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Waste Management

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

Assessment of biotechnological strategies for the valorization of metal bearing wastes

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ARTICLE INFO

Article history:

Received 7 June 2011

Accepted 13 October 2011

Available online 15 November 2011

Keywords:

Bioleaching

Metal

Sediment

Catalyst

Fluorescent powders

Fe/S oxidizing bacteria

ABSTRACT

The present work deals with the application of biotechnology for the mobilization of metals from different solid wastes: end of life industrial catalysts, heavy metal contaminated marine sediments and fluorescent powders coming from a cathode ray tube glass recycling process. Performed experiments were aimed at assessing the performance of acidophilic chemoautotrophic Fe/S-oxidizing bacteria for such different solid matrices, also focusing on the effect of solid concentration and of different substrata. The achieved results have evidenced that metal solubilization seems to be strongly influenced by the metal speciation and partitioning in the solid matrix. No biological effect was observed for Ni, Zn, As, Cr mobilization from marine sediments (34%, 44%, 15%, 10% yields, respectively) due to metal partitioning. On the other hand, for spent refinery catalysts (Ni, V, Mo extractions of 83%, 90% and 40%, respectively) and fluorescent powders (Zn and Y extraction of 55% and 70%, respectively), the improvement in metal extraction observed in the presence of a microbial activity confirms the key role of Fe/S oxidizing bacteria and ferrous iron. A negative effect of solid concentration was in general observed on bioleaching performances, due to the toxicity of dissolved metals and/or to the solid organic component.

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1. Introduction

Waste generation is a side effect of consumption and production and it tends to increase with economic advance. As a consequence, toxic chemicals are produced and may reach environmental matrices, like air, water, soils or sediments. Pollutants may be present with high concentrations at the points of discharge, or may remain low but highly toxic for the receiving bodies (Juwarkar et al., 2010). Furthermore, some substances may be subjected to bioaccumulation or biomagnification, rising up the food web (Kelly et al., 2007).

Nowadays, waste production, management and disposal are object of interest by scientific community. Considering the progressive raw materials scarcity, the new interest in waste is not only related to their management or health hazardousness but also to their economic value.

Spent hydroprocessing catalysts contain middle-high concentration of base valuable metals, such as nickel (Ni), vanadium (V) and molybdenum (Mo). They represent a large amount of refinery solid waste and have been classified as hazardous waste by the Environmental Protection Agency in the USA (Dufresne, 2007; Furimsky, 1996; Marafi and Stanislaus, 2008). Cathode Ray Tube (CRT) fluorescent powders are waste coming from recycling process of cathode ray tube unit, by the diamond cut technology

(Méar et al., 2006). At the moment they are disposed of in landfills, nevertheless they may be considered as an important source of raw materials due to their content in zinc and yttrium. Dredged marine sediment coming from harbors and contaminated sites represent a large volume waste that should be properly managed. In fact, sediments represent the main repositories of anthropogenic pollutants at global scale; as a consequence, in the last decade, a particular attention has been paid to identify the most effective treatments for sediment deposit decontamination, with the final aim to re-use treated sediments either in building industries or in beaches nourishment. Metal concentration in harbor sediments is usually so high that dredged sediments are included within hazardous and toxic waste (US EPA, 2005).

Bioleaching is a commercial application of bio-hydrometallurgy approach to process mineral ores (Brierley and Brierley, 2001). In comparison with conventional technologies, this biological approach can offer attractive features, especially considering environmental issues: processes are more cost efficient, simpler and more environmentally friendly than their chemical counterparts. In fact, bioleaching is today considered a novel approach for metal mobilization from various solid matrices: fly ash, sewage sludge, spent batteries, electronic scrap materials, spent catalysts are only some of the solid wastes that can be potentially treated by means of bioleaching (Marafi and Stanislaus, 2008; Santhiya and Ting, 2005). At the same time, bioleaching has been applied for the bioremediation of soils (Chen and Lin, 2010; Gadd, 2004) and several

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studies have been recently focused on sediment bioremediation (Beolchini et al., 2009; 2010a; Chen and Lin, 2004; Dell'Anno et al., 2009; Löser et al., 2001). Bioleaching approach is based on the ability of microorganisms, bacteria or fungi, to produce leaching agents. In particular, Fe/S oxidizing bacteria are acidophilic autotrophic microorganisms able to acidify their habitat by means of their metabolism (Gadd, 2004). Fe/S oxidizing bacteria can directly bio-oxidize metal sulfides onto the solid matrix (direct mechanism) and/or act by bio-oxidizing ferrous iron in its reduced form, a strong oxidative agent, or elemental sulfur and its reduced compounds, producing leaching agents such as sulfuric acid and sulfur oxidation intermediates (indirect mechanism; Sand et al., 2001). The major advantage of bioleaching, if compared to chemical leaching, is that the necessary reagents for metal mobilization are biologically produced and there's no need of a continuous delivery to the plant; this implies obvious advantages both for the process economics and environmental impact in terms of carbon emissions. In fact, raw materials have been reported to form a significant part (52.2%) of the total process costs for chemical leaching of spent hydrogenation catalysts (Yang et al., 2011) and they are known to have the major contribution, together with energy, on the process impact in terms of global warming potential. In conclusion, technological trends at the moment are moving from thermal processes, really fast and effective, to more eco-compatible solutions, slower but as effective as the first, due to environmental constraints fixed by the Kyoto Protocol and its emission targets for carbon dioxide.

In the present work, Fe/S oxidizing bacteria were applied to spent refinery catalysts, CRT powders and dredged marine sediments in order to assess the performance of bioleaching in metal mobilization. The effect of different substrata, elemental sulfur and ferrous iron, of solid content and of redox potential was investigated. No previous works have been found in the literature for bioleaching applied to CRT powders; furthermore spent refinery catalysts and sediments bioleaching was aimed at assessing the eventual scale up of the process, investigating relatively high solid concentration if compared with values usually present in the scientific literature.

2. Material and methods

2.1. Waste

2.1.1. Spent refinery catalyst

Spent refinery catalysts were provided by Orim SpA (Macerata, Italy) and consisted of small cylinder shape elements. Table 1 shows metal content in the catalyst (Beolchini et al., 2010b). XRD analysis evidenced that vanadium and nickel were present in reduced form, as NiV_2S_4 and Ni_3S_4 ; while molybdenum was in oxidized form (Mo_4O_{11}). In order to lower the hydrocarbon amount, exhaust catalysts were pretreated by washing with water 20% (solid w/v) for 4 h.

2.1.2. Dredged marine sediment

Sediment samples were collected in the Ancona harbor (13,49915° E, 43,62495° N; Adriatic basin, Mediterranean Sea) by

Table 1
Waste metal content.

Spent catalyst		Harbor sediment		CRT powder	
Metal	% (w/w)	Metal	ppm	Metal	% (w/w)
Ni	4.5 ± 0.4	Ni	45 ± 4	Zn	43 ± 3
Mo	4.4 ± 0.4	Zn	100 ± 20	Y	24 ± 2
V	9.4 ± 0.9	As	12 ± 1		
		Cr	73 ± 7		

means of a modified Van Veen grab (about 14 L). Total organic matter and carbonates content was 28.2 ± 0.7 and 460 ± 10 mg/g, respectively. Water content was about 41%. Metal content is reported in Table 1. XRD analysis revealed that about 90% of the sediment was composed of silicates and carbonates, 5% by iron and manganese oxides, and less than 5% by sulfides. Sediment was pretreated by washing with deionized water for 4 h, to remove salts.

2.1.3. Cathode Ray Tube (CRT) powders

Fluorescent powders were provided by the company Relight Srl (Milano, Italy). Table 1 shows Zn and Y concentration in the powder; data reported were calculated as an average of different samples received from Relight in different periods of time. The standard deviation is quite high (15%) due to samples heterogeneity. XRD analysis revealed that Zn was present as sulfide, while Y both as sulfide and oxide.

2.2. Microorganisms

An environmental sample of Fe/S oxidizing bacteria (i.e., *Acidithiobacillus ferrooxidans*, *At. thiooxidans* and *Leptospirillum ferrooxidans*) was kindly provided by Prof. Stoyan Groudev (Department of Engineering and Geocology, University of Mining and Geology "Saint Ivan Rilski", Sofia, Bulgaria). This mixed culture was cultivated under acidic condition (pH 2) in the 9 K medium (Silverman and Lundgren, 1959). Aliquots of the mixed culture were sampled during the exponential growth phase and used to inoculate the biological treatments.

2.3. Bioleaching experiments

Experiments were performed in autoclaved 250 mL Pyrex flasks filled to a volume of 100 mL, in order to assure oxygen transfer. Incubation was carried out at 30 °C and at 175 rpm horizontal shaking, for a maximum of 21 days. The medium was the 9 K medium (par. 2.2), modified according to experimental conditions (Table 2). During the bioleaching experiments, periodical measurements of pH and Eh were carried out and aliquots were sampled for the determination of prokaryotic abundances and metal concentration in the suspension (Beolchini et al., 2010b).

Two sets of experiments have been performed (Table 2). The first one was aimed at assessing the effect of substrata (Fe^{2+} and S^0) on metal extraction by bioleaching from exhaust refinery catalysts and from contaminated marine sediments. A second set of experiments was aimed at finding the best operating conditions for catalysts and cathode ray tube powder. Main factors investigated were solid concentration and redox potential (in terms of $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio). In particular redox potential was 350 mV at the beginning of experiments with no ferric iron addition and 450 mV with ferric iron. For each bioleaching treatment in Table 2, a chemical control test was also performed with no bacteria inoculum.

2.4. Analytical determinations

pH and Eh measurements were carried out by Inolab Multi 720 (WTW).

The total prokaryotic number was determined by epifluorescence microscopy, after staining with Acridine orange (Hobbie et al., 1977).

Solubilized metals and metalloids were determined by atomic absorption spectrometry; samples were conveniently diluted with distilled water at pH 2 (with HNO_3) before atomic absorption determinations.

Total organic matter in sediments was determined as the difference between dry weight (60 °C, 24 h) and the weight of the resi-

Table 2
Operating conditions and extraction yields at the end of the bioleaching treatments for metal extraction from wastes.

Treatments	Waste	Solid (%)	S ⁰ (g/L)	Fe ²⁺ (g/L)	Fe ³⁺ (g/L)	Time (days)
<i>SET I</i>						
1	Spent catalyst	1	0	0%	–	21
2	Spent catalyst	1	5	0%	–	21
3	Spent catalyst	1	0	8.88	–	21
4	Spent catalyst	1	5	8.88	–	21
5	Sediment	10	0	0	–	14
6	Sediment	10	1	0	–	14
7	Sediment	10	0	8.88	–	14
8	Sediment	10	1	8.88	–	14
<i>SET II</i>						
9	Spent catalyst	2	–	8.88	–	16
10	Spent catalyst	2	–	4.44	8.88	16
11	Spent catalyst	10	–	8.88	–	16
12	Spent catalyst	10	–	4.44	8.88	16
13	CRT powder	10	–	8.88	–	16
14	CRT powder	10	–	4.44	8.88	16

due obtained after combustion for 2 h at 450 °C, after a treatment with 10% HCl finalized at removing carbonates.

Metal partitioning in the different geochemical fractions of sediment were determined by means of sequential selective extraction, according to European Union protocol (Quevauviller, 1998). Four different fractions were considered: (i) exchangeable fraction and carbonate fraction, extracted by incubating sediment with acetic acid 0.11 M; (ii) iron and manganese oxide fraction (reducible), extracted with hydroxylamine hydrochloride 0.1 M at pH 2; (iii) organic and sulfide fraction (oxidizable), extracted by a first step with 30% hydrogen peroxide and a subsequent passage with ammonium acetate at pH 2; (iv) residual fraction, that remains in the residual solid, determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), after acid digestion (US EPA, 2001).

Total metal content in the solid samples were determined by Inductively Coupled Plasma spectrometer, after acid digestion (US EPA, 1997). In particular, for the CRT powder and the spent catalyst, 1 g of the solid was treated for 2 h using hydrochloric acid and nitric acid, in a ratio of 3:1. After filtration by Whatman 41 filter paper, the resulting liquor was made up to 100 mL by using 50% HCl. Metals were analyzed by means of Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). As regards the marine sediments, 0.5 g of dry solid were treated at 150 °C, for 90 min, with hydrochloric acid and nitric acid in a ratio of 3:1. The sample extract was added with 5 mL of 10% boric acid and analyzed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES; US EPA, 2001).

3. Results

The first set of experiments (SET I in Table 2) was aimed at investigating the role of different substrata (i.e., elemental sulfur and ferrous ion) on the biologically mediated solubilization of heavy metals from marine dredged sediments and spent refinery catalysts. Many studies have been based on the effect of substrata addition on the bioleaching of several metal bearing matrices (Chen and Lin, 2004; Ravishankar et al., 1994; Zhao et al., 1981). In particular, sulfur is extensively used in bioleaching processes, as a cheap growth substrate for Fe/S oxidizing bacteria and for its role in sulfuric acid production by these strains (Rulkens et al., 1995; Tyagi et al., 1994). Iron has been mostly studied in terms of biological oxidation performances; furthermore iron is not only a growth substrate for Fe/S oxidizing bacteria (as ferrous iron) but also a strong oxidizing agent, when biooxidized in its ferric form (Silverman and Ehrlich, 1964). Moreover, Fe²⁺ enhances bacteria

resistance to high metal levels (Sugio et al., 1984). Previous studies (Beolchini et al., 2009) already demonstrated the effectiveness of bioleaching on metal mobilization from dredged marine sediments, when the solid content in the microcosms was 2%. For such a reason, experiments with sediment were performed with a 10% solid content. Iron and sulfur concentration was fixed according to the solid content in each treatment.

Extraction yields at the end of the treatment with spent catalyst are shown in Fig. 1a. It can be observed that the highest extraction yields for Ni and V (83% and 90%, respectively) were achieved in biological treatments 3 and 4 (Table 2), which are those in the presence of iron, while their respective abiotic controls reached significantly lower yields. Treatments in the absence of iron (1 and 2 in Table 2) showed no statistically different yields between inoculated flasks (biological treatments, Fig. 1a) and not inoculated ones (controls, Fig. 1a), both in the presence and in the absence of elemental sulfur (Beolchini et al., 2010b). Molybdenum dissolution did not reach values as high as Ni and V: the highest extraction yield was in the range 35–40% and was recorded in the inoculated microcosms with either ferrous ion and/or elemental sulfur (treatments 2–4 in Table 2). In contrast with its effect on Ni and V dissolution, ferrous iron did not seem to enhance molybdenum recovery, even if a significant difference between biological treatments and chemical controls was remarkable also for molybdenum. The effect of ferrous iron on the biological activity of the microbial consortia is also confirmed by pH profiles (data not shown here).

Fig. 1b shows Ni, Zn, As and Cr extraction yields at the end of bioleaching experiments with dredged marine sediment. The highest dissolution yields were obtained for Zn and Ni, with 44% and 34%, in treatments 7 and 8, respectively (Table 2). On the contrary, As and Cr were scarcely mobilized (Table 2). Data reported in Fig. 2b also evidence no significant difference between biological treatments and their controls. In order to assess the presence of biological activity, microbial abundances and pH profiles were also monitored. Fig. 2a shows microbial abundances after one week of incubation for treatments 5–8 in Table 2 and Fig. 2b the pH temporal profiles for treatments 6 and 8. As observed for the spent catalyst, the presence of ferrous iron was essential to have pH profiles corresponding to a good biological activity of Fe/S oxidizing bacteria. If no iron was added, in fact, pH could reach values around 7 (Fig. 2b), as a consequence of the strong buffer action of marine sediments, which are very rich in carbonates. The adaptation of acidophilic strains to circum-neutral and strongly buffered pH of marine sediments is a key passage; for such a reason, the analysis of cellular growth during time was also performed. It is possible to note in Fig. 2a that biological treatments performed in the presence of iron gave cellular abundances significantly higher than in the

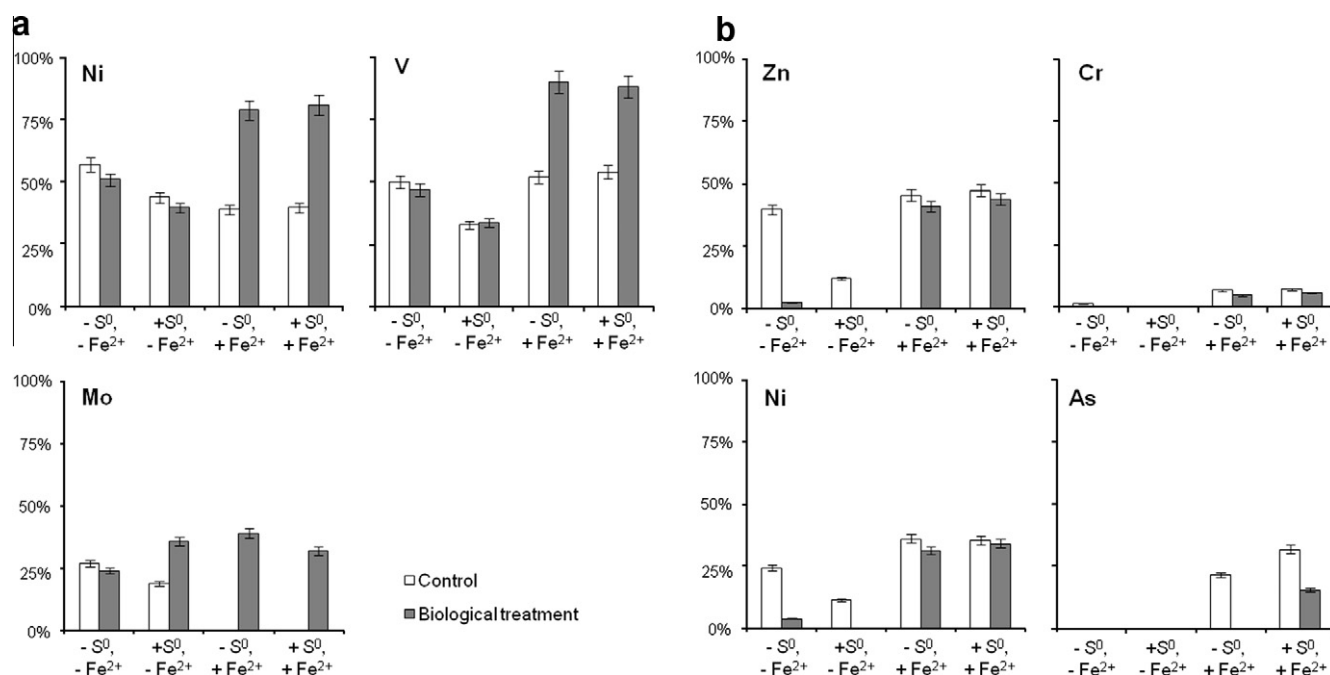


Fig. 1. Effect of substrata (Fe and S) and of biological activity on metal extraction by bioleaching from catalysts (a) and sediment (b).

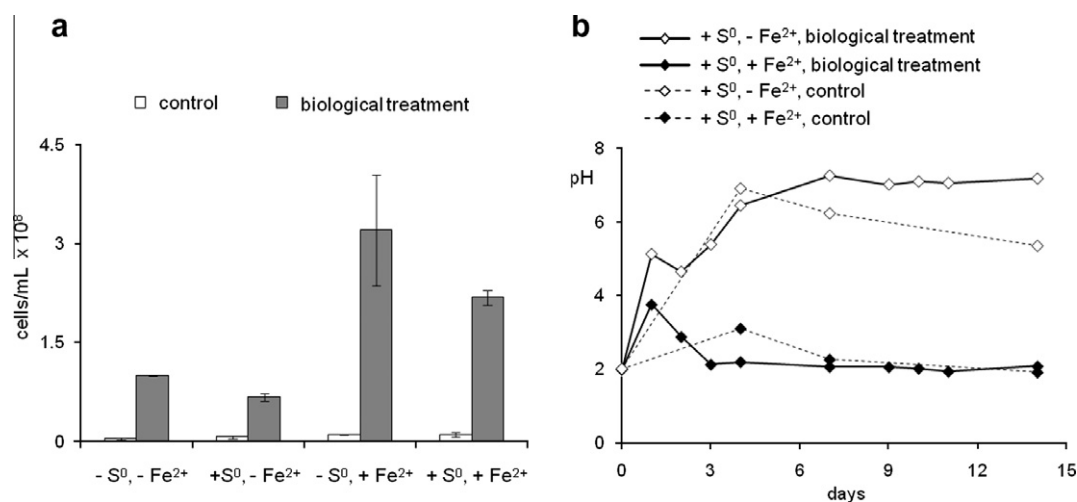


Fig. 2. Temporal profiles of pH (a) and microbial abundances (b) after one week of incubation during bioleaching of marine sediments (Treatments 1–4 in Table 2).

absence of such substrate. Elemental sulfur seems not to have a significant effect on cell growth. As expected, no cellular growth was recorded in abiotic controls (i.e., no inoculated flasks). In conclusion, no biological effect on metal mobilization was recorded and consequently Zn, As, Ni and Cr solubilization was probably due to the acid conditions. Metal partitioning in the geochemical fraction was also determined, aiming at a better understanding of their mobilization during bioleaching. Fig. 3a illustrates Zn, Cr, Ni and As partitioning in the Ancona harbor sediment before the treatment. As an example, zinc partitioning after bioleaching is also reported (Fig. 3b). It is confirmed that in the absence of Fe²⁺ no significant mobilization of zinc took place, while in the presence of iron, an overall solubilization of about 40% was achieved, as already shown in Fig. 1b. Fig. 3 suggests that this mobilization interests mainly the three mobile fractions of zinc (exchangeable/carbonatic, reducible, oxidizable), while a light decrement of metal associated with the residual phase was also observed. Also

for the other investigated metals, the geochemical partitioning (not reported here) showed that the residual fractions were nearly stable.

Results obtained in this first set of experiments were considered for planning new experiments, finalized to the individuation of the best operating conditions for metal extraction by bioleaching from spent refinery catalysts and from fluorescent powders, residue of cathode ray tubes treatment (CRT powders). Sediments were no more considered in the present optimization step, considering that bioleaching resulted not to be effective with the tested samples.

Investigated factors were the solid content and the presence of ferric iron. Experiments were set up with 2% and 10% solid content (w/v), in order to investigate solid concentration more similar to full scale applications. The presence of ferric iron was chosen as factor in order to assess the effect of redox potential on bioleaching performances. Ferric ions can keep sufficiently high the redox potential during leaching and it was demonstrated, for example

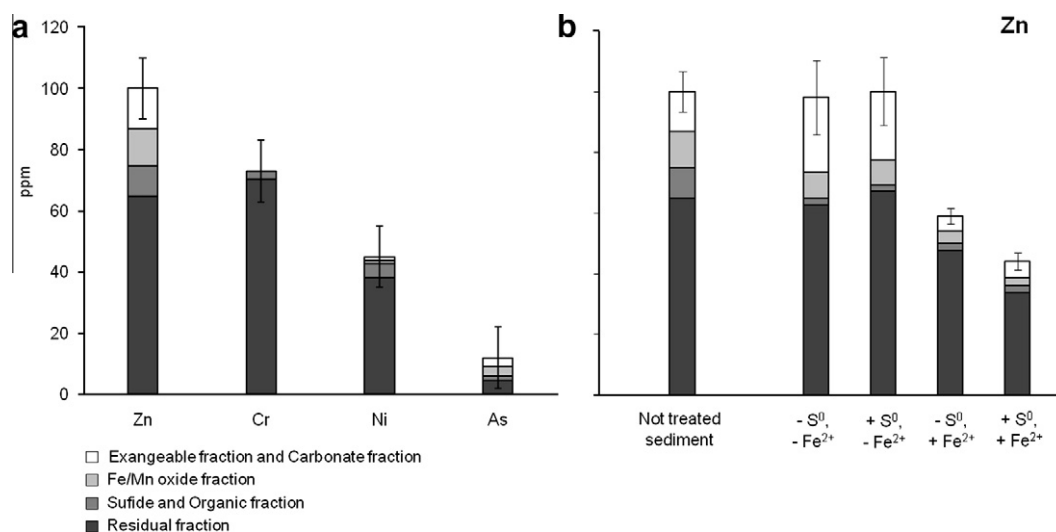


Fig. 3. Metal partitioning before (a) and after (b) 16 days bioleaching on marine harbor sediment.

for chalcopyrite, that metal dissolution rate is strictly dependent on the redox potential, controlled by the Fe^{3+}/Fe^{2+} ratio (Córdoba et al., 2008).

Fig. 4a shows Ni, V and Mo extraction yield profiles during time. Results are comparable to those observed in the presence of spent catalyst at 1% solid content (Fig. 1a): at the end of 16 days experiment, Ni and V reached the highest solubilization and Mo never exceeded 32% yield. It can also be observed in Fig. 4a that nickel and vanadium dissolution kinetics were significantly higher than molybdenum ones. In fact, high extraction yields were already obtained for Ni and V after 7 days of treatment. Molybdenum dissolution showed a different dissolution kinetic with a progressive increase during time and reaching only one fifth of nickel and vanadium extraction values. The analysis of variance (two ways ANOVA; data not shown) revealed that only Ni and Mo extraction were negatively influenced by high solid content, while the presence of Fe^{3+} seemed to play a positive role on V extraction, both

as concerns process kinetics and final extraction yields. Fig. 5a shows temporal profiles of bacterial abundances. Fe/S oxidizing bacteria showed a good adaptation to the catalyst only with the lowest solid content (2% w/v) and without ferric iron. In fact, only the treatment 9 (2% spent catalyst and no ferric iron adding, Table 2) presented significant cell growth. This was also confirmed by temporal profiles of pH and redox potential (data not shown). During such treatment, bacterial number rose and Eh increased significantly, as a consequence of active biooxidation of ferrous iron: the maximum cell number (4.5×10^8 cells/mL) was recorded after 7 days of incubation, with the highest observed redox potential (540–550 mV). A little cell growth (7.6×10^7 cells/mL) was also recorded at the beginning of the experiment in treatment 11 (10% spent catalyst and no ferric iron adding, Table 2), but the initial biological growth was not sufficient to maintain the microcosm oxidative conditions (Fig. 5a). In fact, redox potential was lower than 350 mV during the whole experiment. Profiles in

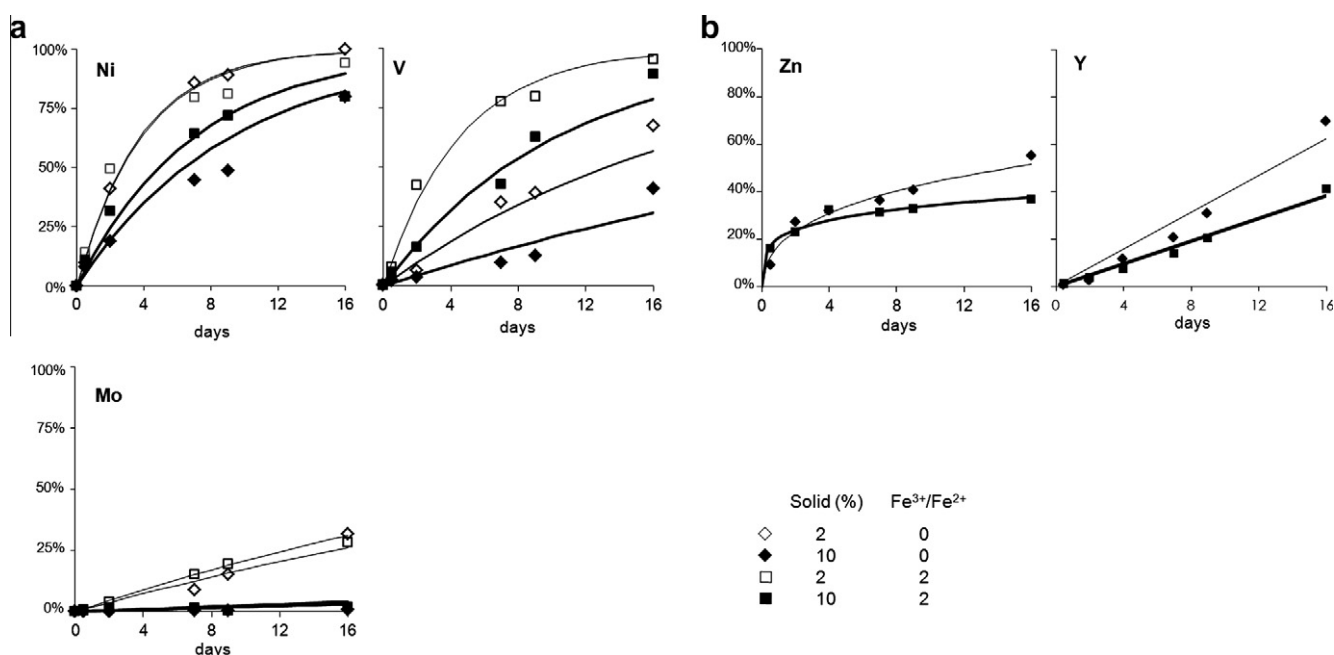


Fig. 4. Temporal profiles of metal extraction yield during bioleaching: effect of solid concentration and of redox potential for catalysts (a) and cathode ray tube powder (b).

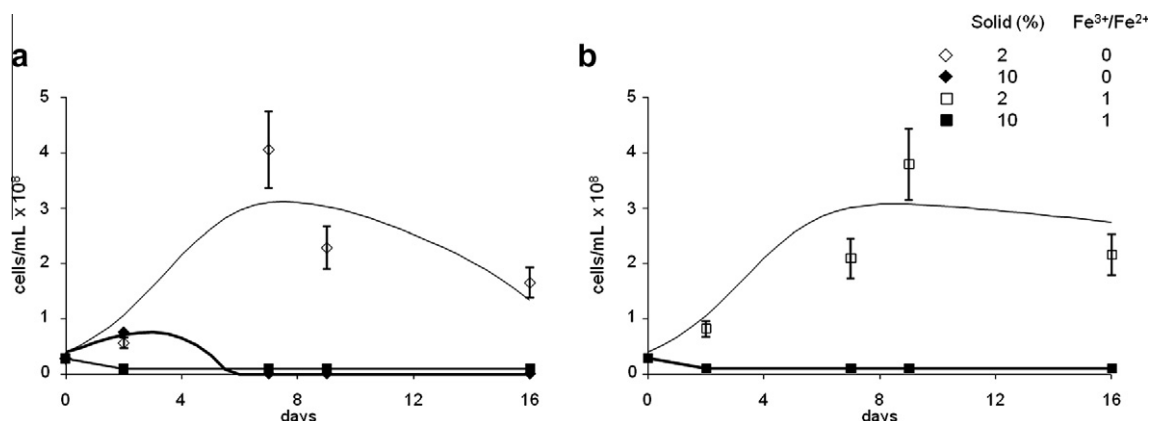


Fig. 5. Temporal profiles of bacterial abundances during bioleaching of spent catalysts (a) and CRT powders (b).

Fig. 5a also show that treatments carried out in the presence of Fe³⁺ (treatments 10 and 12 in Table 2) never showed a significant bacterial growth.

Fig. 4b shows temporal profiles of zinc and yttrium extraction yields from CRT fluorescent powders. It can be observed that zinc solubilization reached a stationary value of 30–35% after three days of incubation in the presence of ferric iron, while it progressively increased to 55% in the absence of the latter during the whole treatment. As concerns yttrium, its extraction yield linearly increased with time and reached the highest values at the end of the experiment (16 days): 70% and 40% in the absence and in the presence of ferric iron, respectively. An analysis of such data together with profiles of microbial abundances (Fig. 5b), pH and Eh (not shown here) suggests that the improvement in metal extraction yields may be associated to bacterial activity. In fact, a good bacterial adaptation was also observed in the same treatment with the highest metal extraction: microbial abundances reached about 3.0 × 10⁸ cells/mL after 1 week of incubation in the experiment with no ferric iron, while no significant growth was observed in the presence of such element (treatment 13 in Table 2). This was also confirmed by Eh and pH profiles (not shown here).

A first order kinetic model (Beolchini et al., 2010b) was fitted to the experimental data displayed in Fig. 4a:

$$\frac{dY}{dt} = k(100 - Y) \quad (1)$$

where Y is the metal extraction yields (%), t is time (days) and k is the first order rate constant (day⁻¹). Integration of Eq. (1), with the initial condition $t = 0$ and $Y = 0$, yields:

$$Y = 100(1 - e^{-kt}) \quad (2)$$

As concerns CRT fluorescent powders (Fig. 4b), yttrium extraction yield was very well fitted by linear regression:

$$Y = mt \quad (3)$$

with m (d⁻¹) as adjustable parameter, while for zinc extraction yields the simple Eq. (2) was modified introducing a further adjustable parameter, n (dimensionless), in order to ameliorate data fitting:

$$Y = 100(1 - e^{-k_1 t^n}) \quad (4)$$

Parameters k , k_1 , m and n have been estimated through linear/nonlinear regression technique [30], minimizing the following objective function:

$$\varphi = \sum_i (Y_{i,calc} - Y_{i,exp})^2 \quad (5)$$

where index i refers to the experimental observations. The estimated values for the different parameters and regression coefficients are reported in Table 3.

The following semi-empirical equation was fitted to experimental data describing temporal changes of prokaryotic abundance during bioleaching of both catalysts and CRT powders (Beolchini et al., 2010a):

$$\frac{dX}{dt} = k_2 X(1 - \beta X) + K_0 \left| \int_0^t X(t) dt \right| \quad (6)$$

$X(0) = X_0$

Table 3
Estimated values for parameters in kinetic models.

Spent catalyst						CRT powders						
Solid (%)	Fe ²⁺ (g/L)	Fe ³⁺ (g/L)	Eq. (2), k			Eq. (6)	Solid (%)	Fe ²⁺ (g/L)	Fe ³⁺ (g/L)	Eq. (3)	Eq. (4)	Eq. (6)
			Ni	V	Mo							
2	4.44	0	0.26	0.05	0.019	k_2 0.69 β 0.26 K_0 -0.03 R^2 0.93	10	4.44	0	m 0.04	k_1 0.19 n 0.48 R^2 0.95	k_2 0.68 β 0.30 K_0 -0.01 R^2 0.83
	4.44	8.88	0.11	0.02	0.002	k_2 0.45 β 0.02 K_0 -0.21 R^2 0.82						
10	4.44	0	0.26	0.22	0.023	-	10	4.44	0	m 0.04	k_1 0.19 n 0.48 R^2 0.95	k_2 0.68 β 0.30 K_0 -0.01 R^2 0.83
	4.44	8.88	0.14	0.10	0.002	-						

where X is the prokaryotic abundance (cell/mL), t is time (days) and k_2 is the rate constant (day^{-1}). Eq. (4) is a modified version of the empirical logistic equation (Simkins and Alexander, 1984) which takes into account also the potential decrease of microbial abundance after the stationary phase. Eq. (4) has been fitted to experimental data (Fig. 5a) and the three adjustable parameters k_2 , K_0 , and β have been estimated through a nonlinear regression technique (Himmelblau, 1970), as previously reported, during resolution of Eq. (4) by Runge–Kutta algorithm. The estimated values for the different parameters and the regression coefficients are reported in Table 3.

The relatively high values of the regression coefficients in Table 3 confirm the suitability of Eqs. (2), (3), (4), and (6) to mathematically describe the temporal changes of metal extraction and microbial abundance in the different experimental conditions investigated in the present study. Such equations are relatively simple, despite the system complexity due to physical, chemical and biological processes taking place simultaneously, and represent a useful tool for the prediction of process performances during bioleaching.

4. Discussion

The achieved results in the first set of experiments (Table 2, Figs. 1–3) have evidenced a key role of ferrous iron and a poor role of elemental sulfur for Fe/S oxidizing bacteria growth in the presence of the different solid wastes. This is probably due to the physiology of such strains: several studies have confirmed that Fe^{2+} enhances *At. ferrooxidans* resistance to some metals (Sugio et al., 1984; Tuovinen et al., 1971) and metal concentration in the spent catalyst and in the CRT powders were very high, even for highly resistant bacteria such as Fe/S oxidizing strains. Ferrous iron resulted to be determinant also for the strain adaptation to a complex, strongly buffered, environmental matrix such as marine harbor sediment (Fig. 2). The good strain adaptation to the solid was not always associated to high metal solubilization and the considered metals showed different patterns. Although in the presence of ferrous iron Fe/S oxidizing bacteria could grow and adapt to sediment samples, no biological effect was observed in metal solubilization from dredged marine sediments (Fig. 1b). The obtained low extraction yields for marine harbor sediments need a more detailed discussion. Marine harbor sediment is probably not suitable as substrate for the physiology of the used strains: sediment from Ancona harbor is a strongly buffered matrix (as a consequence of the high carbonate content) and in general harbor sediments are rich in organic matter, which may inhibit autotrophic bacteria activity. Furthermore the metal partitioning is also important in the assessment of bioleaching potential. In fact this is a site specific characteristic (Hlavay et al., 2004) and in the tested samples, metals are mostly associated with the residual fraction (Fig. 3), which is supposed to be quite immobilized (Tessier et al., 1979). The poor performance of bioleaching can furthermore be attributed to a very low metal association with the sulfide fraction, which is the one interested within Fe/S oxidizing bacteria metabolism.

The second set of experiments (Figs. 4 and 5) has allowed to assess, for two different metal bearing wastes, the effect of solid concentration and the role of ferric iron on the biologically mediated dissolution of metals.

As regards spent refinery catalysts, the role of Fe/S oxidizing bacteria was evident and determinant for Ni, V and Mo dissolution, when Fe^{2+} was added and the catalyst content in the flasks was relatively low (i.e., 1% and 2%, w/v). Ni and V extraction yields were high also with a 10% solid concentration in the presence of Fe^{3+} and with no observed bacterial growth, while molybdenum extraction was not significant at such concentration. These aspects

suggest that different mechanisms are behind nickel (and vanadium) dissolution and molybdenum one. Ni and V were mainly present in the catalyst as sulfides while Mo was in oxide form, as confirmed by XRD analyses. At the same way, nickel and vanadium dissolution kinetics were significantly faster than molybdenum ones (Fig. 4a). This difference might be another aspect that suggests that molybdenum has different solubilization mechanisms. Nickel and vanadium solubilization is probably favored by bio-oxidation of iron to ferric ion, a strong oxidative agent: the presence of Fe^{2+} can be responsible of a cycle triggered by Fe/S oxidizing bacteria metabolism, which can grow in the presence of the waste and oxidize Fe^{2+} to Fe^{3+} . On the contrary, molybdenum dissolution might request the presence of reducing agents (e.g. thiosulfate), which are not added to microcosms at the beginning of the process and are produced during Fe/S oxidizing bacteria growth.

Bioleaching experiments with CRT powders showed an improvement of metal extraction associated with a good bacterial adaptation, in the absence of ferric iron (treatment 13 in Table 2). Zinc and yttrium extraction yields around 30–40% were observed with no bacterial activity, and this may be due to the oxidizing action of ferric iron. The improvement in metal extraction observed in the presence of a microbial activity confirms the key role of Fe/S oxidizing bacteria and ferrous iron as promoters of zinc and yttrium mobilization. This aspect needs a further investigation, considering the poor information in the literature, in order to have a better understanding of mechanisms behind this process, taking into account also metal speciation in the solid matrix.

The solid concentration showed a negative effect on bacterial growth in the presence of spent catalysts (Fig. 5) and in some cases on metal solubilization. Similarly, the comparison with data coming from other works (Beolchini et al., 2009) suggests a negative effect of solid concentration also on bioleaching performances in the presence of marine sediments. This aspect, due to the toxicity of dissolved metals and/or to the solid organic component, represents a limit of the technological application of bioleaching at full scale.

As concerns the role of ferric iron on the biologically mediated dissolution of metals, it is known that bacteria are able to maintain a high $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio, and consequently high redox potential, by cyclic regeneration, with better rates of leaching (Kodali et al., 2004). Nevertheless Fe^{3+} is also toxic for Fe/S oxidizing bacteria if present at high concentration (Das et al., 1997; Dopson et al., 2003). Furthermore, ferric iron is a very strong oxidative reagent and a number of recent studies have been focused on its role and application in the dissolution of metals from solid matrices. Fe^{3+} has been demonstrated to enhance Cu leaching from chalcopyrite (Córdoba et al., 2008), while in another study (Li et al., 2009) it was demonstrated that Fe^{3+} offers a valid alternative for the pre-treatment of High sulfur and high arsenic refractory Gold Concentration (HGC) ores. Considering the results obtained in the present work, it is evident that the dual action of ferric iron (i.e., oxidative for metals but toxic for Fe/S bacteria) may influence solubilization with a metal specific pattern. On the one hand, experiments with CRT powders and spent catalysts demonstrated that ferric iron (i.e., $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio of 2) inhibited the microbial growth but, on the other hand, V mobilization from spent catalysts was positively influenced by the presence of Fe^{3+} . For such metal, microbial consortia and Fe^{3+} seemed to act both as oxidative agents at the same way. Zn and Y dissolution from CRT powders and Ni and Mo from spent refinery catalysts seemed to be mostly associated to the bacterial metabolism.

Summarizing, metal solubilization from wastes results to be strongly influenced by the metal speciation and partitioning in the solid matrix. While no biological effect was observed for Ni, Zn, As, Cr mobilization from marine sediments, an improvement in metal extraction was observed for spent refinery catalysts and fluorescent powders in the presence of a microbial activity. On

the other hand, a negative effect of solid concentration was in general observed on bioleaching performances, probably associated to the toxicity of dissolved metals and/or to the solid organic component.

5. Conclusion

The present work assessed the performance of bioleaching for metal extraction from different typologies of wastes: spent catalysts, contaminated sediments, fluorescent powders residual of the cathode ray tube (CRT) recycling process. The management of such wastes is of extreme importance for environmental protection and a biotechnological approach may represent an environmentally friendly strategy for both solving the disposal problem and also recovering secondary raw materials. The studied approach results to be promising for yttrium and zinc recovery from CRT powders and from nickel, vanadium and molybdenum extraction from exhaust catalysts, also at relatively high concentration of waste, suitable for scale up purposes. On the other hand, no biological effect was observed for nickel, zinc, arsenic and chromium mobilization from marine sediments sampled in the Central Adriatic coast at a solid concentration compatible with process full scale application. Nevertheless, before affirming that bioleaching is not applicable for marine sediments, further work will be addressed at sediments with different mineralogical characteristics, where metals have a different partitioning. Sustainability studies will also be performed aimed at quantifying the environmental impact of the biotechnological approach proposed here and comparing it to its chemical counterpart.

Acknowledgements

Authors are grateful to companies Orim (Macerata, Italy) and Relight (Milano, Italy) for providing catalysts and cathode tube powders, respectively, and to Ms. Alisia Giuliani, Mr. Selcuk Ciftlik and Mr. Ismail Handiri for their helpful collaboration in the experimental work. Research on CRT powders was performed under FP7 program grant agreement 231962 (Hydro WEEE). Research on sediment was performed within a scientific collaboration with Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA).

References

- Beolchini, F., Dell'Anno, A., De Propris, L., Ubaldini, S., Cerrone, F., Danovaro, R., 2009. Auto- and heterotrophic acidophilic bacteria enhance the bioremediation efficiency of sediments contaminated by heavy metals. *Chemosphere* 74, 1321–1326.
- Beolchini, F., Rocchetti, L., Regoli, F., Dell'Anno, A., 2010a. Bioremediation of marine sediments contaminated by hydrocarbons: experimental analysis and kinetic modeling. *J. Hazard. Mater.* 182, 403–407.
- Beolchini, F., Fonti, V., Ferella, F., Vegliò, F., 2010b. Metal recovery from spent refinery catalysts by means of biotechnological strategies. *J. Hazard. Mater.* 178, 529–534.
- Brierley, J.A., Brierley, C.L., 2001. Present and future commercial applications of biohydrometallurgy. *Hydrometallurgy* 59, 233–239.
- Chen, S.Y., Lin, P.L., 2004. Bioleaching of heavy metals from contaminated sediment by indigenous sulfur-oxidizing bacteria in an air-lift bioreactor: effects of sulfur concentration. *Water Res.* 38, 3205–3214.
- Chen, S.Y., Lin, P.L., 2010. Optimization of operating parameters for the metal bioleaching process of contaminated soil. *Sep. Purif. Technol.* 71, 178–185.
- Córdoba, E.M., Muñoz, J.A., Blázquez, M.L., González, F., Ballester, A., 2008. Leaching of chalcopyrite with ferric ion. Part II: Effect of redox potential. *Hydrometallurgy* 93, 88–96.
- Das, A., Modak, J.M., Natarajan, K.A., 1997. Technical note studies on multi-metal ion tolerance of *Thiobacillus ferrooxidans*. *Miner. Eng.* 10, 743–749.
- Dell'Anno, A., Beolchini, F., Gabellini, M., Rocchetti, L., Pusceddu, A., Danovaro, R., 2009. Bioremediation of petroleum hydrocarbons in anoxic marine sediments: consequences on the speciation of heavy metals. *Mar. Pollut. Bull.* 58, 1808–1814.
- Dopson, M., Baker-Austin, C., Koppineedi, P.R., Bond, P.L., 2003. Growth in sulfidic mineral environments: metal resistance mechanisms in acidophilic microorganisms. *Microbiology* 149, 1959–1970.
- Dufresne, P., 2007. Hydroprocessing catalysts regeneration and recycling. *Appl. Catal. A* 322, 67–75.
- Furimsky, E., 1996. Spent refinery catalysts: environment, safety and utilization. *Catal. Today* 30, 223–286.
- Gadd, G.M., 2004. Microbial influence on metal mobility and application for bioremediation. *Geoderma* 122, 109–119.
- Himmelblau, D.M., 1970. *Process Analysis by Statistical Methods*. John Wiley and Sons, New York.
- Hlavay, J., Prohaska, T., Weiz, M., Wenzel, W.W., Stinger, G., 2004. Determination of trace elements bound to soils and sediment fractions, IUPAC Technical Report. *Pure Appl. Chem.* 76, 415–442.
- Hobbie, J.E., Daley, R.J., Jasper, S., 1977. Use of Nucleopore Filters for Counting Bacteria by Fluorescence Microscopy. *Appl. Environ. Microbiol.* 33, 1225–1228.
- Juwarkar, A.A., Singh, S.K., Mudhoo, A., 2010. A comprehensive overview of elements in bioremediation. *Rev. Environ. Sci. Biotechnol.* 9, 215–288.
- Kelly, B.C., Ikononou, M.G., Blair, J.D., Morin, A.E., Gobas, F.A.P.C., 2007. Food web specific biomagnification of persistent organic pollutants. *Science* 317, 236–239.
- Kodali, B., Rao, B.M., Narasu, L.M., Pogaku, R., 2004. Effect of biochemical reactions in enhancement of rate of leaching. *Chem. Eng. Sci.* 59, 5069–5073.
- Li, Q., Li, D., Qian, F., 2009. Pre-oxidation of high-sulfur and high-arsenic refractory gold concentrate by ozone and ferric ions in acidic media. *Hydrometallurgy* 97, 61–66.
- Löser, C., Seidel, H., Hoffmann, P., Zehndorf, A., 2001. Remediation of heavy metal-contaminated sediments by solid-bed bioleaching. *Environ. Geol.* 40, 643–650.
- Marafi, M., Stanislaus, A., 2008. Spent hydroprocessing catalyst management: a review. Part II. Advances in metal recovery and safe disposal methods. *Resour. Conserv. Recycl.* 53, 1–26.
- Méar, F., Yot, P., Cambon, M., Ribes, M., 2006. The characterization of waste cathode ray tube glass. *Waste Manage.* 26, 1468–1476.
- Quevauviller, Ph., 1998. Operationally defined extraction procedures for soil and sediment analysis I. Standardization. *Trends Anal. Chem.* 17, 289–298.
- Ravishankar, B.R., Blais, J.F., Benmoussa, H., Tyagi, R.D., 1994. Bioleaching of metals from sewage sludge: elemental sulfur recovery. *J. Environ. Eng.* 120, 462–470.
- Rulkens, W.H., Grotenhuis, J.T.C., Tichy, R., 1995. Methods of cleaning contaminated soils and sediments. In: Salomons, W., Förstner, U., Mader, P. (Eds.), *Heavy Metals*. Springer, Berlin, pp. 151–191.
- Sand, W., Gehrke, T., Jozsa, P.G., Schippers, A., 2001. (Bio)chemistry of bacterial leaching – Direct vs. indirect bioleaching. *Hydrometallurgy* 59, 159–175.
- Santhiya, D., Ting, Y.P., 2005. Bioleaching of spent refinery processing catalyst using *Aspergillus niger* with high yield oxalic acid. *J. Biotechnol.* 116, 171–184.
- Silverman, M.P., Ehrlich, H.L., 1964. Microbial formation and degradation of minerals. *Adv. Appl. Microbiol.* 6, 181–183.
- Silverman, M.P., Lundgren, D.G., 1959. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields. *J. Bacteriol.* 77, 642–647.
- Simkins, S., Alexander, M., 1984. Models for mineralization kinetics with the variables of substrate concentration and population density. *Appl. Environ. Microbiol.* 47, 1299–1306.
- Sugio, T., Domatsu, C., Tano, T., Imai, K., 1984. Role of ferrous ions in synthetic cobaltous sulfide leaching of *Thiobacillus ferrooxidans*. *Appl. Environ. Microbiol.* 48, 461–467.
- Tessier, A., Campbell, P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.* 51, 844–851.
- Tuovinen, O.H., Niemelä, S.I., Gyllenberg, H.G., 1971. Tolerance of *Thiobacillus ferrooxidans* to some metals. *Antonie Leeuwenhoek* 37, 484–496.
- Tyagi, R.D., Blais, J.F., Deschenes, L., Lafrance, P., Villeneuve, J.P., 1994. Comparison of microbial sulfuric acid production in sewage sludge from added sulfur and thiosulfate. *J. Environ. Qual.* 23, 1065–1070.
- US EPA (United States Environmental Protection Agency), 1997. *Microwave Assisted Acid Dissolution of Sediments, Sludges, Soils, and Oils*. Method 3051 A, Washington, DC 20460. U.S. Gov. Print. Office.
- US EPA (United States Environmental Protection Agency), 2001. *Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry*. Environmental monitoring systems laboratory. Method 200.7, Office of research and development. Cincinnati, Ohio, 45268.
- US EPA (United States Environmental Protection Agency), 2005. *Contaminated Sediment Remediation Guidance for Hazardous Waste Sites*. EPA 540 R 05 012, Office of Solid Waste and Emergency Response OSWER 9355 0-85.
- Yang, Q.Z., Qi, G.J., Low, H.C., Song, B., 2011. Sustainable recovery of nickel from spent hydrogenation catalyst: economics, emissions and wastes assessment. *J. Cleaner Prod.* 19 (4), 365–375.
- Zhao, L., Zhu, N.W., Wang, X.H., 1981. Comparison of bio-dissolution of spent Ni–Cd batteries by sewage sludge using ferrous ions and elemental sulfur as substrate. *Chemosphere* 70, 974–981.