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Cyanide degradation in gold ore leach effluent by native *Pseudomonas pseudoalcaligenes*

Degradación de cianuro en efluente de lixiviación de minerales auríferos, mediante *Pseudomonas pseudoalcaligenes* nativas

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RESUMEN

The generation of cyanide-containing tailings is increasing; therefore, the search for methodologies and microorganisms to counteract their negative impact on the environment is constant. In this study, Pseudomonas pseudoalcaligenes strains isolated from gold ore cyanidation effluents were used. Initially, tolerance tests were carried out in solutions containing 250 ppm sodium cyanide at different pH values, using an initial population of 4.00 x 10^7 CFU/mL and agitation for 54 hours. A degradation of 39.2 % free cyanide (CN^-) was observed at pH 9.8. Subsequently, CN^- degradation was evaluated for 144 hours in media consisting of cyanidation process effluent solutions at pH 10.5, an initial bacterial population of 9.94 x 10^8 CFU/mL and concentrations of 80 to 700 ppm CN^-. The results showed cyanide reductions ranging from 55.89 to 84.79 %, represented by the equations: y_{80} = -0.2393x + 73.05 and y_{700} = -3.6439x + 617.98, respectively. Therefore, it was determined that the degradation of cyanide is directly related to its concentration in the solution, the higher the concentration of cyanide, the higher the percentage of abatement.

Palabras claves: bioremediation, cyanidation, cyanide degradation, gold ores, *P. pseudoalcaligenes.*

ABSTRACT

La generación de relaves con contenido de cianuro está en aumento; por ello, la búsqueda de metodologías y microorganismos para contrarrestar su impacto negativo en el medio ambiente es constante. En este estudio se utilizaron cepas de Pseudomonas pseudoalcaligenes aisladas de efluentes del proceso de cianuración de minerales auríferos. Inicialmente, se llevaron a cabo ensayos de tolerancia en soluciones con 250 ppm de cianuro de sodio a diferentes valores de pH, empleando una población inicial de 4.00 x 10^7 UFC/mL y con agitación durante 54 horas. Se observó una degradación del 39,2 % de cianuro libre (CN^-) a un pH de 9,8. Posteriormente, se evaluó la degradación del CN^- durante 144 horas en medios constituidos por soluciones de efluentes del proceso de cianuración con pH de 10,5, una población bacteriana inicial de 9.94 x 10^8 UFC/mL y concentraciones de 80 a 700 ppm de CN^-. Los resultados mostraron reducciones de cianuro en un rango del 55,89 al 84,79 %, representadas por las ecuaciones: y_{80} = -0,2393x + 73,05 y y_{700} = -3,6439x + 617,98, respectivamente. Por lo tanto, se determinó que la degradación del cianuro tiene relación directa con su concentración en la solución; es decir, a mayor concentración de cianuro, mayor es el porcentaje de abatimiento.

Keywords: biorremediación, cianuración, degradación de cianuro, minerales auríferos, *P. pseudoalcaligenes.*

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I. INTRODUCTION

Hundreds of millions of tonnes of waste containing cyanide (a highly toxic compound), along with other valuable elements, are generated and discharged into tailings dams each year (Dong et al., 2021). Furthermore, diverse bacterial communities are formed, both culturable and non-culturable, from which it is possible to isolate and characterise strains of different genera, such as *Pseudomonas, Bacillus, Acinetobacter*, among others (Sernaque et al., 2019). In order to preserve the ecosystem from polluting effects, various microorganisms tolerant to extreme conditions are found in nature, capable of biodegrading toxic metals and other pollutants, such as cyanide (Begum et al., 2022).

Sodium cyanide (NaCN) is a chemical compound widely used in gold ore processing plants; consequently, abundant solid and liquid effluents containing cyanide in various forms and compounds are generated, resulting in negative impacts on the environment (Anning et al., 2019; Anand & Pandey, 2022). Microorganisms prominent in cyanide biodegradation include *Streptococcus sp. C33* and Bacillus sp. A74 (Marin, Ochoa & Prado, 2010), *Pseudomonas parafulva* NBRC 16636 (Moradkhani et al., 2018), *Escherichia coli and Pseudomonas fluorescens* (Martinez & Hernandez, 2022). With the use of the *Bacillus subtilis* strain, it has been possible to degrade 100 % of free cyanide in synthetic solution with a concentration of 500 mg/L (Rosario et al., 2023).

Bioremediation or biodegradation involves reactions that convert the cyanide radical into simpler organic or inorganic molecules, which can be easily metabolised to ammonium and carbon dioxide or methane (Sáez et al., 2019). Many microbial species (bacteria, fungi and algae), as well as plants, can catalyse the degradation of cyanide and/or facilitate the formation of less harmful by-products, up to acceptable limits (Gurbuz et al., 2004). There are various enzymes produced by microorganisms that use cyanide as a substrate (Gupta et al., 2010; Tuya, 2014; Tiong et al., 2015). All these options are presented as clean and alternative technologies for environmental protection (Del Carpio et al., 2007).

Several microbial species can degrade cyanide compounds into less toxic products through different metabolic pathways, whose enzymes are produced by microorganisms that use cyanide as a substrate. Biological methods for cyanide treatment, besides being cost-effective, do not generate environmental pollution (Dwivedi et al., 2018). *Alkalophilic Pseudomonads* are being extensively studied for their application in the remediation of various effluents, especially those from gold mining, where NaCN is widely used. Therefore, it is essential to define their tolerance to various cyanide concentrations and to factors such as pH, temperature and inoculum size (Luque-Almagro, 2004; Khamar & Mahmudy, 2015). The identification of oxidation and reduction reactions, as well as aerobic and anaerobic microorganisms, has made it possible to evaluate the advantages and disadvantages of cyanide bioremediation in soils and waters (Gordillo, 2018). Microbiological degradation of cyanide has often been proposed as an alternative to conventional methods, with economic and environmental benefits (Mosher & Figueroa, 1996). Alvarez et al. (2023) evaluated the biodegradation potential of cyanide under alkaline conditions using a native strain of Bacillus subtilis isolated from a gold mine. Similarly, Alvarado et al. (2022) identified several genera of bacteria capable of degrading cyanide under alkaline conditions.

To counteract the negative effects of cyanide, there are several methodologies and microorganisms whose effectiveness depends on various environmental factors (Agurto & Arzapalo, 2021). With the bacterium *Klebsiella sp*. ART1 in aerated reactors, it was possible to degrade up to 98 % of cyanide to less toxic products (Copari et al., 2020). Mekuto et al. (2014) stated that all microorganisms isolated from cyanide-containing wastewater belonged to the genus *Bacillus*. They describe the ability of these microorganisms to tolerate and degrade high concentrations of CN^-, partially converting it into ammonium. However, they also point out the inHibitory effect on cyanide degradation, as the microorganisms prefer to use ammonium over cyanide, which reduces the efficiency of biodegradation (Panay et al., 2020). In the study by Cotrina and Mamani (2022), high degradation rates of cyanide in cyanide effluents were achieved by Pseudomonas *fluorescens.*

Pseudomonas pseudoalcaligenes can use cyanide and its derivatives as a source of nitrogen for their growth, allowing the biodegradation of cyanide industrial waste (Luque-Almagro et al., 2016; Ibáñez et al., 2017) and the abatement of cyanide in wastewater from the jewellery industry (Cabello et al., 2018; Ibáñez, 2019).

II. MATERIALS AND METHOD

The study was carried out with effluents from the cyanidation processes of gold and silver ores, deposited in the tailings pond of Compañía Minera Aurífera Cuatro de Enero S.A., located in Caravelí, Arequipa, Peru (Arias et al., 2023).

2.1. Source of effluent and sampling

Ten samples were extracted, four at feeding points and six at different points of the tailings dam, with a total volume of approximately 30 litres. The samples were transported following the recommendations of Ashbolt (2015). The determination of free cyanide content was carried out by volumetric titration, using a solution of silver nitrate and potassium iodide.

2.2. Evaluation of the tolerance of the activated strain

Tolerance assessment was performed on M9 minimal medium (Luque-Almagro, 2004), used in several previous studies. Khamar et al. (2015) isolated various microorganisms from gold ore processing residues and determined their tolerance in solutions up to 350 ppm cyanide. In the present study, tolerance is evaluated by subjecting the strain to a minimal medium composed of 0.005 % K_2HPO_4, 0.2 % Na_2SO_4, 0.003 % NaCl and 0. 1 % of $MgSO_4$ (Morillo & Guevara, 2015), supplemented with sodium cyanide until reaching 250 ppm CN- and pH values of 9.8, 10.0, 10.5, 11.0 and 11.3.

The assays were performed in flasks shaken on a platform (Orbit Shaker) at 150 rpm for 54 hours, in a medium

composed of 100 mL of M9C and 10 mL of active strain, with an initial bacterial population of 4.00 x 10^7 CFU/mL. Population growth and cyanide degradation were evaluated.

2.3. Cyanide degradation in the cyanidation effluent

The knowledge and management of the variables largely determine the degree of destruction of the cyanide radical in aqueous media. In studies conducted by Morillo & Guevara (2015), it is mentioned that CN^- degradation at high concentrations is influenced by temperature and pH, being very favourable at 36 ºC and at a pH of 9.5.

The degradation tests were performed in 500 mL flasks on platform shakers at 150 rpm, adding 200 mL of cyanidation process solution with different concentrations of CN^- and 20 mL of active strain with a population of 9.94 x 10^8 CFU/mL. The cyanide concentrations used were: 80, 235, 390, 545 and 700 ppm.

III. RESULTS

3.1. Tolerance with 250 ppm CN^-

During the cyanide tolerance tests of the bacteria, population increases were observed during the first hours of bacterial contact, going from 4.00 x 10^7 to 2.52 x 10^8 CFU/mL at pH 9.8 in approximately 18 hours. Subsequently, the population decreased progressively. This behaviour was similar at higher pH values. At pH 11.3, the population was reduced to 6.50 x 10^6 CFU/mL after 54 hours of processing. The dormancy and growth phases were unified, showing a growth trend; the stationary phase was not observed, but the death or decline phase was observed (see Figure 1).

The kinetics of cyanide degradation, by the action of *P. pseudoalcaligenes*, in a minimal salt medium, supplemented with NaCN solution (250 ppm), was evaluated at pH: 9.8, 10.0, 10.5, 11.0 and 11.3 , during the period of 54 hours, reducing to: 152, 162, 170, 171 and to 183 ppm CN^- , respectively. The negative control (without strain inoculation) for the same concentration of CN^- was carried out at pH 10.5, showing the degradation of cyanide from 250 to 230 or a reduction of 8 % in the same period.

Under the conditions evaluated, the degradation of the CN^- in the period tested were: 39.22, 35.21, 32.02, 34.07 and 27.03%, from solutions with pH of: 9.8, 10.0, 10.5, 11.0 and 11.3, respectively.

Figure 1

Population variation of P. pseudoalcaligenes in a minimal salt medium, supplemented with 250 ppm CN^-

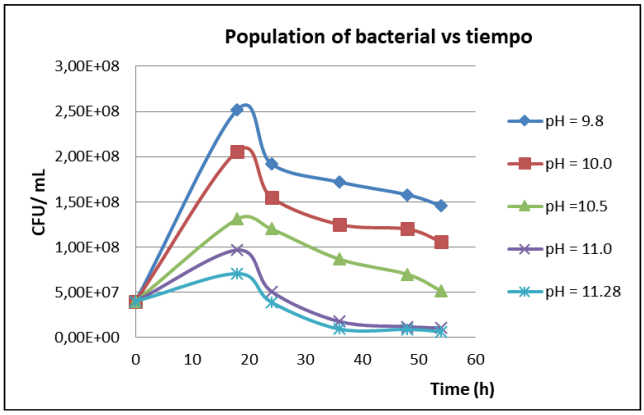


Figure 2

Degradation of CN^- by P. pseudoalcaligenes bacteria

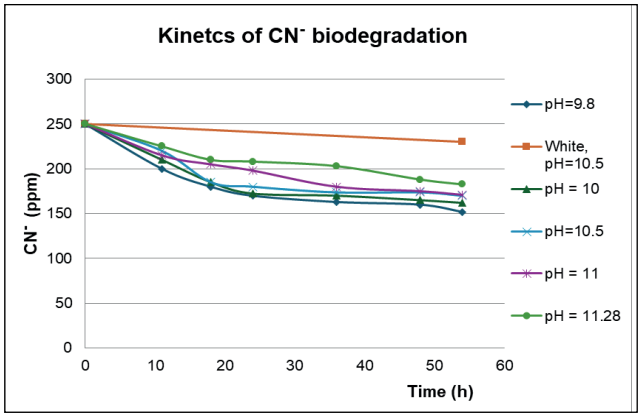
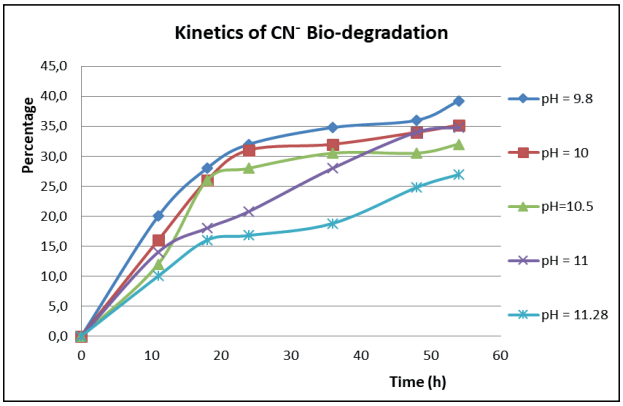


Figure 3

Degradation of CN^- by P. pseudoalcaligenes from an initial content of 250 ppm, at different pH values and for 54 hours.



3.2. Degradation of CN^- in cyanidation process effluent

For cyanide degradation, the strains were initially cultured in a minimal salt medium for 48 hours in order to prepare liquid solutions. These were composed of effluent from the mineral leaching process with different concentrations of cyanide, the bacterial strain *Pseudomonas pseudoalcaligenes* and adjusted to a pH of 10.5.

In all cases the bacterial population decline is very rapid. In the first 48 hours of treatment, about 80% of the population is lost, reaching 2.68 x 10^6 and 5.40 x 10^6 CFU/mL in the media initially containing 80 and 700 ppm CN^- , respectively.

The biodegradation kinetics of cyanide is directly related to its initial concentration in solution. Figure 5 shows a reduction from 700 to 106.5 ppm, which corresponds to a reduction of 84.79 %. At the other extreme, the concentration decreased from 80 to 35.3 ppm, with a reduction of 55.89 %. These results are represented by linear equations (1) and (2), both with negative slopes.

$$y_{700} = -3.6439x + 617.98 \quad (1)$$

$$y_{80} = -0.2393x + 73.05 \quad (2)$$

In the negative control, performed without the inoculation of the bacterial strain, under the same parameters, the reduction from 700 to 614 ppm CN^- equivalent to 12.28% reduction was observed.

Cyanide degradation over 144 hours was: 55.89, 76.43, 79.07, 82.36 and 84.79 %, tested at initial concentrations of: 80, 235, 390, 545 and 700 ppm CN^-, respectively.

Figure 4
Population decline of P. pseudoalcaligenes in cyanidation process effluent

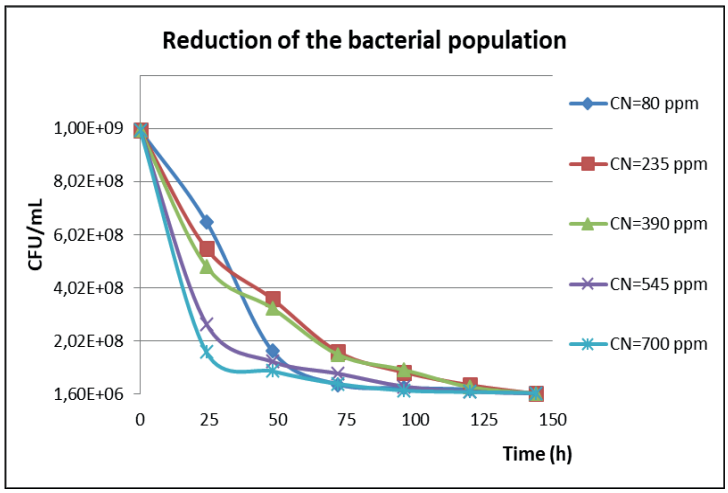


Figure 5
Linear representation of cyanide abatement in leach waste effluents containing different concentrations of CN^-

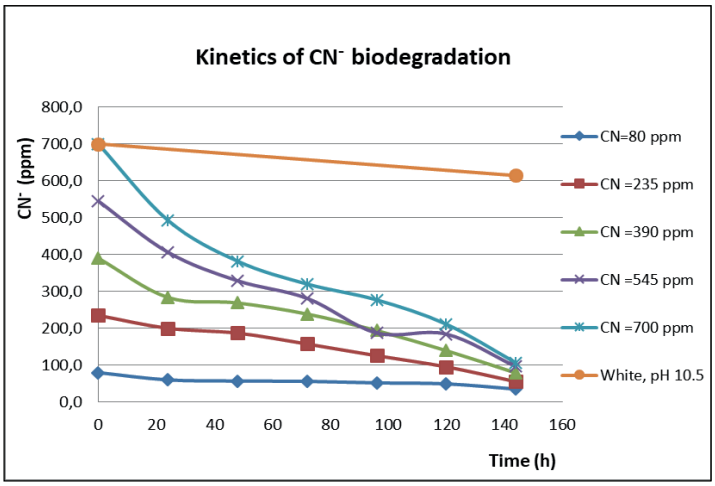
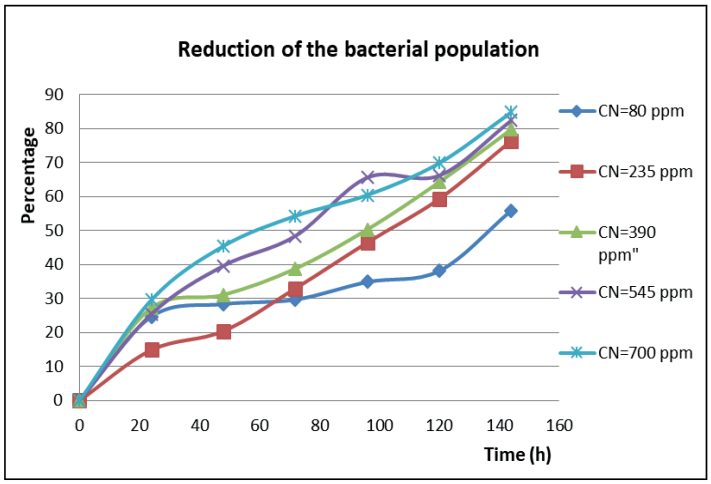


Figure 6
Degradation of CN^- in solutions of pH 10.5



IV. DISCUSSION

Physicochemical treatment processes allow degrading the cyanide radical to its simple forms at relatively low costs; however, microbiological methods offer highly viable and environmentally friendly alternatives (Akcil et al., 2003; Cabello et al., 2018; Alvillo et al., 2021). In waste generated by industrial activity, it is of interest to identify nitrilases capable of degrading cyanide, as these do not require secondary substrates or cofactors for the hydrolysis of cyanide organic derivatives (Park et al., 2017). However, this mechanism has not been widely addressed.

It has been observed that the most favourable kinetics for CN^- degradation occurs in the first hours of processing, as evidenced in the study by Marín et al. (2010). This process, however, leads to a significant reduction in the bacterial population, which decreases over time, so it is necessary to identify the causes of this decrease and seek to optimize the process. The ability of bacteria to tolerate high concentrations of cyanide is not yet fully defined, but it is known to be directly related to the content of this compound in the solution. Variables of interest include temperature, pH (Morillo & Guevara, 2015) and the concentration of organic nutrients (Moradkhani et al., 2018).

In the case of *Pseudomonas pseudoalcaligenes*, the only source of nitrogen for its metabolic activity is the cyanide or cyanate radical. Its degradation involves the identification of an enzyme that facilitates its hydrolyzation and/or conversion to ammonium and carbon dioxide (Sáez et al., 2019). Omics approaches, such as genomics, transcriptomics, metabolomics and proteomics, are enabling better regulation and optimization of remediation processes (Kaur et al., 2021).

V. CONCLUSIONS

Under the parameters evaluated, during the tolerance test of the bacterium *Pseudomonas pseudoalcaligenes*, it was observed that the highest degradation of cyanide occurred at pH 9.8, reaching 39.2 %, which represented a reduction from 250 to 152 ppm. At pH 11.3, the degradation was 27.0 %, reducing from 250 to 183 ppm CN^-. This is evidence that, at more alkaline pH values, the degradation of free cyanide is affected.

At the different cyanide concentrations evaluated, the decrease in bacterial population was very rapid during the first 48 hours, reaching values of 2.68×10^6 and 5.40×10^6 CFU/mL in solutions initially containing 80 and 700 ppm CN^-, respectively. A direct relationship between bacterial population and free cyanide was determined, suggesting possible self-destruction due to depletion of organic nutrients in the medium.

The abatement of free cyanide showed a direct relationship with its initial concentration in solution. In media with an initial concentration of 700 ppm, it was reduced to 106.5 ppm (84.79 % reduction), while in solutions with an initial 80 ppm, the final concentration was 35.3 ppm (55.89 % reduction). Represented by the following equations: $y_{700} = -3.6439x + 617.98$ and $y_{80} = -0.2393x + 73.05$, respectively.

On the other hand, negative control tests performed at different concentrations of free cyanide and at pH 10.5 revealed a natural degradation of CN^-. In this case, an 8 % reduction was recorded over a period of 54 hours and 12.28 % over 144 hours, with a clear tendency to increase over longer periods and under extreme conditions.

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Conflict of interests

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