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Microbial Treatment of Cyanide and Heavy Metals Containing Waste Water from Gold Mining

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ABSTRACT: Cyanide-bearing waste water from'gold mining is usually decontaminated by oxidation so that the dissolved metals are precipitated as hydroxides. The disadvantages of this method are high consumption of chemicals and the formation and liberation of toxic compounds. Various bacterial and fungal strains capable of degrading cyanides or having a high potential for metal adsorption were isolated from water and sediment samples from a wastewater deposit in Romania. Some of the isolates were characterised and identified. For those strains showing the most rapid degradation or the greatest adsorption capacity, important parameters were analysed using synthetic media and process water and waste water from the cyanidation plant. Optimum pH and temperature levels, maximum tolerable cyanide and metal concentrations, as well as possibilities for the addition of required substances, such as hydrocarbon sources or phosphate, were determined. With a view towards the development of a pilot plant, the immobilisation of microorganisms was achieved on a natural zeolite. With a view to a technical application, further improvements are *to* follow *as* part of the development of a pilot plant.

1 INTRODUCTION

The cyanide spill in Baia Mare at the beginning of last year has been called the worst ecological disaster to hit Eastern Europe since the fallout from the Chernobyl nuclear plant disaster in the Ukraine in 1986. Regarding what happened, the question arises: "Was this catastrophe avoidable?"

For about one hundred years, cyanide solutions have been used to extract gold and silver from ores. This process is known as cyanidation and is summarised by the reaction shown İn Equation (1):

$$4\,Au + 8\,NaCN + O_2 + 2\,H_2O \rightarrow 4\,Na[Au(CN)_2] + 4\,NaOH \quad (1)$$

The reaction with silver is similar. In the absence of other metals which form cyanide complexes, relatively weak cyanide solutions can be used because both noble metals form strong complexes with cyanide. However most gold-bearing ore deposits contain other metals which react with cyanide to form metallo-cyanide complexes, resulting in higher cyanide consumption rates and a residual solution which contains a wide range of cyanide species and complexes.

Gold is recovered from the cyariidation solution by cementation with zinc (Equation 2) or by adsorption on activated carbon, but there are many variations of each of these two procedures, leaving a solution which bears cyanide, metallo-cyanide complexes, cyanates, thiocyanates (SCN), and thiocyanate complexes along with other chemical species dissolved from the ore (Mudder 1997).

$$2\,Na[Au(CN)_2] + Zn \rightarrow 2\,Au + Na_2[Zn(CN)_4] \quad (2)$$

Depending on the constituents in the remaining solution and their respective concentrations, die process waste water is hazardous to the environment and needs special treatment and remediation. There are tfiree principal forms of cyanide present in the process solutions: free cyanide, weakly complexed cyanide, and strongly bound or complexed cyanide. The free form of cyanide, which exists as either molecular hydrogen cyanide HCN or the cyanide anion CN~, is considered the most toxic compound and is most readily removed from water through natural attenuation and chemical, physical, and biological treatment processes. The conventional treatment processes can reduce the levels of free cyanide in solution to eitiier non-detectable or environmentally acceptable concentrations.

The toxicity of the weakly metallo-cyanide complexes with copper, nickel or zinc, the second form of cyanide present İn metallurgical processes, primarily arises from the free cyanide produced through dissociation of the complex and secondarily from the complex itself and any free metal yielded through breakdown of the complex.

The third form of cyanide includes the strongly bound metal cyanide complexes of iron and cobalt, which themselves are non-toxic and become toxic only after breakdown (Mudder 1997).

Analytically, cyanide is quantified either by the total cyanide or the WAD (weak acid dissociable) cyanide procedures. The total cyanide procedure reports all forms of free cyanide and metal-bound cyanides, including the non-toxic and stable iron cyanides. The WAD cyanide, the toxicologically important form, reports all forms of cyanide except cyanide-bound iron. For drinking water, the standard of total cyanide is 0.20 mg/l (U.S.EPA). For aquatic life, the limits are in the range of 0.08-0.10 mg/l WAD cyanide. In the case of aquatic life, fish, particularly die salmonids, are more sensitive to the effects of cyanide tfian other aquatic residents like insects, bacteria or algae.

Cyanide-bearing waste water from gold mining is usually decontaminated chemically by oxidation, e.g., with alkaline hypochlorite (Smith & Mudder 1991, Smith & Mudder 1995) so that dissolved metals precipitate as hydroxides. The disadvantages of this method are high consumption of chemicals and the formation and liberation of toxic compounds. Remediation is necessary because aquatic organisms in particular are affected by the toxic effects of the cyanide emitted, even in small concentrations (micrograms/litre).

Within the scope of scientific co-operation with the Mining Research and Design Institute (ICPM) at Baia Mare, Romania, a biotechnological process is being developed to replace the present chemical process (Blumenroth et al. 1999). In this process, microorganisms that can decompose cyanide and adsorb heavy metals are used. This ability has already been shown at several laboratories for different microbes (Boucabeille et al. 1994, Chaptawala et al. 1998, Dubey & Holmes 1995, Figueira et al. 1995, Gadd 1993, Raybuck 1992, Stoll & Duncan 1996, Tsezos 1990, Volesky 1994). The major advantages of biological treatment are that operating costs are low, metals are removed by adsorption, and the accumulation of toxic intermediate metabolites and final products is avoided. The only commercial plant currently known that treats waste water by cyanide bio-degradation is at the Homestake Mine, USA (Whitlock & Mudder 1986, Whitlock 1990).

The pilot plant scheduled for Baia Mare has to be adapted to the local conditions. Therefore, several microorganisms were enriched and isolated from waste water and sediment samples, and degradation and sorption tests with local waste waters were performed (Blumenroth et al. 1997, Blumenroth & Bosecker 2000).

2 RESULTS

2.1 *Water analysis*

A pond near Baia Mare İs used as a basin for waste water from the central flotation and cyanidation plant and for the acid mine water from the Sasar mine. The water in this pond has an average total cyanide concentration of 10-15 mg/I and iron, copper and zinc concentrations of about 40 mg/l each (waste water A). Contamination with organic compounds was found to be rather poor. The daily total cyanide output amounts to about 300 kg. The main components in the heavily contaminated water from the cyanidation plant (waste water B) are cyanide (approx. 1.500 mg/l) as well as copper and zinc. Both waste waters have a high level of calcium (> 200 mg/l) because during the cyanidation process the pH level İs controlled by the addition of lime. A list of some of the data İs given in Table 1.

Table I. Composition of water samples taken from the Bozinta pond (A) and the cyanide leaching plant in the Sasar Mine (B)

Parameters (all [mg/l] besides pH and cell numbers)	Waste water A (mean values)	Waste water B (mean values)
pH value	7 2	11.6
Cyanide, total	7.4	1160
Chloride	8.9	36.8
Nitrate	4.4	6.1
Sulphate	846	280
Phosphate	0.24	< 0.1
Copper	5.0	209
Iron	< 0.1	1.3
Zinc	2.5	269
Calcium	310	218
Cell numbers/ml (medium R2A/FP)	$1\,4\,10^4/3\,8\,10^4$.	0/0

2.2 *Isolates*

Several bacteria and fungi were selectively enriched and isolated from water and sediment samples from the Bozinta pond. The best cyanide-degrading bacteria and the strongest metal-adsorbing fungi were identified by die German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig). The most important bactena were *Pseudomonas spec,* and *Burkholderia cepacia,* both of which are common aquatic organisms. The fungal isolates were identified as strains of the genera *Trichoderma, Epicoccum* and *Aspergillus.*

2.3 *Cyanide degradation*

Initial investigations on cyanide degradation by bacteria were performed using an artificially contaminated, standardised culture medium. The isolated bacteria were analysed for their effectiveness based on the use of cyanide as the sole nitrogen source. The quickest growing strains were used in further experiments. Two very good degraders were found, belonging to the species *Burkholderia cepacia.* Kinetic experiments were carried out at different cyanide concentrations (Fig. 1) and at 30°C or 15°C. Depending on the cyanide concentration, degradation was completed after 1-22 hours and was not influenced by a high sulfate concentration. The degradation was faster at 30°C than at 15°C.

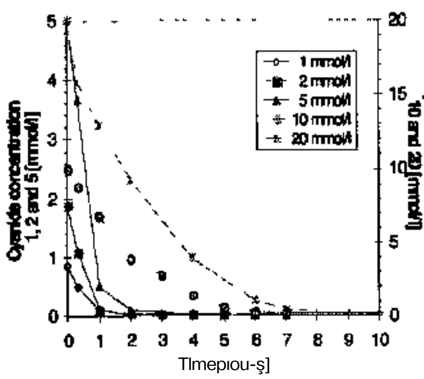

Figure 1 Degradation of different cyanide concentrations at 30°C by *Burkholdena cepacia.*

The degradation rates achieved at pH 7 and pH 9 were more or less the same. The maximum tolerated, degradable cyanide concentration was 520 mg/l CN~, which is several times me concentration in the pond. Therefore, treatment of highly contaminated waste water direct from the cyanidation plant might also be considered. Ammonia arising as a metabolite is assimilated by the microbes. For cyanide decomposition as well as for ammonia assimilation, the optimum pH value was found to be 8.

The degradation of cyanide did not require a carbon source, but for the basic metabolism of the organisms a carbon source was necessary. Besides glucose, the classic carbon source, cheap alternatives such as molasses, whey and liquid residues from beer and juice production were examined for their suitability. The addition of juice and beer residues (4%; v/v) led to successful growth results compared to the glucose concentration of 0.4 % used otherwise.

The bacteria present in the waste water from the Bozinta reservoir did not seriously contribute to the degradation of cyanide because of the small quantity of cells. As the cyanide concentration was very low, waste water A was artificially contaminated in most of the experiments (26-130 mg/l CN~). In both waste waters A and B, the addition of phosphate as a nutrient was vital for the degradation of cyanide. The rate of degradation depended on the level of phosphate present (Fig. 2). Calcium was bound by the addition of EDTA to prevent precipitation with phosphate. Without the addition of EDTA, the cyanide degradation slowed down considerably.

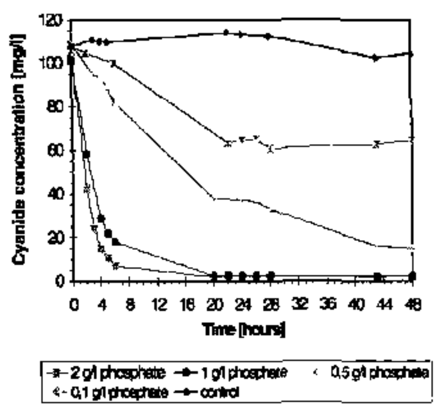

Figure 2 Cyanide degradation in waste water in relation to phosphate addition (5 *cepacia)*

The maximum metal tolerance was determined İn various dilutions of waste water B. According to the isolates being tested, the metal concentrations were in the range of 5-43 mg/l copper or 9-64 mg/l zinc (when present simultaneously). These results can only provide an approximate tolerance area because the metals mentioned are present not just as ions but also as different complexes, and for this reason they have a lower toxicity.

Experiments with diluted effluent from the cyanidation plant (waste water B) resulted in lower degradation rates, which is explained by, among other factors, high contents of zinc and copper, which complex with cyanide and inhibit the growth of the microorganisms.

2.4 *Immobilization of bacteria*

If the bacteria tested in the laboratory are to be used on a technical scale, the immobilisation of the organisms on a suitable layer seems appropriate in order to obtain a larger biologically active surface area and to prevent the microorganisms being washed away by huge amounts of waste water, Apart from commercially available plastic layers, the growth on zeolite rock was examined. The use of zeolites has been reported for waste water treatment (Dictor et al. 1997, Klein & Ziehr 1987, White & Schnabel 1998, Marlon & Liebmann 1994, Suh et al. 1994) and provides a cheap alternative, as the zeolites can be obtained near the processing plant. Growth on the plastic materials was not successful, but the bacteria did attach themselves to zeolite. This was determined by cell staining with acridme orange. Investigations with an electron microscope are as follows.

There are various types of zeolite. When zeolite type 1 (mainly philippsite) was used, the degradation of cyanide followed at the same speed as with suspended cells (< 1 mg/1 after 4 hours). Growth on zeolite type 2 (mainly clinoptilolite) was less efficient and the reduction in cyanide correspondingly slower (< lmg/1 after 29 hours; Fig. 3).

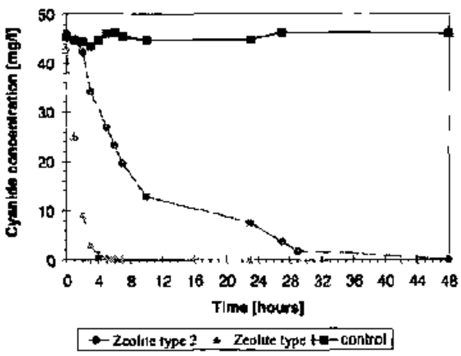

Figure 3. Degradation of cyanide by immobilised cells of *Burkhoidena cepacia.*

Recent long-term column experiments (3 weeks) with zeolite type 2 and repeated addition of glucose (0.2%) showed total degradation of all cyanides in waste water B (10-fold dilution, pH 8).

2.5 *Metal biosorption byfitngi*

Filamentous fungi with high cyanide resistance and metal tolerance, isolated from sediment samples from the Bozinta pond, were chosen for the biosorption experiments. Artificially contaminated synthetic medium or deionised water served as a matrix for the sorption tests. Metals were used separately or as a mixture of iron, copper and zinc in various concentrations (e.g. 25, 50 or 100 mg/I each) and sorption capacities were calculated under different conditions.

It turned out that different fungi had different preferences for the sorption of a particular metal (iron, copper or zinc), but the sorption rates depended on whether the metals were offered separately or in a mixture. Furthermore, the sorption capacity depended on the composition of the growth medium and the glucose concentration m the pre-culture. The fungal mycelium growing on the residue from juice production adsorbed the metals much better than biomass cultivated with molasses or glucose ("juice" 1% > molasses 1% > glucose 0.4% > glucose 4%; Fig. 4).

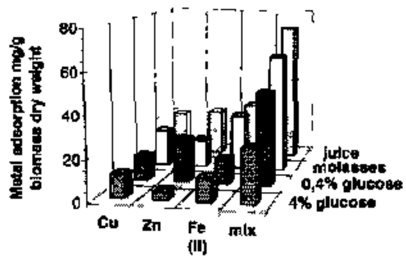

Figure 4. Adsorption levels depending on pre-cultunng conditions *(A fumigatus).*

The sorption rates of living biomass were greater than those of dead biomass. Dried mycelium showed the least effect. (Fig. 5). The incubation generally lasted for seven days, but after two to four days most of the metal was already adsorbed.

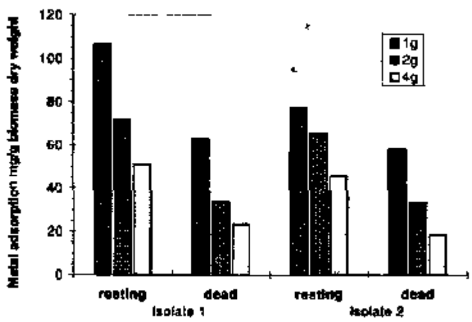

Figure 5 Comparison of metal sorption (mix) by different amounts of dead and resting biomass

3 CONCLUSIONS

Various bacteria and fungi capable of degrading cyanides or having a high potential for adsorbing heavy metals were isolated from water and sediment samples from a mining wastewater deposit in Baia Mare. Some of the isolates were characterised and identified. For those strains showing the most rapid degradation or the greatest adsorption capacity, important parameters were analysed in synthetic media and in process waters from the Baia Mare pond and the Sasar cyanidation plant. Optimal pH and temperature levels, maximum tolerable cyanide and metal concentrations, as well as possible additional-substances required, such as hydrocarbon sources or phosphate, were determined. With a view towards the development of a pilot plant, the immobilisation of microorganisms was tested on different support materials.

The results presented here show a high potential for developing a pilot plant on a technical scale for the biotreatment of cyanide and heavy metal containing waste water from mining and metallurgical processes. The chemistry, toxicology, and environmental fate of the various cyanide compounds are well understood. There are reliable and proven technologies for the destruction of cyanide to discharge treated mining and process" water into the environment. The application of appropriate technologies is no longer limited by science and engineering. More often it is a lack of knowledge, and mismanagement and inadequate legislation for environmental protection which cause environmental problems.

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REFERENCES

Blumenroth, P. & Bosecker K. 2000 Mikrobieller Abbau von Cyanid in Prozeß wässern der Goldgewinnung. In. Wippennann, Th. (Hrsg.). *Bergbau und Umweit,* 183-197, Springer Verlag Berlin.

Blumenroth, P., Bosecker, K., Michnea, A & Sasaran, N. 1999. Development of a bioremediation process for mining waste waters. In. Amils, R. & Ballester, A. (eds) • *Biokydrometallurgy and the environment towards the mining of the 21st century,* pp.547-558, Elsevier (Amsterdam).

Blumenroth, P, Dettmers, H. & Bosecker K. 1997. Isolation of microorganisms from mining wastewaters for cyanide degradation and biosorption of heavy metals *Proceed Internationa} Biohydrometallurgy Symposium IBS'97* - Biomine '97, 4-6 August, Sydney, Australia; Australian Mineral Foundation, Glenside, South Australia

Boucabeille, C, Bories, A., Ollivier, P. & Michel, G. 1994. Microbial degradation of metal complexed cyanides and thiocyanate from mining wastewaters. *Environ Pollut* 84 59-67

Chapatwala, K D, Babu, G R V , Vıjaya, O K., Kumar, K.P & Wolfram, J H. 1998 Biodegradation of cyanides, cyanates and thiocyanales lo ammonia and carbon dioxide by immobilized cells of Pseudomonas punda J Ind *Microbiol & Biotechno!* 20 28-33

Dıctor, M C, Battaglia-Brunet, F, Monn, D., Bones, A & Clarens, M 1997 Biological treatment of gold ore cyanidation wastewater tn Fixed bed reactors. *Environ Poll* 97(3). 287-294

Dubey, SK & Holmes, D.S 1995 Biological cyanide destruction mediated by microorganisms World *Journal of Microbiology & Biotechnology'* 11 * 257-265

Figueira, M M , Ciftiinelli, V S T & Lmardi, V R. 1995 Bacterial degradation of metal cyanide complexes In. C A Jerez, T. Vargas, H Toledo & J.V. Wiertz- *Biohvdrometallurgical Processing,* pp 333-339, University of Chile.

Gadd, G.M. 1993. Tansley Review No. 47 Interaclion of fungi wilh toxic metals *NewPhytol* 124-25-60

Klein, J. & Ziehr, H 1987 Immobilisierung von Mikroorganismen durch Adsorption *BioEngineering* 3 8-16

Marton, S I. & Liebmann, A. 1994 Possible uses of naiural zeolites in wastewater treatment procedure 2 Internat Symp and Exhibition on Environm Contam in Central and Eastern Europe *Proc Budapest* f20 -23 9 94)

Mudder, Terry 1997 Cyanide Monograph Mining Environmental Management, *Mining Journal Ltd,* London, UK.

Raybuck, S A 1992. Microbes and microbial enzymes for cyanide degradation *Biodegradation* 3: 3-18.

Smith, A & Mudder, T. 1991 Chemistry and Treatment of Cyanidation Wastes. *Mining Journal Books Ltd.,* London, England.

Smith, A & Mudder, T. 1995 Cyanide: dispelling the myths. *Mining Environmental Management,* June 1995,4-16.

Stall, A. & Duncan, J.R. 1996. Enhanced heavy metal removal from waste water by viable, glucose pretreated Saccharomyces cerevisiae cells *Biotechnol Lett* 18(10). 1209-1212.

Suh, Yeung-Jean, Park, Jong Mong & Yang, Ji-Won 1994 Biodegradation of cyanide compounds by Pseudomonas fluorescens immobilized on zeolite. *Enzvme Microb. Technol* (June) 16" 529-533

Tsezos, M 1990 Engineering aspects of metal binding by biomass. In: *Microbial Mineral Recovery.* Chapter 14 Eds. H.L Ehrlich *&* *C* L Brierley. McGraw Hill, Inc. USA

Volesky, B 1994. Advances m biosorption of metals- Selection ofbiomass types. *FEMS Microbiol* Rev. 14 291-302.

White, Daniel M. & Schnabel, William I998. Treatment of cyanide waste in a sequencing batch biofilm reactor *Wat Res* 32(1). 254-257.

Whitlock, J.L. 1990. Biological detoxification of precious metal processing wastewaters. *Geomicrobiol }* 8'241-249.

Whitlock, J.L. & Mudder, T.I. 1986. The Homestake wastewater treatment process Biological removal of toxic parameters from cyanidation wastewaters and bioassay effluent evaluation. In Lawrence, R.W , Bran ion, R.M R. & Ebner, H.G. (eds.): Fundamental and Applied Biohydrometallurgy, pp 327-339; Elsevier, Amsterdam, *Proceedings 6th Internat Symp Biohydrometallurgy,* Vancouver.