

Performance of Various Cyanide Degrading Bacteria on the Biodegradation of Free Cyanide

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Abstract—This study reports on the biodegradation of free cyanide (FCN) by cyanide degrading bacteria (CDB) that were isolated from mining wastewater and in thiocyanate containing wastewater. The performance of these isolates was compared to cryopreserved CDBs that were used in precious studies. The performance of the isolates to degrade FCN was studied in batch cultures. It was observed that the CDB from the thiocyanate wastewater showed higher biodegradation rates ($2.114 \text{ g CN}^- \cdot \text{L}^{-1} \cdot \text{O.D}_{600\text{nm}}^{-1} \cdot \text{h}^{-1}$) compared to the isolates from the mining wastewater. The isolates from the cryopreserved CDBs and from the mining wastewater achieved a biodegradation rate of $1.285 \text{ g CN}^- \cdot \text{L}^{-1} \cdot \text{O.D}_{600\text{nm}}^{-1} \cdot \text{h}^{-1}$ and $1.209 \text{ g CN}^- \cdot \text{L}^{-1} \cdot \text{O.D}_{600\text{nm}}^{-1} \cdot \text{h}^{-1}$, respectively. This study demonstrated that the source of the organisms plays a significant role on FCN biodegradation.

Keywords—Cyanide degrading bacteria; Free cyanide; Mining wastewater; Thiocyanate.

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I. INTRODUCTION

Free cyanide (FCN) is the most toxic form of cyanide compounds [1]. This chemical compound is also the main product that is used in metallurgical processes for precious metal recovery [2].

Effluents containing FCN should be treated before they are realised into the environment to avoid detrimental effects on the environment medium, such as the contamination of fresh water sources and soil pollution [3], which would culminate in deleterious human health outcomes such as: metabolic acidosis, coma, seizures, bradycardia, and lack of response to oxygen treatment [4].

Physical, chemical and biological treatments are the different treatment methods available for FCN [5]. Nevertheless, biological methods are the most applied as the technology is inexpensive and due to the absence of hazards by-products from the process, as seen in most other treatment methods [6]. Microorganisms that are used to degrade FCN, have an ability to break the triple bond between the carbon and nitrogen atoms ($\text{C}\equiv\text{N}$) in FCN and use these elements as a carbon or/and nitrogen source for their metabolism [7]. Microorganisms use enzyme to accelerate the bond destruction of FCN [8] with the biodegradation by-products formed, being dependent on the enzyme type that the microorganisms use and the conditions of the bioreactor used, i.e. aerobic or anaerobic conditions [9]. Several pathways are available to biodegrade FCN but the hydrolytic pathway is the most commonly used process [10]. Enzymes for hydrolytic conversion of FCN include nitrogenase, cyanide hydratase, nitrile hydratase, thiocyanate hydrolase, nitrilase, and cyanidase [11] among others. Depending on these enzymes, various by-products from FCN biodegradation could be formed such as: ammonium nitrogen ($\text{NH}_4^+\text{-N}$), ammoniac (NH_3), formate (HCOO^-), Formamide (HCNOH_2), Carbonyl sulphide (COS), sulphide (SO_3^{2-}) and hydrogen sulphide (H_2S) [12]–[16].

However the environmental conditions could affect the

enzyme activity from the microorganisms known for FCN conversion [17]. These external factors include pH and temperature, which makes it difficult to choose suitable microorganisms for bioreactors used to biodegrade FCN [18]. However, microorganisms, depending on their species, have an ability to acclimate to new environmental conditions during their latency phase [19]. Huertas et al. [20] have reported a FCN biodegradation latency phase of 20 h for *Pseudomonas pseudoalcaligenes* CECT5344 isolated from river water. Generally, most of FCN biodegradation technologies, use different microorganisms sourced the wastewater containing the pollutant of interest, from which a consortia can be engineered. Mekuto et al. [12] have studied FCN biodegradation in FCN contaminated wastewater by using a consortium of different *Bacillus* sp. and found a similar trend of FCN biodegradation when compared to other studies. Nevertheless, few studies focused on the FCN biodegradation performance by each CDB collected from different sources.

This research was aimed at evaluating the performance of bacteria when their source medium different. This paper also supports a decision on the choice of the suitable source of the CDB for FCN biodegradation for large-scale operations.

II. MATERIALS AND METHODS

A. Materials Sources

Microorganisms (n=5) were tested for their performance to degrade FCN. These microorganisms were collected from different sources.

Three (n = 3) bacterial strains (C_1 , C_2 and C_3) conserved at -80°C were obtained from of the *BioERG* laboratories at the Department of Biotechnology, CPUT, were recultured in Tryptone Soy broth and incubated at 37°C for 24h before isolation.

Two (n= 2) microorganisms, each from mining wastewater (C_m) and thiocyanate containing wastewater (C_t) were collected from from a mining company in South Africa.

The thiocyanate wastewater was also mixed with wastewater from a previous study in which the effluent was treated [21].

B. CDB Isolation

Two different media, without nutrients (SN) and with nutrients (AN) were prepared. The SN medium was enriched using nutrient agar (Merck, South Africa) and 2 g $\text{CN}^- \text{L}^{-1}$ from KCN (Merck, South Africa). AN medium had the same composition as the SN, with several supplemental nutrient sources and trace metals being added as highlighted in [3]. A volume (200 μL) of the mining wastewater and thiocyanate wastewater was spread plated in agar plates constituted by different media, subsequent to incubation at 37°C for 7 days to assess microbial growth. The visible colonies on agar plates were grown in Tryptone Soy Broth (TSB) solution at 37°C overnight. Microorganisms imaging using a Scanning Electron Microscopy (SEM) was prepared according to [21] but hexamethyldisilazane (HDMS) solution was replaced by silicon tetrachloride solution before SEM visualisation. Microbial samples in TSB solution were centrifuged at 10 000 g for 5 min. and fixation in 2.5 % glutaraldehyde for 24 h at 4°C . The

glutaraldehyde solution was discarded and the microbial pellets were washed twice by using a phosphate buffer (pH 7) before dehydration in an ethanol series of 50%, 70% and 100% during a 12h period at 4°C . Thereafter, silicon tetrachloride solution was used for drying the samples and the final microorganisms pellets were sent for SEM analysis.

C. FCN Biodegradation Tests

The observable colonies from the agar plates containing 2 g $\text{CN}^- \text{L}^{-1}$ were streaked and grown in Tryptone Soy broth (Merck, Germany) and thereafter incubated at 37°C overnight. The isolates were recultured and tested for their ability to biodegrade free cyanide as KCN (Sigma Aldrich, Germany), in solutions containing 1, 2 and 3 g $\text{CN}^- \text{L}^{-1}$ subsequent to use in mining wastewater biodegradation studies. A volume (1 mL) of the bacterial isolates was added in 99 mL of the KCN solution and in 99 mL of the mining wastewater; an inoculum concentration equivalent to 1% v/v. The pH was set at 8.5 and the temperature was kept at 25°C , with a constant incubator rotation of 120 rpm. FCN, NH_4^+ concentrations and bacterial growth were quantified every 2h over a 24h period. Photometric methods were used to measure the FCN and NH_4^+ concentrations according to the analytical methods reported in Mekuto et al. [22], in which Merck tests kits 09701 and 00683 were used to measure FCN and NH_4^+ concentrations by using Merck Spectroquant Nova 60 instrument, respectively. Bacteria density was quantified at a wavelength of 600 nm using spectrophotometer (JENWAY 7305 series). At the end of the biodegradation tests, i.e. when the FCN concentration was below the detection limit ($<0.010 \text{ mg CN}^- \text{L}^{-1}$), a small volume of the solution was recovered from each test subsequent to drying at 60°C for 30 min, samples which were sent for XRD analyses.

D. CDB Performance Evaluation

FCN biodegradation performance (P_{FCN}) for each CDB was calculated according the following equation:

$$P_{\text{FCN}} = [\text{FCN}_i] \times 1/\text{BD}_i \times 1/t \quad (1)$$

Where:

$[\text{FCN}_i]$: FCN concentration initial (g. L^{-1}),

BD_i : Initially Bacterial Density (O.D._{600nm}), and

t: degradation duration (h).

III. RESULTS AND DISCUSSION

A. FCN Biodegradation

FCN biodegradation by various sources of the CDBs have shown a similar trend as presented by the FCN profiles in Figure.1.

Fig.1 (a) shows O.D._{600nm} of the CDB decreased during the first 4h and increased to the optimal value before decreasing again. The initial O.D._{600nm} of the CDB was respectively 0.055; 0.048; 0.050; and 0.035 for C_1 , C_2 , C_3 , C_m and C_t . After the first 4h, the O.D._{600nm} values increased from 6×10^{-3} to 5×10^{-2} ; 4×10^{-3} to 10^{-2} ; 5×10^{-3} to 2×10^{-2} ; 5×10^{-3} to 10^{-2} ; and 8×10^{-3} to 7×10^{-2} for C_1 , C_2 , C_3 , C_m and C_t , respectively. All CDBs tested were able to grow on the medium containing various FCN concentrations. Nevertheless, from the medium with an FCN

concentration of 3g CNL^{-1} , the CDB growth was minimal, with the achieved maximum O.D._{600nm} of 0.007; 0.008; 0.008; 0.01 and 0.014 for C_1 , C_2 , C_3 , C_m and C_t , being observed respectively. FCN biodegradation rates of 99% were obtained for all tests after 28 h, 37h and 72 h in 1g, 2g and 3g CNL^{-1} solutions, respectively.

In addition, NH_4^+ was produced during the FCN biodegradation tests a by-product. The optimal NH_4^+ concentration varied in function of the CDB types (0.12 mgL^{-1} for C_1 , 0.08 mgL^{-1} for C_2 , 0.10 mgL^{-1} for C_3 , 0.05 mgL^{-1} for C_m , and 0.16 mgL^{-1} for C_t). The NH_4^+ concentration produced was initially very high than its value at the end of the tests. NH_4^+ was used by the CDB for their growth as highlighted in [23]; as nitrifying CDB could also convert NH_4^+ to nitrate and nitrite. Biodegradation of the FCN leads to NH_4^+ formation as reported by various previous studies on FCN biodegradation [24][25]. The formation of NH_4^+ reveals a FCN biodegradation by hydrolytic pathway[5].

FCN biodegradation in mining wastewater was achieved in a short period of less than 10 h, i.e. 5 h for C_t (Fig.1 b) and in 6h for C_1 , C_2 , C_3 and C_m . The exponential phase of the bacterial growth was observed after 1 h of contact time between CDB and the mining wastewater. Then, CDB growth declined proportionally with the decreases in the pollutants (NH_4^+ and FCN).

The CDB and FCN substrate equilibrium time was 2 and 8 h for KCN solution and the mining wastewater, respectively.

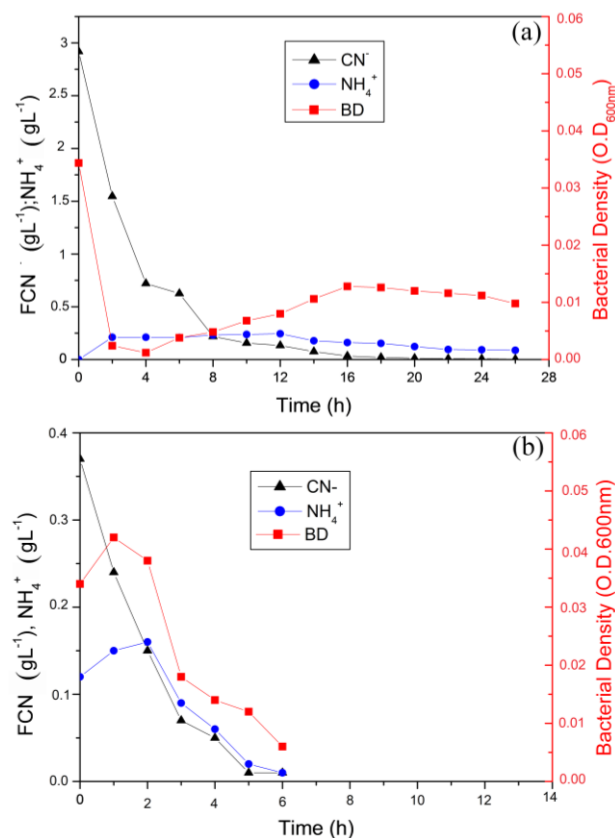


Fig. 1: FCN Biodegradation by C_t : (a) in KCN solution synthesised containing 3g CNL^{-1} and (b) in Mining wastewater containing 0.37g CNL^{-1}

Each CDB has its own unique behaviour and performance to degrade FCN. Figure 2 presents the topographic surface of the biomass from different CDBs used after growing in the medium containing FCN. Two different surfaces were observed for all biomass such as the background surface and the front surface. The background surface formed by the irregular holes represents the biomass support. Same background surface was observed for all biomass. The front surface is specifically for each type of CDB biomass. More gums are observed on the front surface for C_t biomass followed by C_m and C_2 biomass. C_1 and C_3 biomass had minimal gums. These gums represents the nutrient and FCN uptake in the biomass [5]. Biofilm biomass embedded in these pollutants could affect the topographic surface of the biomass [5]. Therefore, C_t embedded highest FCN concentration than C_m and C_2 . C_1 and C_3 have the lowest ability to uptake FCN.

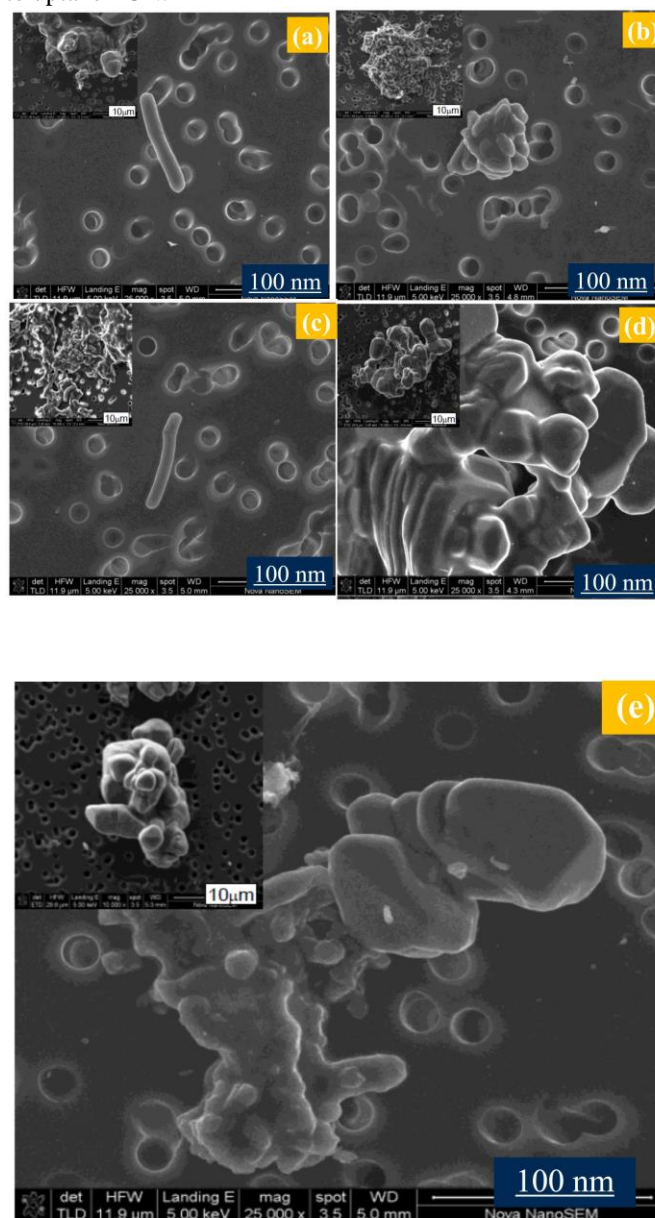


Fig. 2: SEM images: (a) C_1 , (b) C_2 , (c) C_3 , (d) C_m and (e) C_t

Besides the by-product i.e. NH_4^+ , some crystallographic materials have been detected in the mixture of FCN and CDB solution, shown as deposits as illustrated in Figure 3.

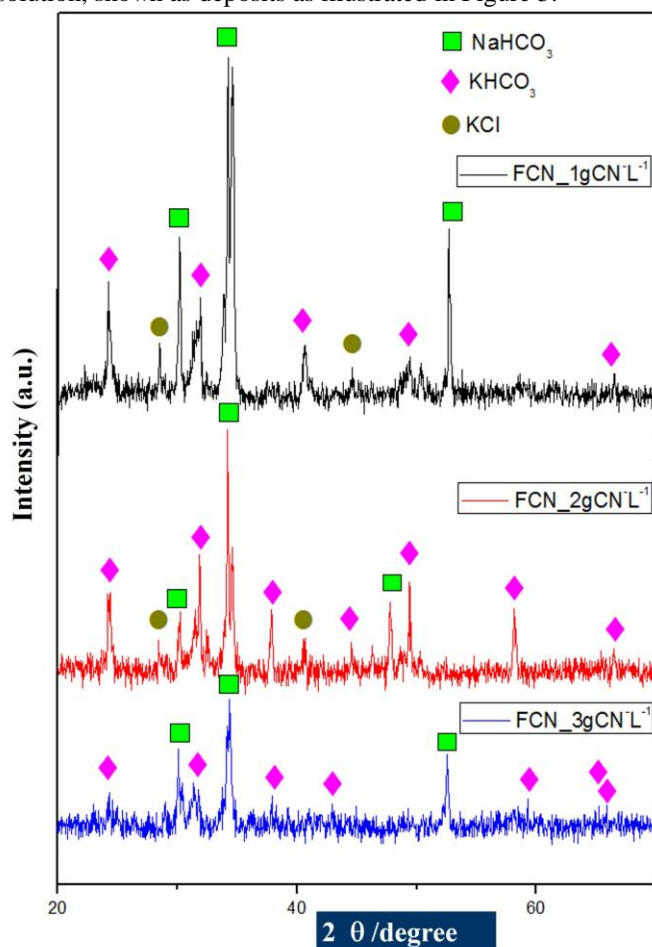


Fig. 3: XRD patterns of FCN solution deposit

Three kind of crystallographic materials Nacholite (NaHCO_3), Kalicinite (KHCO_3) and Sylvite (KCl) were produced. The first two products have a monoclinic facial and the third one has a face-centered cubic. The by-product depends on the enzyme used by the CDB to degrade FCN [9]. In addition, Figure 3 shows the absence of the CN compound as a complex. CN chemical compounds were determined to have been consumed and/or converted by the CDB since the CDBs can utilise the CN in their metabolism as reported in [9]. Also, FCN biodegradation by-product peaks are an inverse to the initial FCN concentration being observed in solution; albeit, CDBs had a difficulty in degrading FCN at the highest concentration.

From the XRD profiles, the KCl disappearance at FCN concentration of 3 g $\text{CN}\cdot\text{L}^{-1}$ was evident. This was hypothesised to be a resultant toxicity of the FCN that enabled the non-formation of this by-product at higher concentrations as reported by Kuyucak and Akcil [26].

B. CDB Performance

CDB performance to degrade FCN is presented in Figure 4. The isolate labelled as C_1 had the highest P_{FCN} value (i.e. 1.905 g $\text{CN}^{-1}\cdot\text{L}^{-1}\cdot\text{O.D}_{600\text{nm}}^{-1}\cdot\text{h}^{-1}$ for the KCN solution and 2.114 g $\text{CN}^{-1}\cdot\text{L}^{-1}\cdot\text{O.D}_{600\text{nm}}^{-1}\cdot\text{h}^{-1}$ for the mining wastewater). Isolates C_2 , C_3

and C_1 had similar P_{FCN} value of 1.190 g $\text{CN}^{-1}\cdot\text{L}^{-1}\cdot\text{O.D}_{600\text{nm}}^{-1}\cdot\text{h}^{-1}$; 1.176 g $\text{CN}^{-1}\cdot\text{L}^{-1}\cdot\text{O.D}_{600\text{nm}}^{-1}\cdot\text{h}^{-1}$ and 1.102 g $\text{CN}^{-1}\cdot\text{L}^{-1}\cdot\text{O.D}_{600\text{nm}}^{-1}\cdot\text{h}^{-1}$ for the KCN solution and 1.285 g $\text{CN}^{-1}\cdot\text{L}^{-1}\cdot\text{O.D}_{600\text{nm}}^{-1}\cdot\text{h}^{-1}$; 1.233 g $\text{CN}^{-1}\cdot\text{L}^{-1}\cdot\text{O.D}_{600\text{nm}}^{-1}\cdot\text{h}^{-1}$ and 1.121 g $\text{CN}^{-1}\cdot\text{L}^{-1}\cdot\text{O.D}_{600\text{nm}}^{-1}\cdot\text{h}^{-1}$ for the mining wastewater, respectively. Isolate C_m had the lowest P_{FCN} value of 1.06 g $\text{CN}^{-1}\cdot\text{L}^{-1}\cdot\text{O.D}_{600\text{nm}}^{-1}\cdot\text{h}^{-1}$ in the KCN solution and 1.209 g $\text{CN}^{-1}\cdot\text{L}^{-1}\cdot\text{O.D}_{600\text{nm}}^{-1}\cdot\text{h}^{-1}$ for the mining wastewater.

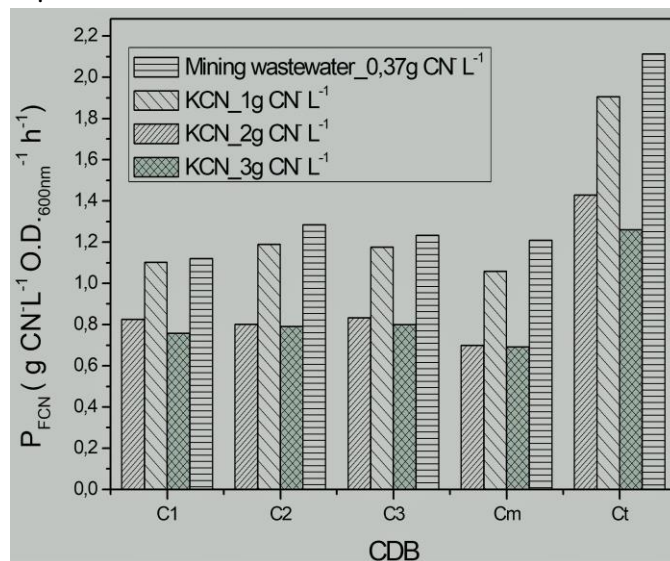


Fig. 4: FCN biodegradation performance(P_{FCN}) by various CDB

These results are similar to biomass SEM images, which revealed the biomass surface of C_t , which had a highest P_{FCN} value, thus containing the highest gums than C_m and C_2 . Biomass surface of C_1 and C_3 , that had the lowest P_{FCN} value, contained the lowest gums. Only the biomass C_m , which had very low P_{FCN} had shown more gum than C_1 , C_2 and C_3 .

P_{FCN} for all CDB was very higher for the lowest initially FCN concentration than the highest initially FCN concentration. FCN concentration of 3g $\text{CN}\cdot\text{L}^{-1}$ was the highest FCN concentration biodegraded by all CDB types used.

Overall, the initial concentration of FCN could affect its biodegradation [19]

FCN in mining wastewater was easily biodegraded by all CDBs. Mining wastewater liquid medium is rich in trace nutrients such as metals and had an initial FCN concentration that was low (0.37 g $\text{CN}\cdot\text{L}^{-1}$), thus the ability of the CDBs to effectively remove the pollutant in the wastewater.

IV. CONCLUSION

CDB from different sources were tested for the biodegradation of free cyanide (FCN). CDBs that were isolated from the thiocyanate solution (C_t) have shown their potential to degrade the FCN at a higher degradation rate ($P_{\text{FCN}} = 2.114$ g $\text{CN}^{-1}\cdot\text{L}^{-1}\cdot\text{O.D}_{600\text{nm}}^{-1}\cdot\text{h}^{-1}$) as confirmed by the SEM images with a highest gums concentration embedded within the C_t biomass. Besides NH_4^+ , various crystallographic by-products were also detected after the FCN biodegradation process. Further research will determine the enzymes that the CDBs utilized, in order to explain the reaction mechanism that ensued during FCN biodegradation and evaluate whether they can be recovered for further applications.

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