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Microbial Biofilm as a Methodology for Treatment of Cyanide-Contaminated Water

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Cyanide-contaminated wastewater is a very important problem of negative environmental impact. The objective of the research was the reduction of cyanide in the contaminated water emitted in a gold recovery process in the district of Chala-Arequipa after being used in mining processes, the method used was the formation of a microbial biofilm on suitable supports for its growth. The presence of cyanide in two water samples before treatment was 1,352 mg/L respectively. The treatment was performed in bioreactors with biofilms of *Pseudomona aeruginosa and Escherichia coli* supported on polypropylene and polyurethane for biofilm formation and growth. The tests were conducted over a period of 5, 6 and 7 days, obtaining the following results: in test 1, with *Pseudomona aeruginosa* biofilms, the cyanide concentration was reduced to 38.98 mg/L (97.11 %), with a pH of 10.77, total suspended solids at 10.79 mg/L, electrical conductivity at 10. 63 mg/L and turbidity at 7.46 NTU; test 2, in bioreactors with *Escherichia coli* biofilms was also favourable, decreasing cyanide to 325.1 mg/L (75.95 %), with a pH of 8.7, total suspended solids at 1.43 mg/L, dissolved oxygen at 6.70 mg/L, electrical conductivity at 51.13 μS/cm and turbidity at 7.13 NTU. Thus, it was established that the biofilms of *Pseudomona aeruginosa and Escherichia coli* constitute an environmentally sustainable methodology for the reduction of cyanide in water, which can be complemented with other technology to culminate with the elimination or removal of cyanide to levels below the maximum permissible limits of the environmental quality standards, avoiding the negative impacts that these discharges produce to ecosystems and human beings.

1. Introduction

Presence of cyanide in wastewater has become a crucial problem to solve in the world, as is the case of the population of Chala - Arequipa (Peru) where the Organismo de Evaluación y Fiscalización-OEFA found cyanide concentrations above the environmental quality standards; Therefore, the need to remediate the water resource has made it possible to seek new technologies in the detoxification and destruction of contaminants such as cyanide, lead, chromium, etc., replacing the traditional approach of disposal and confinement (Garzón et al., 2017), methods such as electrochemical precipitation (Li et al., 2022), photoelectrocatalytic oxidation (Wei et al., 2022), using quaternary polyethylenimine - cellulose fibers (Lin et al., 2022), combined methods of electrocoagulation, adsorption and wetlands (Pratiwi et al., 2021) and natural adsorbents (up to 89 %) (Sain et al., 2021).

The forms of environmentally friendly technologies are bioremediation using microorganisms, fungi, plants enzymes produced by them (Muñoz and Guillén, 2015). Bioremediation of water using microorganisms is a method that is being investigated more frequently in order to find the best efficiency of their metabolization to degrade pollutants such as cyanide. This biological process carried out by certain microorganisms with the capacity to use cyanide to obtain carbon or nitrogen results in a less toxic by-product (Restrepo et al., 2006). For cyanide-contaminated wastewater contaminated with cyanide have used as bioremediation medium *Pseudomonas pseudoalcaligenes* which is able to grow in cyanide medium as the only source of nitrogen, they synthesize by metabolism the enzymes nitrilases and oxidases that provide the carbon to transform cyanide, at alkaline pH, it was possible to biodegrade up to 90 % in 8 days (Carmona, 2016); something similar was done with the biological detoxification of water from a reactor, with the same bacterial strain *Pseudomonas pseudoalcaligenes* in alkaline medium, controlling the pH (at 10) to avoid volatilizing hydrogen cyanide (HCN)

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and dissolved oxygen (10%) decreasing cyanide by 2. 8 mg CN/L (Huertas et al., 2010). In gold mining waste with in vitro assays and using the bacteria *pseudomonas fluorescens, pseudomonas alcaligenes, acinetobacter, enterobacter and Bacillus*, reduced cyanide up to 2.8 mg CN/L (Huertas et al., 2010). *Bacillus*, reduced up to 96 % of cyanide in 9 days (Cardona, 2015). Another bacterium used was *Chromobacterium violaceum* together with activated sludge that reduced from 50 mg/L cyanide to the amount of 7.5 mg/L (Shin et al., 2019); in a study using a biofilm-forming bacterium to act a degrading bacterium *Rhodococcus rhodochrous BX2* reduced organic cyanide from groundwater (An et al., 2018). In other cases, microorganisms in the form of cells reduce pollution and can also be a source of energy (Sawasdee V. and Pisutpaisal N., 2018). In another research Pb was reduced with a consortium of microbial (Peens J. et al., 2018).
Bioremediation provides a solution to the problem of contamination by chemical compounds in water and soil, being a viable and promising alternative (Garzón et al., 2017); therefore, this study provides new basic knowledge about this biotechnological treatment using a microbial biofilm with microorganisms for remediation of cyanide contaminated wastewater, verifying the feasibility and efficiency of the method to recover cyanide wastewater, avoiding negative impacts on the environment.

2. Methodology

The experimental process of the research followed the following phases:

- Obtaining 60 liters of contaminated water sample from a gold processing plant in the town of Chala-Arequipa, following the normative protocol RS 004-94-EM-DGAA and subsequent evaluation to determine the level of cyanide compared with the Maximum Permissible Limits stipulated in the DS. 010-2010-MINAN.
- Isolation of microorganisms from the water sample in the laboratory, which were subjected to adaptation and purification in high cyanide medium.
- Identification of *Pseudomona aeruginosa* and *Escherichia coli* microorganisms by biochemical Gram stain test.

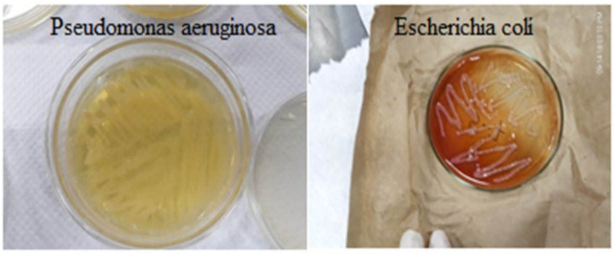

Figure 1: Microorganisms identified, Pseudomona aeruginosa y Escherichia coli

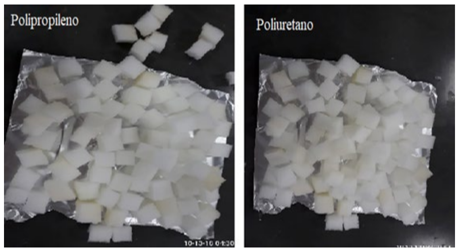

Figure 2: Biofilm supports

- Design and construction of 0.30 m long, 0.25 m wide and 0.35 m deep bioreactor cells (Figure 3).

Figure 3: Bioreactor in operation

- Preparation of supports for the microbial biofilm of polypropylene and polyurethane material in cubic form of 1 cm side (Figure 2).
- Treatment of the cyanide contaminated water samples in the bioreactor cells coded and numbered as indicated in the matrix presented in Table 1.
- Finally, the characterization of the treated water samples was carried out.

Table 1: Treatment factor matrix

Type of microorganism for the biofilm	Amount of inoculum (mg/L)	Treatment time (days)	Amount of carriers (units)	Repeat code
Pseudomona aeruginosa	1500	5	100	R1 (3 repetitions in reactor 1)
		6	100	R2 (3 repetitions in reactor 2)
		7	100	R3 (3 repetitions in reactor 3)
Escherichia coli	1500	5	100	R1 (3 repetitions in reactor 1)
		6	100	R2 (3 repetitions in reactor 2)
		7	100	R3 (3 repetitions in reactor 3)

3. Resulted and discussion

3.1 Initial characteristics of the water sample for treatment

Table 2 shows that the industrial wastewater sample contained 1,352 mg/L of cyanide; this value exceeds the maximum permissible limit (MPL) for discharges of liquid effluents from mining-metallurgical activities, established by Peruvian regulations (MINAM, 2010), whose values are 1 mg/L at any time and 0.8 mg/L for the annual average. Likewise, the pH exceeds the MPL, which establishes that it must be between 6 and 9, as well as total suspended solids, which is set at 50 mg/L (limit at any given time).

Table 2: Initial characteristics of contaminated water

Parameters	Valour	units
Total cyanide	1,352	mg/L
pH	13.4	pH units
Temperature	24.5	°C
DBO	70	mg/L
Dissolved Oxygen	1	mg/L
Electrical conductivity	60	µS/cm
Total suspended solids	70	mg/L
Turbidity	90	NTU

3.2 Kinetics of biofilm inoculum growth with Pseudomonas aeruginosa and Escherichia coli

The growth of the biofilm of the bacteria Pseudomonas aeruginosa and Escherichia coli was verified as shown in Table 3, observing an increase in the volume of the inoculum due to the kinetics of growth over time due to the generation or reproduction of the bacteria. bacteria, having favourable temperature and pH for it (Ramírez et al., 2006)

Table 3: Initial characteristic of contaminated water

Biofilm	Time (h)									
	0	1	2	3	4	5	6	7	8	9
N° of *Pseudomonas aeruginosa* (CFU/mg/L)	0.001	0.016	0.024	0.033	0.039	0.043	0.048	0.039	0.055	0.059
N° of *Escherichia coli* (CFU/mg/L)	0.001	0.019	0.03	0.038	0.043	0.048	0.038	0.039	0.048	0.069

3.3 Results of growth kinetics in the treatments

With the result of the verification of bacterial growth, the contaminated water samples were treated. Figure 4 shows the growth kinetics of the *Pseudomonas aeruginosa* biofilm in three bioreactors, it is observed that there is an increase in the bacterial population due to the favourable conditions presented such as alkaline pH (Carmona, 2016), the temperature also favoured bacterial growth although the most optimal is around 35 °C (PAHO/WHO, 2015).

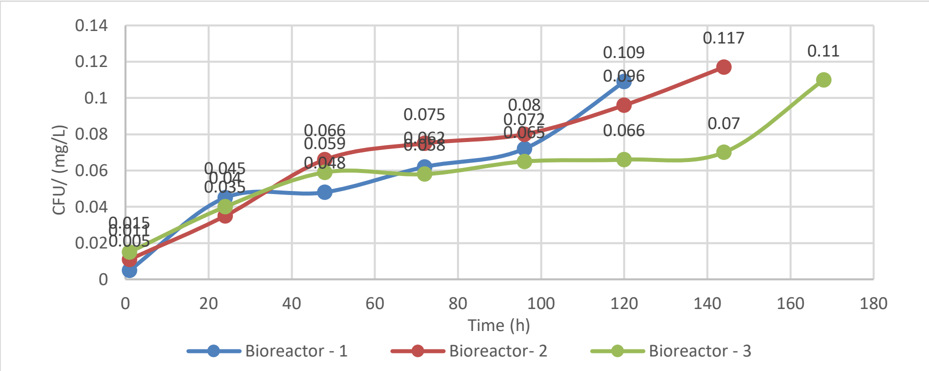

Figure 4: Growth kinetics of Pseudomas aeruginosa biofilm.

Figure 5 shows the growth kinetics for the *Escherichia coli* biofilm, tested in three bioreactors, also verifying the adaptability and growth of the bacteria.

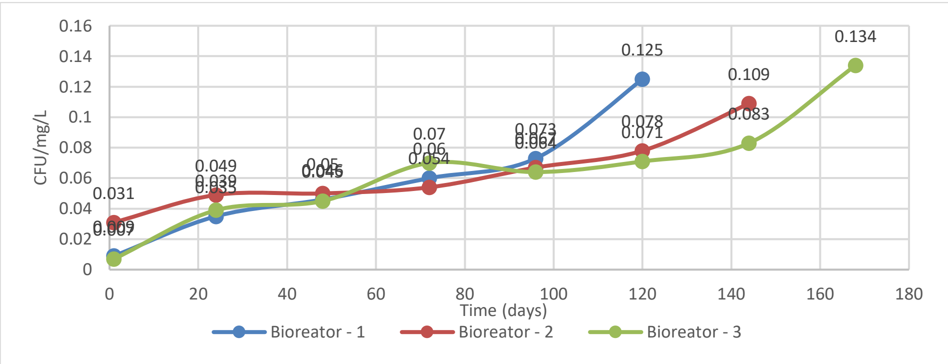

Figure 4: Kinetics of Escherichia coli biofilm growth

Table 4 shows the quantification of the biofilm population of *Pseudomona aeruginosa* and *Escherichia coli* at the beginning and end of the treatment of the cyanide-contaminated water samples. Similarly, Table 5 shows the quantification for *Escherichia coli*. In both cases, the increase in the microbial population was determined, which will intervene in the reduction of the cyanide present in the water samples.

Table 4: Initial and final population of Pseudomona aeruginosa in bioreactors during treatment

Reactor	Initial Population (CFU/mL)	Final Population (CFU/mL)
R1	550 x 10^6	1.48 x 10^{12}
R2	385 x 10^6	1.44 x 10^{12}
R3	522.5 x10^6	1.5 x 10^{12}

Table 5: Initial and final population of Escherichia coli microorganisms in the bioreactors during treatment

Reactor	Initial Population (CFU/mL)	Final (CFU/mL)
R1	6.05 x 10^{10}	1.65 x 10^{13}
R2	4.29 x 10^{10}	1.83 x 10^{13}
R3	3.465 x10^{10}	1.72 x 10^{13}

3.4 Characteristics of contaminated water samples after treatment

Figure 6 shows the reduction of cyanide in water after treatment with a biofilm of *Pseudomonas aeruginosa* microorganisms. It is observed that after 5 days cyanide was reduced from 1,352 mg/L to 38.98 mg/L (97.11%)

in reactor R1, while in the other reactors R2 and R3 the reduction percentage was lower.This possibly happens due to the operating conditions, mainly due to the activity of the microbial population as established by the scientific literature, which indicates that it can reach between 90% and 96% (Carmona, 2016) and Cardona, 2015). Using immobilized Psudomona fluorescens, cyanide was reduced by 99.12% in 48 hours with a degradation of 1.0445 mg/L-h (Naranjo and Manuel, 2006).Figure 7 shows the reduction of cyanide in water after biofilm treatment of *Escherichia coli* microorganisms. It is established that after 6 days the cyanide was reduced from 1,352 mg/L to 325.1 mg/L (75.95%) in the R2 reactor, while in the other R1 and R3 reactors the reduction percentage was lower. This biofilm was less efficient than the Pseudomonas aeruginosa biofilm in reducing cyanide; however, these reductions do not reach the level of the maximum permissible limits of the Peruvian standard, which is 1 mg/L (MINAM, 2010), taking into account that cyanide is lethal with an intake of 2-5 mg/kg of body weight (Agudelo C. et al., 2010)

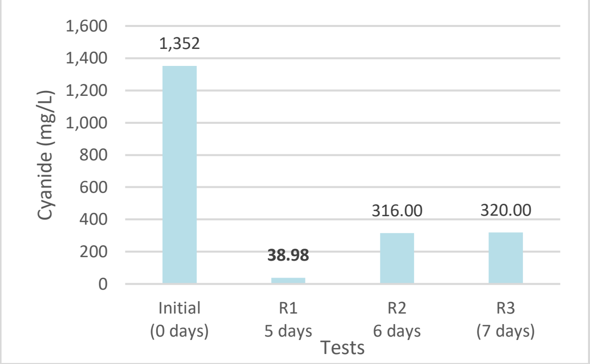

Figure 6: Reduction of cyanide with Pseudomona aeruginosa biofilm

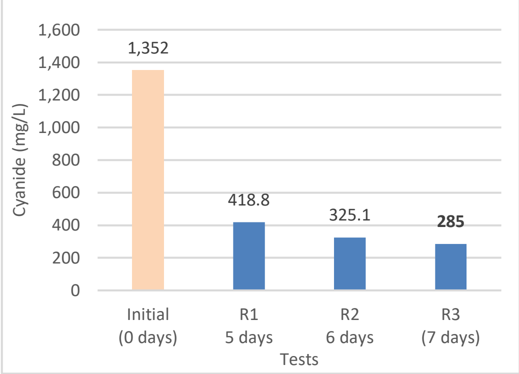

Figure 7: Cyanide reduction with Escherichia coli biofilm

The physicochemical properties of the wastewater after being treated with the biofilms are presented in Table 6, the pH decreased due to the acids generated by the bacteria but remains in the optimal range for bacterial population growth. It was found that the pH and total suspended solids in the wastewater were within the maximum permissible limits to be disposed of in the receiving body, in accordance with Peruvian environmental regulations (MINAM, 2010). The pH is very important in the recovery of aqueous cyanide compounds since at pH greater than 10.2 more than 90% of the total cyanide is as CN- ion and its recovery is more feasible, when the pH is less than 8.4 it is as HCN with a high volatility due to its high vapor pressure (100 kPa at 26°C), causing a decrease in the concentration of cyanide in the solution (Pérez and Higuera, 2008); consequently, this could have been reflected in the result of the investigation, since in the reactor with *Psudomona aeruginosa* the pH was 10.77.

Table 6: physicochemical characteristics of treated water

Treatment	pH	Temperature (°C)	Electrical conductivity (µS/cm)	DBO_5 (mg/L)	Dissolved Oxygen (mg/L)	Turbidity (NTU)	Total Suspended Solids (mg/L)
No treatment	13.4	24.5	60	70	1	90	70
With Pseudomonas aeruginosa	10.77	25.97	10.63	10.10	5.76	7.46	10.79
With Escherichia coli	8.70	23.10	51.13	38.20	6.70	7.13	13.43

4. Conclusion

With microbial biofilms of *Pseudomonas aeruginosa* and *Escherichia coli*, supported on polypropylene and polyurethane material, the concentration of cyanide in contaminated industrial wastewater was reduced by 97.11% and 75.95% for each biofilm in 5 and 7 days of treatment ; Therefore, biotechnology with microorganisms is an environmentally advantageous alternative to other methods that use chemicals and more research is needed to scale the methodology to larger volumes of effluents of this type of pollutant, which is very dangerous, lethal to life and the environment.

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