MICROBIAL DEGRADATION OF CYANIDES

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ABSTRACT

Cyanides are extremely toxic (poisons) for most living cells. Cyanides pollution is of high impact in every ecosystem and must be destroyed by all the available technological ways. The best way to control environmental cyanides is clearly to prevent its accumulation. Tails and effluents derived from mining activities carry on CN^-, SCN^- and $[M(CN)]^+$ complexes; being the two latest of hard removal by common chemical methods. The availability of microorganisms capable to degradate cyanides rapidly to non-toxic end products is, therefore, of considerable significance in environmental biotechnology. In the first instance, we had obtained a mixed microbial cultures selected (but not identified) by its capacity to develop using CN^-, SCN^- or $[M(CN)]^+$ complexes, being each one a source of carbon, nitrogen and energetic substrate. This objective would generate, from the very beginning, technical and economical advantages, due to the possibility of degradating these three types of cyanide compounds in one process and to avoid the addition of others substrates, allowing the development of a general application technology. Kinetics studies are presented for determining the effect of different parameters: pH, inoculum quantity, temperature, initial cyanide concentration, agitation and medium salinity, on the microbial activity of cyanide degradation. The results obtained with the mixed selected microbial culture demonstrate the application potentiality and constitute a base for operating a bioreactor under the best conditions.

INTRODUCTION

The principal consumer of cyanide is the mineral processing industry. Due to its toxicity it is of special environmental concern. Cyanidation of gold ores is a common and widespread process for gold extraction. The resulting effluents carry on cyanide, thiocyanate and metal-cyanide complexes. Since all of them are toxic, they must be removed from waste waters (Smith and Mudder, 1991).

Different chemical processes have been applied to decompose cyanide, the most important being alkaline chlorination, copper-catalysed hydrogen peroxide oxidation and the INCO (SO_2 based) process (Smith and Mudder, 1991). These processes may require special equipment and, in many cases, do not degrade thiocyanate and some metal-cyanide complexes. During this century soil microbiologists by scientific interest had been reporting the isolation of different microorganisms capable to degradate any type of cyanide, fundamentally using them as the nitrogen source (Knowles, 1976; Harris and Knowles, 1983; Stafford and Callely, 1969; Silva-Avalos et al., 1990). In recent years the subject has acquired environmental and economical significance.

The main known biochemical mechanisms (Knowles, 1976) associated to cyanide degradation by aerobic microorganisms are:

- The utilisation of any type of cyanide compound as the nitrogen source. Harris and Knowles (1976) developed a method for isolating bacterial strains utilising cyanide as nitrogen source and glucose as carbon and energy source. An heterotrophic bacterium using thiocyanate as nitrogen source has been isolated (Stafford and Callely, 1969), but require phenol as carbon and energy source. A high number of microorganisms utilising any cyanide compound as nitrogen source have been reported (Silva-Ava-

los et al., 1990), but from a technological point of view their application for degradating cyanides depends on the addition and consumption of other organic substrates.

- The utilisation of some cyanide compound as nitrogen, carbon and energy source. Happold et al. (1954) isolated a micro-organism capable of growing degradating thiocyanate by such biochemical mechanism. Ware and Painter (1955) reported the isolation of a bacterium capable of degradating cyanide by this biochemical mechanism. Unfortunately few studies there have been about these microorganisms. The isolation and studies of the best conditions for microbial growth of strains with this type of biochemical mechanism are of greater interest for achieving low cost and more general applicability technologies.

We have reported (Paños and Bellini, 1998) the obtention of microbial enrichment cultures selected by their capacity to degradate CN^-, SCN^- and $[M(CN)]^+$ using each of them as nitrogen, carbon and energy source. These selected cultures would generate, from the very beginning, technical and economical advantages for a technological development. From the mixed selected cultures two strains called BIIMC 1 and BIIMC 2 have been isolated as pure strains and characterised as being able to grow using cyanide, thiocyanate or metal-cyanide complexes independently. Each of them is used as nitrogen, carbon and energy source. The BIIMC 1 strain is a rod- shaped bacterium, forming circular smooth colonies in agarized medium as it is shown in Figure 1. The BIIMC 2 strain is a filamentous organism forming highly ramificated colonies in agarized medium as it is shown in Figure 2. Both morphologies are majority in the mixed selected cultures. Each strain capacity for using any one of the three cyanide compounds has been proved by successive subculturing each isolated strain in synthetic media containing each one of the cyanide compounds and any other compound that could act as a source of nitrogen, carbon or energy was added. Each strain has maintained its morphology and colony features in the different media. Notwithstanding, the kinetic studies presented here have been carried out by inoculating each assay with an aliquot of the corresponding "mixed selected culture" coming from a previous assay carried out under the same conditions. It is a way to obtain kinetics results more approximated to those that could be obtained in an industrial application.

Figure 1. Photography of the BIIMC 1 strain (a rod-shaped bacterium) forming circular smooth colonies in agarized medium.

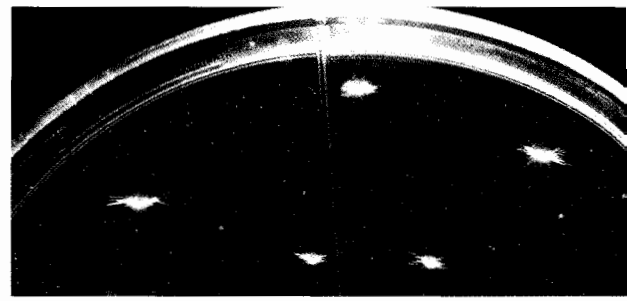

Figure 2. Photography of the BIIMC 2 strain (afilamentous organism) forming highly ramificated colonies in agarized medium.

The objective of this work is to determine the influence of variables such as pH, inoculum quantity, substrate concentration, temperature, agitation and medium salinity, in the mixed selected culture microbial activity of cyanide degradation. These studies are aimed at operating a bioreactor in the best conditions.

MATERIALS AND METHODS

Preparation of cyanide medium

The medium was prepared at the time to carry out each assay, by adding to the basal medium (Solution A) the necessary quantity of the cyanide mother solution (Solution B) for obtaining the cyanide concentration required in each assay. Solution A was prepared by dissolving the following salts in one litter of distilled water: KH_2PO_4 (0.5 g), $CaCl_2.2H_2O$ (0.1 g), $MgSO_4.7H_2O$ (0.5 g). Solution B was prepared dissolving NaCN (7.54 g) in 100 ml of distilled water at a previously adjusted pH:10 with a NaOH 1N solution. Cyanide concentration in Solution B is 40,000 ppm. After mixing Solutions A and B, pH was adjusted to the required by each assay with NaOH 0.001, 0.01, 0.05, 0.10 or 1.0 N solutions, in an Accumet Model 50 pH/Ion/Conductivity Meter.

Inoculation and sterile controls

Inoculated assays were done by adding to the fresh cyanide medium the percentage (V:V) indicated in each case of an inoculum coming from a prior assay (48-72 hrs) carried out under the same conditions of the assay to be analysed. The microbial composition of the inocula corresponds to the mixed selected microbial culture, containing as majority the strains BIIMC 1 and BIIMC 2. The microbial development and the presence as majority of the morphologies corresponding to these two strains were periodically checked out during all the experiences by microscopic observations in a CARL ZEISS microscope Model AXIOLAB with a Model MC80 microphotography camera. The sterile controls corresponding to each experience were performed without inoculation and adding to the fresh cyanide medium 4% (V:V) of a 5% (W:V) thymol solution in alcohol. The microscopic observations of samples coming from the sterile controls did not show any evidence of microbial development.

Cyanide microbial degradation in different conditions

All the assays were performed in cylindrical flasks, so as to obtain a good liquid-air contact surface even without agitation. The plastic screw caps of the flasks were highly perforated for ensuring the contact with air. The assays were incubated statically, that is without agitation, or in the same flasks but stirring in magnetic stirrers HANNA HI 190 M at mean speed (stirred assays). According to the indications in each experience, the assays were incubated at 4 ºC (refrigerator), ambient temperature (20-25 ºC) or 37 ºC in a regulable temperature incubator Binder Model BD. Cyanide concentration was measured with a cyanide specific electrode Orion ionplus connected to one channel of a pH/Ion/Conductivity Meter Accumet Model 50. In the other channel a pH electrode was connected to check pH periodically during the experiences time, in such a way as to detect any possible decrease of pH below 8 and prevent a cyanidric acid loss. In all the inoculated assays, during the experiences there was a tendency to lightly increase the pH over the initial pH, probably by ammonia production as an intermediate metabolite to be assimilated by the microbial cells. pH decrease was not detected at any case.

The cyanide microbial degradation kinetic is expressed as the percentage of the remaining cyanide concentration with respect to the initial cyanide concentration versus time as a function of the different variables.

RESULTS AND DISCUSSION

Effect of pH on cyanide microbial degradation

The initial cyanide concentration in the medium was 400 ppm. Assays were inoculated with 4 ml of the mixed selected microbial culture for each 100 ml of fresh medium. Inoculated assays and the corresponding sterile controls were performed at pHs adjusted to 8, 9, 10 and 11. All the flasks were statically incubated at ambient temperature.

The cyanide microbial degradation as a function of pH is shown in Figure 3. In the non-inoculated and sterile with thymol controls, the initial cyanide concentration remained constant during the experience time period and microbial development was not detected by periodical microscopic observations. Results show the cyanide microbial degradation in a wide range of pH from 8 to 11, being the pH of most mining cyanide effluents in such range. However, the cyanide microbial degradation increases at pH 9. The cyanide concentration percentages over 100 % are not real, but result from the smooth connecting curves.

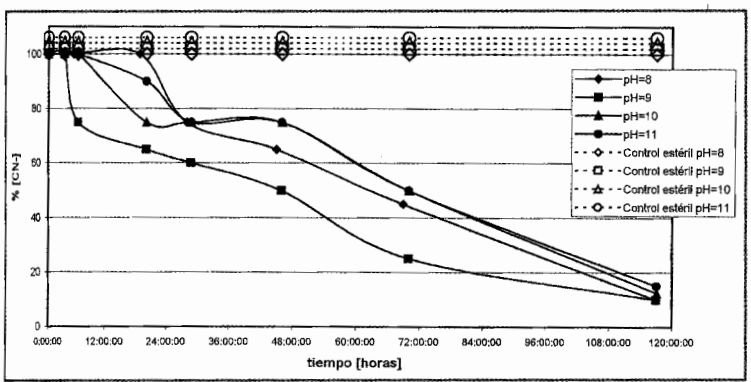

Figure 3. Cyanide concentration percentage versus time as a function of pH. Initial cyanide concentration: 400 ppm. Statically incubated at ambient temperature.

Effect of the inoculum quantity on cyanide microbial degradation

A potential industrial process should be inoculated by recycling a percentage of treated effluent carry on a good biomass produced from cyanides degradation. Assays were performed at initial cyanide concentration 400 ppm, inoculating with 4, 8 and 12 ml of the mixed selected culture, coming from a prior assay carried out in the same conditions to each present assay, for each 100 ml of fresh cyanide medium. Assays were performed at pH 8 and 9 with the corresponding non-inoculated sterile controls and all of them were statically incubated to ambient temperature.

The cyanide microbial degradation at pH 8 and 9 as a function of the inoculum quantity is shown in Figure 4. The results indicate as the inoculum quantity is increased, the adaptation time and the time for accelerated cyanide degradation starting are decreased. However, during the last stages of the experience, results tend to be equivalent, probably due to a direct relation between microbial multiplication velocity and cyanide concentration (substrate), below a certain cyanide concentration.

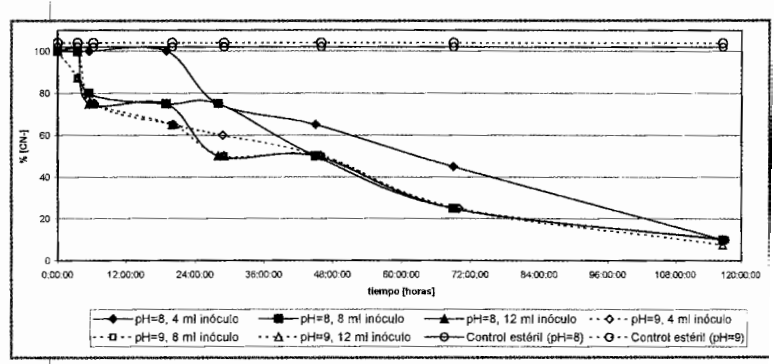

Figure 4. Cyanide concentration percentage versus time as a function of the inoculum quantity added to each 100 ml of fresh solution at pH 8 and 9. Initial cyanide concentration 400 ppm. Statically incubated at ambient temperature.

Effect of temperature on cyanide microbial degradation

Assays were performed in parallel incubating statically at 4 ºC, ambient temperature and 37 ºC. The initial cyanide concentration was 400 ppm. The pH were adjusted to 8 and 9 and 4 ml of the mixed selected culture were added for each 100 ml of fresh medium. The corresponding non-inoculated sterile controls were performed at each pH and temperature.

The cyanide microbial degradation as a function of temperature is shown in the graphic of Figure 5. The results show the cyanide microbial activity increase as the temperature is increased, at least up to 37 ºC. Temperature markedly affects the cyanide microbial degradation. As it is shown in the graphic,

at 37 ºC and pH 8 as well as 9, the accelerated cyanide microbial degradation starts immediately. At pH 9 in 5.5 hours, 50 % of cyanide concentration was degradated, while at 4 ºC the cyanide concentration was maintained during 48 hours at values in the order of the initial and sterile controls cyanide concentrations. It is apparent the importance of temperature in the microbial activity.

Microscopic observations done during the experiences shown that at ambient temperature as at 37 ºC, the same microbial morphologies were developed being majority those corresponding to BIIMC 1 and BIIMC 2 microbial strains. They were also present in assays carried out at 4 ºC, but in minor proportions as regards to assays at higher temperatures.

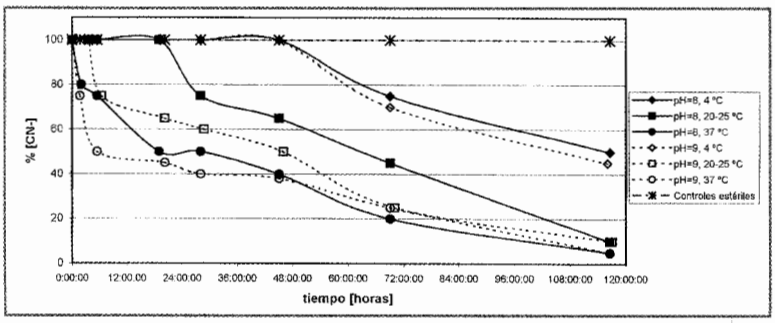

Figure 5. Cyanide concentration percentage versus time as a function of temperature at pH 8 and 9. Initial cyanide concentration: 400 ppm. Statically incubated.

Effect of initial cyanide concentration on cyanide microbial degradation

Cyanide concentration in effluents resulting from mining industry is in general at most 200 ppm. It is noticed the prior assays were performed at initial cyanide concentration of 400 ppm because the selected microorganisms had been initially selected and grown at 400 ppm. Kinetics studies were done at initial cyanides concentrations : 200, 400 and 600 ppm, so as to detect a probable inhibitor effect caused by very high cyanide concentrations.

Fresh media prepared at cyanide concentrations of 200, 400 and 600 ppm and adjusted to pH 9, were inoculated with 4 ml of the mixed selected culture. The corresponding non-inoculated sterile controls were done. All the assays were statically incubated in parallel at ambient temperature and at 37 ºC.

Cyanide microbial degradation at ambient temperature and at 37 ºC, as a function of cyanide initial concentration is shown in Figure 6. By clearing reasons the values corresponding to the non-inoculated sterile controls were not represented, but as it was in the prior experiences, the values of these controls were in the order of 100% with respect to the initial cyanide concentration during the experience time. The graphic shows the cyanide microbial degradation decreases as the initial cyanide concentration increases. Particularly, it is observed the period corresponding to the initial microbial adaptation phase, before starting the cyanide degradation, significantly increases as the initial cyanide concentration is increased. It is strongly suggesting a microbial inhibitor effect at high cyanide concentrations. The inhibitor effect is more noticeable at ambient temperature than at 37 ºC.

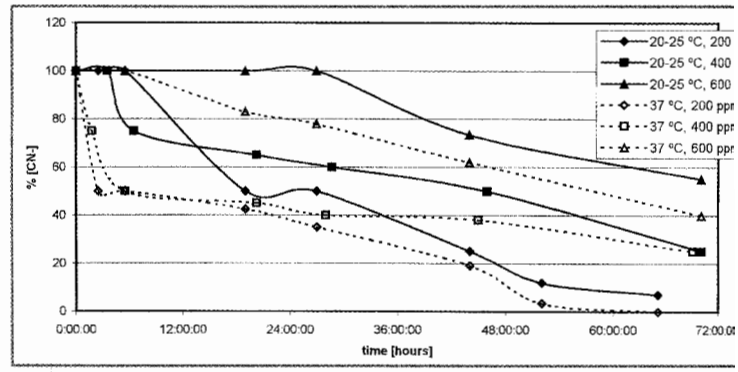

Figure 6. Cyanide concentration percentage versus time as a function of the initial cyanide concentration at pH 9. Statically incubated at ambient temperature and at 37 ºC.

Effect of oxygen dissolution (agitation) on cyanide microbial degradation

The prior experiences were done in static manner, in such a way as the oxygen availability was constant in all of them, and given by the oxygen dissolved through the liquid-air contact surface in the cylinder flasks. The mixed selected culture is aerobic. So, the oxygen availability should be an important factor. To prove this effect, assays in parallel were carried out in static manner (without stirring) and stirred as it was indicated in Materials and Methods. Assays were performed at initial cyanide concentrations of 200 and 400 ppm and adjusted to pH 9. They were inoculated with 4 ml of the mixed selected culture coming from a prior assay made in the same conditions. The corresponding non-inoculated sterile controls were performed in the same conditions. All the assays were incubated at ambient temperature (20-25 ºC).

The cyanide microbial degradation at initial cyanide concentrations 200 and 400 ppm, as a function of agitation: static or stirred, is shown in Figure 7. It is observed that at both initial cyanide concentrations, the cyanide microbial degradation increases in stirred assays compared to static assays, confirming that the oxygen availability is an important factor for microbial development and the consequently cyanide degradation. It is noted that at initial cyanide concentration of 200 ppm and in the stirred assay, during the first 2.5 hours 50% of the initial cyanide was degradated. The effect of oxygen availability is more notable at 200 ppm than at 400 ppm, suggesting the inhibitor effect given by high cyanide concentration made to decrease the positive effect of other factors.

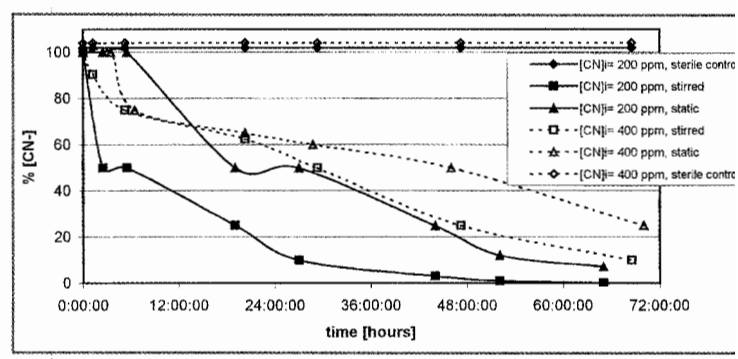

Figure 7: Cyanide concentration percentage versus time as a function of agitation: statically or stirred, at cyanide initial concentrations (200 and 400 ppm). pH 9, incubated at ambient temperature.

Effect of medium salinity on cyanide microbial degradation

For analysing the salinity effect on the microbial activity of cyanide degradation, assays were done using tap water in place of Solution A, or adding to tap water NaCl in the necessary quantity for obtaining 10 and 20 g/l as the end salt concentrations. Tap water generally contains the elements required as traces for microbial development. Solutions were adjusted to pH 9 and assays were performed at initial cyanide concentrations of 200 and 400 ppm. Assays were inoculated with 4 ml of the mixed selected culture coming from assays priory done under the same conditions. The corresponding non-inoculated sterile controls were performed in the same conditions. All the assays were statically incubated at 37 ºC.

The cyanide microbial degradation at initial cyanide concentrations of 200 and 400 ppm, as a function of the salt content in the medium (NaCl: 0, 10 and 20 g/l) is shown in Figure 8. The values corresponding to the six non-inoculated sterile controls have not been graphicated, but the cyanide percentages were maintained in the order of 100% during the experience time. It is observed that cyanide microbial degradation increases as the medium salinity is increased.

Microscopic observations were periodically done during the experience taking small samples from the different assays. At NaCl concentrations 0, 10 and 20 g/l the majority morphologies of the present microorganisms correspond to the BIIMC 1 and BIIMC 2 strains morphologies, which were also developed in the medium prepared with solution A. The corresponding colonies as the shown in Figures 1 and 2 were obtained in media prepared with Solution A (without NaCl). Also in the graphic of Figure 8, it is shown there is cyanide microbial degradation without addition of NaCl. Considering the term halophilic is correctly applied to a specialised group of bacteria that requires specifically a high NaCl concentration for growth, the probability these strains were halophilic microorganisms is limited.

The microbial behaviour is more similar to osmophylic microorganisms (Hocking, 1988), which increase their microbial activity as the osmotic pressure increases, but the osmotic pressure is given by non-specific solutes. However, more microbiological studies are necessary to assure it. It is noted that, in general mining effluents are of relatively high salinity given by the dissolution of salts contained in minerals ores.

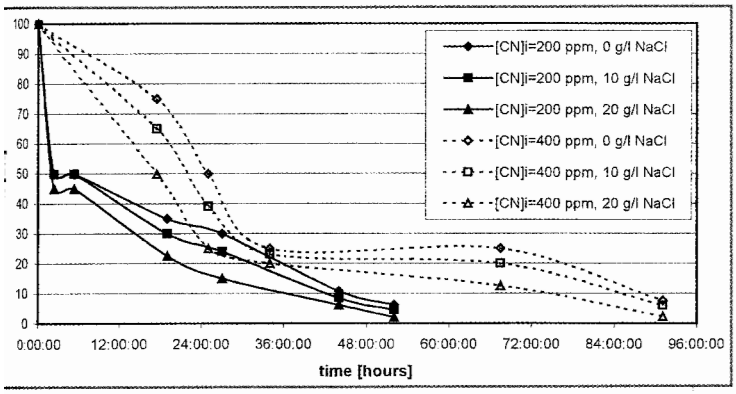

Figure 8. Cyanide concentration percentage versus time as a function of the medium salinity at initial cyanide concentrations: 200 and 400 ppm. pH 9. Statically incubated at 37 ºC.

CONCLUSIONS

The following conclusions can be drawn:

- The application potentiality of cyanides microbial degradation by the aerobic mixed selected culture has been demonstrated.
- The cyanide microbial degradation may be carried out in a wide range of pH from 8 to 11, being most of cyaniding mining effluents in this pH range. The cyanide microbial degradation velocity increases at pH 9.
- The increase of the inoculum quantity decreases the initial microbial adaptation period and the starting of the accelerated cyanide microbial degradation.
- The cyanide microbial degradation increases as the temperature is increased, unless up to 37 ºC.
- The cyanide microbial degradation increases as the initial cyanide concentration is decreased from 600 to 200 ppm, suggesting an inhibitor effect at very high cyanide concentrations. Nevertheless, in most of cyaniding mining effluents, the cyanide concentration is at most 200 ppm.
- The oxygen availability for this aerobic selected culture is an important factor. The agitation notably increases the cyanide microbial degradation.
- The medium salinity increases the cyanide microbial degradation.
- The results obtained in this investigation constitutes a base for designing and operating a bioreactor under the best conditions.

ACKNOWLEDGEMENTS

We acknowledge to CICYTCA - San Juan National University, for supporting this work. We also want to acknowledge the support given by the Mining Research Institute at National University of San Juan, for this research.

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