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A New Approach for Evaluating Wastes: Bioleaching

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Abstract

Bioleaching is a biological process where metals and microorganisms have been interaction. At this processes microorganisms prove recovery of metals by dissolving insoluble forms of them. However this biological solubilization is often used at recovering metals from ore mining, wastes have also considerable economic importance at metal gaining for their cheap raw material. At the recent years, it has been reported that important amount of aluminum, zinc, nickel and copper can be extracted from different kind of municipal and industrial wastes.

Key Words: Bioleaching, Metal recovery, Waste.

Introduction

The waste problem has risen together with rapidly increasing world population and improvement at industry by developing technology. For this reason evaluating the wastes by recovering the valuable metals or detoxifying these materials for environmentally safe deposition are the main targets of waste management. To date at the scope of waste management many systems have been tested. However, in the recent years, it has been shown that bioleaching is a novel promising technology for recovering some minerals from municipal and industrial wastes (Bajirova and Velkova, 2001; Bosecker, 2001; Soliso et al., 2002; Picher et al., 2002; Aung and Ting, 2005; Ishigaki et al., 2005; Santhiya and Ting, 2005).

Bioleaching is a biological process where metals and microorganisms have been interactions. At this process insoluble metals are recovered by transforming them into soluble form with several microorganisms (Brown, 1994; Trehan, 1996; Bosecker, 1997; Krebs, et al., 1997). This solubilization is the result of transformation of organic and inorganic acids, by oxidation and reduction reactions and secretion of complex making agents. Microorganisms can manage the solubilization not only interacting with solid phase directly but also oxidation of Fe^{+2} to Fe^{+3} as an electron acceptor, indirectly (Barrett et al., 1993; Bosecker, 1997; Sand et al., 2001; Tribustch, 2001). Three main groups of microorganisms are participating at bioleaching process. These microorganisms are autotrophic or heterotrophic bacteria and molds. Bacteria including autotrophic group have some advantages due to their low nutrition necessity. On the other side heterotrophic molds are important for alcalic materials because of their ability to grow at higher pHs values. However, the chemolitotrophic *Thiobacillus ferrooxidans* and *T. thiooxidans* belonging to the bacilliacea have the most importance for industrial applications (Schinner and Burgstaller, 1989; Barrett et al., 1993; Leduc and Ferroni, 1994; Bosecker, 1997; Castro et al., 2000; Aung and Ting, 2005; Santhinya and Ting, 2005).

At the process of bioleaching; fly ash obtained by waste incineration is mostly used. Because it has been known that fly ash has concentrated Al, Zn, Cd, Cr, Ni metals (Bosecker, 2001; Krebs et al., 1997; Brombacker et al., 1998; Ishigaki et al., 2005). Moreover, many metal recovery and detoxification researches have been done on other industrial waste materials such as galvanic sludge, filter dust and ore's (Bosecker, 2001; Bojinova and Velkova, 2001; Solisio et al., 2002; Aung and Ting, 2005).

In the recent years, bioleaching has become an interesting field for scientists due to increasing volume of municipal and industrial waste. Really, occurrence of this biological phenomenon spontaneously in the nature has important potential at recovering metals from wastes. At this review, studies on leaching with waste materials will be brought together by evaluating the mechanisms and the microorganisms, used in the bioleaching technique. Thus these information might have beneficial at scaling-up this application from laboratory phase to practical and at developing more effective reactor systems.

MICROORGANISMS USED IN THE TECHNIQUE

The active role of microorganisms in the bioleaching process can be understood after the study of Temple and Colmer in 1951 by isolation and definition of *T. Ferrooxidans*, which were capable of oxidation iron and sulphur (Olsen et al., 2003). In later studies, it was observed that other microorganisms as well as *Thiobacillus* group were influential. Those microorganisms were divided into four groups; *Thiobacillus, Leptospirilum, Termophilic bacteria* and *heterophilic microorganisms* (Bosecker, 1997; Krebs et al, 1997). Microorganisms with an active role in bioleaching and some of their features are presented in Table 1.

Thiobacillus

Members of this group that are capable of oxidation sulphur were first discovered by Beijerinck in 1902. These bacteria play a role at the cycle of iron and sulphur in biosphere and carry out leaching of metals from minerals. Although there are different strains in this group, the most important ones are *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* (Barrett et al. 1993).

Thiobacillus are gram negative, non-spore forming, rod shape microorganisms which can grow under aerobic conditions. Their cells are between 0.3 and 0.8 µm in diameter, and between 0.9 and 2 µm in length. Moreover,

Table 1. Some microorganisms and their properties at bioleaching processes.

Microorganisms	Type	Fermentation type	Leaching solution	pH Range	Optimum pH	Temperature
Aspergillus niger	Mold	Heterotrophic	Oxalate, citrate, gluconate, lactate	-*	-	-
Bacillus megaterium	Bacteria	Heterotrophic	Citrate, amino acids	-	-	-
Leptospirillum ferrooxidans	Bacteria	Chemolitotrophic	Ferric iron	-	2,5-3,0	30
Leptospirillum thermoferrooxidans	Bacteria	Chemolitotrophic	Ferric iron	-	1,7-1,9	45-50
Sulfobacillus thermosulfioxidans	Archea	Chemolitotrophic	Ferric iron, sulfuric acid	Acidophilic	-	37-42
Sulfolobus acidocaldarius	Archea	Chemolitotrophic	Ferric iron, sulfuric acid	0,9-5,8	2,0-3,0	55-85
Sulfolobus ambivalens	Archea	Chemolitotrophic	Ferric iron	-	-	Absolute thermophilic
Sulfolobus themosülfioxidans	Archea	Chemolitotrophic	Ferric iron, sulfuric acid	-	-	Absolute thermophilic
Thiobacillus ferrooxidans	Bacteria	Chemolitotrophic	Ferric iron, sulfuric acid	1,4-6,0	2,4	28-35
Thiobacillus thiooxidans	Bacteria	Chemolitotrophic	Sulfuric acid	0,5-6,0	2,0-3,5	10-37
Thiobacillus acidophilus	Bacteria	Chemolitotrophic	Sulfuric acid	1,5-6,0	3,0	25-30
Thiobacillus albertis	Bacteria	Chemolitotrophic	Sulfuric acid	2,0-4,5	3,5-4,0	28-30
Thiobacillus thioparus	Bacteria	Chemolitotrophic	Sulfuric acid	4,5-10	6,6-7,2	11-25

* undetermined

they are able to move with their polar filagellums. Many of them are of chemolitotrophic, since they can use CO_2 as an energy source for their own cell metabolism. They can obtain energy from relatively oxided sulphur composites (Leduc and Ferroni, 1994).

Bacterial leaching is carried out in the range of 1.5-3 pHs, where all metals are included. Therefore, *Thiobacillus ferrooxidans* and *T. thiooxidans* have much more importance than others because of their tolerance to mediums having high acid levels. *T. thiooxidans* was first noticed in 1922, for its ability to oxide elemental sulphur very quickly. Although the other strains included in this group are able to oxide sulphur and sulphides, they develop at higher pH levels. However, sulphuric acid produced in the medium helps metals to diffuse into the solution through metal sources (Barrett et al., 1993; Bosecker, 1997).

Morphologically, *T. ferrooxidans* is very similar to *T. thiooxidans*. However, it differs from *T. thiooxidans* because of its oxidation of elemental sulphur quite slowly. Another important feature of the bacteria is that it obtains energy from oxidation of reduced sulphur composites. In the absence of oxygen, *T. ferrooxidans* can grow on reduced inorganic sulphur composites, using ferric iron as an alternative electron acceptor (Leduc and Ferroni, 1994).

Apart form these, acidophilic *thiobacillius* strains were also discovered. *T. prosperus* is one of the new members able to metabolize halotolerant metals. *T. cuprinus* is another bacterium that oxides facultative chemolitotrophic metal sulphides, but not ferros iron (Krebs et al., 1997).

Leptospirillium

Leptospirillum type bacteria are the members of Spirillaceae family. All leptospirillums are absolutely aerob and have polar filagellums. *Leptosprillium ferrooxidans* is acidophilic and obligatorily chemolitotrophic bacteria that can oxides iron. This microorganism can tolerate low pH and high concentrations of uranium, molybdenum and silver, but it is very sensitive to copper and cannot oxide sulphur and sulphur composites. They are inefficient over metal sulphides on their own. Furthermore, these bacteria have mesophilic character (Barrett et al., 1993; Leduc and Ferroni, 1994; Krebs et al., 1997).

Thermophilic bacteria

These bacteria can grow on various metals at 50°C and use ferros iron as an energy source. However, their development can be observed in case of yeast extract. One of the important member of this group; *Acidionous bricleyi* is categorized under Sulfolobus spp. group, and display chemolitotrophic, facultative aerob, absolute acidic features. Under anaerob conditions, they can use elemental sulphur as electron acceptors and reduced it to H_2S. Members of *Sulfolobus* spp. are aerobic, facultative and chemolitotrophic bacteria, and they can also tolerate sulphide minerals, elemental sulphur and ferros iron (Bosecker, 1997; Krebs et al., 1997).

Heterotrophic microorganisms

Heterotrophic bacteria and fungi that need organic support for their development and energy supply have also ability to leach metals. Just as, it was reported that, manganese recovery was attributed to microbial enzymatic reduction of oxidized metal composites or effects of organic acids production, such as lactic acid, oxalic acid and citric acid. At this biologic reaction heterotrophilic microorganisms do not have any utility. Among the members of this group, *Bacillus spp*. from bacteria and *Aspergillus spp.* and *Penicillium spp*. from moulds play an important role in metal recovery (Krebs et al., 1997; Castro et al., 2000; Santhiya and Ting, 2005).

BIOLEACHING MECHANISMS

T. ferrooxidans, T. thiooxidans and L. ferrooxidans are microorganisms most widely used in bioleaching process (Bosecker, 1997; Krebs et al., 1997). By these microorganisms, slowly dissolving metal sulphides can be transformed into the water soluble metal sulphides through biochemical oxidation. These transformations can occur in two ways. These are direct and indirect bacterial bioleaching (Sand et al., 2001; Tribustch, 2001).

Direct bacterial leaching

In this mechanism, there is a physical contact between mineral sulphide and bacterial surface. To obtain transformations into sulphate, oxidation is realized through a few enzymatic reactions. As it can be seen in the following reaction (1), metal reserve is oxidized to Iron III sulphate. Therefore, metals in dissoluble forms are transformed into soluble sulphate forms (Barrett et al., 1993; Bosecker, 1997).

$$4FeS_2 + 15O_2 + 2H_2O \xrightarrow{\text{Bacteria}} 2Fe_2(SO_4)_3 + 2H_2SO_4 \qquad (1)$$

Reported studies have shown that sulphites that do not include iron (CuS, Cu_2S, ZnS, PbS, MoS_2, Sb_2S_3 CoS) could be also oxidized by *T. Ferrooxidans* (2). Thus, the following reaction may be suggested for all sulphite metals (Bosecker, 1997).

$$MeS + 2O_2 \xrightarrow{\text{Bacteria}} MeSO_4 \qquad (2)$$

In this mechanism bacteria should always be in contact with mineral surface. However, adsorbing of minerals to the bacterial surface and mechanism of metal solubility has not been clearly explained yet. According to the evaluations, bacteria is not attach on the whole mineral surface, it only preferred specific areas where crystal is not occur, so, it was stated that metal solubility is depended on electrochemical interactions (Bosecker, 1997; Sand et al., 2001; Tribustch, 2001).

Indirect bacterial leaching

At indirect leaching, ferric ions (Fe^{+3}) act as an oxidation agent for minerals. In this mechanism, the main function Of bacteria is to use ferros iron in the reaction again by transforming into the ferric iron. Therefore, metal is recovered as a result of a chemical reaction with ferric iron (3). Consequently, bacteria are included in bioleaching indirectly (Barrett et al., 1993; Trehan, 1996; Bosecker, 1997).

According to the reported hypotheses, there is no direct contact between bacteria and minerals in this mechanism. However, it has been shown that ferros iron is transformed into ferric iron within polymers found on the cell walls of bacteria. Furthermore, leaching solution is influential on this kind of reaction (Sand et al., 2001).

$$MeS + Fe_2(SO_4)_3 \xrightarrow{\text{Bacteria}} 2FeSO_4 + S^0 \qquad (3)$$

When these findings have been considered, it is understood that bioleaching is an interaction between processes of biological and chemical oxidation. Some researchers claimed that the mechanism of direct leaching proceeds through the mechanism of indirect bioleaching method. It has been also stated that ferros iron is transformed into ferric iron within the exopolymeric structure released by bacteria at the direct leaching like indirect leaching that bacteria seems to be adsorbed on the metal (Sand et al., 2001; Tribustch, 2001).

METAL RECOVERY FROM MUNICIPAL AND INDUSTRIAL WASTE MATERIALS

Bioleaching was first used in order to separate valuable minerals such as cadmium, cobalt, copper, gold, manganese, nickel, platinum, silver, uranium and zinc from ores. However, use of this method in industry is for production of especially copper, gold and uranium (Barrett et al., 1993; Bosecker, 1997). In the recent studies it was also conducted that different waste materials have really economical potential resources for bioleaching (Ebner, 1977; Schinner et al., 1989; Brombacher et al., 1998; Bojinova and Velkova, 2001; Bosecker, 2001; Solisio et al., 2002; Picher et al., 2002; Aung and Ting, 2005; Ishigaki et al., 2005; Santhiya and

Table 2. The metals recovered from different wastes and microorganisms.

Waste Type	Recovered Metals	Microorganisms	References
Sewer sludge	Heavy Metals	*Thiobacillus sp.*	Bosecker, 2001; Picher et al., 2002;
Filter Dusts	Cu, Cr, Zn	*Thiobacillus thiooxidans*	Krebs et al., 1997
Fly ash from municipal waste	Zn, Ni, Cu, Zn, Cd, Cr, As	*Thiobacillus thiooxidans* *Aspergillus niger*	Ishigaki et al., 2005;
Pig manure	Cu, Fe, Zn	*Acidothiobacillus ferrooxidans*	Picher et al., 2002
Mine waste	Al, Na, Mg, K, Zn,	*Thiobacillus ferrooxidans*	Bojinova ve Velkova, 2001; Solisio et al., 2002
Paper factory waste	Fe, Cu, Zn	*Acidothiobacillus ferrooxidans*	Picher et al., 2002
Petroleum refinery waste	Al, Mo, Ni,	*Aspergillus niger*	Aung ve Ting, 2005; Santhiya ve Ting, 2005

Ting, 2005). Specifically in many studies it was determined that copper and zinc can be recovered approximately 100 % (Ebner, 1977; Solisio et al., 2002; Ishigaki et al., 2005). At the same time, another use of this process is detoxification of waste materials. Because heavy metals in waste materials are mobilized by microorganisms and separated in that way, which leads to safer forms of waste materials for the environment. Metals recovered from some waste materials and the microorganisms being used at the bioleaching processes are presented in Table 2 (Bosecker, 2001).

Bioleaching can be occurred easily in the case of waste materials being as solid that microorganisms can adsorb (Krebs et al., 1997). For this reason fly ash is the most widely used solid material gained by incineration of waste (Ebner, 1977; Brombacher et al., 1998; Ishigaki et al., 2005). As it is well known, one of the most important problems in eradication of waste materials is that these materials occupy a considerably large volume. Incineration is the most important process in order to solve this problem. When waste materials are burnt, metals become concentrated in content and become essential sources for bioleaching. On the other hand, dust held in different filters, waste materials from metal industry and refineries and animal feces as well as ashes from waste materials are some of the waste materials used in studies (Bojinova and Velkova, 2001;Solisio et al., 2002; Picher etal., 2002; Aung and Ting, 2005; Santhiya and Ting, 2005).

The first study to extract metals from waste materials was conducted by Ebner (1977). The researcher tested 30 different *Thiobacillus* sp. strains on waste materials collected from different plants which processed copper and zinc ore's. At the end of the study it was reported that copper could be extracted from waste materials by 95 %, and zinc 70 %. Similarly, It was determined that *T. thiooxidans* (DSM504) strain, used in discontinuous system for metal extraction by using fly ash from waste materials, could separate more than 80% of cadmium, copper and zinc (Krebs et al., 1997). In another study the actions of *T. thiooxidans* TM-32; and *Acidothiobacillus ferroxidans* ATCC 23270 that could oxide sulphur and iron, were followed, and 67 % copper, 78 % zinc and 100 % chrome and cadmium extraction values could be obtained when the two bacteria were used together

(Ishigaki et al., 2005). The mud forms of dust held in different filters was used in the another study where aluminium and zinc were extracted. In the discontinuous fermentor system, recovery of 76 % and 78% zinc and aluminum respectively was recorded, by using *T. ferroxidans* (Solisio et al., 2002). Metal recovery was also examined on sewer, waste from paper factories and pig feces, by using *A. ferrooxidans*. Generally, values obtained for waste water mud, waste from paper factories and pig feces were 43 %, 43 %, 37 % for copper; 41 %, 45 %, 33 % for iron and 29 %, 35 %, 29 % for zinc (Picher et al., 2002).

Heterotrophic microorganisms were used to recover metals from waste materials as well as autotrophic Thiobacillus (Aung and Ting, 2005; Santhiya and Ting, 2005). *Aspergillus niger* was attempted to recover metals from refinary waste materials and it was observed that there was a decrease in cell density, depending on the amount of refinery waste materials, whereas there was an increase in oxalic acid amount. Level of metal extraction increased as well as the oxalic acid amount. After the 60 day of bioleaching period, the highest metal extraction values recorded were 54.5 % Al, 58.2 % Ni and 82.3 % Mo (Santhiya and Aung, 2005). In another study where a similar organism was used, 9 % Ni, 23 % Fe, 30 % Al, 36 % V and 64 % Sb were transformed into soluble forms after mobilization (Aung and Ting, 2005).

Although discontinuous systems are generally used in studies to recover metals from waste materials, there have been attempts to suggest new model systems to an increase leaching productivity and to shorten its duration (Brombacher et al., 1998; Aung and Ting, 2005). It takes bacteria functioning in bioleaching process a long time (1-3 months) to adapt themselves to the media and to mobilize metals (Bosecker, 1997; Krebs et al., 1997). That's why, the model system designed by Brombacher et al., (1998) increased efficiency of bioleaching and shortened the time of the process. The system is formed with three consequtive fermenters lined after one another. Thus, bacteria used do not face waste materials at a toxic level for themselves and the transformation is slow. 81 % of zinc, 52 % of aluminum and all of the toxic cadmium (100 %), 89 % of copper and 64 % of nickel were extracted, using that model system. Moreover, leaching was completed in a 6-day-period (Brombacher et al, 1998). In another application, *A. niger* cells were first multiplied in a media including sucrose and then metal recovery was obtained, treating those cells with waste materials. Also, extraction was realized at a single phase. After the study, it was seen that a double phased extraction was more successful. As a matter of fact, there was an increase in all the metals searched (Al, V, Sb, Fe, Ni) (Aung and Ting, 2005). In another study by the same group, 2[*N*-Morpholina]ethanesulfonic acid (MES) was used to increase productivity of extraction. After the study, it was observed that there was an increase in Al by 3.5 % and in Ni by 4.6 % when compared to normal applications in metal recycling and the process took 30 days, which typically lasts for 60 days (Santhiya and Ting, 2005).

One of the main issues in metal extraction from waste materials is the concentration of the waste amount in the reaction media. Because, it was reported that non-diluted waste materials had an inhibiting effect on bioleaching organisms (Picher et al., 2002; Solisio et al., 2002; Aung and Ting, 2005; Ishigaki et al., 2005; Santhiya and Ting, 2005). When the beginning concentration exceeded 1%, inhibition effect started and extraction values decreased rapidly, although the most efficient concentration generally depends on the type and composition of waste materials.

In order to determine effects of concentration, initial amounts of waste materials different initial waste concentrations were studied and it was observed that the most influential extraction was 1 %. When the initial concentration increased from 1% to 10%, zinc extraction decreased to 70% from 76%, and concentration of aluminum to undetectable level from 78 % (Solisio et al., 2002). It was shown that concentration more than %1 inhibited bacteria, especially when the fly ash from waste materials was used. It should be noted that metals become more concentrated when waste materials are incinerated (Aung and Ting, 2005). However, in case of using iron and sulphur oxidation bacteria together, it was seen that extraction continued even at a concentration of 3% of waste materials whereas extraction was halt when these strains were used indivually (Ishigaki et al., 2005). Besides, it was emphasized that the amount of dissolved organic carbon has to be decreased where waste water sludge and pig manure materials were used. This was because of the fact that leaching bacteria were not able to develop over the amount of 180 and 500 mg/L dissolved organic carbon levels (Picher et al., 2002).

In addition to an active role in metal recovery, it was also stated that bioleaching method could be used also to detoxify waste materials (Bosecker, 2001). As it is well known, some industrial waste materials include considerable amounts of toxic metals. The possibility of these metals to diffuse into the underground water and environment has carrying serious danger. In fact Chemolitotrophic thiobasillus strains, capable of growing in the waste water sludge and doing detoxification, can be isolated (Tyag and Couillard, 1991).

CONCLUSION

Bioleaching applied with Chemolitotrophic and heterotrophic microorganisms is an important biological process for industrial and municipal waste in order to recover valuable metals and to detoxify them. Especially because of the low prices of raw material waste increase this importance much more.

According to the results of studies this technique has more potential at extracting Al, Zn and Cu. But the concentration level of waste material in the leaching medium is important matter to be taken into the consideration. Therefore due to the composition of wastes 1% of concentration is limit level for applications. Moreover it has been reported that gradated fermentation systems has given higher yields for the leaching processes.

In future, for improving the yield of metal recovery from wastes, new strains have to be identified that are capable of working under higher concentrations. For this purpose, strains genetically modified or selected by mutations can be applied. Besides, for shortening the fermentation time, constructing more effective and economic systems are some of the other features.

REFERENCES

Aung, K.M. and Ting, Y. P. (2005). Bioleaching of spent fluid catalytic cracking catalyst using *Aspergillus niger.* J*ournal of Biotechnology. 116, 159-170.*

Barrett, J., Hughes, M. N., Karavaiko, G.I. And Spencer, P.A. (1993). Metal extraction by bacterial oxidation of minerals. ss. 1-153, Ellis harwood limited, England.

Bojinova, D. Y., And Velkova, R. G. (2001). Bioleaching of metals from mineral waste product. Acta Biotechnol. 21, 275-282.

Bosecker, K. (1997). Bioleaching; solubilization by microorganisms. FEMS Microbiology reviews. 20, 591-604.

Bosecker, K. (2001). Microbial leaching in environmental clean-up programmes. Hydrometallurgy. 59, 245-248.

Brombacher, C., Bachopen, R. and Brandl, (1998). Development of a laboratory-scale leaching plant for metal extraction from fly ash by *Thiobacillus* strains. Appl. Environ. Microbiol. 64, 1237-1241.

Brown, M. J. (1994). Metal recovery and processing. In Biotechnology the science and the business. Eds: Moses, V. ve Cape, R. E., ss. 567-579, Harvood academic publishers, Switzerland.

Castro I.M., Fietto J.L.R.,. Vieira R.X, Tropia M.J.M., Campos L.M.M., Paniago E.B., and Brandao R.L. (2000). Bioleaching of zinc and nickel from silicates using *Aspergillus niger* cultures. Hydrometallurgy. 57, 39-49.

Ebner, H. G. (1977). Metal extraction from industrial waste with

Thiobacilli. In: Bacterial leaching. Ed: Schwartz, W., Verlag, ss. 217-222, Chemie, Weinheim.

Ishigaki, T., Nakanishi, A., Tateda, M., Ike, M. and Fujita, M. (2005). Bioleaching of metal from municipal waste incineration fly ash using a mixed culture of sulfur-oxidizing and iron-oxidizing bacteria. Chemosphere. (In press)

Krebs, W., Brombacher, C., Bosshard, P. P., Bachofen, R. and Brandl, H. (1997). Microbial recovery of metals from solids. FEMS Microbiology reviews. 20, 605-617.

Leduc, L. G. and Ferroni, G. D. (1994). The chemolithotrophic bacterium *Thiobacillus ferrooxidans*. FEMS Microbiology reviews. 14, 103-120.

Olsen, G. J., Brierley, J. A. and Brierley C. L. (2003). Bioleaching review part B: Progress in bioleaching: application of microbial processes by the minerals industries. Appl. Microbiol. Biotechnol. 63, 249-257.

Picher, S., Drogui , P., Guay , R. and Blais J.F. (2002). Wastewater sludge and pig manure used as culture media for bioleaching of metal sulphides. Hydrometallurgy. 65, 177-186.

Sand W., Gehrke T., Jozsa P. And Schippers A. (2001). Biochemistry of bacterial leaching-direct vs. indirect bioleaching. Hydrometallurgy. 59, 159-175.

Santhiya, D. and Ting, Yen-Peng (2005). Bioleaching of spent refinery processing catalyst using *Aspergillus niger* with high-yield oxalic acid. Journal of Biotechnology. 116, 171-184.

Schinner, F. and Burgstaller, W. (1989). Extraction of zinc from industrial waste by a *Penicillium* sp. Appl. Environ. Microbiol. 55, 1153-1156.

Solisio, C., Lodi, A. and Veglio F. (2002). Bioleaching of zinc and aluminium from industrial waste sludges by means of *Thiobacillus ferrooxidans.* Waste Management. 22, 667-675.

Trehan, K. (1996). Mining and metal biotechnology. In biotechnology, New age international (P) limited publishers London. 88-101.

Tribustch, H. (2001). Direct versus indirect bioleaching. Hydrometallurgy. 59, 175-185.

Tyagi, R. D. And Couillard, D. (1991). An innovative biological process for heavy metals removal from municipal sludge. In Biological degradation of wastes. Ed. Martin, A.M., Pages 307-322. Elsevier, Amsterdam.