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Optimization of Factors for the Biological Treatment of Free and Complexed Cyanide

Angelica Julieta Alvillo-Rivera [1], Sofia Esperanza Garrido-Hoyos [2,*] and Genoveva Rosano-Ortega [3]

[1] Polytechnic University of the State of Morelos, Boulevard Cuauhnáhuac 566, Progreso C.P. 48485, Mexico
[2] Mexican Institute of Water Technology, Blvd. Paseo Cuauhnáhuac 8532, Progreso C.P. 62550, Mexico
[3] Decanato de Ciencias Biológicas, Universidad Popular Autónoma del Estado de Puebla, 21 Sur #1103, Barrio de Santiago C.P. 72410, Mexico
* Correspondence: sgarrido@tlaloc.imta.mx

Abstract: Mexico is characterized as a mining country since it is the world's main silver producer. During its extraction, wastewater (mining tailings) is generated which contains cyanide and heavy metals. The purpose of this research was to determine whether a bacterial consortium isolated from a tailings dam can use cyanide as a source of nitrogen and carbon to carry out its biodegradation. The study determined the effects of three physicochemical factors (pH, temperature and inoculum concentration) and three metals (copper, iron and nickel) on cyanide biodegradation. The results showed that the highest cyanide removals were obtained when working with a pH of 9.5, a temperature of 25 °C and 15% *v/v* of inoculum (88%), while the optimum values for copper, iron and nickel were 0, 7.7 and 0.46 mg·L^{-1}, respectively, showing that copper causes an inhibitory effect (cyanide biodegradation of 68%) on the bacteria and consequently on the biological degradation of cyanide and that iron can promote the biodegradation of the pollutant by 91%.

Keywords: cyanide; heavy metals; biodegradation; alkalophilic bacteria

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1. Introduction

Cyanide is the general term applied to a group of chemicals containing a carbon atom with a triple bond to a nitrogen atom (:CΞN:); organic compounds possessing a -CΞN functional group attached to an alkyl residue are called nitriles, according to IUPAC nomenclature [1]. The main man-made forms of cyanide are gaseous hydrogen cyanide (HCN) and sodium cyanide (NaCN) and potassium cyanide (KCN), the latter two existing in a solid state [2]. However, there are many other forms of cyanide derived from anthropogenic activities, such as manufacturing, chemical industries, pesticide use, mining, metal finishing, coke plant operations, paint production, petroleum refining, and explosives and automobile manufacturing [3].

The classification of the different cyanide compounds depends on the concentration of the free cyanide ion [4,5]; the compounds can be classified as: (a) weak acid dissociable complexes (WADs), which are dissociable at pH 4. 5, which in turn are divided into: (1) free cyanide (CN^- and HCN); (2) simple cyanides: easily soluble (NaCN) and insoluble neutral salts (KCN, $Ca(CN)_2$, $Hg(CN)_2$, $Zn(CN)_2$, $Cd(CN)_2$, Cu(CN), $Ni(CN)_2$ and Ag(CN)); and (3) weak complex cyanides ($Zn(CN)_4^{2-}$, $Cd(CN)_3^-$ and $Cd(CN)_4^{2-}$); and (b) strong acid dissociable complexes (SADs), which are characterized by being dissociable at a pH of 2 and include: (1) cyanides with moderately strong complexes ($Cu(CN)_2^-$, $Cu(CN)_3^2$, $Cu(CN)_4^{3-}$, $Ni(CN)_4^{2-}$ and $Ag(CN)_2^-$), (2) cyanides with strong complexes ($Fe(CN)_6^{4-}$, $Co(CN)_6^{4-}$, $Au(CN)_2^-$ and $Fe(CN)_6^{3-}$), (3) Thiocyanate (SCN^-) and (4) Cyanate (CNO^-).

Cyanide does not cause chronic diseases because it is not cumulative [6–8]. However, there is a risk to human health, mammals, reptiles, amphibians, birds, fish, and other aquatic lifeforms upon exposure [9]. since it enters the body through various routes (inhalation, ingestion, or absorption) and is a non-specific enzyme inhibitor, since it can

act by inhibiting several enzymes, such as succinyldeshydrogenase, superoxide dismutase, carbonic anhydrase and cytochrome oxidase [10].

The degree and rate at which these complexes dissociate depends on several factors; largely, these are the pH of the solution, the initial concentration of the cyanide complex, the temperature, the UV radiation, the REDOX potential (Eh) of the solution and the formation constants [11].

Different physical, chemical, and biological techniques have been studied for cyanide treatment, each type of process removing specific forms of cyanide. In the case of adsorption, free cyanide is not treated, while membranes and biological processes remove all forms of cyanide. As for chemical oxidation, this involves removing mainly free cyanide and its weak bonds [12]. The decision about which is best will depend on factors such as the cost of the treatment, the available space to be used, the initial concentrations of the contaminant and the amount of waste to be treated, among others [13].

The biological treatment of mining tailings has been less well studied than the physicochemical processes already established; however, they are considered of great importance due to the generation of secondary compounds that are less harmful to humans and the environment compared to those mentioned above (physicochemical processes) [14].

Biological methods use living organisms (biomass), cellular components or free enzymes to transform waste or pollutants, as well as to alter the redox state of metals. Depending on the form of microbial agglomeration used by the biological processes, these can be divided into: (a) suspended biomass systems and (b) immobilized biomass (biofilm) systems [3,15], which in turn can be divided into two types: (1) stationary or fixed media systems (water moves through a stationary medium) and (2) moving media systems or moving biological bed reactors (solid media move through liquid) [16,17]. In turn, they can be classified according to the availability of oxygen in them as aerobic, anaerobic, or anoxic [18].

There are many materials that can be used to carry out biomass immobilization; however, the most-studied packaging materials are stones (tezontle and granite), clay and synthetic materials (polypropylene, polyethylene, polyurethane, polystyrene, and plastic waste) [19]. These packings must possess certain characteristics, such as being structurally resistant (resistant to degradation by UV, erosion or degradation, and fungal and bacterial attack), non-toxic to microorganisms, lightweight, having a large surface area (influenced by the size and configuration of the packaging), having rough surfaces, and being low-cost and conducive to rapid colonization by microorganisms [18,20–23].

Microorganisms use cyanide as a source of carbon and nitrogen [9,24], which is why it is important to take into account treatments that use nitrifying bacteria (which use nitrogen in their growth and development processes), since these types of bacteria are also capable of metabolizing a wide variety of organic pollutants [25]. Bacteria have developed several mechanisms to tolerate the harmful effects of metals which involve (a) cellular components that capture ions, neutralizing their toxicity; (b) enzymes that modify the redox state of metals or metalloids, converting them into less toxic forms; and (c) membrane transporters that expel harmful species from the cell cytoplasm [26].

The vast majority of studies that have been carried out on the treatment of free cyanide have been at the laboratory level and in steady-state systems, either suspended biomass systems or immobilized biomass systems, using different types of bacteria, such as *Bacillus* (*B. cereus*, among others [27,28], *Pseudomonas* (*P. resinovorans* [1], *P. fluorencens* [29], and *P. pseudoalcaligenes* [30], *Alcaligenes* [31], *Rhodococcus* sp. [32], *Klebsiella pneumoniae* [33] and *Ralstonia* sp. [34]; however, the use of fungi in this type of treatment has also been reported, for example, *Trametes versicolor* [35].

In the case of immobilized biomass systems, various types of packaging and operating conditions have been studied. The most commonly used packings are natural (granite rock, citrus peels, cellulose and gravel) and synthetic (stainless steel, geotextiles, alginate and plastics), which have shown effects on biomass concentrations in treatments varying from 1×10^6–1×10^7 [28,36] or 3500 mg·L^{-1} to 10,000 mg·L^{-1} [37]. The retention time

and agitation or flow rate depend on the configurations of the reactors and can range from 8 h [36] to 200 days [38], while pH can fluctuate between 6.5 and 10.5 units.

On the other hand, several studies have been conducted in batch reactors on the degradation of cyanide complexes with metals, which are extremely stable, using bacteria of the genera *Pseudomonas, Burkholderia, Acinetobacter, Bacillus* and *Azotobacter* and fungi of the genera *Fusarium, Trichoderma, Trametes* and *Penicillium* [29,31,39], generally using an external carbon source, such as cassava, citrus residues or glucose [40], providing promising results, which is the reason for the elaboration of the present research.

2. Objective

The objective of this work was to study the influence of factors such as pH, temperature, and inoculum concentration in batch systems with suspended biomass for the biodegradation of free cyanide, as well as the interferences that occur in its biological treatment when cyanide is complexed with three different metals.

3. Materials and Methods

3.1. Experimental System

All experiments were carried out for 35 days in 500 mL amber bottles. Each bottle was filled with 450 mL of sterilized synthetic water (mineral medium), which was found to be constituted as stated by Khamar et al. (2015) [41] ($g \cdot L^{-1}$) with analytical grade reagents and was produced by J.T. Baker: $Na_2HP_4.7H_2O$, 12.8; KH_2PO_4, 3; NaCl, 0.5; $MgSO_4.7H_2O$, 0.5; $CaCl_2$, 0.1; and 1% (v/v) mineral salts ($ZnSO_4.7H_2O$, 0.05; $MnCl_2.4H_2O$, 0.05; $CuCl_2.2H_2O$, 0.005; $Na_2MoO_4.2H_2O$, 0.005; $Na_2B_4O_7.10H_2O$, 0.002; $CoCl_2.6H_2O$, 0.0003).

3.2. Bacterial Strains/Bacterial Consortia

The first step was to obtain the microorganisms. We worked with two different microbial consortia, the first one previously acclimatized to cyanide (MC10), while the second had not been in contact with the contaminant (MC0).

MC10 was obtained from a reactor previously worked with 10 $mg \cdot L^{-1}$ CN- by Ortiz-Sayavedra and Tejeda-Gil (2018) [42], which, in turn, came from a soil sample from the "Mastrantos II" tailings dam of the "El Cubo" mine located in the state of Guanajuato, Mexico. Meanwhile, MC0 was obtained from the mixed liquor (activated sludge) of the Domestic Wastewater Treatment Plant of the Mexican Institute of Water Technology (IMTA), located in Jiutepec, Morelos, Mexico, whose waters are free from cyanide in any of its forms.

3.3. Inoculation, Propagation and Maintenance of Bacteria

To obtain MC0, 100 mL of the water sample was taken and placed in flasks with 500 mL of deionized water, shaken for one hour, and after 5 min of rest, 10 mL of the supernatant was inoculated, under sterile conditions, in 90 mL of Meyer brand peptone broth (1:10, previously sterilized) for 60 h at 35 ± 2 °C and with orbital agitation at 120 rpm.

The propagation and maintenance of bacteria was carried out by means of biweekly reseeding in peptone broth at pH 7.2 in 250 mL Erlenmeyer flasks and incubated at 32 °C with orbital shaking at 120 rpm for 60 h in a water bath, using the Lab Company orbital shaker model B5-11, and subsequently maintained at a temperature of 32 °C in a Precision model 6 incubator.

3.4. Development of the Biomass Quantification Method

Prior to experimentation, growth curves were generated to determine the correlations that exist between colony forming units (CFU), optical density (OD) measurement and dry mass determination [43–45]. The methods of dilution and plate casting described in NOM-110-SSA1-1994 [46] and NOM-092-SSA1-1994 [47] and cited by the authors Monballiu et al., 2015 [48] and Madigan et al., 2009 [49] were used to count CFUs. The wavelength at which the measurements were performed was 600 nm [49–51] and the target to be used was the uninoculated culture medium [52], while, for the determination of the dry weight of the

microorganisms, the procedure of NMX-AA-034-SCFI-2015 [53] for total suspended solids and volatile suspended solids was taken into account.

3.5. Evaluation of Different Inocula in the Biodegradation of Free Cyanide

The evaluations of the inocula were based on the tests cited by OECD (1992) [54] 301 A and 310 were performed at a temperature of 25 °C, cyanide being added as the only source of carbon and energy, with an inoculum that had not been in contact with the contaminant (MC0) and one that had been acclimated (MC10); however, the pH was modified from 7 to 9.5 units to suit the contaminant to be analyzed (cyanide) and to avoid its volatilization [55]. An important condition for the tests to be considered positive was that 10% biodegradation had to be achieved within the first ten days (a ten-day window) and maximum biodegradation after 29 days (latency phase (tL)). The solutions used to carry out the biodegradability study were three: (a) an inoculum control (IC), which consisted of a mineral medium (without the addition of cyanide and with glucose as a carbon source) to ensure microbial activity; (b) a test substance (TS) composed of mineral medium, inoculum and cyanide, to evaluate cyanide biodegradation; and (c) a volatilization control (VC), whose composition was mineral medium and cyanide (pH = 9.5), to ensure that the results were not due to contaminant volatilization [56,57].

3.6. Evaluation of Different Carbon Sources and Inhibition Tests

The evaluation of two different carbon sources was carried out: one was sodium acetate (SPFC) and the other was glucose (SPFCG), these being added to the mineral medium to compare the cyanide biodegradation efficiency by having an extra carbon source; additionally, the volatilization (CV), inoculum (CI) and cyanide without carbon source (SP) controls were run. The concentrations used to evaluate the different carbon sources were 10 $mg \cdot L^{-1}$ for each of the compounds (cyanide, sodium acetate and glucose). For the statistical analysis, comparisons of means, multiple comparisons and analysis of variance (ANOVA) tests were performed with a confidence level of 95% to determine the factors with a statistically significant difference. The program used to carry out the statistical analysis was Statgraphics Centurion XV, version 15.2.06.

Once the appropriate composition of the mineral medium for cyanide biodegradation (with or without an extra carbon source) was obtained, tests were carried out with different concentrations of cyanide (15, 20, 30 and 50 $mg \cdot L^{-1}$) to determine the inhibitory effect of the contaminant on the microorganisms.

3.7. Influence of pH, Temperature and Inoculum Concentration on Free Cyanide Biodegradation

To evaluate the effects of various conditions on cyanide biodegradation, physicochemical growth conditions were varied. A 33 factorial design was performed, where the factors evaluated and their respective levels were: pH, 9.5 (−1), 10.5 (0) and 11.5 (+1); temperature, 25 °C (−1), 30 °C (0) and 35 °C (+1); and inoculum concentration, 10% (−1), 12.5% (0) and 15% (+1). The initial concentration of free cyanide was 30 $mg \cdot L^{-1}$. The 3^3 factorial design was chosen because we wanted to determine whether the chosen factors had an influence on and the relationship that exists between all the possible combinations of levels of the response variable, which was the biodegradation of cyanide [58].

3.8. Sorption Tests

Prior to the evaluation of the metals complexed with cyanide for their biodegradation, the equilibrium time and sorption coefficients were determined for each metal. Inactivated biomass was used to determine the equilibrium time of the metals to be used. The biomass was inactivated according to the methodology cited by Flores-Velázquez (2017) [59], Torres-Bojorges (2012) [60] and Mijaylova-Nacheva et al. (2014) [61], which consists of adding 200 $mg \cdot L^{-1}$ of Hg_2SO_4 (HACH brand) to the biomass and leaving it exposed to the toxic compound for 24 h, subsequently rinsing it with water. A 0.01 M $CaCl_2$ solution (450 mL) was used as the liquid phase with a concentration of 6 $mg \cdot L^{-1}$ copper, 10 $mg \cdot L^{-1}$ iron

and 1.5 mg·L^{-1} nickel, and a volume of inactive biomass was added to provide a TSS concentration of 1000 mg·L^{-1}. The tests were carried out at room temperature and under orbital shaking at 100 rpm. Samples were taken after 1, 2.5, 5, 10, 12.5, 15, 30, 30, 60, 90, 180, 270, 360, 540, 720, 1080, 1440, 2160 and 2880 min, and the equilibrium time was established when the values of the metals were constant with respect to time.

Subsequently, tests were carried out for the determination of the sorption coefficient through the analysis of sorption isotherms, evaluating six different metal concentrations (copper = 1, 2, 3, 3, 4, 5 and 6 mg·L^{-1}; iron = 1, 2, 4, 6, 8 and 10 mg·L^{-1}; and nickel = 0.25, 0.5, 0.75, 1, 1.25 and 1.5 mg·L^{-1}). All tests were carried out at room temperature and under orbital shaking at 100 rpm in a Lab Company model B5–11 incubator. The tests had a duration equal to the equilibration time. The amount of the sorbed compound in the biomass was calculated with Equation (1).

$$q_e = \frac{(C_o - C_e)V}{m} \quad (1)$$

where q_e = the sorbed concentration of the compound after equilibrium (mg metal g lodo^{-1}); m = the mass of the sludge (g); C_o = the initial concentration of the compound in the liquid phase (mg·L^{-1}); C_e = the final concentration in the liquid phase of the compound at equilibrium (mg·L^{-1}); and V = the volume of the solution (L).

3.9. Determination of the Interference of Cyanide Complexed with Metals in Its Biodegradation

Subsequently, and with the optimum values for pH, temperature and inoculum, the interference that exists in the biodegradation of cyanide when it is complexed with three different metals, either individually or in groups, when the biomass is suspended, was evaluated. The metals and their concentrations were chosen based on the tolerance of certain bacteria to these metals and on the characterization of mine tailings reported in the literature [62–65] and because they are characterized as forming strong complexes with cyanide [4,5]. The initial concentration of free cyanide was 30 mg·L^{-1}. In this case, a 3^3 factorial design was also performed, in which the metals studied and their respective concentrations (levels) were: copper, 0 mg·L^{-1} (−1), 3 mg·L^{-1} (0) and 6 mg·L^{-1} (+1); iron, 0 mg·L^{-1} (−1), 5 mg·L^{-1} (0) and 10 mg·L^{-1} (+1); and nickel, 0 mg·L^{-1} (−1), 0.75 mg·L^{-1} (0) and 1.75 mg·L^{-1} (+1).

3.10. Analytical Techniques

Bacterial growth was measured by optical density at a wavelength of 600 nm [49–51,66] using a Shimadzu brand spectrophotometer model UV-1800. The blank used was uninoculated culture medium [52]. The pH was measured with an Orion Model Star A211 Potentiometer, which was calibrated daily using three calibration points (4.01, 7.00 and 10.01), while free cyanide and ammonium were measured using a HANNA Instruments ion selective electrode, using five calibration points: 1, 5, 15, 15, 30 and 50; and 50, 100, 250, 500 and 1000 mg·L^{-1}, respectively. The pH was adjusted using sodium hydroxide (1 M) (NaOH, J.T. Baker brand) or sulfuric acid (1 M) (H_2SO_4, J.T. Baker brand). The metals were analyzed by atomic absorption; for the preservation of the samples, Meyer brand concentrated nitric acid was added until a pH < 2 was reached, while the atomic absorption equipment was produced by Perkin Elmer and the lamps were hollow cathode lamps with wavelengths of 324.7 nm for copper, 239.6 nm for iron and 232 nm for nickel, while the carrier gas was a mixture of air and acetylene.

The cyanide consumption efficiency (biodegradation, %) was calculated according to Equation (2). The ammonium production yield (YN-NH_4^+, g N-NH_4^+/CN^- consumed) (the amount of ammonium formed from the cyanide (substrate) used) was calculated

according to Equation (3). Equation (4) was used to determine the biomass/substrate yield (YBM, g N-biomass/g CN^- consumed).

$$Biodegradation\ (\%) = \left(\frac{[starting\ substrate] - [final\ substrate]}{[starting\ substrate]}\right)100 \quad (2)$$

$$Yield_{YN-NH_4^+} = \frac{mg\ final\ product}{mg\ substrate\ used} \quad (3)$$

$$Yield_{YBM}\left(\frac{mg\ cells}{mg\ substrate\ used}\right) = \frac{biomass\ produced}{substract\ used} = \frac{BM_{final}exponential\ phase - BM_{inicial}exponential\ phase}{[starting\ substrate] - [final\ substrate]} \quad (4)$$

The mathematical model used to describe cell growth was the Monod kinetic model that describes the growth of biomass when there is a limited substrate. Equation (5) is a mathematical expression of the Monod model; once it has been linearized, it is expressed as follows (Lineweaver–Burk):

$$\mu = \frac{\mu_{max} S}{K_s + S} \quad (5)$$

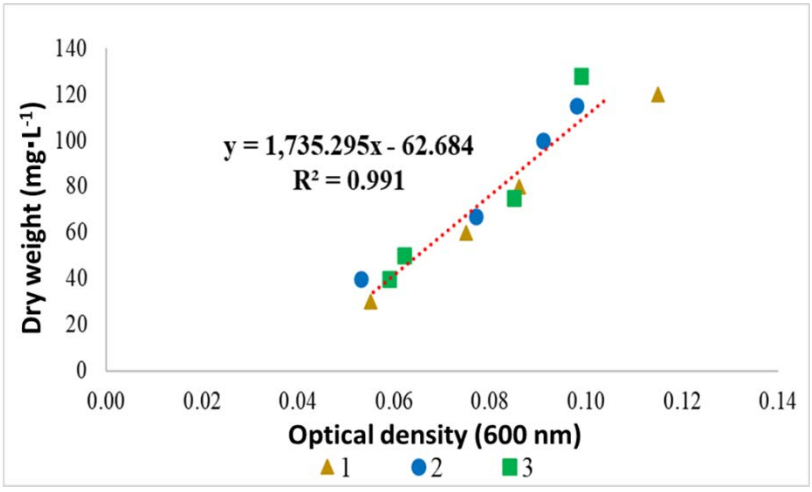
$$\frac{1}{\mu} = \frac{1}{\frac{(S_0 - S)}{t}} = \frac{K_s}{\mu_{max}}\frac{1}{S} + \frac{1}{\mu_{max}} \quad (6)$$

where μ = the specific growth velocity (d^{-1}); μ_{max} = the maximum specific growth rate (d^{-1}); K_s = the concentration of the substrate at which the reaction rate is half its maximum rate ($mg{\cdot}L^{-1}$); and S = the substrate concentration ($mg{\cdot}L^{-1}$)

4. Results

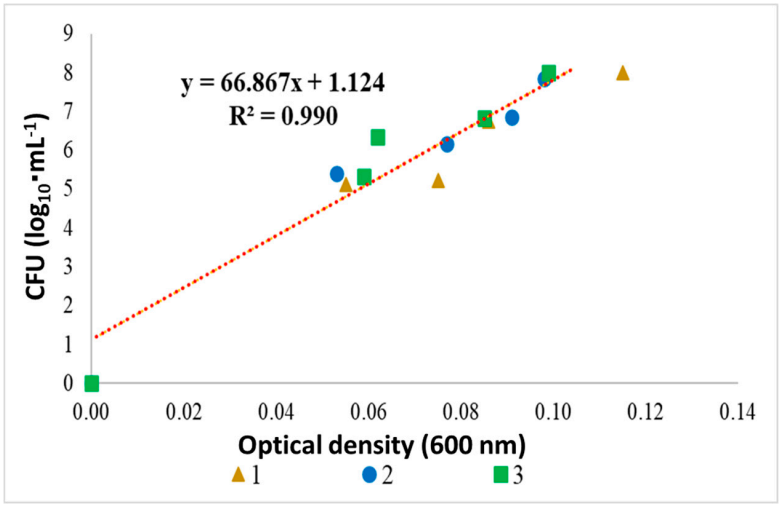
4.1. Biomass Quantification Method Development

Figure 1 shows the correlations that exist between colony forming units (CFUs) and optical density measurements, as well as dry weight (total suspended solids) and optical density measurements. The coefficient of determination is greater than 0.99, which determines the linearity of optical density versus dry weight and CFUs. Optical density measurement has quite a few advantages over solid culture media seeding or mass determination, such as its low cost and speed and the fact that it is a non-destructive test [50]. The points in the figure (yellow triangles, blue circles, and green squares) are the points for each measurement (performed in triplicate).



(a)

Figure 1. *Cont.*



(b)

Figure 1. Correlations between optical density and (**a**) dry weight and (**b**) cell count in microbial growth.

4.2. Evaluation of Different Inocula in the Biodegradation of Free Cyanide

When evaluating the previously acclimatized (MC10) and non-acclimatized (MC0) inocula, it was observed that biodegradation within the first ten days was higher than 10% in both cases (MC0 = 20% (9.20 mg CN^- L^{-1}) and MC10 = 47% (6.20 mg CN^- L^{-1})). On the other hand, for the second condition cited by the OECD (100% biodegradation of the contaminant in 29 days), the toxicity of cyanide is attributed to the inability of the bacterial consortium to reach the maximum biodegradation at 29 days; however, the contaminant is considered a biodegradable substance (according to the OECD), since in the time previously mentioned, 80% biodegradation of free cyanide was reached (2.32 mg CN_f^- L^{-1}) in the medium that was inoculated with the previously acclimatized bacteria (MC10), in comparison with the one obtained from the activated sludge (MC0) that had a biodegradation of 56% (5.07 mg CN_f^- L^{-1}). In addition, it is possible to appreciate that the volatilization loss was less than 5% and that the growth and biomass growth in MC0 was 6% and in MC10 16%, with CI 5% (Figure 2).

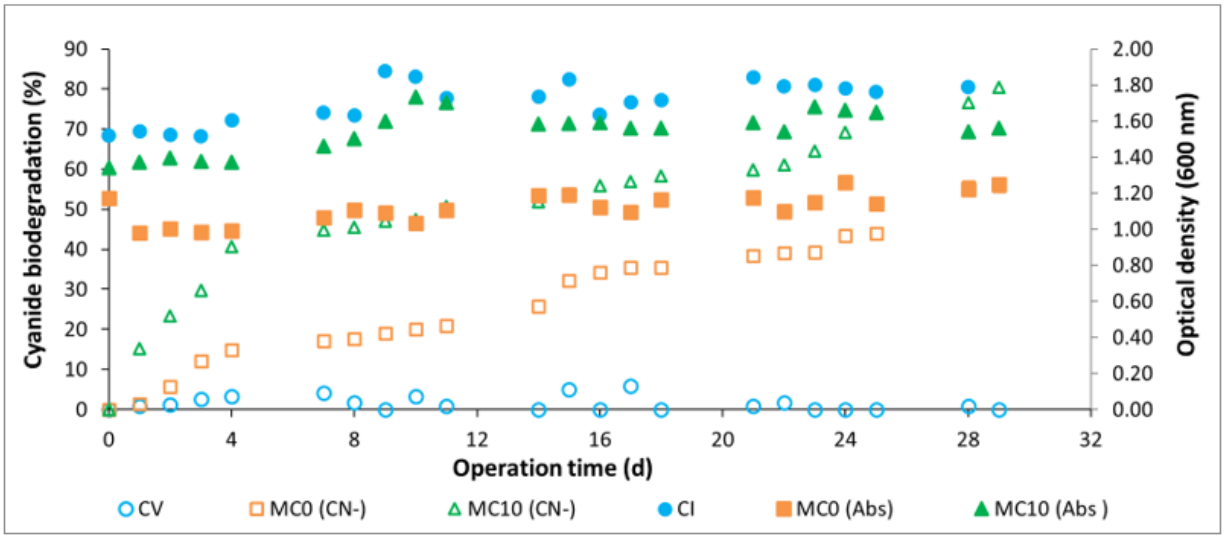


Figure 2. Biodegradation of free cyanide and bacterial growth in cyanide biodegradability tests (pH = 9.5 and T = 25 °C).

The Y_{BM} yield rates were 0.012 and 0.023 mg cells · mg substrate consumed^{-1} and the maximum growth rates were 0.435 and 1.569 d^{-1} for MC0 and MC10, respectively.

The formation of ammonium from the degradation of cyanide is based on the following two chemical reactions (Equations (7) and (8)), in which cyanide is first converted to cyanate ions, which are then transformed to ammonium ions and carbonate.

$$2CN^- + O_2 \rightarrow 2CNO^- \quad (7)$$

$$CNO^- + 2H_2O \leftrightarrow NH_4^+ + CO_3^{2-} \quad (8)$$

Effective ammonium formation is attributed to the ability of the bacterial consortium to carry out its adaptation to the contaminant, generating, in turn, its biodegradation. The ammonium production yield rates were 0.64 and 0.69 mg CN^- mg NH_4^{-1} for MC0 and MC10, respectively, (Figure 3) and the maximum rate of substrate consumption was 0.09 for MC0 and 0.11 for MC10.

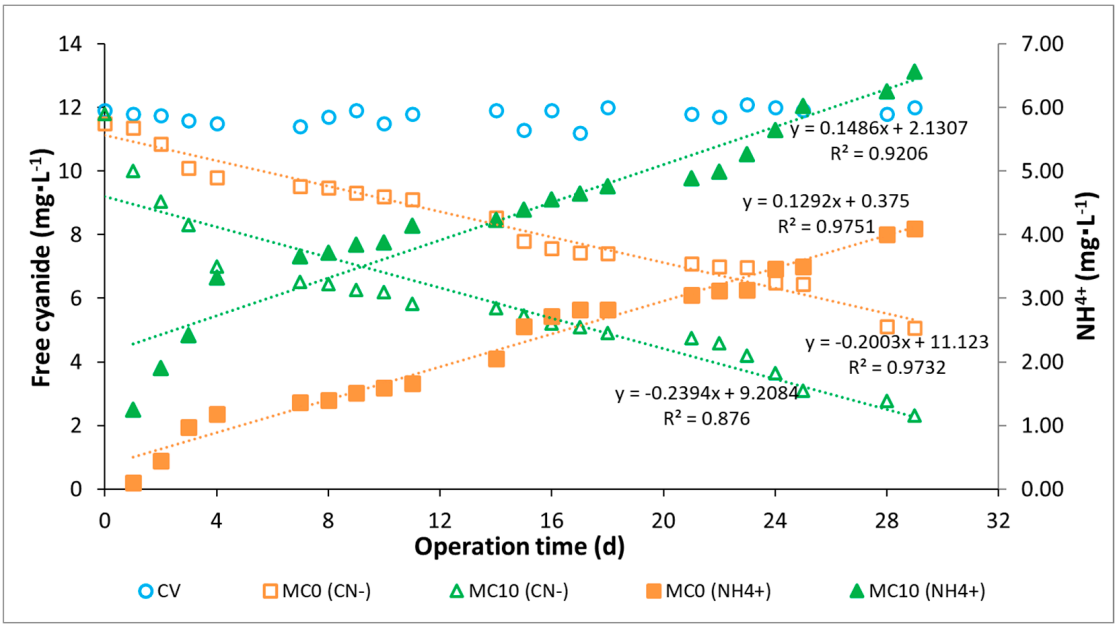


Figure 3. Zero-order kinetics for free cyanide and ammonium in biodegradability tests (pH = 9.5 and T = 25 °C).

Studies by Kao et al. (2003) [50] and Luque-Almagro et al. (2005) [24] reported the generation of ammonium from cyanide degradation in aqueous media. Ammonium production indicates the degradation pathway of free cyanide, which is oxidative [67]. High ammonium values are attributed to high bacterial activity in cyanide degradation and the increased availability of hydrogen ions, while a decrease in this compound may be indicative of a reduction in nitrogen use from cyanide and the conversion of ammonium to nitrite and nitrate [1,63].

4.3. Evaluation of Different Carbon Sources and Inhibition Tests

The data obtained were fitted to the Monod model, both for the case of reactors containing only cyanide as a carbon source and those using another extra source (Figure 4). The cyanide degradation rates were favored when working with the SPFC medium (3.308 d^{-1}), followed by SP (2.660 d^{-1}) and, finally, SPFCG (1.879 d^{-1}); however, the differences were not statistically significant.

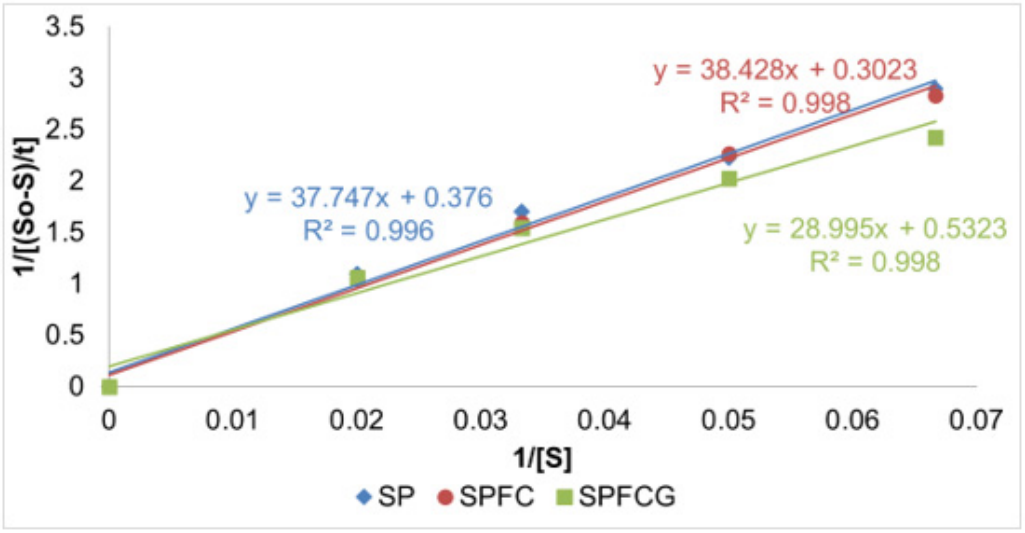


Figure 4. Reaction kinetics for the biodegradation of free cyanide in inhibition tests with different concentrations and carbon sources applying the Monod model.

The maximum degradation rates and saturation constants for SP, SPFC and SPGCG media and for the different concentrations (15, 20, 30 and 50 mg·L^{-1}) of free cyanide were calculated according to Monod kinetics (Table 1). The maximum rates were determined when working with an initial free cyanide concentration of 30 mg·L^{-1} in SP medium, while when working with SPFC and SPFCG media, the highest rate was determined when working with 20 mg·L^{-1} of initial free cyanide. The rates obtained in this study were higher than those obtained by Abdoul-Raimi (2013): 0.0067, 0.0058, 0.0072 and 0.0087 h^{-1}, using different initial concentrations of glucose (0.5, 1, 2 and 4 g·L^{-1}). As for the saturation constant, it was favored when working with the lowest concentrations of the pollutant.

Table 1. Maximum degradation rates and saturation constants for four different cyanide concentrations and three different mineral media.

[CN$^-$] (mg L^{-1})	μ_{max} (d^{-1})			K_s (mg·L^{-1})		
	SP	SPFC	SPFCG	SP	SPFC	SPFCG
15	1.22	1.32	1.89	11.48	10.08	11.60
20	1.37	1.83	1.95	16.08	17.59	16.27
30	1.60	1.64	1.29	23.94	25.53	25.06
50	1.01	1.11	1.73	38.24	37.55	31.23

Table 2 shows the final cyanide concentrations and the corresponding removals using three different mineral media (with cyanide (SP), with cyanide and sodium acetate (SPFC), and with cyanide and glucose (SPFCG)) and four different cyanide concentrations. In the reactors operated with 15 mg·L^{-1} of initial free cyanide, effluents with concentrations lower than 3 mg·L^{-1} were obtained in the SPFC after 21 days of operation and in the SP and SPFCG after 25 days of operation. On the other hand, when working with an initial concentration of 20 mg·L^{-1} of the pollutant, 3 mg·L^{-1} of free cyanide in the effluent was obtained after 29 days of operation in the SPFCG reactor and after 35 days with the SP and SPFC reactors. Meanwhile, for concentrations of 30 and 50 mg·L^{-1}, it took more than 35 days to reach values below 3 mg·L^{-1}.

When evaluating the loss of cyanide in the volatilization controls for the different concentrations used, it was found that this was less than 5%, giving final free cyanide concentrations of 13.88 ± 0.21, 21.40 ± 0.20, 30.40 ± 0.57 and 46.99 ± 0.20 mg·L^{-1}; therefore, it was considered negligible, and the decrease in cyanide in the inoculated reactors was attributed to the action of the microorganisms through oxidative mechanisms working on the pollutant.

Table 2. Free cyanide concentrations after 35 days of operation and corresponding biodegradation levels using different cyanide concentrations and mineral media.

$[CN^-]_i$ (mg·L^{-1})	SP $[CN^-]_f$ (mg·L^{-1})	SPFC $[CN^-]_f$ (mg·L^{-1})	SPFCG $[CN^-]_f$ (mg·L^{-1})
15	1.72 (87%)	1.65 (88%)	1.37 (91%)
20	1.23 (87%)	2.32 (87%)	2.51 (88%)
30	5.76 (78%)	6.13 (78%)	6.32 (82%)
50	15.70 (67%)	15.19 (66%)	15.15 (72%)

On the other hand, to evaluate the biodegradation of cyanide using different carbon sources, a comparison of means for each of the concentrations was generated, with a significance value of 95%. Since the significance value P was greater than 0.05, 0.9315, 0.6017, 0.9738 and 0.7987 for 15, 20, 30 and 50 mg·L^{-1}, respectively, it was concluded that there was no statistically significant difference between them and that cyanide biodegradation for each of the concentrations was not affected by the composition of the medium.

To analyze the inhibition of the microorganisms, mean comparisons were made between the concentrations (15, 20, 30 and 50 mg·L^{-1}) for each of the different media, which showed that there was a statistically significant difference, with a confidence level of 95%. Therefore, the tests were analyzed using the least significant difference procedure to determine which concentrations were significantly different from the others. The results showed that there was no statistically significant difference between the initial cyanide concentrations of 15, 20 and 30 mg·L^{-1}, nor, in turn, between the cyanide concentrations of 30 and 50 mg·L^{-1}; however, the final cyanide concentrations were favored when working with 30 mg·L^{-1}, these being 5. 76, 6.13 and 6.32 mg·L^{-1}, compared to final cyanide concentrations of 15.70, 15.19 and 15.15 mg·L^{-1} when working with 50 mg·L^{-1} of initial cyanide for SP, SPFC and SPFCG media, respectively.

Tables 3 and 4 show the yields obtained for biomass/substrate and ammonium production, respectively. It can be observed that in both cases these were increased when working with lower cyanide concentrations. When performing a statistical analysis with a significance value of 95% between the different initial cyanide concentrations, it was determined that there was no statistically significant difference when working with 15, 20 or 30 mg·L^{-1} CN^-; however, by increasing the free cyanide to 50 mg·L^{-1}, the difference became statistically significant in comparison with the previously mentioned concentrations.

Table 3. Biomass/substrate yields with different media (Y_{BM}).

$[CN^-]_i$ (mg·L^{-1})	SP	SPFC	SPFCG
15	0.0134	0.0244	0.0062
20	0.0052	0.0158	0.0042
30	0.0042	0.0050	0.0018
50	0.0027	0.0009	0.0004

Table 4. Ammonium production yields with different media ($Y_{N\text{-}NH4+}$).

$[CN^-]_i$ (mg·L^{-1})	SP	SPFC	SPFCG
15	27.84	28.00	31.15
20	27.87	27.64	27.46
30	24.96	24.98	26.32
50	21.41	21.07	23.34

4.4. Influence of pH, Temperature and Inoculum Concentration on Free Cyanide Biodegradation

To evaluate the effect of pH, temperature and initial inoculum concentration on free cyanide biodegradation, an analysis of variance was performed, whereby it was determined

that the three factors studied had a statistically significant effect on cyanide biodegradation, since the P values were less than the preset significance value ($\alpha = 0.05$), these being 0.0000, 0.0370 and 0.0035, respectively. The F-ratio value of 31.22 implies that the model was significant [Mekuto, L.; Ntwampe].

Figure 5 shows the main effects on cyanide removal, both for pH and inoculum. The optimum values for cyanide biodegradation were not reached, as it was not possible to vary the pH values below 9 units due to the volatilization of the contaminant [Logsdon], while at a pH higher than 11.5 it is difficult to carry out the growth of microorganisms. However, the optimum temperature value is 28.5 °C.

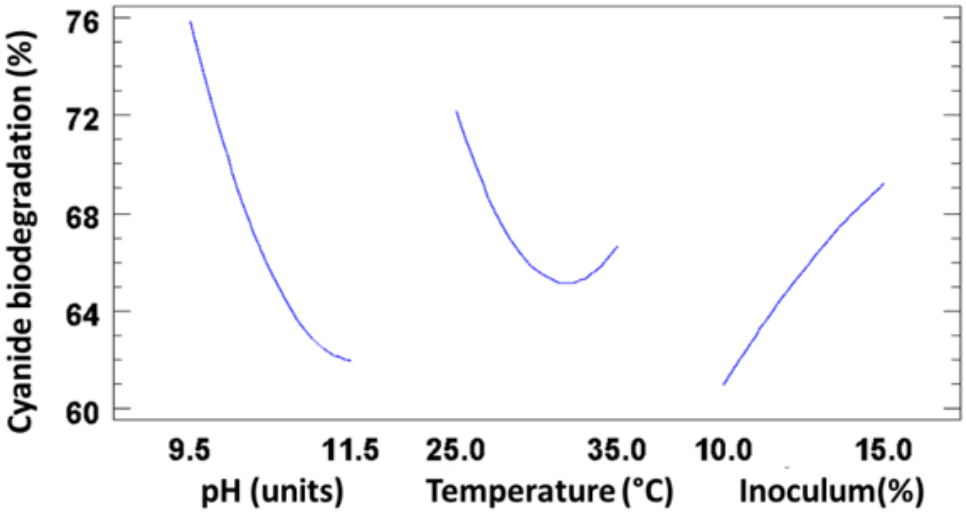


Figure 5. Main effects in cyanide removal.

The factor with the greatest effect was pH, followed by inoculum concentration and, finally, temperature. The R-squared coefficient of determination was 72.93%, which is above the recommended limit (70%) to carry out a good interpretation of the variability present [Gutierrez, Villegas-Mendoza I]. This means that the values studied were responsible for cyanide biodegradation. The regression equation of the fitted model is:

$$\begin{aligned} &\textit{Cyanide biodegradation} \\ &\quad = 303.741 - 29.7778 \times pH - 4.00556 \times Temperature \\ &\quad + 2.74444 \times Inoculum + 0.566667 \times pH \times Temperature \\ &\quad + 0.466667 \times pH \times Inoculum \\ &\quad - 0.2 \times Temperature \times Inoculum. \end{aligned}$$

The best results obtained in this study were obtained when working with the low pH level (9.5 units), high inoculum concentration (15% *v/v*) and low temperature (25 °C), with which a maximum absorbance of 0.913 (1551 mg TSS L^{-1}) and a cyanide biodegradation of 88% were achieved. On the other hand, Khamar et al. (2015) [41] obtained the highest cyanide biodegradation percentages (66%) when working with a temperature of 25 °C, a pH of 9.5 and an inoculum concentration of 15% *v/v*, as compared with the results obtained by Mekuto et al. (2015) [68], where the optimum temperature was 33.6 °C and a pH of 9.88 was used with a Bacillus genus strain, and those obtained by Wu et al. (2014) [69], whose optimum temperature was 31 °C and who used a pH of 10.3 with a Bacillus species.

4.5. Sorption Tests

Metal sorption tests on biomass showed equilibrium times of 270 min for copper and nickel and 5 min for iron. The sorption coefficients (qe) are shown in Table 5.

Table 5. Sorption coefficients of metals in biomass.

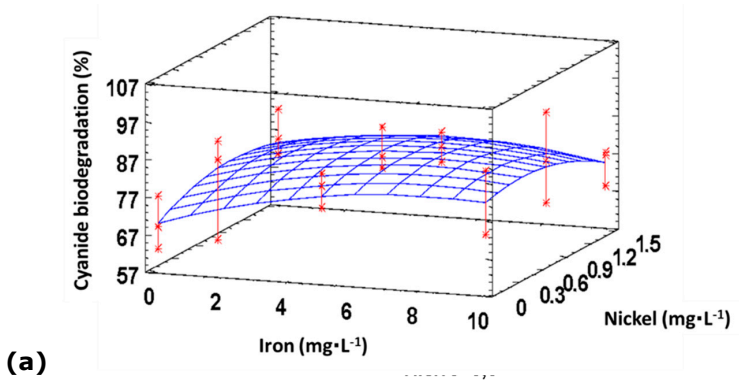
[Cu] (mg·L^{-1})	q_e	[Fe] (mg·L^{-1})	q_e	[Ni] (mg·L^{-1})	q_e
1	0.35	1	0.46	0.25	0
2	0.62	2	0.90	0.50	0
3	0.79	4	1.98	0.75	0.14
4	1.15	6	2.60	1.00	0.08
5	1.04	8	3.70	1.25	0.54
6	1.78	10	4.26	1.50	0.81

4.6. Determination of the Interference of Cyanide Complexed with Metals in Its Biodegradation

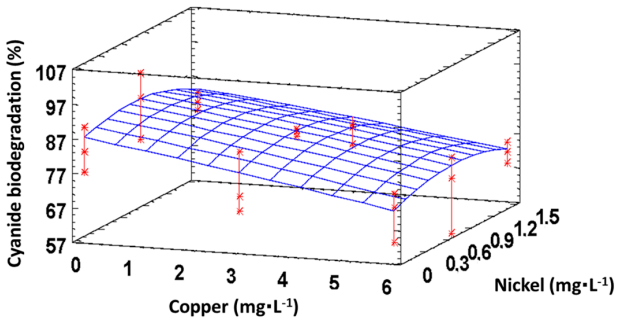
The ANOVA tests of the results obtained for the evaluation of cyanide biodegradation when complexed with metals showed that copper and iron were the factors that had a significant effect on the final concentration of cyanide, since the P values were less than the preset significance value ($\alpha = 0.05$), being 0.0002 and 0.0051, respectively.

The R-squared coefficient of determination was 63.88%, which is below the recommended limit (70%) that can be attributed to the existence of other factors that are influencing the model. Copper is the factor that has the greatest influence on the biological degradation of cyanide, since it presents an inhibitory effect [70] to carry out the biodegradation of the contaminant, while iron presents statistically significant effects on its biodegradation and is benefited when working with values of 10 mg·L^{-1}. On the other hand, there was no statistically significant effect when working with nickel (0, 0.75 and 1.5 mg·L^{-1}), nor were there statistically significant differences between the interactions of the three metals.

The response surface plots (Figure 6) show that when working with a concentration of 3 mg·L^{-1} copper, the best cyanide removals were presented at high iron values and that nickel did not show a significant effect (Figure 6a); on the other hand, when working with an iron concentration of 5 mg·L^{-1} (Figure 6b), nickel did not show a significant effect either; however, cyanide removal was favored at low copper values, while when working with 0. 75 mg·L^{-1} of nickel (Figure 6c), cyanide removal was higher with low copper and high iron values.

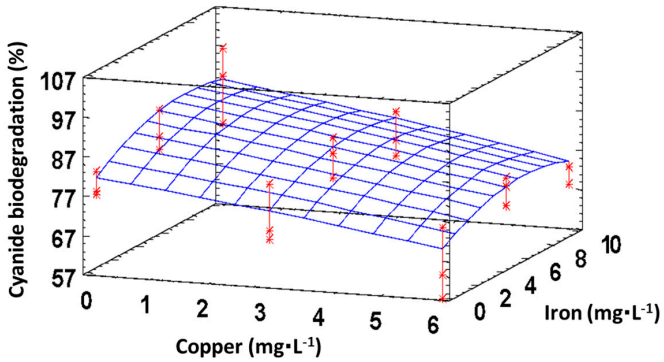


(a)



(b)

Figure 6. *Cont.*



(c)

Figure 6. Response surface plot (estimated) for cyanide removal (%): (**a**) copper (3 mg·L^{-1}), (**b**) iron (5 mg·L^{-1}) and (**c**) nickel (0.75 mg·L^{-1}).

The regression equation of the fitted model is:

$$\begin{aligned} &Cyanide\ biodegradation \\ &\quad = 73.3148 - 1.46296 \times Copper + 1.9222 \times Iron \\ &\quad + 4.14815 \times Nickel - 0.122222 \times Copper \times Iron \\ &\quad - 0.22222 \times Copper \times Nickel - 0.84444 \times Iron \times Nickel \end{aligned}$$

The optimum value for copper could not be obtained since it has a tendency to be less than zero, which is not experimentally possible. On the other hand, the optimum values for the biodegradation of cyanide complexed with iron and nickel were 7.7 and 0.46 mg·L^{-1}, respectively. The average values for metals during the whole experimentation were: for copper, 2.88 ± 0.55 and 5.91 ± 0.67 mg·L^{-1}; for iron, 3.91 ± 1.33 and 7.80 ± 3.44 mg·L^{-1}; and for nickel, 0.81 ± 0.14 and 1.47 ± 0.23 mg·L^{-1}.

The initial concentrations of metals with which a greater biodegradation of cyanide (91%) was obtained at day 36 of operation with an initial concentration of the contaminant of 30 mg·L^{-1} were as follows: copper, 0 mg·L^{-1}; iron, 10 mg·L^{-1}; and nickel, 0 mg·L^{-1}. The maximum biomass growth under the above operating conditions was 1500 Abs (2540 mg TSS L^{-1}).

5. Conclusions

In this study, it was possible to identify the optimal values to carry out the biodegradation of free cyanide, in addition to determining the concentrations of metals at which bacteria can treat the complexed cyanide.

The degradation of cyanide in the inhibition tests was carried out by the metabolism of the microorganisms inoculated in the reactors (biodegradation). The volatilization of the contaminant as hydrocyanic acid was less than 5%. The most appropriate culture to biodegrade cyanide was MC10, since the YBM yield rate was higher (0.023 mg cells mg substrate consumed^{-1}) compared to that obtained with MC0 (0.012 mg cells mg substrate consumed^{-1}), as were the maximum degradation rates, which were 0.435 and 1.569 d^{-1} for MC0 and MC10, respectively.

The compositions of the mineral media (cyanide, cyanide and sodium acetate, or cyanide and glucose) at constant conditions with respect to pH, temperature and inoculum concentration did not present statistically significant differences in cyanide biodegradation, so the mineral medium that did not have an extra carbon source was chosen as the most suitable to carry out cyanide biodegradation, with maximum degradation rates of 1.30 ± 0.25, 1.48 ± 0.32 and 1.37 ± 0.30 d^{-1}, respectively.

The optimum values for carrying out free cyanide biodegradation using a previously acclimatized consortium were: 9.5, 25 °C and 15% *v/v*, for pH, temperature, and inoculum concentration, respectively. The adsorption of metals on the biomass was 100% for iron, 50%

for nickel and 70% for copper, while the optimum values to carry out the biodegradation of complexed cyanide were 0, 7.7 and 0.46 mg·L^{-1} for copper, iron, and nickel, respectively.

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References

1. Abdoul-Raimi, B.; Shamim, A.; Ynoussa, M.; Nosa, O. Biodegradation of Cyanide Complex Compounds in Aqueos Media by *Pseudomonas resinovorans*. *Environ. Eng. Sci.* **2013**, *30*, 757–764. [CrossRef]
2. Dash, R.; Gaur, A.; Balomajumder, C. Cyanide in industrial wastewaters and its removal: A review on biotreatment. *J. Hazard. Mater.* **2009**, *163*, 1–11. [CrossRef] [PubMed]
3. Dash, R.; Balomajumder, C.; Kumar, A. Treatment of metal cyanide bearing wastewater by simultaneous adsorption and biodegradation (SAB). *J. Hazard. Mater.* **2008**, *152*, 387–396. [CrossRef] [PubMed]
4. Fernández-Pérez, B. Development of a New Method for the Removal of Cyanide from Mine Wastewater. Ph.D. Thesis, Universidad de Oviedo, Oviedo, Spain, 2007.
5. Lottermoser, B. *Mine Wastes: Characterization, Treatment, Environmental Impacts*, 2nd ed.; Springer: Berlin/Heidelberg, Germany, 2007; ISBN -10: 3-540-4869-1.
6. NJHealth. *Hoja Informativa Sobre Sustancias Peligrosas*; New Jersey Department of Health: Trenton, NJ, USA, 2009. Available online: http://nj.gov/health/eoh/rtkweb/documents/fs/1013sp.pdf (accessed on 26 October 2017).
7. Astete, J.; Cáceres, W.; Gastañaga, M.; Lucero, M.; Sabastizagal, I.; Oblitas, T.; Pari, J.; Rodríguez, F. Intoxicación por plomo y otros problemas de salud en niños de poblaciones aledañas a relaves mineros. *Rev. Peru. de Med. Exp. y Salud Pública* **2009**, *26*, 15–19.
8. Ramírez, A. *Toxicidad del Cianuro. Investigación Bibliográfica Sobre Los Efectos en Animales y en el Hombre. Anales de la Facultad de Medicina*; UNMSM, Facultad de Medicina: Lima, Peru, 2010; Volume 71, pp. 54–61.
9. Luque-Almagro, V.; Cabello, P.; Sáez, L.; Olaya-Abril, A.; Moreno-Vivián, C.; Roldán, M. Exploring anaerobic environments for cyanide and cyano-derivatives microbial degradation. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 1067–1074. [CrossRef]
10. Luque-Almagro, V.; Moreno-Vivián, C.; Roldán, M. Biodegradation of cyanide wastes from mining and jewellery industries. *Curr. Opin. Biotechnol.* **2016**, *38*, 9–13. [CrossRef]
11. Álvarez-García, R. Application of Passive Systems for the Treatment of Residual Solutions from Cyanidation Processes in Gold Mining. Ph.D. Thesis, Universidad de Oviedo, Oviedo, Spain, 2005.
12. Akcil, A. Destruction of cyanide in gold mill effluents: Biological versus chemical treatments. *Biotechnol. Adv.* **2003**, *21*, 501. [CrossRef]
13. Alvillo-Rivera, A.J.; Garrido-Hoyos, S.E.; Buitrón, G. Cyanide treatment of mining tailings using suspended biomass and moving bed biomass reactors. *Environ. Sci. Pollut. Res.* **2022**, *29*, 37458–37470. [CrossRef]
14. Alvillo Rivera, A.J. Biodegradación de Cianuro en Relaves Mineros Mediante Reactores de Biomasa Suspendida y Biomasa de Lecho Móvil. Ph.D. Thesis, Universidad Nacional Autónoma de México, Mexico City, Mexico, 2022.
15. Nava-Urrego, L.; Gasperín-Sanchéz, R.; Durán-Moreno, A. Comparison of a suspended biomass reactor and a bonded biomass reactor for the biodegradation of toxic compounds present in wastewater from oil refineries. *Rev. Int. Contam. Ambient.* **2014**, *30*, 101–112.
16. Bassin, J.; Kleerebezem, R.; Rosado, A.; Vvan Loosdrecht, M.; Dezotti, M. Effect of Different Operational Conditions on Biofilm Development, Nitrification, and Nitrifying Microbial Population in Moving-Bed Biofilm Reactors. *Environ. Sci. Technol.* **2011**, *46*, 1546–1555. [CrossRef]
17. Ibrahim, H.T.; Qiang, H.; Al-Rekabi, W.S.; Qiqi, Y. Improvements in Biofilm Processes for Wastewater Treatment. *Pak. J. Nutr.* **2012**, *11*, 610–636. [CrossRef]
18. Metcalf & Eddy. *Wastewater Engineering. Treatment and Reuse*, 4th ed.; Mc Graw Hill Education: New York, NY, USA, 2003.
19. Smith-Nava, H. Treatment of Wastewater from Residential Marine Platforms Using Immobilized Biomass Systems. Master's Thesis, Universidad Nacional Autónoma de México, Mexico City, Mexico, 2016.
20. Quan, F.; Yuxiao, W.; Tianmin, W.; Hao, Z.; Libing, C.; Chong, Z.; Hongzhang, C.; Xiuqin, K.; Xin-Hiu, X. Effects of packing rates of cubic-shaped polyurethane foam carriers on the microbial community and the removal of organics and nitrogen in moving bed biofilm reactors. *Bioresour. Technol.* **2012**, *117*, 201–207.

21. CONAGUA. *Manual de Agua Potable, Alcantarillado y Saneamiento, Diseño de Plantas de Tratamiento de Aguas Residuales Municipales: Filtros Anaerobios de Flujo Ascendente*; Comisión Nacional del Agua: Mexico City, Mexico, 2014; ISBN 978-607-626-017-3.
22. Camargo-Rodríguez, M. *Tesis de Maestría: Effect of Aeration on the Removal of Organic Matter and Nitrogen in Moving Bed Bioreactors*; Universidad Nacional Autónoma de México: Mexico City, Mexico, 2011.
23. Rusten, B.; Eikebrokk, B.; Ulgenes, Y.; Lygren, E. Design and operations of the Kaldnes moving bed biofilm reactors. *Aquac. Eng.* **2006**, *34*, 322–331. [CrossRef]
24. Luque-Almagro, V.; Huertas, M.; Martínez-Luque, M.; Moreno-Vivián, C.; Roldán, M.; García-Gil, J.; Castillo, F.; Blasco, R. Bacterial degradation of cyanide and its metal complexes under alkaline conditions. *Appl. Environ. Microbiol.* **2005**, *71*, 940–947. [CrossRef] [PubMed]
25. Batt, A.L.; Kim, S.; Aga, D.S. Enhanced Biodegradation of Iopromide and Trimethoprim in Nitrifying Activated Sludge. *Environ. Sci. Technol.* **2006**, *40*, 7367–7373. [CrossRef] [PubMed]
26. Cervantes, C.; Espino-Saldaña, A.; Acevedo-Aguilar, F.; León-Rodríguez, I.; Rivera-Cano, M.; Avila-Rodríguez, M.; Wróbel-Kaczmarczyk, K.; Wróbel-Zasada, K.; Gutiérrez-Corona, J.; Rodríguez-Zavala, J.; et al. Microbial interactions with heavy metals. *Rev. Latinoam. de Microbiol.* **2006**, *48*, 203–210.
27. Dwivedi, N.; Balomajumder, C.; Mondal, P. Comparative evaluation of cyanide removal by adsorption, biodegradation, and simultaneous adsorption and biodegradation (SAB) process using *Bacillus cereus* and almond Shell. *J. Environ. Biol.* **2016**, *37*, 551–556. [PubMed]
28. Guamán-Guadalima, M.P.; Nieto-Monteros, D.A. Evaluation of the rotational speed and carbon source on the biological removal of free cyanide present on gold mine wastewater, using a rotating biological contactor. *J. Water Process Eng.* **2018**, *23*, 84–90. [CrossRef]
29. Deloya, M. Cyanide waste treatment by bioremediation. *Tecnol. Marcha* **2012**, *25*, 61–72.
30. Huertas, M.; Sáez, L.; Roldán, M.; Luque-Almagro, V.; Martínez-Luque, M.; Blaxo, E.; Castillo, F.; Moreno-Vivián, C.; García-García, I. Alkaline cyanide degradation by *Pseudomonas pseudoalcaligenes* CECT5344 in a batch reactor. Influence of pH. *J. Hazard. Mater.* **2010**, *179*, 72–78. [CrossRef]
31. Cornejo, M. Bioremediation of Mining Tailings with a Molecularly Characterized Native Microbial Consortium and Producer of Cyanide Degrading Enzymes and Derivatives. Master's Thesis, Universidad Nacional de Tumbes, Tumbes, Peru, 2016.
32. Maniyan, M.; Sjahrir, F.; Ibrahim, A.; Cass, A. Biodegradation of cyanide by *Rhodococcus* UKMP-5M. *Biología* **2013**, *68*, 177–185.
33. Avcioglu, N.; Seyis-Bilkay, I. Biological Treatment of Cyanide by Using *Klebsiella pneumoniae* Species. *Food Technol. Biotechnol.* **2016**, *54*, 450–454. [CrossRef] [PubMed]
34. Chaudhari, A.U.; Kodam, K.M. Biodegradation of thiocyanate using co-culture of *Klebsiella pneumoniae* and *Ralstonia* sp. *Appl. Microbiol. Biotechnol.* **2010**, *85*, 1167–1174. [CrossRef] [PubMed]
35. Cabuk, A.; Unal, A.; Kolankaya, N. Biodegradation of cyanide by a white rot fungus, *Trametes versicolor*. *Biotechnol. Lett.* **2006**, *28*, 1313–1317. [CrossRef] [PubMed]
36. Santos, B.; Obed-Ntwampe, S.; Hamuel-Doughari, J. *Continuos Biotechnological Treatment of Cyanide Contaminated Waters by Using a Cyanide Resistant Species of Aspergillus awamori*; Intech: London, UK, 2013; pp. 123–146.
37. White, D.M.; Pilon, T.A.; Woolard, C. Biological treatment of cyanide containing wastewater. *Water Res.* **2000**, *34*, 2105–2109. [CrossRef]
38. Vincent, S.; Dale, C.; Young, B.; Laliberté, M.; Pouzenc, A. Gold mine effluent treatment: Cyanide compounds and total nitrogen biodegradation using moving bed biofilm. *Proc. Water Environ. Fed.* **2016**, *2016*, 5636–5651. [CrossRef]
39. Tuya, J. Evaluation of the Degradative Capacity of Cyanide by Alkaliphilic Bacteria Isolated from the Tailings of the Metal Concentrator Plant Mesapata Cathac-Ancash. Licentiate Thesis, Universidad Nacional Mayor de San Marcos, Lima, Peru, 2014.
40. Mekuto, L.; Ntwampe, S.; Akcil, A. An integrated biological approach for treatment of cyanidation wastewater. *Sci. Total Environ.* **2016**, *571*, 711–720. [CrossRef]
41. Khamar, Z.; Makhdoumi-Kakhki, A.; Mahmudy, M. Remediation of cyanide from the gold mine tailing pond by a novel bacterial co-culture. *Int. Biodeterior. Biodegrad.* **2015**, *99*, 123–128. [CrossRef]
42. Ortiz-Sayavedra, K.; y Tejeda-Gil, A. Biodegradability Tests of Mining Tailings Product of Cyanidation in the Extraction of Au and Ag. Bachelor's Thesis, Unversidad Popular Autónoma de Puebla, Puebla, Mexico, 2018.
43. Caballero-Armenta, M.; González-González, M. *Microbiología Ambiental. Cuaderno de Prácticas*; Universidad Nacional de Educación a Distancia: Madrid, Spain, 2006; ISBN 84-362-5287-X.
44. Dos-Santos, A. Study of the Kinetic Behavior of Microorganisms of Interest in Food Safety with Mathematical Models. Ph.D. Thesis, Universidad Autónoma de Barcelona, Barcelona, Spain, 2007.
45. Podder, M.; Majumder, C. A comparative investigation on the inhibition kinetics of bioaccumulation of As(III) and As(V) ions using Bacillus arsenicus MTCC 4380. *Ecohydrol. Hydrobiol.* **2017**, *17*, 148–163. [CrossRef]
46. *NOM-110-SSA1-1994*; Norma Oficial Mexicana, Bienes y Servicios. Preparación y Dilución de Muestras de Alimentos Para su Análisis Microbiológico. Diario Oficial de la Federación: Mexico City, Mexico, 1994.
47. *NOM-092-SSA1-1994*; Norma Oficial Mexicana, Bienes y Servicios. Método Para la Cuenta de Bacterias Aerobias en Placa. Diario Oficial de la Federación: Mexico City, Mexico, 1994.
48. Monballiu, A.; Cardon, N.; Nguyen, M.; Cornelly, C.; Meesschaert, B.; Chiang, Y. Tolerance of Chemoorganotrophic Bioleaching Microorganisms to Heavy Metal and Alkaline Stresses. *Bioinorg. Chem. Appl.* **2015**, *2015*, 861874. [CrossRef]

49. Madigan, M.; Martinko, J.; Parker, J. *Broke Biología de los Microorganismos*; Editorial Prentice Hall International, Inc.: Hoboken, NJ, USA, 2009; pp. 149–177.
50. Kao, C.; Liu, J.; Lou, H.; Lin, C.; Chen, S. Biotransformation of cyanide to methane and ammonia by *Klebsiella oxytoca*. *Chemosphere* **2003**, *50*, 1055–1061. [CrossRef] [PubMed]
51. Kim, D.; Chung, S.; Lee, S.; Choi, J. Relation of microbial biomass to counting units for *Pseudomonas aeruginosa*. *Afr. J. Microbiol. Res.* **2012**, *6*, 4620–4622.
52. Chapatwala, K.; Babu, G.; Vijaya, O.; Kumar, K.; Wolfram, J. Biodegradation of cyanides, cyanates and thiocyanates to ammonia and carbón dioxide by inmobilized cells of *Pseudomonas putida*. *J. Ind. Microbiol. Biotechnol.* **1998**, *20*, 28–33. [CrossRef] [PubMed]
53. *NMX-AA-034-SCFI-2015*; Análisis de Agua. Medición de Sólidos y Sales Disueltas en Aguas Naturales, Residuales y Residuales Tratadas. Método de Prueba. Diario Oficial de la Federación: México: Mexico City, Mexico, 2015.
54. OCDE. *Guidelines for the Testing of Chemicals*; Organisation for Economic Cooperation and Development: Paris, France, 1992.
55. Logsdon, M.; Hagelstein, K.; Mudder, T. *El Manejo del Cianuro en la Extracción de Oro. Consejo Internacional de Metales y Medio Ambiente*; Primera Impresión: Toronto, ON, Canada, 2001; ISBN 1-895720-35-4.
56. García-González, S. Chemical Oxidation of Spent Amines from Gas Sweetening Processes in Refineries. Master's Thesis, Universidad Nacional Autónoma de México, Mexico City, Mexico, 2008.
57. Vázquez-Rodríguez, G.; Beltrán-Hernández, R. Pruebas normalizadas para la evaluación de la biodegradabilidad de sustancias químicas. *Interciencia* **2004**, *29*, 568–573.
58. Villegas-Mendoza, I.E. Influence of Hydraulic and Physicochemical Parameters on the Efficiency of Silica Removal by an Electrocoagulation System. Master's Thesis, Universidad Nacional Autónoma de México, Mexico City, Mexico, 2011.
59. Flores-Velázquez, Y. Remoción de Compuestos Farmacéuticos en Reactores Con Biomasa Inmovilizada. Ph.D. Thesis, Universidad Nacional Autónoma de México, Mexico City, Mexico, 2017.
60. Torres-Borjorges, A. Degradation of Nonylphenol Isomers by Means of a Nitrifying Consortium in a Submerged Membrane Reactor. Ph.D. Thesis, Universidad Nacional Autónoma de México, Mexico City, Mexico, 2012.
61. Mijaylova-Nacheva, P.; Estrada-Arriaga, E.; Ramírez-Camperos, E. *Tecnologías Para la Remoción de Contaminantes Emergentes, Nutrients y Producción de Energía en Aguas y Lodos Residuals Para Cuencas Hidrográficas del Estado de Morelos. Informe final TC1404.1. Subcoordinación de Tratamiento de Aguas Residuales*; Instituto Mexicano de Tecnología del Agua: Progreso, Mexico, 2014.
62. Acheampong, M.; Meulepas, R.; Lens, P. Removal of heavy metals and cyanide from gold mine wastewater. *J. Chem. Technol. Biotechnol.* **2010**, *85*, 590–613. [CrossRef]
63. Akcil, A. First application of cyanidation process in Turkish gold mining and its environmental impacts. *Miner. Eng.* **2002**, *15*, 695–699. [CrossRef]
64. Gurbuz, F.; Ciftci, H.; Akcil, A. Biodegradation of cyanide containing effluents by *Scenedesmus obliquus*. *J. Hazard. Mater.* **2009**, *162*, 74–79. [CrossRef]
65. Zuluaga, M.; Hernández-Roldán, D. Pilot Scale Cyanide Treatment (1.0 m^3/day) of Tailings from the Au and Ag Mining Industry by Means of Oxidation with Ozone and Physicochemical Process. Master's Thesis, Universidad Popular Autónoma del Estado de Puebla, Puebla, Mexico, 2020.
66. Dumestre, A.; Chone, T.; Portal, J.; Gerard, M.; Berthelin, J. Cyanide Degradataion under Alkaline Conditions by a Strain *Fusarium solani* Isolated from Contaminated Soils. *Appl. Environ. Microbiol.* **1997**, *63*, 2729–2734. [CrossRef]
67. Kuyucak, N.; Akcil, A. Cyanide and removal options from effluents in gold mining and metallurgical processes. *Miner. Eng.* **2013**, *50–51*, 13–29. [CrossRef]
68. Mekuto, L.; Ntwampe, S.; Jackson, V. Biodegradation of free cyanide and subsequent utilisation of biodegradation by-products by Bacillus consortia: Optimisation using response suface methodology. *Environ. Sci. Pollut. Res.* **2015**, *22*, 10434–10443. [CrossRef]
69. Wu, C.-F.; Xu, X.-M.; Zhu, Q.; Deng, M.-C.; Feng, L.; Peng, J.; Yuan, J.-P.; Wang, J.-H. An effective method for the detoxification of cyanide rich wastewater by *Bacillus* sp. CN-22. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 3801–3807. [CrossRef] [PubMed]
70. Prado, V.; Esparza, M.; Vidal, R.; Durán, C. Actividad bactericida de superficies de cobre frente a bacterias asociadas a infecciones nosocomiales, en un modelo in vitro de adherencia y sobrevivencia. *Rev. Med. Chile* **2013**, *141*, 291–297. [CrossRef] [PubMed]