Biodegradation of free cyanide by bacterial species isolated from cyanide-contaminated artisanal gold mining catchment area under Sahelian climate in Burkina Faso

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Abstract

Soil and water samples were collected from a catchment area affected by illegal artisanal cyanidation activities for gold extraction in Burkina Faso to evaluate cyanide contamination and the presence of Cyanide Degrading Bacteria (CDB). Free cyanide (F-CN) and potential CDB were detected in all samples, with F-CN concentrations varying from 0.023 to 0.563 mg Kg^{-1}, and 0.7 to 23 µg L^{-1} in soil and water samples, respectively. The isolated species were then grown in liquid medium containing 40, 60 and 80 mg F-CN L^{-1}, with and without nutrients addition, at pH 9.5 and room temperature to test their effective F-CN degradation capacity. It was found that more than 95% of F-CN was removed within 25 hours, and that F-CN removal was associated with bacterial growth and ammonium production. However, F-CN initial concentrations higher than 100 mg L^{-1} have inhibited bacterial growth and cyanide degradation. Finally, abiotic tests have shown that less than 3% of F-CN was removed probably due to volatilization.

It was concluded that the removal of F-CN was mainly biologically mediated and suggested as primarily for detoxification. The locally isolated bacterial species have high application potential for the bioremediation of cyanide-contaminated artisanal gold sites under Sahelian climate.

Keywords: soil, water, toxic chemicals, contamination, bioremediation.

1. Introduction

Cyanide is a strong toxic chemical frequently used in various industrial processes, including synthetic fiber production, extraction of gold and silver, coal processing and extractive metallurgy (Boucabeille et al., 1994). In the mining industry, it is also widely used to recover gold residues after mercury amalgamation (Carling et al., 2013). In the environment, it can be present in three forms: free cyanide (F-CN), cyanide weak acid dissociable (WAD-CN) and strong acid dissociable (SAD-CN) (Botz et al., 1995); with hydrogen cyanide (HCN), a F-CN component, being the predominant one in most natural waters (Doudoroff, 1976; Moran, 1998; Souren, 2000). HCN and other cyano-compounds that liberate F-CN ions are highly toxic to almost all living organisms, and their presence in natural waters and soils can be harmful to aquatic ecosystems and to human health (Clarke and Morna, 2009; Hijosa-Valsero et al., 2013). Most of the cyanide contamination is linked with gold mining activities (Clarke and Morna, 2009). Numerous chemical and physical processes have been developed to remove cyanide from contaminated water and soil. However their applications were often limited, due to environmental variations, operational costs and the production of hazardous by-products (Akcil, 2003; Dzombak et al., 1996; Kumar et al., 2013). On the other hand, biological processes are known to be environment-friendly and cost-effective. Kumar et al. (2013) have suggested that the use of microorganisms could be the best alternative to transform cyanide compounds into less toxic ones in the environment. Many studies have demonstrated the capacity of microorganisms to degrade efficiently F-CN (Boucabeille et al., 1994). Mekuto et al. (2013) have tested bacteria collected from electroplating wastewater. Besides, Boucabeille et al. (1994) investigated the capacity of bacteria from mining wastewater storage basin sludge. Other tests have been conducted with known species of bacteria not directly related with soil or water surrounding mining activities (Dash et al., 2008; Ezzi and Lynch, 2005; Gurbuz et al., 2009).

Though officially prohibited, the use of cyanide in artisanal small scale gold mining (ASGM) is widespread in West-Africa, particularly in Burkina Faso (Butaré and Keita, 2009). However, due to the illegal aspect of the activity, it is extremely difficult to access ASGM cyanidation sites. As such, very few data are available on the cyanide use and environmental distribution in ASGM in the literature. During a baseline survey conducted in 2014 at the “Zougnazagmiline” ASGM site, located in the northern part of Burkina Faso,

authors have found that the quantity of cyanide used in only one ASGM site could reach easily 20 Kg per week. Then, the cyanide-containing leachate was released to the environment without any treatment or control.

The objectives of the present study were then to look for the distribution of F-CN in water and soil samples collected from the Zougnazagmiline site, Burkina Faso, to understand F-CN transport at catchment level; then, to search for potential CDB in the contaminated site; and finally, to test at lab-scale the biodegradation capacity of these potentially cyanide degraders. The work was focused on F-CN as it is the most toxic form of cyanide in the environment (Botz et al., 2005).

2. Material and methods

2.1 Site description

The "Zougnazagmiline" site was identified in 2014 during a baseline survey on the quantification of toxic chemicals used in ASGM and the perception of mine workers on health risks. The catchment with an area of 22 Km^2, is located in the Center-North Region of Burkina Faso, in the District of Bouroum, about 300 Km far from Ouagadougou, the capital city (Fig.1). The climate is characterized by Sahelian climate type with an average annual rainfall of 400 mm and two seasons, the wet season from June to October and the dry season from November to May (Sivakumar and Gnoumou, 1987).

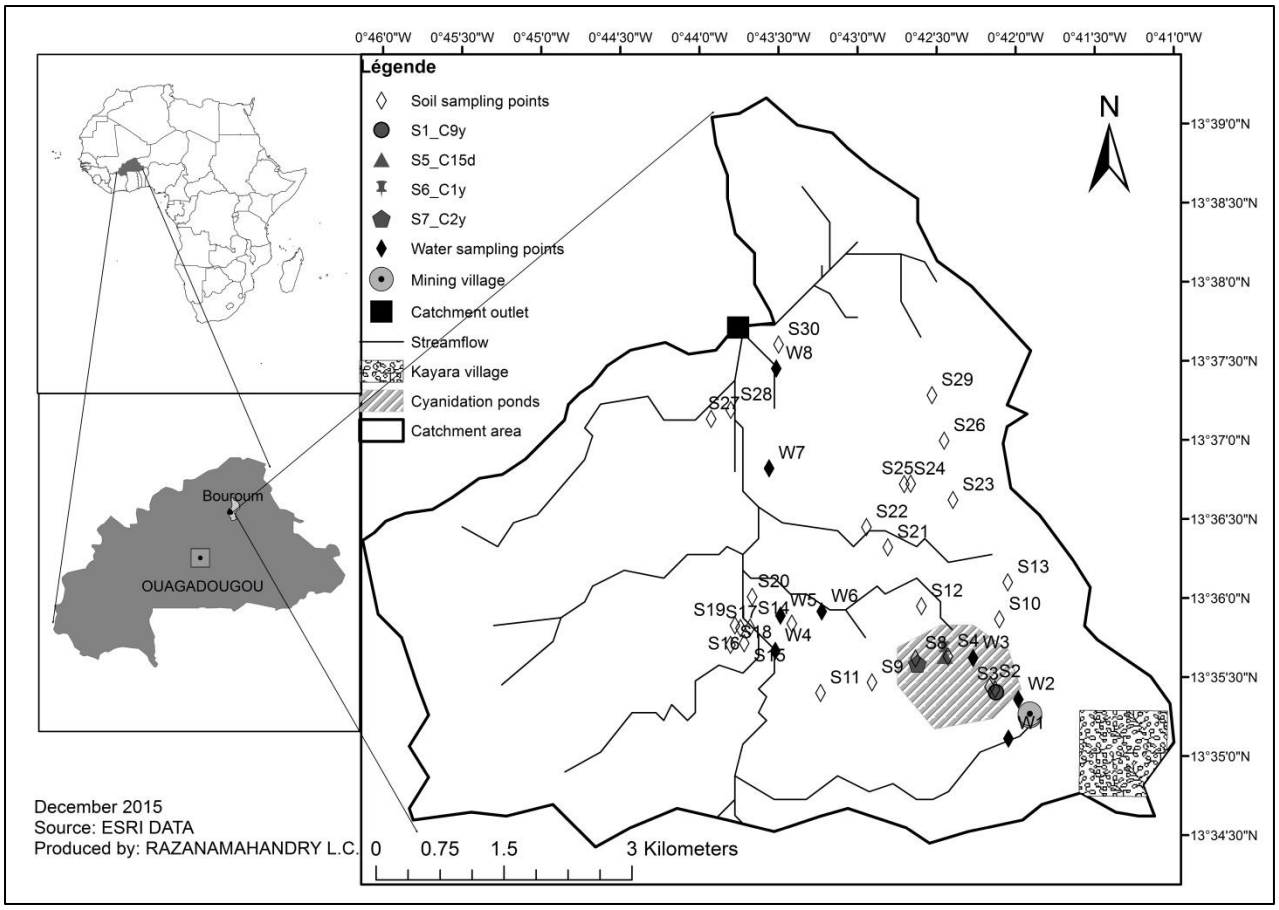

Fig. 1 : Site location and sampling points distribution

ASGM activity in the site started late in the nineties. The majority of current mine workers were initially farmers that started to be involved in the activity to complete their revenues during dry season and later as resilience to climate change (Luning, 2006). They have no official authorization to work on the site and are using rudimentary tools and techniques, without any protection. During a baseline survey conducted in 2014 at the "Zougnazagmiline" ASGM site, the full gold extraction process chain was found. First, mineral blocs from gold hole are recovered by dynamite pile. Then, they are manually crushed before mechanical grinding to obtain mineral powders which were washed with detergent. After that, the washed mineral powders were amalgamated with metallic mercury with a ratio of 14.4 g of mercury per 50 Kg of powder to recover the majority of gold present in the ore. The residual sludge was then mixed with sodium cyanide pellets to recover the remaining gold. Independently to the gold residue content, every 4.5 m^3 of recuperated sludge was mixed with 1Kg of sodium cyanide pellets (NaCN) and 200 L of water. These

activities are indeed officially prohibited so the cyanidation basins are constructed out of the "mining village" for security reason. After the treatment, cyanide leachates are directly released to the environment.

2.2 Soil and water sampling location and procedures

The boundaries of the catchment area are shown in Fig.1. One sampling campaign was conducted between 24 to 28th of March, in 2015, during the dry season. Water and sampling points were selected in such a way that it will give good insight of pollutant transfer from the gold processing sites to the catchment outlet.

Water samples were collected from a total of 8 sampling points as shown in Fig. 1. W1 was collected from a gold hole at 80 m depth. W2 to W8 were taken at public drinking water taps from groundwater sources. W2 was the nearest point to the cyanidation site whereas W8 was the nearest to the catchment outlet. No surface water was collected because the rivers within the catchment area were completely dry during the sampling campaign. Water samples were collected in sterilized non-transparent 500 mL glass bottles. Each sample was preserved by addition of NaOH pellets according to Standard Methods SM-4500-CN-F (American Public Health Association, 1998) and immediately stored into cooler containers at 4°C to prevent F-CN transformations during samples transport. Upon arrival to the laboratory, the samples were refrigerated and analyzed within 48 hours. .

Soil samples were collected from a total of 30 points (S1 to S30) at 20 cm depth from the surface. S5_C15d, S6_C1y, S7_C2y and S1_C9y were collected from abandoned cyanidation sites for 15 days, 1 year, 2 years and 9 years, respectively. Soil samples were collected into plastic bags without preservative addition and immediately stored in cooler containers at 4^{o}C.

It was assumed that the presence of F-CN in the soil samples at 20 cm depth would be due to surface run-off whereas the presence of F-CN in the groundwater samples would suggest an infiltration process.

2.3 Stock solutions

Stock solutions of F-CN [KCN, 1 g L^{-1}] from VWR (France) and 1 M stock solutions of: $NaH_2PO_4.H_2O$ [137.99 g mol^{-1}; 100 mL]; K_2HPO_4[174.18 g mol^{-1}; 100 mL]; $(NH_4)_2SO_4$ [132.14 g mol-1; 100 mL]; $MgSO_4.7H_2O$ [246.48 g mol^{-1}; 100 mL]; $NaNO_3$ [84.99 g mol-1; 100 mL] from Sigma Aldrich Chemie GmbH (Germany) were prepared with ultra-pure water (Milli-Q, Merck Millipore). The stock solutions were stored at 4°C.

2.4 Culture medium and isolation of potential CDB

A culture medium (CM) based on the work of Oudjehani et al. (2002) was used to isolate the cyanide degrading bacteria (CDB) from soil and water samples, but NaCl was replaced by Water Peptone (WP) and pH was adjusted to 9.5 with 10N NaOH to minimize volatilization of HCN. Then, the CM was autoclaved at 121°C. After that, 20 mL of the autoclaved solution was dispersed onto a Petri Dish and stored at 4°C during 24 hours.

Soil samples were pretreated before isolating the CDB. Total bacteria from each soil sample were extracted by WP according to Taylor et al. (1972). 10g of soil was mixed with 90 mL of autoclaved PW (Amresco, USA) during 5 min. After physical separation, the bacteria were recovered from the supernatant. Then, 100 µL of water samples and soil extracts supernatants were separately poured and dispersed on the solidified CM by using U tube. The mixture was finally incubated during 7 days at 28°C (Memmert incubator). Visible colonies after incubation were assumed to be potential CDB They were multiplied with nutrient broth at 30°C for 24 hours and then refrigerated at 4°C until use.

2.5 Biodegradation tests

6 liquid medium containing glucose and salts including (9.6 mL of 1M $NaH_2PO_4.H_2O$, 19.49 mL of 1M K_2HPO_4, 12.49 mL of 1M $(NH_4)_2SO_4$, 0.59 mL of 1M $MgSO_4.7H_2O$ and 17.69 mL of 1M $NaNO_3$) as nutrients were prepared in 1L Erlenmeyer. Each solution was then spiked with 40, 60, 80, 100, 200 and 300 mg L^{-1} of KCN respectively (Medium M2). After that, 0.062 ufc L^{-1} of bacterial species from the nutrient broth were grown into the solutions on a shaking table for 24 hours, at 200 rpm, room temperature and pH of 9.5 to minimize HCN volatilization, in order to test the effective capacity of the potential CDB to

degrade F-CN. 10 mL of the mixture were collected at the beginning of the experiment, and after 1, 2, 4, 9, 11 and 24 hours to monitor F-CN, bacterial species and ammonium concentrations.

Three additional set of experiments were conducted by following the same experimental protocol. However, no nutrient was added to the solutions (Medium M1). First, alive microorganisms were used to check the presence of autotroph CDB. Secondly, autoclaved bacteria were used to investigate the contribution of adsorption in the removal of F-CN (Medium M4). Finally, various concentrations of F-CN were mixed with ultrapure water only for 24 hours to measure the rate of HCN volatilization (Medium M3). The experiments were conducted under sterilized condition to avoid external bacterial contamination.

2.6 Analytical methods

F-CN and ammonium (NH_4^+) were determined by a spectrophotometer (DR5000, Hach-Company, Germany) with a wavelength of 612 and 425 nm, respectively.

Before analysis, F-CN in soil samples was extracted based on the method reported by Rennert and Mansfeldt (2004). In the present study 1g of soil was mixed with 10 mL of 1M NaOH on a magnetic stirrer during 10 min. The mixture was then centrifuged (Himac CT 6EL, VWR, France) for 10 min at 1000 rpm. It was assumed that F-CN content of the recovered supernatant represented the F-CN content in the selected soil sample.

The pyridine pyrazalone method (Novak et al., 2013) was applied for quantifying F-CN with a range of detection from 0 to 200 mg L^{-1}. Recovery curve of soil extraction was established and shown in Fig.2 with a best value of R^2= 0.994. But, the recovered concentrations values were only 50% due to the cyanide complexation with many compound present in soil (Kjeldsen, 1999).

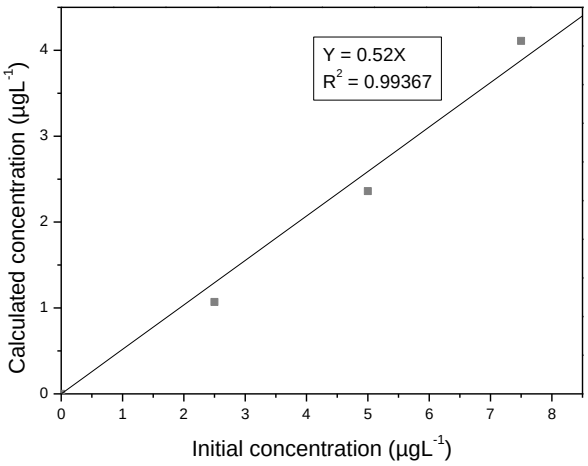

Fig. 2 : Curve of recovery test

Nessler method (Gurbuz et al., 2009; Kjeldsen, 1999) was used for determining NH_4^+ with a range of 0 to 2.50 mg L^{-1}.

Bacterial growth was measured in two ways, namely: (a) via the optical density at 600nm [O.D. (600 nm)] by using spectrophotometer; (b) by estimating the number of colony forming units (CFU) from Malassez cell counts of fourth dilution samples.

The pH of each soil and water sample was measured in 10 mL samples before analysis using a pH meter (pH-meter, WTW 3310, Germany).

All analysis was done in duplicate.

3. Results and discussion

3.1 F-CN distribution in soil

The concentrations of F-CN in the collected soil and water samples and the pH are presented in Fig 3. F-CN was detected in all samples with concentrations varying from 0.023 to 0.563 mg Kg^{-1} in soils and from 0.7 to 23 µg L^{-1} in water. It was observed that F-CN concentrations in water were very high near the cyanidation ponds, then decreased as the sampling point moved away from the ponds, and finally increased at the catchment outlet. On the other hand, the concentrations of F-CN in soils were lower in the cyanidation ponds that have been recently abandoned than in the ones that have been abandoned for longer times (F-CNS5_C15d<F-CNS6_C1y<F-CNS7_C2y<F-CNS1_C9y). CDB is more available for converting

cyanide complexes compounds to F-CN in old gold mining soil than in fresh soil (Oudjehani et al., 2002).However, it was difficult to correlate F-CN concentrations in soils with the distance from the cyanidation sites.

In Burkina Faso, the accepted levels of F-CN are 0.5 mg Kg^{-1} for soil sample and 0.2 mg L^{-1} for water sample (Compaore et al., 2001). Though, only two soil samples and no water sample have exceeded these standards, the detection of F-CN in all samples indicated that F-CN was persistent in the site, that widespread contamination has occurred within the catchment area, and that the F-CN was gradually transported to the outlet by both surface run-off and infiltration, as confirmed by the presence of F-CN in the groundwater samples, particularly that high concentration at W8. The solubility of F-CN in the environment is controlled by several parameters such as the pH, conductivity, the concentrations of metallic ions, and the presence of microorganisms that can degrade cyanide compounds (Dzombak et al., 2006). From the results here, no clear correlation was found between F-CN concentrations and pH. Further investigations, which were not under the scope of the present study, are needed to fully understand the behavior of cyanide compounds in these sites.

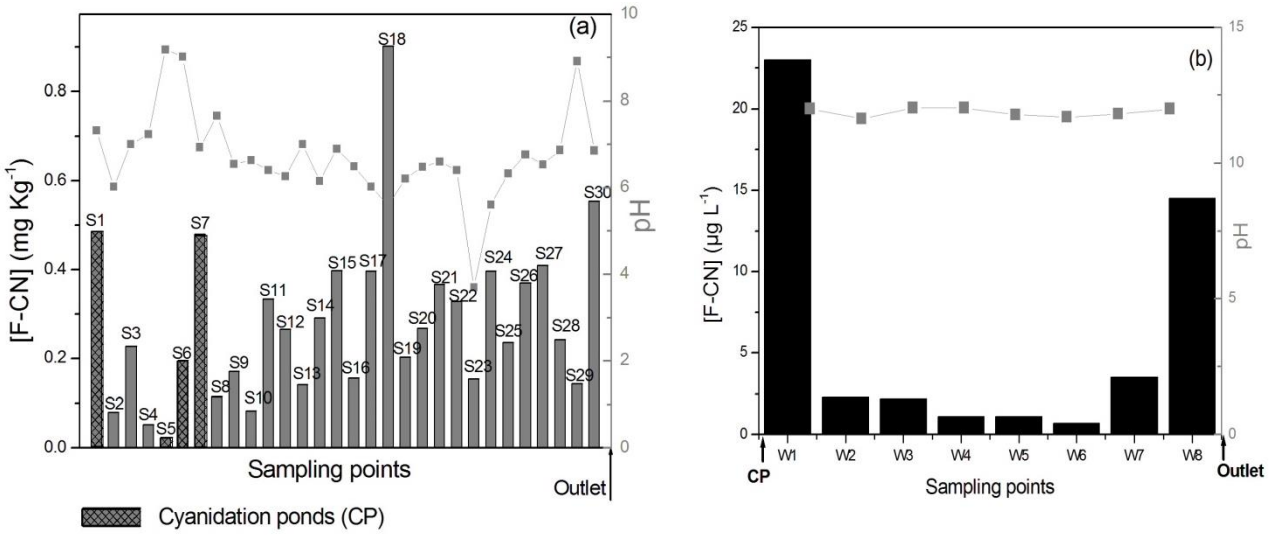

Fig. 3 : Cyanide concentration in (a) soil and (b) water samples

3.2 Presence of CDB in the contaminated site

Fig.4 shows the concentrations of CDB in soil and water samples, which indicates that CDB were present in all samples. Their concentrations varied from 2×10^{3} CFU g^{-1} to $2{,}36 \times 10^{5}$ CFU g^{-1}, and from 200 to

11500 CFU mL^{-1} in soil and water samples, respectively. It was observed that when the F-CN concentrations were increased in the water samples, the concentrations of CDB also increased. However, no clear relationship was found between F-CN in soil samples and CDB concentrations. The discovery of bacterial species that could biodegrade F-CN is not new. However, most of the species tested were collected from GenBank. The presence of these potential CDB in all the collected samples was important. Indeed, the area was characterized by hot and dry climate conditions during 9 months a year. Thus, using these species would be an advantage for the bioremediation of the contaminated sites in the region as they have already developed adaptation mechanisms not only to these environment and climate conditions but also to the presence of F-CN.

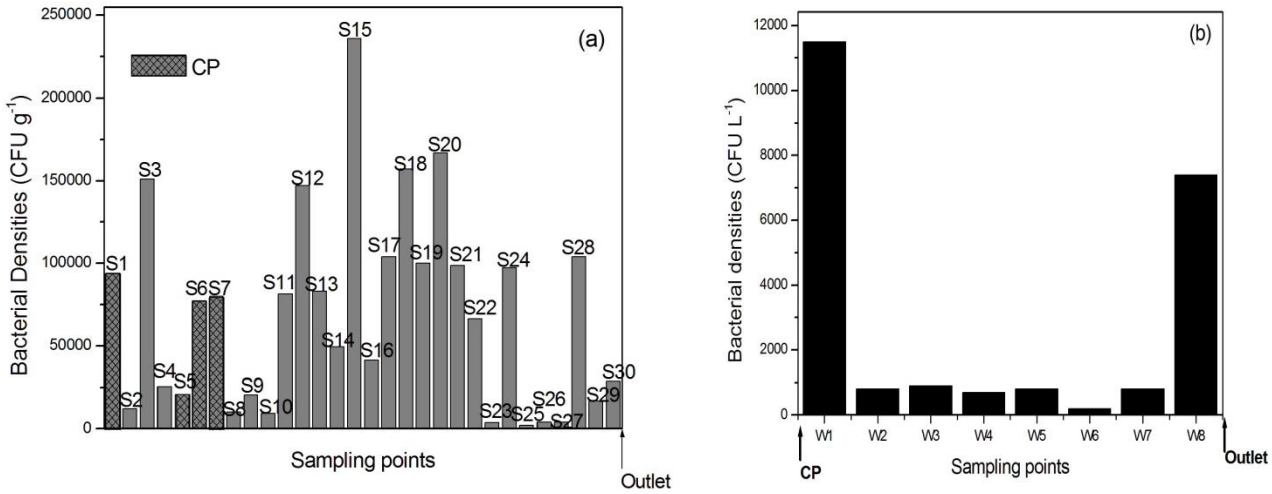

Fig. 4 : Concentrations of bacteria in (a) soil and (b) water samples

3.3 Biodegradation of F-CN by CDB

The effective biodegradation capacity of the isolated bacterial species was tested. The results of the experiments are presented.

The curve of F-CN removal and bacterial growth in M1 (liquid medium without nutrients) and M2 (liquid medium with nutrients) are respectively shown in Fig.5a and in Fig.5b. When F-CN initial concentrations were 40, 60 and 80 mg L^{-1}, F-CN concentrations were first almost stable during the first hour, were decreased slowly from 1 to 2 hours, then greatly from 2 to 9 hours, and finally again slowly from 9 hours to the end of the experiments. More than 95% of F-CN was removed from all the solutions, and the residual F-CN concentrations were lower than 5.8 mg L^{-1} in M1 and 0.4 mg L^{-1} in M2. On the other

hand, bacterial concentration in M1 was first decreased in the first two hours, then was relatively stable until 9 hours and was finally greatly increased to the end of the experiment. In M2 however, where nutrients were initially available, bacterial growth has occurred immediately after the start of the experiment. The growth was slow during the first 9 hours and fast from 9 to 24 hours. At the end, the final concentrations of bacterial species were between OD_{600nm} = 0.39 to OD_{600nm} = 0.45 in M1 and between OD_{600nm} = 0.95 to OD_{600nm} = 1.00 in M2, showing significant difference in bacterial growth between the two medium

When F-CN initial concentration was 100 mg L^{-1}, less than 3 mg L^{-1} of F-CN in both M1 and M2 were removed after 24 hours, and no more bacteria were detected after only 1 hour after the start (Fig.5). Similar results on the removal of F-CN were also observed when the initial concentrations were 200 and 300 mg L^{-1} (data not shown), in ultra-pure water (M3), and in the solution with inactivated bacteria (M4) (Fig.6).

The slight removal of F-CN in M1 and M2 when F-CN initial concentrations were higher than100 mgL^{-1}, and in M3 and M4 suggest the volatilization of F-CN, which was also reported by Mekuto et al. (2013). It is almost impossible to prevent the volatilization of the free cyanide (Huertas et al., 2010). Additionally, F-CN was not adsorbed on the cell surfaces as confirmed when inactivated bacteria were used. Indeed, at pH > 9.5 the predominant form of F-CN is CN^- whereas bacterial surfaces are also negatively charged (Botz et al., 1995). Thus, the results of the experiments conducted with 40, 60 and 80 mg L^{-1} and living microorganisms clearly demonstrate that the removal of F-CN was biologically mediated. However; due to the toxicity of cyanide, none of the species were able to survive in solutions containing too high concentrations of F-CN. Two reasons could explain the bacterial decline in M1 during the first hour. First, the resistance capacity would differ from species. Thus, non-resistant bacteria would have dead in contact with high concentration of F-CN. Or, some of the isolated bacteria were resistant but were not cyanide degrading ones. Without sufficient nutrients, they were not able to survive. A similar trend would have also occurred in M2, but due to the availability of nutrients from the start, the growth was more important than the decline.

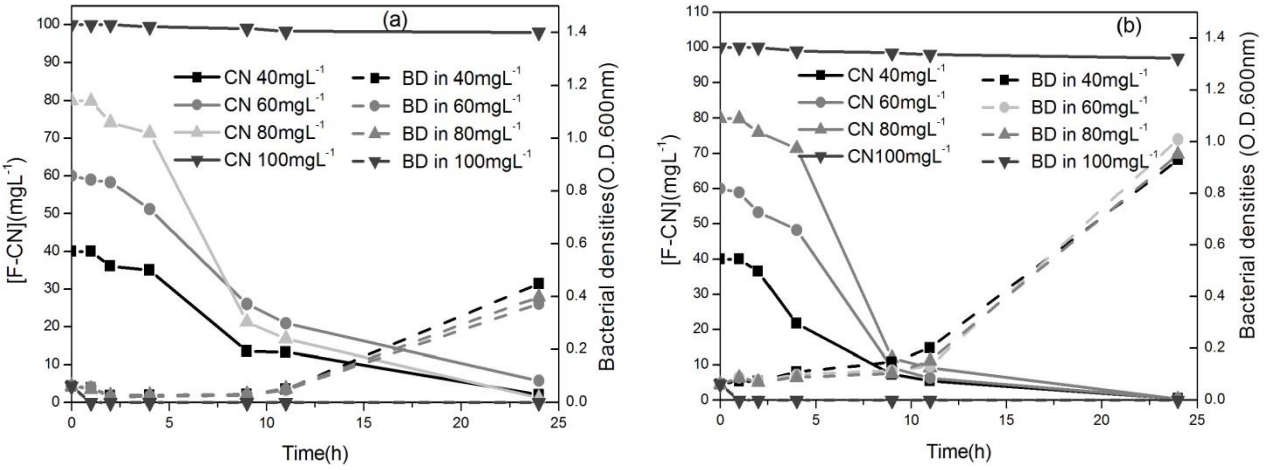

Fig. 5 : F-CN removal and bacterial growth in liquid medium (a) without nutrients, M1 and (b) with nutrients, M2 under aerobic condition

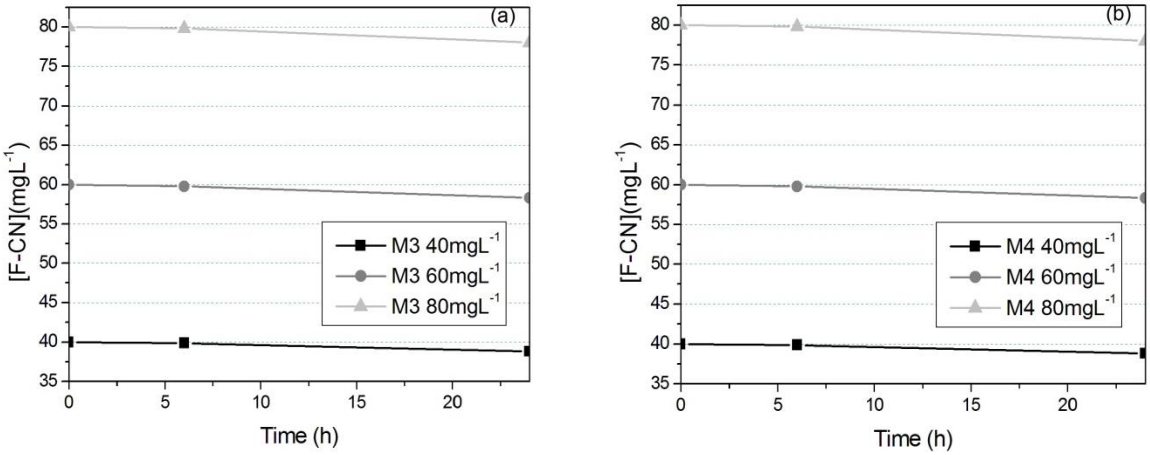

Fig. 6 : Cyanide removal in (a) ultrapure water and in (b) the solution with inactivated bacteria

To understand the fate of biodegraded F-CN, ammonium (NH_4^+) concentrations in M1 and M2 were also monitored (Fig.7). At the start, no NH_4^+ was detected in M1 (Fig. 7a), whereas the initial concentration of NH_4^+ was about 18 mg L^{-1} in M2, corresponding to the ammonium concentration in the liquid medium. In both M1 and M2, rapid and significant increase in NH_4^+ concentrations during the first 2 hours was followed by a decrease from 2 to 9 hours and then an increase from 9 to 24 hours, to almost attend the same concentration as at 2 hours; though the concentrations of NH_4^+ in M2 were always higher than that in M1. NH_4^+ production in M1 and M2 was obviously the result of the degradation of F-CN. The increase in NH_4^+ in M2 would be due also to the degradation of F-CN. Then, NH_4^+ was transformed to NO_2^- under aerobic condition, which explains its decrease from 2 to 9 hours (Akcil, 2003). But, the second increase phase of NH_4^+ concentration from 9 to 24 hours was not clarified yet, though it is thought that

during this phase, the rate of NH_4^+ production from the degradation of residual F-CN would be higher than that of NH_4^+ consumption. These fluctuations in NH_4^+ concentrations were also reported in 2013 by Mekuto et al. (2013) who conducted F-CN biodegradation experiments with mixture of *Bacillus sp*, and who suggested that the selected species were both cyanide-degraders and nitrifiers, and therefore, can be used in the reduction of residual NH_4^+.

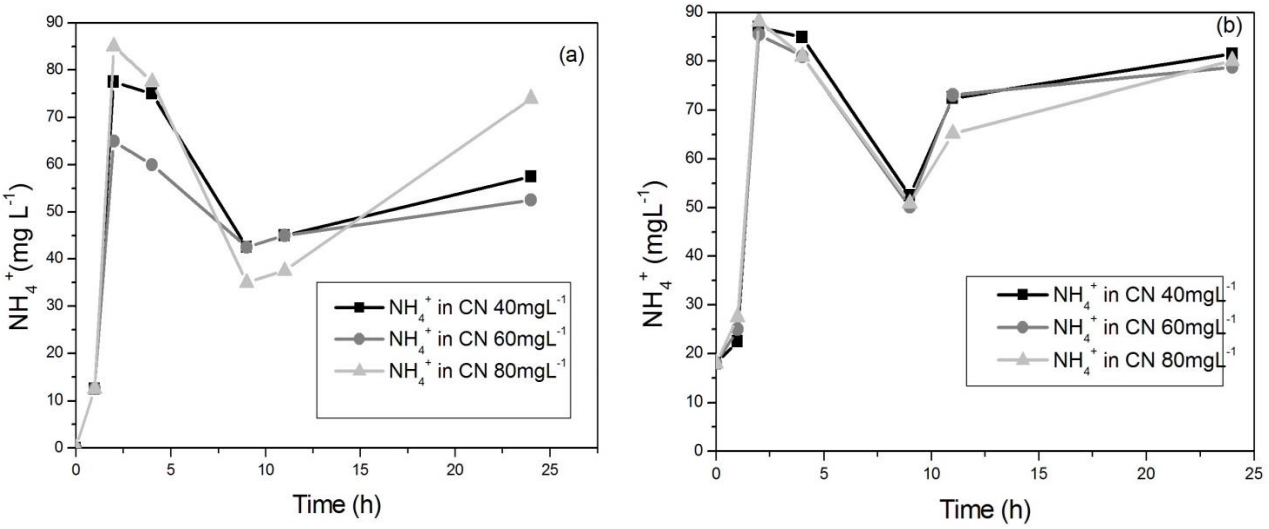

Fig. 7 : NH_4^+ production in (a) M1 and (b) M2

From these results, it is assumed that once in contact with an important concentration of F-CN, non-resistant and non-degrading bacteria will die in the absence of growth nutrients. The surviving ones will then degrade F-CN and use the degradation product for their growth. In the presence of nutrients, the resistant but non-degrading bacteria will immediately use the available nutrients for their growth, whereas the degrading ones will simultaneously use the nutrients for growth and degrade F-CN for energy demand. It is finally suggested that F-CN degradation was a detoxification mechanism rather than for direct bacterial metabolism.

3.4 Performance of CDB

The performance of the CDB in the present study was compared with that of some of the most performing bacterial species reported in the literature. Data are presented in Table 1. It was found that they have reached also good performance in terms of F-CN removal efficiency. Above all, they have shown very high F-CN consumption rate within a relatively short time when compared with the other species. The presence of various species in the culture medium might have accelerated the reactions in the present study.

Table 1 : Comparison in bacterial species performance for the biodegradation of F-CN in the presence of nutrients and under aerobic condition

Bacterial species	Initial [F-CN] (mg L^{-1})	Initial Bacterial density (OD_{600nm})	F-CN consumption rate (mg L^{-1} $O.D^{-1}$ h^{-1})	Removal Efficiency (%)	Experimental conditions (Duration/pH/Temperature)	References
Mixture of bacterial species	80	0.062	54	99	24h 9.5 30°C	Present study
Bacillus sp. CN-22	700	1	9.72	29	72h 10.3 31°C	(Wu et al., 2014)
Pseudomonas pseudoalcaligenes CECT5344	45	1	0.78	100	57.6h 10 30°C	(Huertas et al., 2010)
Rhodococcus sp.	12	0.5	2	50	12h 7 30°C	(Maniyam et al., 2011)
Klebsiella oxytoca	21	1	0.26	99.9	80h 7 30°C	(Kao et al., 2003)

4. Conclusion

In the present study, these following points were carried out:

- The risks associated with illegal cyanidation treatment on the environment at catchment level were quantified and reported for the first time in the present study. Though the cyanidation sites were grouped together, it was found that F-CN persisted in the environment and contaminated almost the entire catchment area through surface run-off and soil infiltration.
- Bacterial species that could biodegrade F-CN were also present and widespread on the contaminated site. Laboratory tests have shown that these species have high application potentials for the bioremediation of sites affected by artisanal gold activities, particularly in West Africa where ASGM impacts are of great environmental issues. Their main advantage is that they were collected on the contaminated site, thus they have already developed faculty to adapt to the difficult environmental condition, due not only to cyanide contamination but also to the hot and arid climate conditions of the region.
- The isolated bacterial species could growth by using only the product from the biodegradation of F-CN. They could be used also for the remediation of sites with limited nutrients such as groundwater.

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