Journal of Hazardous Materials 178 (2010) 529–534

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Metal recovery from spent refinery catalysts by means of biotechnological strategies

F. Beolchini[a,*], V. Fonti[a], F. Ferella[b], F. Vegliò[b]

[a] Department of Marine Sciences, Polytechnic University of Marche, Via Brecce Bianche, 60131 Ancona, Italy
[b] Department of Chemistry, Chemical Engineering and Materials, University of L'Aquila, Monteluco di Roio, 67040 L'Aquila, Italy

ARTICLE INFO

Article history:
Received 1 July 2009
Received in revised form 25 January 2010
Accepted 25 January 2010
Available online 1 February 2010

Keywords:
Metal
Spent catalysts
Bioleaching
Iron/sulphur oxidizing bacteria
Indirect mechanism

ABSTRACT

A bioleaching study aimed at recovering metals from hazardous spent hydroprocessing catalysts was carried out. The exhaust catalyst was rich in nickel (4.5 mg/g), vanadium (9.4 mg/g) and molybdenum (4.4 mg/g). Involved microorganisms were iron/sulphur oxidizing bacteria. Investigated factors were elemental sulphur addition, ferrous iron addition and actions contrasting a possible metal toxicity (either adding powdered activated charcoal or simulating a cross current process by means of periodical filtration). Ferrous iron resulted to be essential for metal extraction: nickel and vanadium extraction yields were 83% and 90%, respectively, while about 50% with no iron. The observed values for molybdenum extraction yields were not as high as Ni and V ones (the highest values were around 30–40%). The investigated actions aimed at contrasting a possible metal toxicity resulted not to be effective; in contrast, sequential filtration of the liquor leach had a significant negative effect on metals extraction. Nickel and vanadium dissolution kinetics resulted to be significantly faster than molybdenum dissolution ones. Furthermore, a simple first order kinetic model was successfully fitted to experimental data. All the observed results supported the important role of the indirect mechanism in bioleaching of LC-Finer catalysts.

1. Introduction

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 [1–3]. During their use, metal sulphides and oxides and metalorganic compounds are deposited on the catalyst surface, causing a loss of the catalytic activity and specificity [1,2]. Spent catalysts can be regenerated and returned to the operation but the number of regeneration/utilization cycles is limited and, at a certain point, catalyst replacement is inevitable [2]. Since their toxic components and their base valuable metal content, replace in discharge this kind of waste represents not only an environmental threat but an important economical loss, too. To avoid pollution in land disposal as well as minimise landfill space, spent catalysts are subjected to metal extraction by various solubilization process. Worldwide, several companies are involved in metal reclamation from spent hydroprocessing catalysts; their technologies are based on two main approaches: either hydrometallurgy or pyrometallurgy. With the hydrometallurgical approach metals are leached by means of catalysis with acids or bases, while pyrometallurgy uses a heat treatment, such as roasting and smelting [3,4].

In this paper, a biotechnological environmentally friendly strategy, involving bioleaching abilities of Fe/S oxidizing bacteria (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*), has been applied on Italian refineries spent hydroprocessing catalysts.

In comparison with conventional technologies, biotechnological leaching processes may not seem competitive: a significantly longer leaching time are required to achieve extraction efficiencies which are comparable to those observed using other leaching methods. Nevertheless, bioleaching can offer attractive features, especially considering environmental issues: processes are more cost efficient, simpler and more environmentally friendly than their chemical counterparts [5,6].

Chemical reactions involved during metal bioleaching from exhaust catalysts can be simplified as follows for a bivalent metal, Me^{2+}, present as a metal sulphide, MeS, in the solid matrix [7]:

$$MeS + (1/2)O_2 + H^{+bacteria} \rightarrow Me^{2+} + S^0 + H_2O \quad (1)$$

$$MeS + Fe^{3+} + H_2O \rightarrow Me^{2+} + Fe^{2+} + S_2O_3{}^{2-} + H^+ \quad (2)$$

$$4Fe^{2+} + O_2 + 4H^{+bacteria} \rightarrow 4Fe^{3+} + 2H_2O \quad (3)$$

$$S^0 + (3/2)O_2 + H_2O^{bacteria} \rightarrow 2H^+ + SO_4{}^{2-} \quad (4)$$

* Corresponding author. Tel.: +39 071 2204225; fax: +39 071 2204650.
E-mail address: f.beolchini@univpm.it (F. Beolchini).

0304-3894/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2010.01.114

Table 1
Factors and levels investigated for exhaust catalysts bioleaching.

Factors	Levels	
S^0	No	Yes
Fe^{2+}	No	Yes
Inhibiting toxicity action	No	PAC[a]/filtration

[a] PAC: powder activated carbon.

$$S_2O_3{}^{2-} + H_2O + 2O_2{}^{\text{bacteria}} \rightarrow 2SO_4{}^{2-} + 2H^+ \quad (5)$$

where Eq. (1) is representative of the "direct mechanism" of bioleaching, namely a direct bio-oxidation of the metal sulphide onto the solid matrix, and Eq. (2) represents the "indirect mechanism" of bioleaching, a chemical oxidation of the metal sulphide by the ferric iron generated by bio-oxidation in solution (Eq. (3)). Eqs. (4) and (5) represent, respectively, the elemental sulphur and thiosulphate bio-oxidation which might lead to sulphate ion in the case of complete reaction [8].

A factorial experiment was implemented where the three following experimental factors were investigated: the presence of elemental sulphur (S^0), the presence of ferrous iron (Fe^{2+}) and the application of strategies inhibiting an eventual toxic effect of the high concentration of metals. The performed experiments were aimed at finding the best operating conditions for nickel, vanadium and molybdenum extraction from exhaust catalyst by bioleaching.

2. Experimental

2.1. The exhaust catalyst

Spent catalysts come from Italian refinery LC-Fining units. Their chemical composition was evaluated by means of inductively coupled plasma optical emission spectrometer (ICP-OES), after acid digestion of spent catalyst. In particular, 1 g of the solid was treated for 2 h using concentrated hydrochloric acid and nitric acid, in a ratio of 3:1. After cooling, the digestate was filtered through Whatman 41 filter paper and made up to 100 mL using 50% HCl to perform the metal analysis: Ni, V and Mo content was $4.5 \pm 0.6\%$, $9.4 \pm 0.6\%$ and $4.4 \pm 0.2\%$ (w/w), respectively.

X-ray diffraction analysis (Siemens D-500 diffractometer) revealed the presence of the following mineralogical forms, in order of abundance: Al_2O_3 (aluminium oxide), NiV_2S_4 (nickel vanadium sulphide), Mo_4O_{11} (molybdenum oxide), Ni_3S_4 (polydymite). Aliphatic hydrocarbon content in the spent catalyst was about 5% [9,10].

In order to remove hydrocarbons, exhaust catalysts were pretreated by washing with a mixture of aqueous solutions of ethyl alcohol 1% (v/v) and Tween 80 0.1% (v/v): 50 g/L of catalysts were suspended in this solution under magnetic agitation, under room temperature for 24 h. The hydrocarbon washing yield was estimated at about 75%. At the end of washing, catalysts were filtered and stored in a water suspension (catalyst: water 1:1) in order to avoid ignition.

2.2. Microorganisms

A mixed culture of three strains of Fe/S oxidizing bacteria (*A. ferrooxidans*, *A. thiooxidans* and *L. ferrooxidans*) isolated from an environmental sample was kindly provided by Prof. Stoyan Groudev (Department of Engineering and Geoecology, University of Mining and Geology "Saint Ivan Rilski", Sofia, Bulgaria). This culture was cultivated under acidic condition (pH 2), in the ideal liquid growth medium 9K [11]: two solutions were prepared separately and blended only after their sterilization by autoclave. The first solution was obtained dissolving the following reagents in 700 mL of distilled water: $(NH_4)_2SO_4$ 3.0 g, KCl 0.1 g/L, H_2HPO_4 0.5 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L and $Ca(NO_3)_2$ 0.01 g/L. The second one was an energetic solution of 49.1 g of $FeSO_4$, dissolved in 300 mL of distilled water. Sulphuric acid 4 M was used to adjust pH to 2, before sterilization of the two solutions.

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 in an orbital thermostated incubator (S150, Stuart), at 30 °C and 175 rpm shaking. Catalyst concentration was 10 g/L and the medium composition was as a 9K [11] modified according to experimental conditions. Periodically pH was monitored and aliquot amounts (1.5 mL) were sampled for metals determination in solution after centrifugation at $8000 \times g \times 5$ min. Table 1 shows factors and levels investigated, while Table 2 shows all treatments in details, according to a factorial experiment. Two 2^3 full factorial plan (3 factors, 2 levels each; [12]) have been implemented rather than a full one with three levels for the "strategy inhibiting metal toxicity" factor, since it was not considered useful to combine filtration and activated carbons. Consequently the number of treatments was 12 rather than 16, and data analysis was performed by means of Yates algorithms [12] considering two separate full factorial plans where the level for the "strategy inhibiting metal toxicity" factor were either "no and activated carbon" or "no and filtration".

The 9K medium above described (Par. 2.2) was also used as medium for bioleaching experiments conducted in the presence of

Table 2
Factorial plan (factors and levels in Table 1 with solubilized concentrations (±5% standard deviation) for nickel, molybdenum and vanadium at the end of the treatments (21 days).

Treatments	S^0	Fe^{2+}	Inhibiting toxicity action	Ni (mg/L)		V (mg/L)		Mo (mg/L)	
				Bio[a]	Ctrl[b]	Bio[a]	Ctrl[b]	Bio[a]	Ctrl[b]
1	No	No	no	22.8	25.8	44.1	46.5	10.6	11.7
2	Yes	No	no	17.9	19.5	31.4	31.3	15.9	8.5
3	No	Yes	no	35.5	17.5	84.2	48.8	17.1	0.0
4	Yes	Yes	no	36.6	18.2	82.6	51.1	13.9	0.0
5	No	No	PAC	17.5	22.5	44.0	63.7	2.6	7.0
6	Yes	No	PAC	14.1	17.4	32.3	41.8	4.0	5.0
7	No	Yes	PAC	37.3	18.5	86.8	53.2	13.9	0.6
8	Yes	Yes	PAC	33.0	17.9	56.7	49.1	14.4	0.6
9	No	No	Filtration	7.6	9.0	20.6	22.5	3.5	3.1
10	Yes	No	Filtration	8.6	8.0	22.4	19.8	2.7	2.7
11	No	Yes	Filtration	13.7	4.7	29.4	12.2	2.7	0.2
12	Yes	Yes	Filtration	16.0	3.9	35.6	9.8	4.0	0.2

[a] Biological treatment.
[b] Chemical control.

iron. For experiments in the absence of iron source, culture was carried out in a modified 9K medium, where ferrous sulphate ($FeSO_4$) was substituted by elemental sulphur (S^0) 5 g/L.

For each bioleaching treatment in Table 2, a chemical control test was also performed with no bacteria inoculum. Where specified, powdered activated carbon (PAC; Powdered Activated Charcoal Norit, 05100, Fluka) was added in a mass ratio 1:10 carbon:catalyst. Filtration was performed as a possible inhibiting toxicity action by filtering (0.22 μm; Millipore membranes) every 7 days the suspension and re-suspending the cake (bearing both catalysts and bacteria) in fresh medium.

2.4. Analytical determinations

Periodical measurement of pH was carried out by Inolab Multi 720 (WTW). Molybdenum, nickel, vanadium and aluminium were determined by atomic absorption spectrometry (Varian Spectra AA 200). Samples were conveniently diluted by deionised water at pH 2 (with HCl) before atomic absorption determinations.

3. Results

The experimental activity was aimed at finding the best operating conditions for nickel, vanadium and molybdenum extraction from exhaust LC-Finer catalysts by bioleaching.

The main factors considered were elemental sulphur, ferrous iron and the application of strategies inhibiting an eventual toxic effect of highly concentrated metals (Table 1). Many studies have been based on the effect of substrate addition, like elemental sulphur [13,14] and ferrous ion [15], on the bioleaching of several metal bearing matrices. Sulphur is extensively used in bioleaching processes: it is a cheap growth substrate for Fe/S oxidizing bacteria [16] and responsible for the production of sulphuric acid, as a consequence of microbial metabolism, favouring metal mobilisation [17]. Iron is not only a growth substrate for Fe/S oxidizing bacteria (as ferrous iron) but it is also a strong oxidizing agent when bio-oxidized in its ferric form [18]. Some action contrasting the toxic effect of heavy metal high concentration consequent to metal solubilisation during bioleaching was supposed to be necessary during the experiments; in fact, metal concentrations might be too high, even for highly resistant bacteria as Fe/S oxidizing strains [19–21].

The strategies chosen for inhibiting the metal toxicity were two: (i) either adding activated carbon as metal adsorbers (like in a carbon-in-leach operation, [22]), ii) or periodically filtering the suspension and resuspending catalysts and bacteria in fresh growth medium (simulating a cross current process, [23]). Table 2 shows in details all experiments performed according to plan in Table 1.

The experiments were carried out on washed spent LC-Finer catalyst, as described in the Material and Method section. The washing pre-treatment was necessary because of the high organic compound content, supposed to inhibit Fe/S oxidizing bacteria metabolism [24].

Fig. 1 shows temporal changes of pH during bioleaching in the presence/absence of ferrous ion and elemental sulphur (treatments from 1 to 4 in Table 2). Chemical controls are also reported. Profiles observed with actions for inhibiting metals toxicity (treatments 5–12 in Table 2) were similar and they are not shown here. Data in Fig. 1 show trends that are well known for the growth of Fe/S oxidizing strains [25]: after a first increase, pH decreases during the time as a proof of bacterial activity. This was particularly evident in those biological treatments with ferrous iron (treatments 3, 4, 7, 8, 11, 12 in Table 2): after 8 days, pH was lower than 2, as a consequence of bacterial growth and activity. These results were supposed to be associated to the bacterial ability to tolerate high metal concentrations if Fe^{2+} is present [26]. In the absence of iron

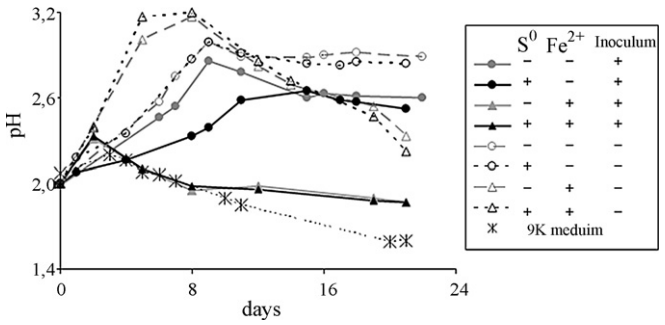

Fig. 1. pH profiles vs. time during bioleaching: effect of Fe^{2+} (160 mM) and S^0 (1 g/L). Dotted lines refer to chemical controls (in which no inoculum was added). Stars show pH profile for the adapted culture in ideal 9K medium, without catalyst.

(treatments 1, 2, 5, 6, 9, 10 in Table 2), pH decrease seemed not to be significant when compared to profiles in ideal conditions (9K medium, no LC-Finer added). As concerns the effect of elemental sulphur on pH temporal changes, this is significant only when no iron was added to bacterial cultures (see Fig. 1) and it seems that the elemental sulphur has a "buffer effect" on pH according to reaction (4), avoiding a too high rising.

Fig. 2 shows nickel, vanadium and molybdenum extraction yields observed in all treatments after 21 days bioleaching. Metal concentrations in the pregnant solution corresponding to those yields are reported in Table 2. These values are important when designing the downstream operations for metals purification and recovery [27]. It can be observed in Fig. 2 that the highest extraction yields for Ni and V ($83 \pm 4\%$ and $90 \pm 5\%$, respectively) were achieved in treatments 3 and 4, which are those in the presence of iron (Table 2) with no application of any strategy aimed at inhibiting metal toxicity. Furthermore, the effect of ferrous iron on the biological activity of the microbial consortia is demonstrated by the significant difference between inoculated flasks (*biological treatments*, Fig. 2) and not inoculated ones (*chemical controls*, Fig. 2). An analysis of the observed extraction yields in treatments 9–12 suggests that the periodical filtration of the liquor leach, aimed at inhibiting metal toxicity, had, on the other hand, a negative effect on metal mobilisation: in fact the highest values experimentally observed for Ni and V extraction yields were not higher than 40% for both metals. This aspect may suggest that filtration (and re-suspension in fresh medium) removes compounds which may have a key role in metal dissolution. Fig. 2 also shows that activated carbon did not favour metal leaching as expected: in fact, no significant differences are apparent between treatments 1–4 and 5–8. As concerns molybdenum dissolution, the observed values for

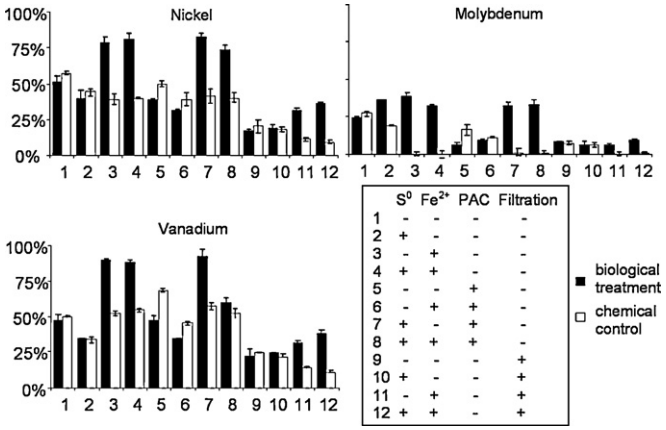

Fig. 2. Metal extraction yields at the end of LC-Finer bioleaching (operating conditions and final metal concentrations are shown in Table 2).

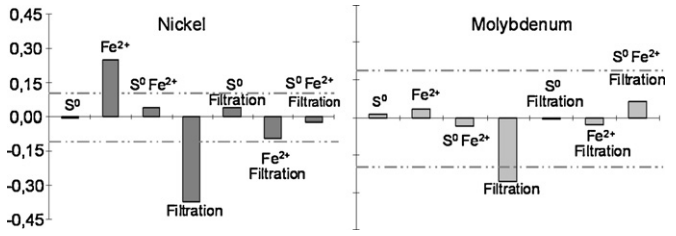

Fig. 3. ANOVA graphs for Ni and Mo extraction yields (dotted lines represent 95% confidence significant effects; MSE = 0.003%^{2} with 2 d.f., 0.007%^{2} with 2 d.f. and 0.007%^{2} with 2 d.f. for Ni, V and Mo, respectively).

Mo extraction yields were not as high as Ni and V ones. In fact, the highest values were around 30–40% for treatments where no strategy inhibiting metal toxicity was applied and around 5–10% when periodically filtering the liquor leach. The so low Mo extraction yields observed in treatments 5 and 6 were supposed to be due to activated carbon adsorption of molybdate ions [28]. In contrast with its effect on nickel and vanadium dissolution, iron did not seem to have a positive effect on molybdenum recovery: Mo extraction yields seem not to be enhanced by iron, even if a significant difference between biological treatments and chemical controls is remarkable also for molybdenum. These aspects suggest that different mechanisms are behind nickel (and vanadium) dissolution and molybdenum one. More details on this hypothesis are given in the discussion section. The statistical significance of factors was evaluated by means of an analysis of variance (ANOVA; [12]) performed considering the metal extraction yields as responses. Fig. 3 shows main effects and interactions as estimated for nickel and molybdenum. Results observed for vanadium where similar to nickel ones, and they are not reported here. It can be observed that ANOVA results confirm that iron had a positive effect on nickel (significance 98%) and vanadium (significance 98%), while it did not show a statistically significant effect on molybdenum extraction yield. Filtration, on the other hand, had a negative effect (significance >98%) on all metal extraction.

Table 3
Estimated values for equation (7) parameter k and R^2.

Metal	k (days^{-1})	R^2
Nickel	0.110	0.88
Vanadium	0.138	0.98
Molybdenum	0.017	0.99

Fig. 4 shows temporal profiles of metal extraction yields achieved in treatments 1–4 and in their respective chemical controls. It can be observed that nickel and vanadium dissolution kinetics are significantly faster than molybdenum dissolution ones. In fact, extraction yields higher than 60% (corresponding to >80% of the 12 days value) are achieved in 7 days bioleaching. This might also give opportunity for industrial application, where 7 days residence time might be reached with recirculation of flows. On the other hand molybdenum dissolution progressively increases during time reaching only one fifth of the 12 days value after 7 days bioleaching. This difference on metal dissolution kinetics might be another aspect that suggests different mechanisms behind nickel (and vanadium) dissolution and molybdenum one. A deeper investigation on the metal dissolution kinetics was performed by fitting a simple first order kinetic model to experimental data related to the highest dissolution yield (i.e., bioleaching treatments 3 and 4):

$$\frac{dY}{dt} = k(100 - Y) \quad (6)$$

where Y is the metal extraction yield (%), t is time (days) and k is the first order rate constant (days^{-1}). Integration of Eq. (6), with the initial condition $t = 0$ and $Y = 0$, yields:

$$Y = 100(1 - e^{-kt}) \quad (7)$$

Fig. 5 shows experimental and calculated profiles and Table 3 shows the estimated values for k and R^2 by non-linear regression analysis [29]. The estimated values for R^2 (0.88, 0.98 and 0.99 for Ni, V and Mo, respectively) indicate that a very simple first order kinetic model, like Eq. (6) seems to be suitable for data fitting, despite the system complexity due to physical, chemical and biological processes taking place simultaneously. The estimated values

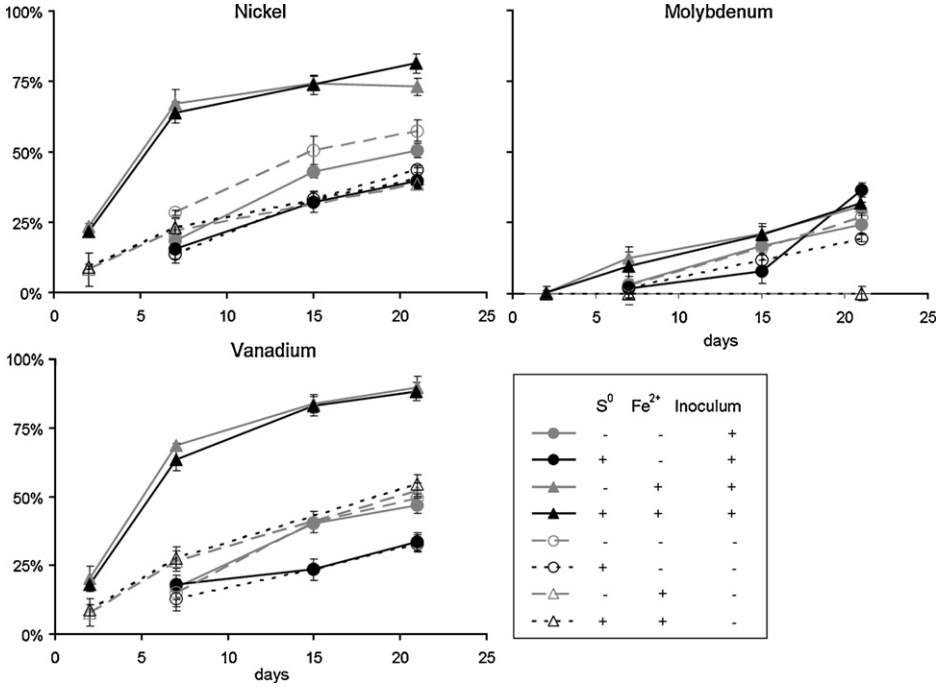

Fig. 4. Metal extraction yields vs. time profiles; microcosms in the presence/absence of Fe^{2+} (160 mM). Dotted lines refer to chemical controls.

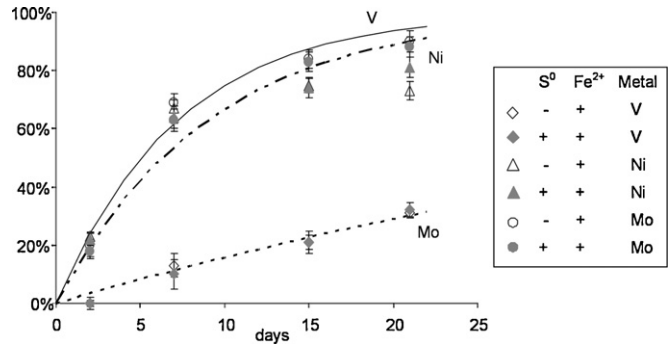

Fig. 5. Temporal change of experimental (treatments 3 and 4 in Table 2) and calculated by Eq. (7) metal extraction yields.

for k were used to calculate the time necessary for reaching 63% of the stationary value at about 9 days for nickel, 7.5 days for vanadium and 59 days for molybdenum, confirming the above reported considerations on metal dissolution kinetics.

4. Discussion

Results displayed in Fig. 2 show that Fe/S oxidizing bacteria are very effective in Ni and V extraction from LC-Finer spent refinery catalysts, especially in the presence of ferrous iron. The effect of this substrate was evident and statistically significant, in agreement with ANOVA results (Fig. 3). The important role of Fe^{2+} may be associated to its dual biological and chemical function. On the one hand, Fe^{2+} is a key substrate for Fe/S oxidizing bacteria: in fact, several studies have confirmed that Fe^{2+} enhances *A. ferrooxidans* resistance to some metals [27,30]. On the other hand, the ferric iron produced by bacteria is a strongly oxidizing agent [30], such as the highest extraction yields for Ni and V in biological treatment containing iron (treatments 3, 4, 7, 8, 11, 12 in Table 2) may be attributed to a chemical oxidation, mediated by the biologically produced ferric iron. The presence of Fe^{2+} can be responsible of a cycle triggered by Fe/S oxidizing bacteria metabolism: ferrous iron favours bacteria adaptation to the high metal content [30,31], they oxidize Fe^{2+} to Fe^{3+}, which dissolves Ni and V sulphides on the spent catalyst by means of an oxidative attack producing other ferrous iron for the bacterial metabolism. Therefore, iron is a key factor for the effectiveness of bioleaching process. These considerations support the important role of the indirect mechanism in bioleaching of heavy metal contaminated matrices, as already demonstrated by other studies [7].

As concerns molybdenum, it showed both extraction yields and process kinetics significantly lower than nickel and vanadium, even in the presence of Fe^{2+} and S^0. This may suggest that molybdenum follows a dissolution pattern not associated to sulphides oxidation and this is also confirmed by XRD spectra, which revealed that molybdenum is present as an oxide rather than molybdenum sulphide (Section 2.1). Consequently molybdenum dissolution might request the presence of reducing agents, which are not added to microcosms at the beginning of the process and which might be produced as metabolites during bacteria fermentation. The 30% extraction yield observed after 12 days bioleaching, was probably due to Mo_4O_{11} reduction by the several intermediate sulphur species with high reducing power (i.e., thiosulphate, polythionate, sulphite), known to be produced by Fe/S oxidizing bacteria [32] and used as leaching agent in gold extraction [33]. The negative effect of periodical filtrations on all metal dissolution is a further confirmation of the indirect involvement of microbial consortia on metal leaching, producing either oxidizing Fe^{3+} for Ni and V extraction or reducing sulphur compounds for Mo extraction. This aspect suggests a possible configuration for metal recovery from LC-Finer exhaust catalysts, which might be more effective with respect to the bioleaching process. A flow diagram of the proposed configuration is shown in Fig. 6. Metal leaching takes place just through the chemical action of ferric iron and intermediate compounds of sulphur bio-oxidation; ferrous iron is subsequently oxidized to ferric iron in a separate biological reactor where Fe/S oxidizing strains have previously been inoculated. Further studies will be performed both aimed at verifying the feasibility of the process showed in Figure 6 and at optimising bioleaching for solid/liquid ratio and iron concentration. In addition, the assessment of reuse options for bioleached spent catalysts would be a further important issue. Some authors have reported that leached catalysts can be used to produce commercial value bricks, cement or glass-ceramic materials [3]. Specific information are not present in the literature for catalysts treated by bioleaching and this could be and interesting aspect for an effective integrated process analysis.

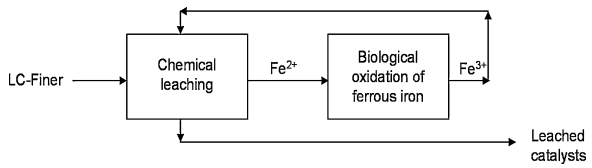

Fig. 6. Flow diagram of a metal recovery process from spent refinery catalyst.

Acknowledgements

This research has been carried out with the financial support of Italian Ministry of Research. Authors are grateful to Mr. Marcello Centofanti for his helpful collaboration in the experimental work.

References

[1] E. Furimsky, Spent refinery catalysts: environment, safety and utilization, Catal. Today 30 (1996) 223–286.
[2] P. Dufresne, Hydroprocessing catalysts regeneration and recycling, Appl. Catal. A 322 (2007) 67–75.
[3] M. Marafi, A. Stanislaus, Spent hydroprocessing catalyst management: a review. Part II. Advances in metal recovery and safe disposal methods, Resources Conserv. Recycl. 53 (2008) 1–26.
[4] M. Marafi, A. Stanislaus, Options and processes for spent catalyst handling and utilization, J. Hazard. Mater. 101 (2003) 123–132.
[5] K.M.M. Aung, Y.P. Ting, Bioleaching of spent fluid catalytic cracking catalyst using *Aspergillus niger*, J. Biotechnol. 116 (2005) 159–170.
[6] D. Santhiya, Y.P. Ting, Bioleaching of spent refinery processing catalyst using *Aspergillus niger* with high-yield oxalic acid, J. Biotechnol. 116 (2005) 171–184.
[7] F. Vegliò, F. Beolchini, A. Nardini, L. Toro, Bioleaching of a pyrrhotite ore by sulfooxidans strain: kinetic analysis, Chem. Eng. Sci. 55 (2000) 783–795.
[8] K. Tang, V. Baskaran, M. Nemati, Bacteria of the sulphur cycle: an overview of microbiology, biokinetics and their role in petroleum and mining industries, Biochem. Eng. J. 44 (2008) 73–94.
[9] EPA, Method 3550C SW-846 manual, in: Test Methods for Evaluating Solid Waste, Physical/Chemical Method, EPA, 2007.
[10] EPA, Method 8270D SW-846 manual, in: Test Methods for Evaluating Solid Waste. Physical/Chemical Method, EPA, 2007.
[11] M.P. Silverman, D.G. Lundgren, Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields, J. Bacteriol. 77 (1959) 642–647.
[12] D.C. Montgomery, Design and Analysis of Experiments, third ed., Wiley, New York, 1991.
[13] B.R. Ravishankar, J.F. Blais, H. Benmoussa, R.D. Tyagi, Bioleaching of metals from sewage sludge: elemental sulfur recovery, J. Environ. Eng. 120 (1994) 462–470.
[14] S.Y. Chen, J.G. Lin, Bioleaching of heavy metals from contaminated sediment by indigenous sulphur-oxidizing bacteria in air-lift bioreactor: effect of sulphur concentration, Water Res. 38 (2004) 3205–3214.
[15] L. Zhao, N.W. Zhu, X.H. Wang, Comparison of bio-dissolution of spent Ni–Cd batteries by sewage sludge using ferrous ions and elemental sulfur as substrate, Chemosphere 70 (2008) 974–981.
[16] R.D. Tyagi, J.F. Blais, L. Deschenes, P. Lafrance, J.P. Villeneuve, Comparison of microbial sulfuric acid production in sewage sludge from added sulfur and thiosulfate, J. Environ. Qual. 23 (1994) 1065–1070.
[17] W.H. Rulkens, J.T.C. Grotenhuis, R. Tichy, Methods of cleaning contaminated soils and sediments, in: W. Salomons, U. Förstner, P. Mader (Eds.), Heavy Metals, Springer, Berlin, 1995, pp. 151–191.

[18] M.P. Silverman, H.L. Ehrlich, Microbial formation and degradation of minerals, Adv. Appl. Microbiol. 6 (1964) 181–183.
[19] O.H. Tuovinen, S.I. Niemelä, H.G. Gyllenberg, Tolerance of *Thiobacillus ferrooxidans* to some metals, Antonie Leeuwenhoek 37 (1971) 484–496.
[20] J.T. Trevors, K.M. Oddie, B.H. Belliveau, Metal resistance in bacteria, FEMS Microbiol. Rev. 32 (1985) 39–54.
[21] M. Dopson, C. Baker-Austin, P.R. Koppineedi, P.L. Bond, Growth in sulfidic mineral environments: metal resistance mechanisms in acidophilic microorganisms, Microbiology 149 (2003) 1959–1970.
[22] N. Gönen, E. Körpe, M.E. Yıldırım, U. Selengil, Leaching and CIL processes in gold recovery from refractory ore with thiourea solutions, Miner. Eng. 20 (2007) 559–565.
[23] I. De Michelis, F. Ferella, F. Beolchini, A. Olivieri, F. Vegliò, Characterisation and classification of solid wastes coming from reductive acid leaching of low-grade manganiferrous ore, J. Hazard. Mater. 162 (2009) 1285–1291.
[24] O.H. Tuovinen, D.P. Kelly, Studies on the growth of *Thiobacillus ferrooxidans*. I. Use of membrane filters and ferrous iron agar to determine viable numbers, and comparison with $^{14}CO_2$-fixation and iron oxidation as measures of growth, Arch. Microbiol. 88 (1973) 285–298.
[25] S. Ubaldini, F. Vegliò, F. Beolchini, L. Toro, C. Abbruzzese, Gold recovery from a refractory pyrrhotite ore by biooxidation, Int. J. Miner. Process. 60 (2000) 247–262.
[26] T. Sugio, C. Domatsu, T. Tano, K. Imai, Role of ferrous ions in synthetic cobaltous sulfide leaching of *Thiobacillus ferrooxidans*, Appl. Environ. Microbiol. 48 (1984) 461–546.
[27] N. Xu, C. Christodoulatos, W. Braida, Adsorption of molybdate and tetrathiomolybdate onto pyrite and goethite: effect of pH and competitive anions, Chemosphere 62 (2006) 1726–1735.
[28] A. Schippers, W. Sand, Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur, Appl. Environ. Microbiol. 65 (1999) 319–321.
[29] D.M. Himmelblau, Process Analysis by Statistical Methods, John Wiley and Sons, New York, 1970.
[30] I. De Michelis, F. Ferella, E. Karakaya, F. Beolchini, F. Vegliò, Recovery of zinc and manganese from alkaline and zinc–carbon spent batteries, J. Power Sources 172 (2007) 975–983.
[31] G.J. Olson, T.R. Clark, Bioleaching of molybdenite, Hydrometallurgy 93 (2008) 10–15.
[32] W. Krebs, C. Brombacher, P.P. Bosshard, R. Bachofen, H. Brandl, Microbial recovery of metals from solids, FEMS Microbiol. Rev. 20 (1997) 605–617.
[33] M.G. Aylmore, D.M. Muir, Thiosulfate leaching of gold—a review, Hydrometallurgy 14 (2001) 135–174.