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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

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ABSTRACT

Wastewater streams generated during the leaching process of precious metals recovery (e.g. gold, silver) contain high concentrations of toxic compounds, being free cyanide (CN^-) one of the most important among them. Free cyanide is a toxic compound that affects cellular respiration and causes irreversible environment problems. Thus, effective wastewater treatment processes should be applied to reduce CN^- without producing other contaminants. This study evaluated the effect of rotational speed and carbon source on the biological removal of CN^- present on gold mine wastewater, using a rotating biological contactor (RBC).

A factorial design (2^3) was developed by triplicate (N = 24) to evaluate: carbon source concentration (commercial sugar cane, 99% sucrose), free cyanide concentration (CN^-), and rotational speed (ω) on the biological removal of free cyanide (%B.R.). The bioreactor worked on continuous system under the following conditions: V_{RBC} = 4.2L, F_I = 0.42L/h (gold mine wastewater at 10 or 300 mg CN^-/L, liquid culture media with or without carbon source, final pH = 10.5 ± 0.5), HRT = 10 h, and ω = 5 or 10 rpm.

According to the factorial analysis: free cyanide concentration ($[CN^-]_i$), carbon source concentration ($[FC]_i$), and rotational speed (ω) affected significantly ($p < 0.05$) during the biological removal of CN^-. Furthermore, the highest biological removal percentage (96.89%) was achieved when the RBC worked under the following conditions: $[CN^-]_i$ = 300 mg/L, $[FC]_i$ = 3.8 g/L, and ω = 5 rpm; even though dissolved oxygen reached levels below 2 mg/L. Thus, biological removal increased when free cyanide and carbon source concentrations were at their highest level 300 mg/L and 3.8 g/L, respectively; however rotational speed (10 rpm) diminished the bioprocess.

1. Introduction

Cyanide has been widely used among different industries such as: plastic, chemistry, photography, and mining [1–3]. The mining industry uses alkaline solutions containing sodium cyanide (NaCN) (100–500 mg/L) as a method to recover precious metals (e. g. gold, silver) through cyanidation or leaching process [1,4–7]. However, wastewater generated from this process contains different forms of cyanide, for example: free cyanide (HCN o CN^-), weak acid dissociable (WAD), strong cyanide complexes, etc.; which as a whole are known as total cyanide (0.5–1000 mg/L) [8–13].

Free cyanide is known by its high toxicity, it inhibits cellular respiration and causes irreversible environmental issues, such as: water pollution (e.g. rivers and groundwater), land and aquatic sediments contamination [7,9,10,14–16]. In consequence, it has been suggested that mining industries should reduce free cyanide concentrations present on wastewater to < 0.1 mg/L before its discharge [17,16], in order to reduce the environmental impact.

Nowadays, physico-chemical processes (e.g. INCO process, hydrogen peroxide process catalyzed by copper, Caro's acid, chlorination, iron precipitation and natural attenuation) have been used to remove free cyanide present on gold mine wastewater [1,2,15]. However, these processes present several disadvantages, including high costs, maintenance difficulty, and production of toxic compounds.

Biological treatments, however, have become an alternative because of their effectiveness, cheaper operating costs, and less environmental impact [8,1,18].

During free cyanide biodegradation, microorganisms convert it to less toxic compounds (e.g. NH_4^+ y CO_3^{2-}) [2,6,9,19–21] through different pathways, such as: oxidative, hydrolytic, reductive, and

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substitution/transfer which enables them to tolerate and degrade the compound [22,21].

Studies have reported different genus of microorganisms able to degrade free cyanide, for example: *Pseudomonas* (e.g. *P. fluorescens, P. pútida, P. aeruginosa*), *Bacillus* (e.g. *B. pulimus* C1), *Acinetobacter*, and *Burkholderia* [23,24,11,15]. Because free cyanide, as a carbon source, represents a highly toxic compound (e.g. growth inhibition) [23,20,25,26], its biological removal is enhanced by the presence of an additional carbon source (e.g. glucose, acetate, starch, dextrose) in the culture media, which are mostly added as pure reactant [27–29,23,13,25].

The most common bioreactors types used to remove free cyanide are: fluidized bed reactor, discontinued reactor, packed bed reactor, and rotating biological contactor (RBC) [30–33]. The RBC system is widely used in the biological treatment of domestic and industrial wastewater because it consumes less energy and its operating conditions are cheaper than physicochemical processes. Moreover, rotational speed enhances the correct function of a RBC system, because it affects the mass transfer of dissolved oxygen, substrate, and nutrients from the liquid to the biofilm, and helps to maintain the biofilm thickness, as well [34,35].

Studies regarding to cyanide biodegradation evaluate one parameter at the time, for example: cyanide concentration [15,20,26,27,36–39], carbon source [26,27,38], hydraulic retention time [36], microorganism [27], pH [37,25,15], and temperature [15]; which means that all the factors are independent of each other. Furthermore, this method require large set of experiments, is time consuming, and its results could not be accurate. Due to these limitations, factorial design (2^n) is commonly used to evaluate the effect of independent factors and its interactions over the response variable within an established set of experiments [40].

Here we used a factorial design (2^3) to evaluate the effect of free cyanide concentration, rotational speed, and carbon source concentration (commercial sugar cane, 99% sucrose) on the biological removal of free cyanide present on gold mine wastewater, using a rotating biological contactor.

2. Materials and methods

2.1. Wastewater samples

The gold mine wastewater samples were collected from ORENAS S. A., located at La Lopez, Camilo Ponce Enriquez, Azuay, Ecuador (UTM: 17′642505 E y 9′658117 N). The first sample was from the cyanidation tanks (675 mg CN^-/L) and the second after the INCO process (SO_2-gas) from the precipitation pool (10 mg CN^-/L) (Table 1).

Table 1
Physicochemical analysis of the gold mine wastewater.

[a]Parameter	Cyanidation tanks	After INCO process	Unit
pH	10.97 ± 0.03	7.10 ± 0.06	–
Temperature	27.03 ± 0.35	26.72 ± 0.42	C
Dissolved oxygen	17.57 ± 0.30	17.16 ± 0.32	mg/L
Free cyanide	675.00 ± 0	9.52 ± 0	mg/L
[b]Cu	113.417 ± 0.012	0.070 ± 0.028	mg/L
[b]Fe	2.214 ± 0.005	0.328 ± 0.015	mg/L
[b]Au	0.561 ± 0.110	0.260 ± 0.041	mg/L
[b]Ag	0.664 ± 0.004	< 0.001	mg/L
[b]Pb	0.679 ± 0.009	0.256 ± 0.011	mg/L
[b]Na	1482.167 ± 0.059	41.603 ± 0.187	mg/L

[a] Mean of 3 ± s.d.
[b] Metals where quantified by atomic absorption at Analytical and Instrumental Laboratory, Chemical and Exact Sciences Department, Tecnica Particular de Loja University.

2.2. Microbial consortium

The microbial consortium used to inoculate the RBC, was isolated from sediment contaminated by wastewater streams at ORENAS S. A. [41] unpublished). Then it was exposed to real gold mine wastewater containing free cyanide in the study developed by Jumbo and Nieto [42]. After that, 16S RNA gen was amplified from the microbial consortium with 1492R primer and sequenced at Macrogen. Finally, the sequence was compared with NCBI database, and *Bacillus* sp. was obtained as the most similar [43] unpublished).

2.3. Rotating biological contactor (RBC)

The rotating biological contactor (Fig. 1a) used in this study was built in stainless steel and with an electrical power engine. The RBC has 5 compartments connected by small channels on its bottom. Compartments 1, 2, and 3 had 6 wood discs for each compartment, which were located on a horizontal axis and submerged 40% in the wastewater during the experiments; and compartments 4 and 5 received the treated wastewater. The rotational speed was adjusted manually and controlled automatically.

2.4. Biological removal of free cyanide

2.4.1. Biofilm growth

A batch culture was developed during 40 days to allow the biofilm growth over the discs. The total working volume was 4.2 L (compartments 1, 2, and 3) and was composed by: 83% of gold mine wastewater (300 mg CN^-/L, was diluted in distilled water), 2% of liquid culture media (Table 2), 15% of the microbial consortium ($2.50E^{+07}$ cells/mL), and pH was kept constant at 10.5 ± 0.5 (8 M NaOH). The rotational speed was 5 rpm and the temperature was 20 ± 5° C.

2.4.2. Experiments

A factorial design 2^3 by triplicate was developed with the Minitab 17.1.0 software (Table 3). The factors evaluated were: rotational speed (ω), carbon source concentration (commercial sugar cane, 99% sucrose, $[FC]_i$), and free cyanide concentration ($[CN^-]_i$), over the response: biological removal (% B. R.).

A total of 24 experiments and 8 controls were ran in a continuous system, under the following conditions: hydraulic retention time (HRT) of 10 h, working volume of 4.2 L (compartments 1–3), influent flux of 0.42 L/h, pH was kept constant at 10.5 ± 0.5, and laboratory temperature of 20 ± 5° C. A Cole-Parmer peristaltic pump was used to transfer the influent at 0.42 L/h (gold mine wastewater at 10 or 300 mgCN^-/L, liquid culture media with or without carbon source, pH = 10.5 ± 0.5) from the reservoir pool to the RBC. The flux entered by the compartment 1 passed through small channels to compartments 2 and 3 (where the biological removal took place) to finally exit in compartments 4 and 5. Control experiments (without biofilm) were performed using gold mine wastewater (10 or 300 mg CN^-/L) and liquid culture media without carbon source (pH = 10.5 ± 0.5) (Fig. 1b).

2.5. Analytical methods

2.5.1. Free cyanide

Free cyanide concentration was quantified every hour at each compartment by two methods: for concentrations < 10 mg CN^-/L, an ion selective cyanide electrode (Thermo Scientific Orion 9606 BNWP) was used. For concentrations > 10 mg CN^-/L titration with silver nitrate ($AgNO_3$) was used with potassium iodide (KI, 5%) as indicator [8].

2.5.2. Dissolved oxygen

A 30 mL sample was taken from each compartment every hour. Then, DO was measured using a Hanna Instruments Dissolved Oxygen/BOD Meter (HI 98186-019) [44].