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Assessment of solid reactive mixtures for the development of biological permeable reactive barriers

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ABSTRACT

Solid reactive mixtures were tested as filling material for the development of biological permeable reactive barriers for the treatment of heavy metals contaminated waters. Mixture selection was performed by taking into account the different mechanisms operating in sulphate and cadmium removal with particular attention to bioprecipitation and sorption onto the organic matrices in the mixtures. Suspensions of eight reactive mixtures were tested for sulphate removal (initial concentration 3 g L^{-1}). Each mixture was made up of four main functional components: a mix of organic sources for bacterial growth, a neutralizing agent, a porous medium and zero-valent iron. The best mixture among the tested ones (M8: 6% leaves, 9% compost, 3% zero-valent iron, 30% silica sand, 30% perlite, 22% limestone) presented optimal conditions for SRB growth (pH 7.8 ± 0.1; E_h = −410 ± 5 mV) and 83% sulphate removal in 22 days (25% due to bioreduction, 32% due to sorption onto compost and 20% onto leaves). M8 mixture allowed the complete abatement of cadmium with a significant contribution of sorption over bioprecipitation (6% Cd removal due to SRB activity). Sorption properties, characterised by potentiometric titrations and related modelling, were mainly due to carboxylic sites of organic components used in reactive mixtures.

1. Introduction

Acid mine drainage (AMD) is an important problem associated with mining and beneficiation of sulphide ores. AMD polluted waters are generally characterised by low pH, elevated concentrations of iron, sulphates and toxic metals. Metallic pollutants can be precipitated using natural lime or biologically produced hydrogen sulphide [1–3].

Biological remediation using sulphate reducing bacteria (SRB) can be performed in active systems such as off-line sulphidogenic bioreactors [4] or passive systems such as biological permeable reactive barriers (PRB) [2]. PRB has been successfully used for the treatment of a variety of contaminants, including the treatment of AMD and the remediation of streams polluted by heavy metals. Treatment in PRBs can be both abiotic and biotic. In the abiotic treatment neutralizing agents, adsorbents and zero-valent iron (ZVI) can be used as reactive filling materials, while in the biotic one SRB activity can be exploited [5,6]. Different studies underlined the efficiency of both abiotic [7–9] and biotic [10,11] PRB to remove high loads of inorganic contaminants from acidic leachates generated at mining and waste disposal sites. In biological PRBs organic mixtures are generally used as electron donor in the dissimilatory reduction of sulphate to sulphide, which generates alkalinity (reaction (1)) and promotes metal precipitation as sulphide (reaction (2)):

$$SO_4^{2-} + 2CH_2O + 2H^+ \rightarrow H_2S + 2H_2CO_3 \quad (1)$$

$$Me^{2+} + H_2S \rightarrow MeS \downarrow + 2H^+ \quad (2)$$

Organics used in biological PBRs are generally mixtures of promptly biodegradable materials (mushroom compost, manure of cow, horse and sheep, municipal compost) and more recalcitrant ones (sawdust, peat, straw, leaf compost) in order to ensure the long-term growth of SRB [12–16].

Full scale applications of biological PRBs are also characterised by the addition of gravel to improve barrier permeability, and limestone to control pH and stimulate SRB growth [17–19].

ZVI can be also used in order to consume oxygen (establishing the anaerobic environment necessary for SRB growth) and generate hydrogen (used as electron donor by SRB). In addition, ZVI can remove efficiently several organic and inorganic contaminants through different mechanisms. Possible removal mechanisms include direct electron exchange between a contaminant and ZVI that is oxidized to ferrous or ferric ions, and sorption onto the surface or onto iron corrosion products formed on the non-reacted metal surface [20].

Table 1 shows some reactive mixtures reported in the literature, which have been used in batch studies for the treatment of AMD and heavy metal's contaminated wastewaters.

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Table 1
Reactive mixtures used in batch studies for treatment of AMD and heavy metal's contaminated wastewater.

Composition	Influent SO_4^{2-} ($g\,L^{-1}$)	SO_4^{2-} abatement (%)	Reference
Municipal compost sawdust Manure Cellulose Sediments with SRB Silica sand Limestone	1.2–4.6	25–100	[10]
Wood chips Composted leaves Chicken manure Silica sand Sediments with SRB Limestone Urea	3	>95	[13]
Compost Sheep manure Oak leaf Limestone	1	80–99	[15]
M8 Leaves Compost ZVI Silica sand Perlite Limestone	3	83	Present study

Literature studies are generally focused on the whole sulphate-reduction process, omitting the identification of the different mechanisms involved in sulphate and metal removal. In particular the significant contribution of sorption onto organic matrices is not isolated from bioreduction. Discrimination between these two mechanisms avoids an overestimation of the sulphate-bioreduction capacity of the system, *which may provide misleading results* in the following phases of PRB design.

In this view a special attention was given to the selection of the carbon source by the identification of the different mechanisms of removal of sulphates and cadmium, namely bioreduction by SRB and sorption onto organic materials.

In addition new organic components were investigated considering their environmental compatibility, namely the characteristic of not introducing harmful by-products into the environment.

The aim of the work *was to test* new reactive mixtures with environmental compatible composition *and identify the mechanisms* of pollutant removal (bioreduction and sorption) for future development and design of biological PRBs for the treatment of heavy metals contaminated waters.

Cadmium was chosen as target-metal because it is one of the most dangerous heavy metals. In addition it is completely soluble at the concentration used in batch tests in the range of pH and E_h conditions that are characteristic of SRB growth (pH 7.5 ± 8.5, $E_h = -300 \pm -400$ mV, temperature 25 °C). Therefore cadmium decrease in solution can be due only to bioprecipitation (as sulphide) and to sorption, but not to other precipitation reactions.

2. Materials and methods

2.1. Sulphate reducing bacteria (SRB)

The SRB inoculum was a consortium, kindly furnished by the research group of Professor Groudev (Department of Engineering Geoecology, University of Mining and Geology, Sofia, Bulgaria), who collected it in the Curilo mine district near Sophia [21]. This area is located near the uranium deposit "C" and it has been contaminated with radioactive elements (uranium, radium, thorium) and heavy metals (copper, zinc, cadmium) as a result of mining and in situ leaching activities carried out for a long period of time.

Bacteria were propagated using standard procedures and a cultivation medium (C Medium) with the following composition: KH_2PO_4 ($0.5\,g\,L^{-1}$), NH_4Cl ($1\,g\,L^{-1}$), Na_2SO_4 ($4.5\,g\,L^{-1}$), $CaCl_2{\cdot}6H_2O$ ($0.06\,g\,L^{-1}$), $MgSO_4{\cdot}7H_2O$ ($0.06\,g\,L^{-1}$), sodium lactate ($6\,g\,L^{-1}$), yeast extract ($1\,g\,L^{-1}$), $FeSO_4{\cdot}7H_2O$ ($0.004\,g\,L^{-1}$) and sodium citrate ($0.3\,g\,L^{-1}$) [22].

2.2. Solid materials for reactive mixtures

Olive pomace samples were collected in Italian olive oil production plants as pressed and *sun* dried disks in November 2006. Solid samples were ground by an electric mixer and held in a stove at 80 °C for 24 h (average diameter of dried particles was 3 mm).

Leaf samples were collected in October/November 2006 at the base of oak trees (major percentage), walnut trees, red maples, cherry trees, pear trees and horse chestnuts. Leaf dimensions were reduced to an average diameter of 1.8 mm by manual grinding and dried in a stove at 80 °C.

Sheep manure samples were collected in Abruzzo pasture lands in October 2006. The manure was digested in order to eliminate pathogens and then was desiccated and dried in a stove at 80 °C.

Limestone (average diameter of dried particles 5 mm) and silica sand (average diameter of dried particles 0.77 mm) were taken from Italian gravel pits.

Commercial samples were used for compost (universal fertilized soil for gardening, Verdemix-CERMECcompost), perlite (Perlite expanded Agri 30; Isoperl) and ZVI (Connelly GPM-Iron aggregate ETI CC-1004; Connelly, Chicago, Illinois).

2.3. Batch growth tests on solid mixtures

Glass reaction flasks (120 mL) with a sampling port were used for all the experiments. Eight reactive mixtures were prepared (Table 2). Each mixture consisted of an organic source (compost, composted sheep manure, olive pomace, and leaves) used as electron donor for SRB *growth*, a neutralizing agent (limestone) to raise the pH of the medium and stimulate bacteria activity, and a porous medium (silica sand or perlite) to increase the medium porosity. In mixtures M4, M5, M6, M7 and M8 ZVI was also added.

5 kg of each mixture were prepared and manually mixed. Batch growth tests were then performed using 20 g sample of each mixture, which was put into a flask, and 80 mL of C Medium prepared without sodium lactate and yeast extract were added. Flasks were then sealed and 20 mL inoculum of bacteria cultivated in C Medium (7-day-old) were added by a syringe through the sampling port. All experiments were conducted at 37 °C under shaking conditions (150 rpm).

A block design was used to isolate the effect of inoculum variability in the selection of reactive mixtures. Reactive mixtures were then tested in two different sets (or blocks) of experiments: I set (M1, M2, M3, M4) and II set (M2, M4, M5, M6, M7, M8). Reactive mixtures investigated in each set were inoculated using the same SRB inoculum (as a block of uniform material) in order to eliminate the possible effects of this source of variability. In the II set those mixtures of the I set with the highest sulphate removal (M2 and M4) were tested again in order to compare their performance with those of M5–M8 after inoculation by the same block of bacterial biomass.

Medium characteristics (H_2S production, pH, E_h, and SO_4^{2-} concentration) were monitored for 22 days. Measurements of pH (by CRISON GLP22), E_h (by CRISON GLP22) and H_2S (by lead acetate paper) were determined immediately after sample collection. Samples were then filtered through 0.45 μm cellulose acetate filters and used for sulphate analysis (see Section 2.7) and cadmium deter-

Table 2
Composition of reactive mixtures (as dry weight percent) and average values of sulphate removal (%), pH and E_h on 22nd day of batch growth.

	Sheep manure	Leaves	Compost	Olive pomace	Iron (0)	Limestone	Silica sand	Perlite	Set	Abiotic controls	Inoculated samples		
										% SO_4^{2-} removal	% SO_4^{2-} removal	pH	E_h (mV)
M1	8	1				8	83		I	72 ± 3	70 ± 4	7.7 ± 0.1	−370 ± 1
M2	15	3	31			15	36		I	80 ± 3	87 ± 4	7.9 ± 0.1	−380 ± 20
									II	84 ± 5	81 ± 5	7.7 ± 0.1	−370 ± 30
M3	11	2		12		11	64		I	65 ± 5	60 ± 2	8.2 ± 0.2	−300 ± 20
M4	7	11			25	7	60		I	85 ± 2	86 ± 1	8.8 ± 0.1	−140 ± 10
									II	89 ± 5	82 ± 6	8.8 ± 0.1	−130 ± 10
M5		1		12	24	7	56		II	63 ± 1	62 ± 12	8.4 ± 0.1	−130 ± 35
M6				12	19	5	64		II	59 ± 2	52 ± 4	8.1 ± 0.2	−160 ± 10
M7			7	15	8	18	52		II	65 ± 5	88 ± 2	8.2 ± 0.1	−390 ± 10
									III	69 ± 3	91 ± 4	8.3 ± 0.1	−400 ± 20
M8		6	9		3	22	30	30	II	55 ± 2	83 ± 3	7.8 ± 0.1	−410 ± 5
									III	58 ± 1	83 ± 3	7.7 ± 0.1	−410 ± 5

mination by an inductively coupled plasma spectrophotometer (ICP).

For each mixture duplicates of inoculated tests and of abiotic controls were performed. Average values ± maximum semidispersion were reported in graphs and tables.

Sulphate removal onto single organic components (compost, olive pomace and leaves) without inoculum were carried out as those previously described, using the same amount of component as in the mixture (Table 2).

2.4. Batch tests of cadmium abatement

Cadmium removal was performed both with and without SRB inoculum. Samples were prepared as those previously described in Section 2.3, but adding metal spikes during SRB growth. A metal ion stock solution (200 mg L^{-1}) was *prepared by dissolving* nitrate salt in distilled water. Five milliliters of Cd stock solution were added to solid suspensions in order to have an initial metal concentration of 10 mg L^{-1}. Every four days a liquid sample was collected (5 mL) to determine the residual metal concentration, and then other 5 mL of metal-bearing stock solution were added to the flask maintaining a constant total volume. This procedure was repeated 5 times *for each sample*. H_2S production, pH, E_h, SO_4^{2-} and Cd concentrations were determined on collected samples. Each test was performed twice and average values were reported.

Single component tests with compost, olive pomace and leaves were carried out as those previously described using the same amount of component as in M7 and M8 mixtures.

2.5. Potentiometric titrations of single organic components

Potentiometric titrations were performed using sample suspensions of single organic components (compost, olive pomace and leaves) in deionised water (2 g in 40 mL).

Suspensions were fluxed by N_2 for 1 h to remove CO_2, and titrated by standard solutions of NaOH 0.1 M (basic branch) and HCl 0.1 M (acid branch) [23]. After each addition of titrant (NaOH or HCl) pH was allowed to reach the equilibrium under magnetic stirring and then measured by a pH-meter using a glass electrode. All potentiometric titrations were performed in duplicate and average values reported.

2.6. Cadmium sorption capacity onto single organic components

Cadmium sorption capacity onto organic matter was investigated in separate equilibrium experiments. Tests were performed using solid suspensions of compost, olive pomace and leaves (0.4 g of solid in 40 mL of 10 mg L^{-1} cadmium solution). pH was adjusted at the values of M7 and M8 mixtures (Table 2) by HNO_3 or NaOH additions. Metal bearing suspensions were kept under magnetic stirring at constant pH until the equilibrium conditions were reached. Solid–liquid separation was performed by centrifugation (5 min at 4000 rpm) and the equilibrium cadmium concentration in liquid phase (C_f) was determined by ICP. For each condition blank tests without solid were also performed to determine the initial metal concentration (C_0).

2.7. Analytical determination of sulphates

Sulphates were determined by a turbidimetric method: 2.5 mL of sample were placed in a test-tube and mixed with 500 μL of a solution of glycerine and 250 μL of a solution of sodium chloride. Glycerine solution was prepared mixing pure glycerine and distilled water (1:1). NaCl solution was prepared mixing 200 g of NaCl + 40 mL of concentrated HCl diluted to 1 L by distilled water. After the addition of glycerine and NaCl solutions each sample

was added for 300 μL of a solution of $BaCl_2$ (prepared with 90 g of $BaCl_2{\cdot}2H_2O$ in 1 L of distilled water). After two minute shaking samples were analysed by spectrophotometer at 390 nm. Instrument calibration was performed by standard solutions of anhydrous Na_2SO_4 (dried for 1 h in an oven at 110 °C).

3. Results and discussion

3.1. SRB batch growth using liquid medium and solid mixtures

Sulphate reduction by SRB inoculum was preliminary tested in batch tests using liquid C Medium. Biomass activity was evaluated by H_2S release and sulphate diminution during time (Fig. 1A).

Experimental data showed 59 ± 1% abatement of sulphates in 22 days with a steep decrease during the first three days, followed by a further slow removal. Experimental results of sulphate abatement and operating conditions of pH and E_h (pH 7.6 ± 0.4; E_h = −330 ± 20 mV) obtained using C Medium can be taken as representative of optimal growth and performance of the SRB inoculum. In fact the optimal pH and E_h values for SRB growth are in the range of 7/8.5 and −150/−350 mV, respectively.

In the I set four reactive mixtures (M1, M2, M3, and M) were used as solid media for bacteria of the same inoculum. For each mixture abiotic controls without inoculum were also performed to determine sulphate removal due to sorption phenomena. In Table 2 the specific composition of each mixture was reported along with the average values of SO_4^{2-} abatement with and without bacteria, pH and E_h on 22nd day of growth. Sulphide formation was observed for all inoculated reactive mixtures, but not in their abiotic controls denoting the activity of SRB metabolism in the tested media. Residual sulphates during time for the mixtures of the I set were reported in Fig. 1A. These data showed that M2 and M4 mixtures allowed SO_4^{2-} abatement even higher than C Medium. Nevertheless, using C Medium sulphate abatement is only due to sulphate bioreduction, while in reactive mixtures two different mechanisms can operate in sulphate removal: bioreduction and sorption onto the solid components of mixtures. Further tests reported in the follow confirmed the presence of both contributions and allowed their identification.

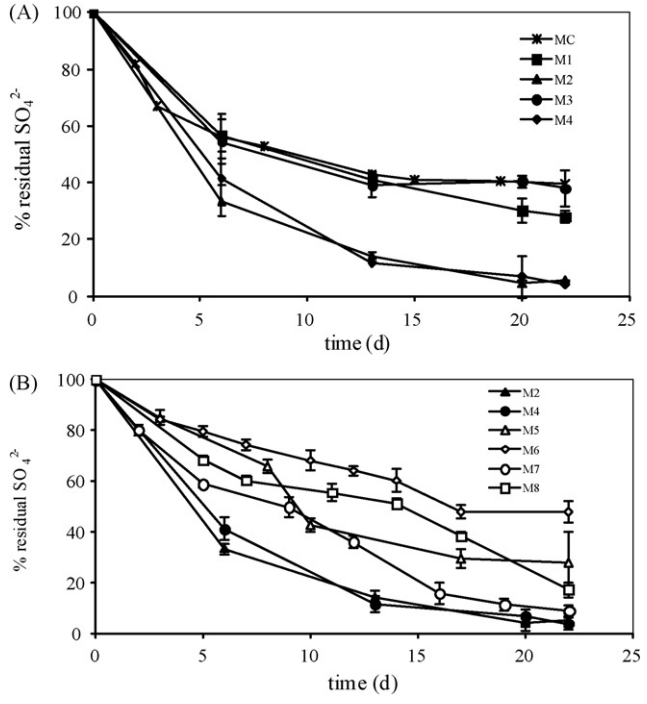

Fig. 1. Residual sulphates for SRB growth in C Medium (MC) and using solid mixtures of I set (A) and II set (B) (Table 1).

In the operating conditions of batch tests, ZVI corrosion and limestone solubilisation should not affect the chemistry of the system. In fact Fe and Ca speciation in solution obtained by a dedicated software for chemical equilibrium modelling (Medusa) [24] showed that Fe precipitates as FeS_2, while Ca is totally soluble.

In the II set another inoculum of bacteria was used to inoculate four other reactive mixtures (M5, M6, M7, and M8) and the mixtures of the I set with the highest sulphate removal (M2 and M4). By this way best performing mixtures of the I set can be compared with those of the II set isolating the possible effects of inoculum variability. Even for the II set abiotic controls were performed for all the mixtures.

Mixtures specifically tested in the II set (M5, M6, M7, and M8) were prepared by using environmental compatible substrates (Table 2) and eliminating manure as potential vehicle of pathogens.

Fig. 1B shows the profiles of residual sulphates during time for all mixtures investigated in the II set. Even in these tests sulphide formation was observed for all inoculated reactive mixtures, but not in abiotic controls.

SO_4^{2-} abatement with and without bacteria, pH and E_h values on 22nd day of growth were reported in Table 2. M7 and M8 showed the highest sulphate removal (M7 performance was significantly better than M8 by a *t*-test with 95% confidence) and suitable values of both pH and E_h.

Initial pH of the different mixtures were buffered (7.2 ± 0.3) due to the presence of limestone. In each mixture of the II set pH final values ranged from 7.8 to 8.4, slightly higher than those of the I set probably because of the presence of ZVI [9]. In fact ZVI can consume hydrogen ions according to the following reaction scheme consuming oxygen (establishing an anaerobic system necessary for SRB growth) and generating hydrogen (used as an electron donor by SRB):

$$Fe^0 + 2H^+ \rightarrow Fe^{2+} + H_2 \quad (3)$$

$$2Fe^0 + O_2 + 4H^+ \rightarrow 2Fe^{2+} + 2H_2O \quad (4)$$

Sulphate abatements obtained for M2 and M4 in I set and II set were compared to evaluate significant differences due to inoculum variability. In particular, a *t*-test was performed on sulphate abatement values on 22nd day. For both mixtures null hypothesis about sulphate abatement cannot be rejected (significance level 95%), meaning that in the investigated conditions the inoculum variability does not significantly affect mixture performance. Consequently a global comparison among all the mixtures of the I and II sets can be performed despite the use of different inocula. It is then possible to note that environmental compatible mixtures of the II set can enable sulphate reduction with comparable performance of manure-containing mixtures of the I set.

In particular mixtures M7 and M8 were the best of the environmental-friendly mixtures both in terms of sulphate abatements and pH and E_h conditions. In addition it can be noticed that their abiotic controls showed the lowest abatement of sulphates. This suggested that for these mixtures sorption was less relevant than in the other ones, and consequently bioreduction is the main mechanism operating in sulphate abatement.

3.2. Mechanisms of sulphate abatement in M7 and M8 mixtures

Sulphate abatement for M7 and M8 mixtures were tested to distinguish the amount of sulphate removal due to bioreduction, and the amount due to sorption onto solid matrices (III set in Table 2). Even in this set, tests with SRB and abiotic controls were performed for each mixture. Sulphide formation was revealed only in tests with

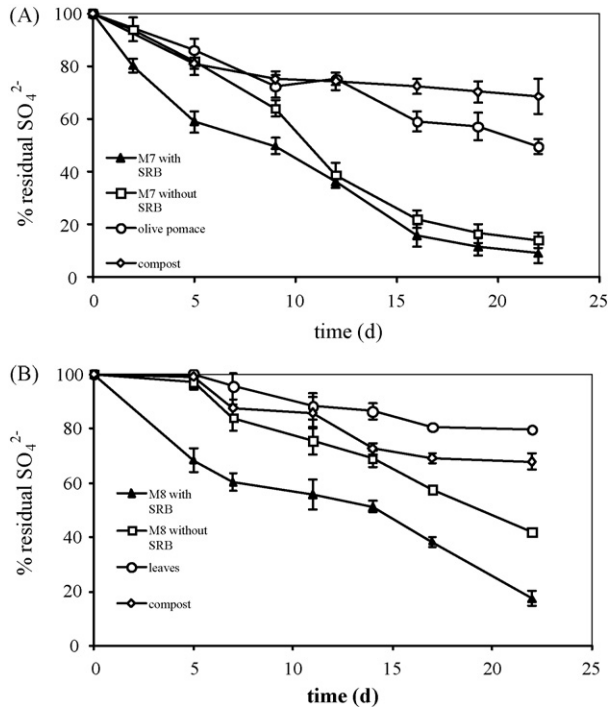

Fig. 2. Residual sulphates for M7 (A) and M8 (B) mixtures with and without SRB, and for single organic components of each mixture without bacteria.

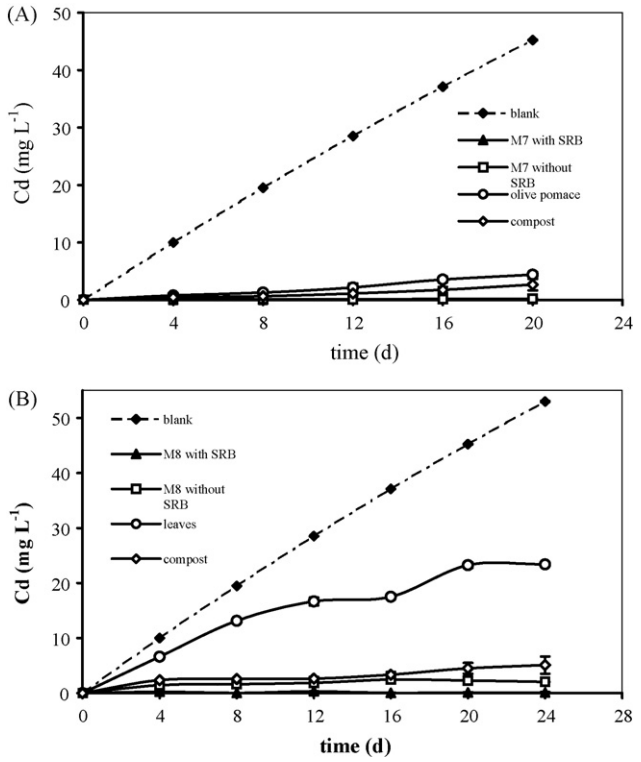

Fig. 3. Cadmium abatement for M7 (A) and M8 (B) mixtures with and without SRB and for single organic components of each mixture without bacteria.

bacteria confirming the absence of significant bioreduction activity without SRB inoculum.

Fig. 2 shows the profiles of residual sulphates for M7 and M8 mixtures with and without inoculated SRB.

Sulphate abatements on 22nd day in mixture M7 with SRB and without SRB were 91 ± 4% and 89 ± 3%, respectively. A *t*-test showed that these values were not statistically different (95% confidence), and then sulphate abatement using M7 mixture with or without bacteria was practically the same.

As regards mixture M8, sulphate abatement on 22nd day with bacteria was 83 ± 3% and without bacteria was 58 ± 1%. A *t*-test showed that these values were statistically different (95% confidence).

Comparison between tests with and without SRB can be used to isolate the contributions of bioreduction and sorption in sulphate removal. In the case of M8 the significant difference between these values was 25 ± 4% which represented SO_4^{2-} removal percentage due to sulphate bioreduction.

Sorption properties of single solid components were then isolated starting from organic components with well-known sorbing efficiency: olive pomace and compost for M7, leaves and compost for M8.

Profiles of residual sulphates for single organic components were reported in Fig. 2A and B.

Sulphate abatements in single component tests with olive pomace and compost were 50 ± 3% and 33 ± 7%, respectively. Their sum (83 ± 10%) is SO_4^{2-} abatement percentage due to sorption onto organic matrices.

A *t*-test denoted that sulphate abatement in mixture M7 without SRB (89 ± 3%) was not statistically different of the sum of these single organic components (95% confidence). This means that main contribution in sorption properties of M7 mixture was just due to the organic components.

As regards M8 mixture, sulphate abatement in single component tests with compost and leaves were 32 ± 3% and 20 ± 2%, respectively. Their sum (52 ± 5%) compared by a *t*-test with the sulphate abatement in mixture M8 without SRB (58 ± 1%) was not statistically different (95% confidence). This means that, even for M8 mixture, main contribution in sorption properties was just due to organic components.

Sulphate abatement in presence of SRB on the 22nd day obtained for M7 and M8 in the III set were compared with experimental data obtained for the same mixtures in the II set. Even in this case different inocula did not cause significant differences in experimental results confirming data reproducibility.

3.3. Mechanisms of cadmium abatement in M7 and M8 mixtures

Mechanisms operating in metal removal (Cd) with SRB growing in solid media were also investigated. Tests of cadmium removal were performed with M7 and M8 mixtures both in presence and in absence of SRB. Single component batch tests were then also performed to isolate the contribution of each organic component.

Fig. 3A shows cadmium concentration in solution for blanks (without solids), for suspensions of M7 mixture with bacteria and without bacteria, and for suspensions of single organic components (olive pomace and compost) without bacteria. Linear trend of blanks confirmed the absence of Cd precipitation during the different additions.

For each test condition, metal abatement was estimated as the integral removal of Cd (q_i, mmol g^{-1}) calculated for the final time of the experiment (20 days):

$$q_i = \frac{V}{PM_{Cd} \cdot m} \int (C_b - C)dt \quad (5)$$

where V (L) is the suspension volume, PM_{Cd} is the molar weight of cadmium, m (g) is the weight of solid, C_b (mg L^{-1}) is cadmium concentration after each addition of metal-bearing stock solution as obtained by the blanks, C (mg L^{-1}) is the residual concentration measured in the suspension four days after each addition (see Section 2.4).

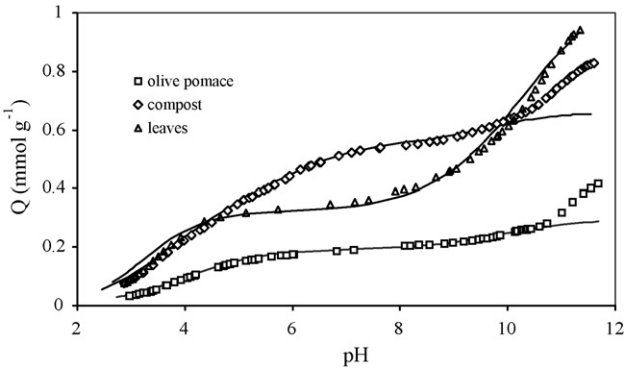

Fig. 4. Experimental data and model predictions for potentiometric titrations of olive pomace, compost and leaves.

M7 presented the same metal abatement both in absence and in presence of bacteria (q_i = 0.2 mmol g^{-1}). As also observed in sulphate removal tests, biomass activity was extremely low in M7 medium and consequently the observed Cd abatement was almost uniquely due to sorption.

The evaluation of single organic contributions by the integral Cd accumulation denoted a double share of compost with respect to pomace (q_i = 1.0 and q_i = 2.3 mmol g^{-1}, respectively).

Fig. 3B shows cadmium concentration in solution for blank test, for suspensions of M8 mixture with bacteria and without bacteria, and for suspensions of single organic components (leaves and compost) without bacteria.

Differently from M7, Cd removal in presence and in absence of biomass was substantially different. In particular, M8 mixture with SRB was characterised by residual Cd concentration that was one magnitude order lower than in the mixture without SRB (mean residual values 0.15 ± 0.05 and 2.0 ± 0.3 mg L^{-1}, respectively). Considering the integral cadmium accumulation values obtained for the mixture with and without SRB a preliminary estimate gives a 6% contribution to bioprecipitation and 94% to sorption. In particular the integral Cd accumulation for the single organic components denoted similar contributions of the sorption capacities of leaves and compost in M8 (q_i = 2.0 and q_i = 2.5 mmol g^{-1}, respectively).

Cadmium abatement tests confirmed that M8 mixture was the optimal reactive mixture for the long term treatment of heavy metal's contaminated wastewater in biological PRBs. In fact in M8 mixture Cd removal was due both to bioprecipitation and sorption, while in M7 mixture Cd removal was only due to sorption.

Table 1 shows a comparison between the batch-optimised mixture (M8) and some reactive mixtures reported in the literature, which have been used in batch studies for the treatment of AMD and heavy metal's contaminated wastewaters. It can be noticed that M8 performance, in terms of sulphate abatement, is comparable with the performance of the other reactive mixtures.

3.4. Characterisation of organic components

Biosorbents of M7 and M8 mixtures were characterised by potentiometric titrations in order to obtain information about nature and concentration of the active sites involved in metal removal. Titration curves of natural organic components generally point out the heterogeneity of this kind of matrices, whose acid–base properties can be represented by the development of mechanistic models based on a set of protonation reactions.

Experimental data of potentiometric titration of single organic components were expressed as concentration of surface charge (Q, mmol g^{-1}) versus bulk solution pH (Fig. 4) according to the charge balance in the system:

$$Q = \frac{C_t V_t + ([H^+] - [OH^-])V_{tot}}{m} \quad (6)$$

where C_t (mmol L^{-1}) is the titrant concentration after each addition, V_t (L) is the titrant volume, V_{tot} (L) is the total suspension volume after each titrant addition, m (g) is the solid weight in suspension, $[H^+]$ (mmol L^{-1}) is the proton concentration in solution obtained by pH measurements, $[OH^-]$ (mmol L^{-1}) is evaluated by $[H^+]$ considering the water dissociation constant.

Titration curves (Fig. 4) denote the complex acid–base behaviour of solid suspensions not showing distinct flex points due to site dissociations. To a first approximation it was assumed that this behaviour was due to acid–base reactions related to the dissociations of weakly acid functional groups. Experimental data expressed as Q vs. pH were smoothed (by a Mathcad algorithm) and the first derivative dQ/dpH was calculated. The maximum points of this derivative coincide with the maximum buffering capacity of the suspensions where pH = log K_H with K_H equilibrium constant of protonation ($S^- + H^+ \overset{K_H}{\leftrightarrow} SH$).

Heterogeneity of acid–base groups in natural matrices can be represented by a continuous approach introducing a probability density function for the logarithm of the equilibrium constant of protonation (K_H). Using the quasi-Gaussian distribution of Sips, charge concentration in the solid phase can be then represented by the following model (Eq. (5)) in which n-types of distributed acidic sites were hypothesised [23]:

$$Q = \sum_{i=1}^{n} \frac{Q_{Max,i}}{1 + (\tilde{K}_{H,i}[H^+])^{m_i}} \quad (7)$$

where $Q_{Max,i}$ (mmol g^{-1}) is the maximum charge on solid for the ith site type characterised by a median value of protonation constant $\tilde{K}_{H,i}$, and m_i is a shape parameter of the distribution.

The analysis of first derivative plots (reported as supplemental material) denoted that the three organic components were characterised by two main ranges of buffering capacities falling in the area of carboxylic (3–7) and phenolic (8–10) dissociations. Both these groups are typical of humic substances of compost and leaves, and of polyphenolic compounds in olive pomace.

The two regions of buffering capacity observed for all organic components were then modelled by assuming two kinds of distributed sites (n = 2 in Eq. (5)).

Adjustable parameters for each type of site ($Q_{Max,i}$: site concentration; $\tilde{K}_{H,i}$: affinity constant; m_i: shape of the affinity distribution) were obtained by simultaneously regressing titration data by Eq. (5) and derivative data by Sips distribution [25]. Parameters were allowed to be changed in selected ranges: the sum of $Q_{Max,i}$ (Q_{TOT} in Table 3) was estimated by the graphical method of Gran [26]; $\tilde{K}_{H,i}$ was allowed to range in the investigated interval of pH ($2 < \tilde{K}_{H,i} < 12$); m_i was changed in the range $0 < m_i \leq 1$, according to Sips distribution.

Table 3
Regressed parameters of titration models.

Parameters	Olive pomace	Leaves	Compost
$Q_{Max,1}$ (mmol g^{-1})	0.20 ± 0.02	0.32 ± 0.02	0.55 ± 0.04
$Log\tilde{K}_{H,1}$	4.0 ± 0.1	3.3 ± 0.2	4.9 ± 0.2
m_1	0.51 ± 0.06	0.8 ± 0.1	0.40 ± 0.05
$Q_{Max,2}$ (mmol g^{-1})	0.10 ± 0.01	0.31 ± 0.01	0.10 ± 0.03
$Log\tilde{K}_{H,2}$	9.9 ± 0.2	10.3 ± 0.1	9.7 ± 0.2
m_2	0.6 ± 0.1	0.55 ± 0.2	0.71 ± 0.03
Q_{TOT} (mmol g^{-1})	0.27	0.72	0.65

Regressed parameters for each organic component were reported in Table 3. Model predictions were compared with experimental data as Q vs. pH in Fig. 4.

For all organic components a good agreement was observed for potentiometric titrations and also for buffering capacity distributions. In addition regressed parameters of $\tilde{K}_{Hi}$ were consistent with the assumed chemical nature of active sites as carboxylic and phenolic groups. Finally the sums of active site concentrations were in agreement with Gran's estimates of total site concentration.

Concentrations of titrated sites were compared with Cd sorption capacities (q_{Cd}) determined for each organic component at the pH value of the reactive mixture (pH 8.2 for M7 and pH 7.7 for M8). Sorption capacity (q_{Cd}) was evaluated by the metal mass balance as:

$$q_{Cd} = \frac{C_0 V_0 - C_f V_f}{m} \quad (8)$$

where C_0 (mmol L^{-1}) is the initial metal concentration, V_0 (L) is the initial suspension volume, C_f (mmol L^{-1}) is the equilibrium metal concentration, V_f (L) is the final suspension volume, and m (g) is the sorbent mass (Section 2.6).

q_{Cd} was 1.1 ± 0.1 mmol g^{-1} for compost, 0.90 ± 0.04 mmol g^{-1} for leaves, and 0.56 ± 0.1 mmol g^{-1} for pomace. These values were highly correlated with the concentration of carboxylic active sites estimated by titration modelling ($Q_{Max,1}$ in Table 3). This correlation suggested the involvement of carboxylic sites in cadmium sorption onto organic components of the matrices.

4. Conclusions

In this study solid reactive mixtures were tested as filling material for the development of biological *PRBs that can be used* in the treatment of heavy metal's contaminated wastewaters.

Discrimination among mixtures was performed considering their ability of sustaining biological activities of sulphate reduction. In this view different mechanisms operating in both sulphate and metal removal were isolated with particular attention to bioprecipitation, due to sulphate reduction, and sorption, due to sorbing properties of organic matrices of the mixtures.

Two best-performing mixtures (M7 and M8) were then compared for specific mechanism operating in sulphate and cadmium removal during batch growth tests. M7 showed sulphate abatements even higher than M8 ($90 \pm 3\%$ and $83 \pm 3\%$, respectively). Nevertheless comparing growth tests with abiotic controls without inocula, only M8 denoted a significant contribution of biological activity in sulphate removal. The optimal mixture among the tested ones was then M8 (6% leaves, 9% compost, 3% zero-valent iron, 30% silica sand, 30% perlite, 22% limestone) which showed good conditions for SRB growth (pH 7.8 ± 0.1; $E_h = -413 \pm 4$ mV) and allowed $83 \pm 3\%$ sulphate abatement in 22 days, with a significant contribution due to bioreduction ($25 \pm 4\%$). As for cadmium abatement complete removal was obtained during batch growth tests but with a predominant contribution of sorption (94%).

Sorption contribution in Cd removal by organic components of M8 mixture was analysed by site characterisation carried out by means of titration tests and relative modelling. The comparison between adsorbent characterisation (potentiometric titrations) and equilibrium sorption tests suggested that carboxylic groups of olive pomace, compost and leaves are the main responsible for metal removal by sorption mechanisms.

Concluding organic mixtures generally used in batch or column lab-studies as solid media for SRB growth present significant sorption contribution in both sulphate and metal removal.

Lab-studies which aim at determining equilibrium and kinetic performance of SRB growing in solid media should isolate sorption contribution from bioreduction of sulphate and bioprecipitation of metals. Otherwise misleading results could be obtained with critical effects in scale-up due to over-estimation of SRB performance.

Anyway, additional studies are required in order to optimize PRB performance in different operative conditions, such as lower pH values, different temperatures and the presence of other elements (both inorganic and organic). In fact a certain number of heavy metals or metalloids may inhibits bacteria activity.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2009.05.081.

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