26 October 2008

Keywords:
Aggregate stability
Bacillus cereus
Labile C fractions
Water-soluble carbohydrates
Wetting–dry cycle

ABSTRACT

Unstable mine tailings are vulnerable to water and air erosion, so it is important to promote their surface stabilisation in order to avoid the spread of heavy metals. In a greenhouse experiment, we assessed the effect of the addition of *Aspergillus niger*-treated sugar beet waste and inoculation with a native bacterium, *Bacillus cereus*, on the stabilisation of soil aggregates of two acidic, semiarid mine tailings, with different acidity degree, during watering and drying periods. Organic amendment raised the pH of both the moderately and highly acidic tailings, whereas the bacterial inoculation increased this parameter in the former. Only the amendment addition increased soil water-soluble carbon in both tailings compared with their controls, under either watering or drying conditions. Both the amendment and *B. cereus* enhanced water-soluble carbohydrates. Both treatments increased dehydrogenase activity and aggregate stability, particularly in the moderately acidic tailing under drying conditions. After soil drying, aggregate stability was increased by the amendment (about 66% higher than the control soil) and by the bacterium (about 45% higher than the control soil) in the moderately acidic tailing. The effectiveness of these treatments as structure-stabilisation methods for degraded, semiarid mine ecosystems appears to be restricted to tailings of moderate acidity.

1. Introduction

Soil surface structure stabilisation of metal mine tailings could reduce erosion, protect soil against degradation and limit the spread of metal contamination (Wong, 2003), particularly when dealing with fragile, semiarid soils which are exposed to a high risk of water erosion (Conesa et al., 2006). Soil structure has a prevailing role in soil infiltration and biogeochemistry processes (Chenu et al., 2001). Therefore, improved soil structure means increased water retention, nutrient uptake, drainage, aeration and root growth. Structural stability is controlled mainly by the soil organic material, iron and aluminium oxides, clay and $CaCO_3$ contents (Caravaca et al., 2004). Numerous studies have addressed the beneficial effect of organic amendments on aggregate stability (Roldán et al., 1994; Caravaca et al., 2003). The organic amendments can improve aggregate stability because of their transformations by soil microbiota and by stimulated microbial activity resulting from a new C input (Roldán et al., 2006). In addition to the evidence of mechanical entanglement by hyphae (Degens et al., 1996), there is speculation that extracellular polysaccharides of fungi or bacteria provide a cementing agent to large, transiently stable aggregates (Abiven et al., 2007). In particular, *Aspergillus niger*-treated sugar beet waste (SB), in the presence of rock phosphate, is an organic amendment rich in polysaccharide compounds and available P (Vassilev et al., 1995) that can be used as energy sources by heterotrophic microorganisms and as cementing and stabilising agents of soil aggregates in the reclamation of degraded mine ecosystems. A number of authors have shown the capacity of SB pulp to bind metals in aqueous solution, due to the carboxyl functions present in its constituents (Reddad et al., 2002). However, there are no data available on the effectiveness of this type of residue with regard to improving the structural stability of semiarid mine tailings.

Toxic metals can adversely affect the number, diversity and activity of soil organisms, inhibiting soil organic matter decomposition and N mineralisation processes (del Val et al., 1999; Misra, 2000). Bacteria isolated from metal-contaminated soils are often more resistant to metals than those collected from uncontaminated environments (Chaudri et al., 1992). Mechanisms allowing the bacteria to persist in metal-polluted media include the formation of organic metal-complexing agents (Higham et al., 1984) and precipitation or redox transformation of metals (Southam, 2002). It has been shown that soil bacteria are associated with the clay and organic particles and the metallic mineral surfaces of the soil microenvironment and these associations may influence the functions and survival of microbes: for example, through storage of water for microbes and buffering against water potential fluctuations (Chenu, 1993). These associations also make an important contribution to the binding of aggregates by soil biota. To the best

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0045-6535/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2008.09.035

of our knowledge, there are no previous studies indicating whether the inoculation of bacterial strains isolated from heavy metal-contaminated soils may improve soil aggregate stability and reduce soil erosion losses.

The Mediterranean climate exposes soil to long and severe drought periods occasionally interspersed with relatively rapid rewetting events. Soil drying and rewetting represents a physiological stress for the microbial communities residing in surface soil. A drying–wetting cycle may induce lysis in a significant proportion of the microbial biomass (Fierer et al., 2003). This decrease in the soil microbiota is largely dependent on its physical stabilisation in the soil matrix or on its ability to survive desiccation in soil. Although the effects of drying–wetting on microbial biomass and activity have been well-studied (Rosacker and Kieft, 1990; Zornoza et al., 2006), few studies have examined the effects of soil drying–wetting on the interaction between the natural microbial population and introduced bacterial species in relation to soil aggregate stability (Roldán et al., 2006).

We hypothesise that the wetting–drying cycle and the degree of acidity of the soil can modify the potential ability of a native bacterium, *Bacillus cereus*, and an organic amendment, *A. niger*-treated SB, to increase the aggregation of acidic mine tailings from a semiarid Mediterranean area.

2. Materials and methods

2.1. Study area

Soil used in this experiment comes from the La Unión mine district (Southeast Spain). The climate is semiarid Mediterranean with an annual rainfall around 250–300 mm and a mean annual temperature of 17.5 °C; the potential evapo-transpiration reaches 1000 mm $year^{-1}$. This zone constituted an important mining nucleus for more than 2500 years. The ore deposits of this zone have iron, lead and zinc as the main metal components. Iron is present in oxides, hydroxides, sulphides, sulphates, carbonates and silicates; lead and zinc occur in galena, sphalerite, carbonates, sulphates, and lead- or zinc-bearing (manganese, iron) oxides (Oen and Fernández, 1975). In this area soil from two mine tailings with an age of about 50 years and with different pH were selected: a extremely acidic mine tailing called "Brunita" (Universal Transverse Mercator (UTM) X686500 Y4164800 Z160, length: 320–400 m, width: 235 m, height: 23 m, volume: 850000 m^3, IGME, 1999) and a moderately acidic mine tailing called "Gorguel" (UTM X687480 Y4162800 Z135, length: 200–300 m, width: 95 m, height: 25 m, volume: 750000 m^3, IGME, 1999). The dominant vegetation of this area is represented by *Lygeum spartum* L. and *Helichrysum decumbens* (Lag.) Camb at the "Brunita" tailing and by *Limonium* sp., *Zygophyllum fabago* L. and *Piptatherum miliaceum* (L.) Cosson at the "Gorguel" tailing. Three soil samples were taken from each tailing. Each soil sample consisted of a mixture of six subsamples randomly taken from the top 20 cm depth of soil. The analytical characteristics of both mine tailings are shown in the Table 1.

2.2. Soil microorganisms

The bacterial strain selected for this study was the most abundant cultivable type in contaminated soils from the experimental area. The bacterial isolation was carried out following the conventional procedure: 1 g of homogenised soil was suspended in 100 mL of sterile water and 1 mL of this suspension was diluted to reach dilutions 10^{-3}–10^{-4}. These were plated in Petri dishes containing plate count agar medium (Oxoid) and cultivated for 48 h at 22 °C. Once selected the most abundant type was grown in nutrient broth medium (Scharlau Chemie) composed of meat and yeast extracts, peptone and sodium chloride, for 2 d at room temperature on a Heidolph Unimax1010 shaker. The bacterial strain was identified as a gram-positive bacterium member of the *B. cereus* group using molecular methods, based on polymerase chain reaction-denaturing gradient gel electrophoresis followed by 16S rDNA cloning and sequencing. For inoculation procedures the bacterial culture was centrifuged at 2287 *g* for 5 min at 2 °C and the sediment was resuspended in sterilised tap water. The bacterial suspension contained 10^8 CFU mL^{-1}.

Table 1
Chemical, biochemical, microbiological and physical characteristics of the soil used in the experiment

	"El Gorguel" tailing	"Brunita" tailing
pH (H_2O)	5.05 ± 0.03*	3.14 ± 0.02
Electrical conductivity (1:5, dS m^{-1})	2.5 ± 0.7	2.8 ± 0.8
$CaCO_3$ (%)	<5	<5
Total organic carbon (%)	0.4 ± 0.1	0.3 ± 0.1
Total N (%)	0.02 ± 0.01	0.03 ± 0.01
Clay (%)	5 ± 2	10 ± 3
Silt (%)	24 ± 5	36 ± 4
Sand (%)	71 ± 6	54 ± 5
Fe_2O_3 (%)	16 ± 3	37 ± 5
Al_2O_3 (%)	8 ± 1	4 ± 1
Total Zn (mg kg^{-1})	6738 ± 2450	1470 ± 567
Total Pb (mg kg^{-1})	4658 ± 2300	3691 ± 1267
Total Cu (mg kg^{-1})	234 ± 67	56 ± 12
Total Cd (mg kg^{-1})	37 ± 9	<1
Total Ni (mg kg^{-1})	31 ± 8	4 ± 2
Total Mg (mg kg^{-1})	8181 ± 1320	941 ± 320
Total Mn (mg kg^{-1})	4421 ± 998	663 ± 134

* Mean ± standard deviation.

2.3. Materials

SB, a lignocellulosic material was dried at 60 °C and then ground to pass a 2-mm-pore screen. Portions of 15 g of SB were mixed with 40 mL of Czapek solution (in g L^{-1}) consisting of agar 15.0; di-potassium hydrogen phosphate 1.0; iron(II) sulfate heptahydrate 0.01; potassium chloride 0.5; magnesium sulfate heptahydrate 0.5; sodium nitrate 3.0; sucrose 30.0; pH = 7.3, for static fermentation in 250 mL Erlenmeyer flasks. Rock phosphate (Morocco fluorapatite, 12.8% P, 1 mm mesh), was added at a rate of 0.75 g per flask. Media were sterilized by autoclaving at 120 °C for 30 min. A spore suspension of *A. niger* NB2 (1.2×10^7 spores mL^{-1}) was spread carefully over the surface of the media. The mixture was allowed to ferment at 30 °C for 20 d without shaking. The characteristics of the SB after fermentation were: pH, 5.3; total P, 224 μg mL^{-1}; total N, 1.2%; cellulose, 11.3%; hemicellulose, 3.1%; lignin, 4.1% and reducing sugar, 0.25 g L^{-1}.

2.4. Experimental design and layout

The experiment was a mesocosm assay, conducted as a completely randomised factorial design with three factors. The first factor had two levels: addition or not of fermented SB to the soil, the second had two levels: non-inoculation or inoculation with *B. cereus*, and the third had two levels: extremely acidic soil or moderately acid soil. Twenty four replicates per treatment were carried out, making a total of 192 pots.

Four-hundred grams of air-dried soil were placed in 600 mL pots. In September 2006, the amendment was mixed manually with the experimental soil at a rate of 5% (w/w). When appropriate, the soil was inoculated with 3×10^9 colony forming units (CFU) of *B. cereus* per pot. The experiment was conducted in a greenhouse, located in the Campus of Espinardo (Murcia, Spain). During the experiment, the average temperature ranged from 20 to 25 °C, and the relative humidity was between 70% and 80%. Midday photosynthetically active radiation averaged 260 μE m^{-2} s^{-1}. Pots

were watered regularly with sterile water maintaining soil moisture adjusted to 60% of water-holding capacity (corresponding with a soil matric potential of −0.2 MPa) for one month. Then, the irrigation was interrupted and the soil was allowed to dry during 1-month until gravimetric water content reached approximately 5% (corresponding with a soil matric potential of −1.2 MPa). Six pots per treatment were sampled at 1, 7 and 30 d of watering period and after the soil drying period. Soil samples were divided into two subsamples. One subsample was sieved at 2 mm and stored at 2 °C for biochemical analysis and the other subsample was air-dried at room temperature and sieved at 2 mm for physical–chemical and chemical analysis or at 0.2–4 mm for aggregate stability.

2.5. Soil physical–chemical and biochemical analyses

Total organic C and total N were determined with an automatic Nitrogen and Carbon Analyzer after pre-treatment with HCl to eliminate carbonates and combustion at 1020 °C. Calcium carbonate was determined using Bernard calcimeter. The texture was determined using the Robinson pipette method combined with sieving. Total metal contents were determined by nitric–perchloric digestion: 1 g of crushed sample was placed in a Kjeldahl flask, and 10 mL of concentrated HNO_3 plus 10 mL of concentrated $HClO_4$ were added. The mixture was heated at 210 °C for 90 min. When cool, the content of the tubes was filtered through an Albert® 145 ashless filter paper, and the volume completed at 50 mL by washing the Kjeldahl flasks with HCl 0.5 N several times and filtering. All metals were quantified using with an Inductively Coupled Plasma Mass Spectrometry (Thermo electron corporation Mod. IRIS intrepid II XDL).

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution, using a Crison GLP 21 pH meter and a Crison GLP 31 conductivity meter (Crison Instruments, Barcelona, Spain), respectively. In soil aqueous extracts, water-soluble carbon was determined in a Shimadzu TOC-5050A analyser of C for liquid samples. Water-soluble carbohydrates were determined by the method of Brink et al. (1960).

Dehydrogenase activity was determined according to García et al. (1997). For this, 1 g of soil at 60% of its field capacity was exposed to 0.2 mL of 0.4% 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride in distilled water for 20 h at 22 °C in the dark. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 mL of methanol by shaking vigorously for 1 min and filtering through Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

2.6. Physical analysis

The percentage of stable aggregates was determined by the method described by Lax et al. (1994). A 4 g aliquot of sieved (0.2–4 mm) soil was placed on a small 0.25 mm sieve and wetted by spray. After 15 min the soil was subjected to an artificial rainfall of 150 mL with energy of 270 J m^{-2}. The remaining soil on the sieve was placed in a previously weighed capsule (T), dried at 105 °C and weighed (P1). Then, the soil was soaked in distilled water and, after 2 h, passed through the same 0.25 mm sieve with the assistance of a small stick to break the remaining aggregates. The residue remaining on the sieve, which was made up of plant debris and sand particles, was dried at 105 °C and weighed (P2). The percentage of stable aggregates with regard to the total aggregates was calculated by (P1 − P2)100/(4 − P2 + T).

2.7. Statistical analysis

Data were log transformed to achieve for normality. SB addition, bacterial inoculation, type of soil and their interactions effects on measured variables were tested by a three-way analysis of variance. Statistical procedures were carried out with the Statistical Package for the Social Sciences 12.0 for Windows.

3. Results

3.1. Physical–chemical and biochemical properties

During the watering period, the addition of fermented SB and bacterial inoculation increased the soil pH of the moderately acidic "El Gorguel" tailing (Table 2), although the interaction residue × bacterial inoculation produced even higher values. The increases produced by the treatments for this physical–chemical parameter remained significant after soil drying. However, both the bacterium and the amendment had no significant effect on the soil electrical conductivity of this tailing, whatever the water regime (Table 2). Only the addition of organic amendment increased significantly the pH of the highly acidic "Brunita" tailing, in both water regimes (Table 3). Neither the residue nor the interaction residue × bacterial inoculation had any significant effect on soil electrical conductivity.

Table 2
Soil physical–chemical properties of the "El Gorguel" tailing in response to bacterial inoculation and composted residue addition during watering and drying periods ($n = 6$)

"El Gorguel" tailing	Well-watered (1 d)	Well-watered (1 week)	Well-watered (1 month)	Non-watered (1 month)
pH (1:5, H_2O)				
Control	5.13 ± 0.01a[a]A[b]	5.47 ± 0.02aB	5.59 ± 0.02aC	5.52 ± 0.01aBC
B. cereus	5.52 ± 0.01bA	5.68 ± 0.01bB	5.75 ± 0.02bB	5.70 ± 0.01bB
Amendment	5.51 ± 0.02bA	5.90 ± 0.02cB	5.91 ± 0.01cB	5.84 ± 0.01cB
B + A	5.67 ± 0.02cA	6.18 ± 0.01dB	6.09 ± 0.01dB	6.05 ± 0.01dB
Electrical conductivity (1:5, dS m^{-1})				
Control	3.73 ± 0.02aA	3.88 ± 0.04aA	4.36 ± 0.03abB	4.20 ± 0.04aB
B. cereus (B)	4.05 ± 0.03bA	3.98 ± 0.01aA	4.17 ± 0.03aA	4.17 ± 0.03aA
Amendment (A)	3.88 ± 0.01abA	4.03 ± 0.01aB	4.38 ± 0.02bC	4.29 ± 0.01aC
B + A	3.86 ± 0.02aA	4.01 ± 0.02aA	4.38 ± 0.01bB	4.31 ± 0.03aB

B = inoculation with *B. cereus*; A = addition of fermented sugar beet; B + A = addition of fermented sugar beet and inoculation with *B. cereus*.
[a] Values in columns, followed by the small same letter, do not differ significantly ($p < 0.05$) as determined by the Tukey test.
[b] Values in rows, followed by the capital same letter, do not differ significantly ($p < 0.05$) as determined by the Tukey test.

Table 3
Soil physical–chemical properties of the "Brunita" tailing in response to bacterial inoculation and composted residue addition during watering and drying periods ($n = 6$)

"Brunita" tailing	Well-watered (1 d)	Well-watered (1 week)	Well-watered (1 month)	Non-watered (1 month)
pH (1:5, H_2O)				
Control	2.67 ± 0.00a[a]A[b]	3.00 ± 0.02aC	2.91 ± 0.01aBC	2.85 ± 0.00aB
B. cereus (B)	2.88 ± 0.01bAB	2.93 ± 0.01aB	2.86 ± 0.01aA	2.84 ± 0.00aA
Amendment (A)	2.96 ± 0.00bcA	3.15 ± 0.01bB	3.20 ± 0.00bC	3.16 ± 0.01bBC
B + A	3.15 ± 0.02cA	3.21 ± 0.01bA	3.21 ± 0.00bA	3.17 ± 0.01bA
Electrical conductivity (1:5, dS m^{-1})				
Control	3.61 ± 0.01bcA	3.41 ± 0.03aA	3.91 ± 0.03bB	3.95 ± 0.03aB
B. cereus (B)	3.67 ± 0.02cA	3.62 ± 0.02bA	3.89 ± 0.01abB	4.08 ± 0.01aC
Amendment (A)	3.48 ± 0.01abA	3.55 ± 0.02abA	3.84 ± 0.01abB	3.98 ± 0.03aB
B + A	3.34 ± 0.02aA	3.45 ± 0.01aA	3.76 ± 0.01aB	4.03 ± 0.03aC

B = inoculation with *B. cereus*; A = addition of fermented sugar beet; B + A = addition of fermented sugar beet and inoculation with *B. cereus*.
[a] Values in columns, followed by the small same letter, do not differ significantly ($p < 0.05$) as determined by the Tukey test.
[b] Values in rows, followed by the capital same letter, do not differ significantly ($p < 0.05$) as determined by the Tukey test.

Table 4
Soil labile C fractions and dehydrogenase activity of the "El Gorguel" tailing in response to bacterial inoculation and composted residue addition during watering and drying periods ($n = 6$)

"El Gorguel" tailing	Well-watered (1 d)	Well-watered (1 week)	Well-watered (1 month)	Non-watered (1 month)
Water-soluble carbon ($\mu g\, g^{-1}$)				
Control	57 ± 1a[a]A[b]	71 ± 3aB	95 ± 0aD	84 ± 3aC
B. cereus (B)	55 ± 5aA	77 ± 2aAB	88 ± 1aB	99 ± 2aB
Amendment (A)	163 ± 3bAB	138 ± 3bA	166 ± 3bB	138 ± 2bA
B + A	151 ± 1bAB	138 ± 2bA	162 ± 3bB	138 ± 1bA
Water-soluble carbohydrates ($\mu g\, g^{-1}$)				
Control	19 ± 1aAB	19 ± 1aA	26 ± 1aB	27 ± 1aB
B. cereus (B)	17 ± 1aA	27 ± 1abA	42 ± 1bB	43 ± 1bB
Amendment (A)	51 ± 2bA	45 ± 2cA	79 ± 2dB	70 ± 1cB
B + A	43 ± 2bA	37 ± 2bcA	68 ± 1cB	63 ± 2cB
Dehydrogenase (μg INTF g^{-1})				
Control	0.44 ± 0.02aB	0.48 ± 0.01aA	0.56 ± 0.03aA	0.46 ± 0.03aA
B. cereus (B)	0.79 ± 0.05aA	0.61 ± 0.03abA	0.68 ± 0.02aA	0.72 ± 0.03bA
Amendment (A)	3.24 ± 0.25bA	4.83 ± 0.07bB	3.11 ± 0.11bA	3.44 ± 0.08cA
B + A	4.52 ± 0.17bA	4.81 ± 0.08bA	3.72 ± 0.02bA	4.73 ± 0.07dA

B = inoculation with *B. cereus*; A = addition of fermented sugar beet; B + A = addition of fermented sugar beet and inoculation with *B. cereus*.
[a] Values in columns, followed by the small same letter, do not differ significantly ($p < 0.05$) as determined by the Tukey test.
[b] Values in rows, followed by the capital same letter, do not differ significantly ($p < 0.05$) as determined by the Tukey test.

Water-soluble C and water-soluble carbohydrates values of control soil and soil inoculated with *B. cereus* increased to the same level during the watering period at the moderately acidic tailing (Table 4). Only the amendment increased significantly the soil water-soluble carbon compared with control soil, under either well-watered or drought-stressed conditions. Application of the fermented residue increased the water-soluble carbohydrates concentration (about 204% higher than control soil) to a greater extent than bacterial inoculation (about 159% higher than control soil), particularly at the end of the watering period.

Addition of fermented residue and inoculation with *B. cereus* increased significantly dehydrogenase activity of the moderately acidic tailing, particularly after drying period (Table 4). There was a significant positive interaction between organic amendment and bacterial inoculation for increasing dehydrogenase activity. The highest increases in dehydrogenase activity were recorded in the combined treatment of fermented residue addition and bacterial inoculation (about 10.3-fold greater than control soil after drying period).

At the highly acidic tailing, control and *B. cereus*-inoculated soils had water-soluble carbon values lower than those of amended soils (Table 5). The differences among treatments diminished at the end of the watering period and after soil drying. Both the amendment and *B. cereus* increased significantly the concentrations of water-soluble carbohydrates. The effect on water-soluble carbohydrates produced by the bacterium was dependent on the soil water regime, this parameter increased only under watering. After the drying period, water-soluble carbohydrates were increased significantly by the addition of fermented residue but not by bacterial inoculation.

Water regime had a significant effect on dehydrogenase activity of the highly acidic tailing. Wetting increased significantly the level of dehydrogenase activity of non-treated and treated soils, particularly in amended soils. In contrast, drought caused a significant decrease in this biochemical parameter. At the end of the drying period, the dehydrogenase activity of the control and *B. cereus*-inoculated soils sharply decreased, reaching similar values than those observed in the same soil under watering.

Table 5
Soil labile C fractions and dehydrogenase activity of the "Brunita" tailing in response to bacterial inoculation and composted residue addition during watering and drying periods ($n = 6$)

"Brunita" tailing	Well-watered (1 d)	Well-watered (1 week)	Well-watered (1 month)	Non-watered (1 month)
Water-soluble carbon ($\mu g\, g^{-1}$)				
Control	30 ± 1a[a]A[b]	64 ± 1aB	78 ± 3aB	75 ± 2aB
B. cereus (B)	60 ± 1aA	70 ± 1aAB	79 ± 2aBC	82 ± 1aC
Amendment (A)	231 ± 6bB	214 ± 2bB	145 ± 2bA	151 ± 2bA
B + A	201 ± 5bBC	209 ± 4bC	158 ± 2bA	170 ± 2cAB
Water-soluble carbohydrates ($\mu g\, g^{-1}$)				
Control	8 ± 0aA	13 ± 0aB	19 ± 0aC	30 ± 1aD
B. cereus (B)	16 ± 1aA	29 ± 2bAB	37 ± 2bB	34 ± 1aB
Amendment (A)	44 ± 1bA	51 ± 2cA	49 ± 2bcA	57 ± 1bA
B + A	40 ± 2bA	53 ± 1cAB	55 ± 2cAB	61 ± 2bB
Dehydrogenase (μg INTF g^{-1})				
Control	0.02 ± 0.01aA	0.19 ± 0.01aB	0.25 ± 0.02aB	0.01 ± 0.01aA
B. cereus (B)	0.02 ± 0.01aA	0.50 ± 0.02bB	0.18 ± 0.01aAB	0.01 ± 0.01aA
Amendment (A)	0.02 ± 0.01aA	0.33 ± 0.02abB	0.71 ± 0.02bC	0.65 ± 0.04bC
B + A	0.03 ± 0.01aA	0.34 ± 0.04abB	0.75 ± 0.03bC	0.71 ± 0.02bC

B = inoculation with *B. cereus*; A = addition of fermented sugar beet; B + A = addition of fermented sugar beet and inoculation with *B. cereus*.
[a] Values in columns, followed by the small same letter, do not differ significantly ($p < 0.05$) as determined by the Tukey test.
[b] Values in rows, followed by the capital same letter, do not differ significantly ($p < 0.05$) as determined by the Tukey test.

3.2. Aggregate stability

Wetting caused a significant increase in the soil aggregate stability of all treatments in the moderately acidic "El Gorguel" tailing (Fig. 1a). During the watering period, the bacterial inoculation and residue addition had hardly any effect on the percentage of soil stable aggregates of this tailing. In contrast, after one month of soil drying the addition of amendment was the most effective treatment for increasing soil aggregate stability (about 66% higher than the control soil), followed by the inoculation with *B. cereus* (about 42% higher than the control soil). At the end of the drying period, the aggregate stability of the control soil sharply decreased, reaching values less than those observed in the same soil under watering.

Bacterial inoculation increased the levels of soil stable aggregates of the highly acidic "Brunita" tailing after 1 week of watering (Fig. 1b). Except for the soil inoculated with *B. cereus*, soil aggregate stability did not vary with the soil wetting. Desiccation caused a significant increase in the aggregate stability of all soils, although there were no statistically significant differences with regard to the control soil.

4. Discussion

The results show that the addition of microbial treated SB and inoculation with an autochthonous bacterial strain improved soil aggregate stability in acidic, semiarid mine tailings and that their effectiveness was dependent on both the acidity degree and the soil water regime.

The cementing effect of organic materials can be attributed to microorganisms themselves and to the products of microbial synthesis and decomposition of organic materials (Lynch and Bragg, 1985). Polysaccharides, which are mostly by-products of the microbial activity developed in the C-containing substrate, are considered as the main temporary aggregate-stabilising agents (Roldán et al., 1994). They have the ability to form complexes with mineral surfaces and then they can improve structural stability by increasing cohesion and restricting swelling (Hafida et al.,

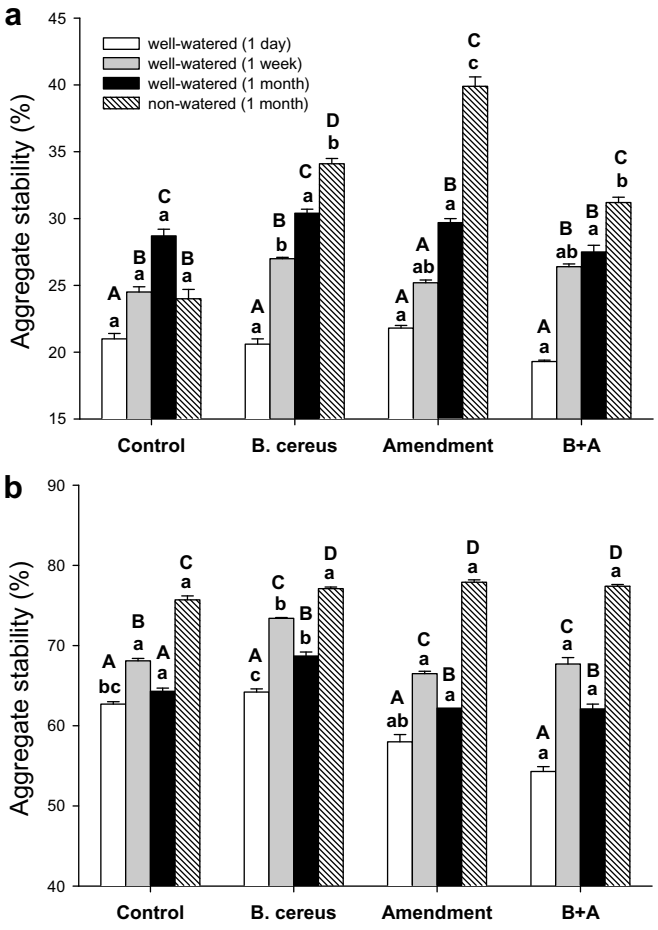

Fig. 1. Percentage of stable aggregates of the moderately acidic "El Gorguel" (A) and highly acidic "Brunita" (B) tailings in response to bacterial inoculation and fermented SB addition during watering and drying periods ($n = 6$). Bars represent standard errors. For each sampling point during wetting–drying cycle, bars with the small same letter are not significant different (Tukey, $p < 0.05$). For each biological treatment, bars with the capital same letter are not significant different (Tukey, $p < 0.05$).

2007). Thus, the levels of water-soluble carbohydrates were higher in the amended soils of both tailings, regardless of the water regime. Other authors have suggested that the addition of organic matter might increase aggregate stability by slowing water uptake by aggregates due to their enhanced hydrophobicity, thereby increasing the resistance of such aggregates to slaking in water (Piccolo and Mbagwu, 1999). In our experiment, the effect of the organic amendment on the stability of aggregates might also be attributed to reactivation of soil microbiota, as suggested by Roldán et al. (1994). Ghani et al. (2003) found that a positive correlation between the labile C fractions and microbial activity exists in soil. So, the soluble C fractions can be used as C and energy sources by soil microflora and may also participate in soil aggregation (Caravaca et al., 2002). In both tailings, there was a very-significant increase in the levels of labile C fractions in the amended soils, pointing to a greater degree of biological activity. In fact, increased biological activity was revealed also by the variations in dehydrogenase activity. Application of organic amendment to soil can increase dehydrogenase activity which has been frequently used as an indicator of soil overall microbial activity (García et al., 1997). The effect of the residue on structural stability was rapid (after 1 week) in the moderately acidic tailing although it had disappeared after 1 month of wetting. Tisdall and Oades (1982) argued that slowly decomposable material like cellulose results in a stability increase which persists for months, in comparison with labile material like glucose, for which the effects on stability are temporary (a few weeks). The positive effect of the organic amendment on the soil structural stability of the moderately acidic tailing was more pronounced after soil drying. Polysaccharides are hygroscopic and therefore may maintain higher water content in the colony microenvironment than in the bulk soil as water potential declines. This increase in water content could increase nutrient availability within the bacterial colonies. Thus, the polysaccharides resulting from the addition of the organic residue could help soil microorganisms to survive desiccation which, in turn, would contribute positively to soil aggregation. In fact, the organic amendment was more effective for increasing dehydrogenase activity of the moderately acidic tailing under drought conditions. In contrast, the soil desiccation produced a decrease in the aggregate stability of the control soil. Some studies have shown that soil drying may represent a significant stress for the soil microbiota, due to the decrease in the diffusion of nutrients to microorganisms, provoking a substantial loss of its biomass and activity (Rosacker and Kieft, 1990). One possible explanation for this observation is that the native soil community has not developed mechanisms to survive desiccation in soil, such as the production of exopolysaccharides. In fact, the levels of water-soluble carbohydrates in the control soil hardly varied during the watering and drying periods. On the other hand, decreased aggregate stability in the control soil of the moderately acidic tailing after drying period could be explained by slaking of aggregates as consequence of the loss of soil moisture. In contrast, the water retained by capillarity in the aggregates during wetting period could increase the internal cohesion and stability of the aggregates.

The secretion of extracellular polymeric substances (EPS) by bacteria, primarily composed of polysaccharides and proteins, is stimulated by the presence of toxic metals and may act as a "sticker", binding adjacent cells due to its adhesive nature (Fang et al., 2002; Guibaud et al., 2005). In addition to their great ability to complex heavy metals, it has been reported that EPS play a significant role in the formation and function of microbial aggregates, including adhesion phenomena, matrix structure formation and microbial physiological processes such as protecting cells against predation, desiccation (Roberson and Firestone, 1992) or harmful effects of heavy metals (Guibaud et al., 2005). The EPS of bacteria can form, with the surrounding mineral particles, an organo-mineral sheath around the cells (Chenu, 1993), which leads to an increase in macroaggregates as an indirect, additional effect. Alami et al. (2000) reported that inoculation with an exopolysaccharide-producing rhizobacterium increased aggregation of sunflower root-adhering soil under water-stress conditions. It has been observed that many species from the genus *Bacillus*, such as *B. megaterium* (Davidson et al., 1996) and *B. subtilis* (Omoike and Chorover, 2006), produce EPS. EPS functional groups are mostly protonated at pH < 2, but become progressively negatively charged with increasing pH. Likewise, proton adsorption and desorption at mineral surface hydroxyl groups exhibit strong pH dependence. Omoike and Chorover (2006) recorded that the adsorption of EPS from *B. subtilis* by goethite decreased as the pH increased from 3 to 9 because of progressive proton dissociation of both goethite and ionisable EPS functional groups. In our experiment, increases in the concentration of water-soluble carbohydrates were detected in the soil inoculated with *B. cereus*, in both soils after 1 week of wetting and only in the less-acidic tailing after soil drying. These results clearly mean that this strain is able to produce EPS in soil, which could be efficient with regard to increasing the percentage of stable aggregates. The fact that increased aggregate stability after soil drying was recorded in the soil of the less-acidic tailing inoculated with *B. cereus* but not in the inoculated soil of the highly acidic tailing could support this idea.

Both the organic amendment and bacterial inoculum were effective at increasing aggregate stability only in the less-acidic tailing. This result reflects the fact that the ability of treatments to act as soil-aggregating agents is highly dependent on environmental pH. The two tailings differed greatly in the percentage of stable aggregates, it being higher in the highly acidic tailing. These tailings are rich in iron oxides, which may contribute to the stabilisation of soil aggregates (Rhoton et al., 2003). One of the key factors that determine the extent to which Fe oxides stabilise soil aggregates is soil pH. At low pH, the Fe oxides develop a net positive charge and thus form a bond with the negatively charged clay and organic matter fractions. The pH increased with the amendment treatment in both soils and this increase could have induced a dispersion of the aggregates formed by Fe oxides in the extremely acidic tailing at the beginning of watering. The differences in aggregate stability between the two tailings were reduced after the addition of the organic amendment, as a consequence of greater improvement in the less-acidic tailing. It is likely that the presence of high levels of iron oxides plays a role in aggregation more relevant than that of added polysaccharides when soil pH is extremely low. On the hand, the role of Fe oxides as soil-aggregating agents is also dependent on redox potential (Rhoton et al., 2003). Thus, the fact of that all soils of the highly acidic tailing were more stable under dry conditions than wet conditions could result from lowering of the redox potential, which favours the dissolution of ferric ions to ferrous ions, and subsequently increases the dispersion of iron oxides, hydroxides or oxyhydroxides.

It can be concluded that the addition of microbially treated SB was more effective than inoculation with an autochthonous bacterium, for stabilising the soil structure of acidic mine tailings during desiccation, although their effectiveness was dependent on the degree of acidity of the tailing. The organic amendment and bacterial inoculation presented little advantage with respect to improvement of soil aggregates stability under well-watered conditions. Under semiarid conditions, the soil remains dry the most part of the year, so it is particularly relevant that the beneficial effect of both treatments occurred mainly after soil drying. The efficiency with which both treatments increased structural stability may be attributed to the production of microbial exopolysaccharides, which also could have counteracted the negative effect of water deficit on microbial activity. The successful application of these treatments as methods of soil aggregate stabilisation, in the reclamation of degraded mine ecosystems, appears to be restricted to tailings of moderate acidity.

Acknowledgement

This research was supported by Plan Nacional (Project AGL2006-09453-CO2-01).

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