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Isolation and Assessment of Cyanide Biodegradation Potential of Indigenous Bacteria from Contaminated Soil

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Abstract

Eight effective bacterial strains were isolated from cyanide-contaminated soil, from which, two (S11 and S13) were tested to determine their minimum inhibitory concentration using potassium cyanide (KCN) and potassium tetracyano nickelate ($K_2Ni(CN)_4$). The isolates were tested for their KCN biodegradation potential (by measuring ammonia production) at neutral and alkaline pHs (7.0 and 9.0). Furthermore, the effect of the initial KCN concentration on biodegradation was evaluated at the optimal pH using nitrogen free M9 medium, supplemented with KCN as nitrogen source. The results showed that both strains tolerated cyanide concentrations of up to 10×10^{-3} mol·l^{-1} (0.651 g·l^{-1} KCN; 2.409 g·l^{-1} $K_2Ni(CN)_4$) which makes them good candidates for cyanide bioremediation. For both strains, a change of initial pH from 7.0 to 9.0 significantly enhanced KCN degradation. S13 grown at pH 9.0 and S11 cultivated at pH 7.0 released the highest and lowest amounts of ammonia, respectively. For both strains, the release of ammonia increased when the initial KCN concentration increased from 10^{-3} to 5×10^{-3} mol·l^{-1}. These findings open prospects for the application of these bacteria for remediation of cyanide-contaminated soils and wastewater at alkaline pH, alkaline pH being conditions that prevent cyanide volatilization.

Keywords

Bioremediation, Biotechnology, Cyanide, Hazardous Waste, Gold Mining, pH

1. Introduction

Cyanide is a triple-bonded carbon-nitrogen molecule which may be found in a wide variety of organic and inorganic compounds. Cyanide is present in small concentrations in some microorganisms and plants; it is produced by algae, bacteria, fungi, and may be found in plants (cassava roots and potato-like tubers) and some foods such as lima beans, almonds and cashew nuts [1]. Nevertheless, its large-scale occurrence in the environment is mainly attributed to human activities, especially metal finishing and mining industries [1].

In West Africa, gold mining is increasingly becoming an engine of development and is characterized by the co-existence of industrial and artisanal mining. This phenomenon has been experienced in many other parts of the world. In Burkina Faso, gold mining activities have experienced an unprecedented growth in the 2010's. Indeed, between 2008 and 2009, the gold production has increased from 5 to 11 metric tons, thus becoming the first domestic product for exportation [2]. Since this period, Burkina Faso has become a major mining country in Africa with a particular increase in gold production ranging from 38.5 tons in 2016 to 52.6 tons in 2018 [3]. With more than 200 active artisanal gold mining sites around the country and more than 700,000 people involved (including men, women and children under 15, most of them living in rural areas), artisanal gold mining is an important source of income for many people in Burkina Faso [4]. The annual artisanal gold production is estimated between 1500 and 2000 kg [4].

This activity however has detrimental effects on human health, the environment and natural resources. For example, the uncontrolled use of cyanide to extract the gold from the ore can lead to its presence in the environment in a variety of toxic forms [5] [6]. Recent reports of water pollution by cyanide have been widely reported around the world including Romania [7], Ghana, [8] Burkina Faso [9], Guyana and China [10]. Short-term exposure to cyanide can cause rapid breathing, tremors and other neurological effects while long-term exposure causes weight loss, thyroid effects, nerve damage and death [1]. To protect humans, animals and the environment including water bodies, wastes containing cyanide from gold mining must be treated to remove cyanide.

Several methods including physical and chemical techniques have been tested for cyanide degradation [11] [12]. However, these processes are not always effective compared to biological treatment methods that can effectively remove a wide range of cyanide compounds [1]. Furthermore, biological methods are economical and reduce the toxicity and corrosiveness of the chemical oxidizers [13]. Therefore, the development of biological processes for the remediation of cyanide contaminated water and soil is of high importance in the context of gold mining.

Attempts to isolate bacteria from polluted sites for cyanide biodegradation have been reported by previous studies [14] [15]. Since bacteria are influenced by several environmental factors and their ability to degrade cyanide is species

and pH specific, further investigations are needed. Accordingly, the aim of this study was to contribute to the development of bioremediation techniques for the detoxification of cyanide contaminated water and soil in gold mining areas. The specific objectives were to: 1) isolate cyanide resistant and degrading bacteria from polluted industrial sites; 2) test the influence of initial pH (7.0 and 9.0) on the ability of the isolated strains to degrade potassium cyanide (KCN) by associated release of ammonia; and 3) evaluate the effect of cyanide concentration on the ability of the strains to degrade KCN.

2. Materiel and Methods

2.1. Soil Sampling Site

In order to isolate cyanide resistant/degrading bacteria, soil samples were collected from a cyanide-contaminated site (the former Revivation and Gulf Coast Metal Finishing Inc., an industrial solvents manufacturing plant located at 2402 North 35th Street, Tampa, Hillsborough County, Florida, USA). Monitoring wells were previously drilled for groundwater quality analysis. Soil samples were collected in the vicinity of monitoring well MW-003, which was fairly close to the historic point of pollution release. Two types of soil samples [named as composite 1 (C1) and composite 2 (C2)] were collected. C1 was collected from 3 subsamples spaced approximately 1.50 m apart. Sample depths varied from 0.50 to 20 cm. Subsamples from composite C2 were collected from the same borings at depths of 20 to 40 cm. In addition, one groundwater sample was collected from the monitoring well MW-12, which was hydraulically down gradient of the release area. The water sample was collected in a glass bottle and preserved with NaOH. All samples were placed into an iced cooler and transported to the laboratory.

2.2. Determination of Soils Characteristics

The characteristics of soil samples C1 and C2 were determined for pH H_2O (1:1 soil: water), pH KCl (1:1 soil: 1 mol·l^{-1} KCl), percent solids (method SM 2540 G), total cyanide (method SW 846 9012). Total cyanide in the groundwater sample was also determined using EPA method 335.4 [16].

2.3. Chemicals and Cyanide Stock Solutions

Two (2) cyanide compounds [potassium cyanide (KCN) and potassium tetracyano nickelate (II) $K_2Ni(CN)_4$, obtained from Fisher Scientific] were used. Stock solutions (400×10^{-3} mol·l^{-1}) were prepared using NaOH for a final pH of 10.0 to avoid the volatilization of hydrogen cyanide as suggested by previous studies [17] [18].

2.4. Isolation of Cyanide Degrading Bacteria from Soil and Groundwater Samples

For the isolation of cyanide resistant bacterial strains, a modified method by

Parmar *et al.* [14] was used. 10 g of each soil sample (C1 and C2) were mixed with 100 ml of sterile distilled water in a 250-ml flask bottle. The mixtures were shaken at 120 rpm for 4 h. After centrifugation at 10,000 g for 10 min, the supernatant was collected and 10 ml were inoculated into 100 ml of sterile M9 minimal salt medium containing 10^{-3} mol·l^{-1} filter sterilized KCN at pH 10.0. The M9 minimal salt medium used in this study contained (g·l^{-1}): monopotassium phosphate (3.0); disodium phosphate (anhydrous) (6.78); sodium chloride (0.5) supplemented with filter sterilized 0.4 ml of 1.0 mol·l^{-1} magnesium sulfate solution and 0.02 ml of 1.0 mol·l^{-1} calcium chloride solution. The medium also contained a carbon source at 4 g·l^{-1} (filter sterilized glucose) but lacked the nitrogen source required for bacterial growth. In this case, the KCN present in the simulated medium served as the sole source of nitrogen. Consequently, only KCN degrading bacteria can grow. Besides, 10 ml of groundwater samples were mixed with 100 ml M9 minimal salt medium supplemented with 10^{-3} mol·l^{-1} KCN as the only nitrogen source.

The mixtures were incubated at 37˚C for 4 days on a shaker at 120 rpm. Next, these enriched cultures were streaked on M9 agar medium supplemented with 10^{-3} mol·l^{-1} KCN at pH 10.0, as the sole nitrogen source. The Petri dishes were incubated at 37˚C for 4 days with daily observation. Colonies differing mainly in the morphology were selected and pure cultures were obtained by continuous sub-culturing. Each pure colony was picked onto nutrient agar and maintained at 4˚C.

2.5. Enrichment Medium for Resting Cells

The bacterial isolates were grown in a complete M9 medium (with NH_4Cl as nitrogen source and glucose as carbon source) at a pH of 7.0 in 250-ml conical flasks. The flasks were incubated at 37˚C for 24 h in a shaker at 120 rpm. The bacteria were then harvested by centrifugation at 10,000 g for 10 min, 4˚C and washed twice in a 50×10^{-3} mol·l^{-1} phosphate buffer solution. The harvested cells were suspended in the same buffer [absorbance at 600 nm (OD600 nm) of about 1.0] and used as resting cells.

2.6. Determination of Minimum Inhibitory Concentrations

The Minimum Inhibitory Concentrations (MIC) were determined using two cyanide compounds: potassium cyanide (KCN) and potassium tetracyano nickelate [$K_2Ni(CN)_4$]. Each isolate was grown in a M9 salt medium containing increasing concentrations of the selected cyanide compound (10×10^{-3}, 20×10^{-3}, 50×10^{-3} and 75×10^{-3} mol·l^{-1}) as the sole nitrogen source. Glucose was used as carbon source. A predetermined quantity of the stock solution was added to 2 ml of the M9 medium in a culture tube to get the desired cyanide concentration for batch tests. About 50 µl of resting cells were added to each sample [19]. The solutions were then placed on a shaker incubator at 37˚C and 120 rpm for 48 h. A control experiment was also set up using the complete M9 medium (with NH_4Cl

as sole nitrogen source). For all of the tubes, the bacterial concentration was determined at the beginning and after 48 h of growth using a nanodrop spectrophotometer. For a given tube, the resulting growth (G) was estimated using Equation (1):

$$G = OD_f - OD_i \quad (1)$$

where OD_f is the OD600 nm after 48 h of growth; OD_i is the initial OD600 nm (0 h).

2.7. Biodegradation Tests

The biodegradation tests were performed in M9 minimal salt medium using KCN as nitrogen source and glucose as carbon source.

2.7.1. Effect of Initial pH on Cyanide Biodegradation Efficiency

To determine the effect of initial pH on the biodegradation potential, the bacterial isolates were grown in a 150-ml Erlenmeyer flask containing 50 ml of M9 salt medium supplemented with KCN at 10^{-3} mol·l^{-1} and glucose at 4 g·l^{-1} as sole sources of nitrogen and carbon respectively. Since acidic conditions promote cyanide volatilization, the initial pHs were adjusted to 7.0 and 9.0 using sterile NaOH or H_2SO_4. The pH was not controlled during the experiments. Resting cells (0.5 ml) were then added to the medium and placed in a shaker incubator at 37˚C, 120 rpm for 102 h. Samples were collected at regular intervals to measure cyanide degradation. Because KCN was the sole nitrogen source and the fact that previous studies have reported ammonia as one of the major products of cyanide degradation [15]-[20], this product was used as an indication of the occurrence of cyanide biodegradation. Ammonia nitrogen was measured (in duplicate) according to method 10023 (salicylate method) for nitrogen, ammonia, low range (0 - 2.50 × 10^{-3} g·l^{-1} NH_3-N) (HACH Company, Loveland USA).

2.7.2. Effect of Cyanide Concentration on the Biodegradation Potential of Effective Bacteria

The effect of cyanide concentration on the biodegradation potential of the effective bacteria was also investigated. The tests were set up in Erlenmeyer flasks using M9 minimal salt medium supplemented with KCN and glucose (4 g·l^{-1}) as sole nitrogen and carbon source respectively. The initial pH was set at the optimum pH obtained from the tests on the effects of pH outlined above. Two KCN concentrations (10^{-3} mol·l^{-1} and 5 × 10^{-3} mol·l^{-1}) were tested after addition of 0.5 ml of resting cells in the 50 ml M9 mineral medium contained in a 150-ml Erlenmeyer. The flasks were incubated in a shaker at 37˚C and 120 rpm for 102 h. Ammonia generation was determined as explained above.

2.8. Statistical Analyses

Data were subjected to analysis of variance (ANOVA) at 95% confidence interval using XLSTAT 2007, to investigate how different pH, cyanide concentrations and the nature of the strains impact the ammonia production. Statistical com-

parisons were made by the Tukey honest significant difference (HSD) test. The significance of observed differences was tested at $P < 0.05$.

3. Results and Discussion

3.1. Characteristics of Soil and Groundwater Samples

Both soil and groundwater samples collected for the isolation of bacterial strains were contaminated with cyanide, the upper section of the soil being slightly more contaminated than the deeper section (Table 1).

The presence of cyanide in the samples enhances the possibility of finding indigenous cyanide resistant bacteria. This presence, despite the long period after the closure of the site, could be explained by the neutral to slightly alkaline conditions of the samples, acidic conditions leading to cyanide volatilization. Indeed, the pH values of the soil samples collected for bacterial isolation were greater than 7.0 for both H_2O and KCl based measurements. The percent solids of C1 and C2 sampling locations were 92.2% and 91.1% of dry weight respectively which assumed that the moisture contents were 7.8% and 8.9% of dry weight respectively. It has been demonstrated that soil water content controls bacterial activity and that lack of water in a soil reduces microbial activity and growth [21].

When comparing the effect of soil water content on bacterial growth, Lovieno and Baath [22] showed that low growth rates were found in air-dried soil that increased rapidly to high stable values in moist soils. Although it remained low, growth was nevertheless possible at moisture content > 4% dry weight. Therefore, the moisture contents of our soil samples are high enough to support the presence and growth of viable indigenous bacteria which is important for bacterial isolation.

Based on the appearance of the colonies, three (3) bacterial strains have been isolated from C1 (designated as S11, S12 and S13) and five (5) from C2 (designated as S21H, S21P, S22, S24 and S26). No colonies were isolated from the water sample. The strains S11 and S13 which gave the largest colonies on the KCN contaminated medium were selected for further investigations (MIC and biodegradation tests).

Table 1. Characteristics of the soil and groundwater samples used for bacterial isolation.

Parameters	Samples		
	Soil composite 1	Soil composite 2	Groundwater
pH_{H2O}	7.59	7.86	-
pH_{KCl}	7.04	7.32	-
Percent Solids (% dry weight)	92.2	91.1	-
Moisture content (% dry weight)	7.8	8.9	-
Total cyanide ($g \cdot kg^{-1}$ dry weight)	1.11×10^{-3}	0.823×10^{-3}	-
Total cyanide ($g \cdot l^{-1}$)			0.99×10^{-3}

3.2. Minimum Inhibitory Concentration Tests

In the control experiment performed with complete M9 medium containing ammonium chloride as sole nitrogen source (control), the growth obtained after 48 h showed that strain S13 grew faster than strain S11 (Figure 1). Indeed, an increase in OD600 nm of 0.143 and 0.230 were obtained for S11 and S13, respectively.

When cyanide compounds were used as sole nitrogen source in the M9 medium, a concentration of 10×10^{-3} mol·l^{-1} allowed the growth of strains S11 and S13, KCN allowing higher growth than $K_2Ni(CN)_4$. For both strains, the growth in the presence of cyanide as N source is lower than the case of ammonium chloride.

S11 increased 0.055 and 0.019 in OD600 nm while S13 increased of 0.094 and 0.015 when KCN or $K_2Ni(CN)_4$ were used, respectively. However, cyanide contents of 20×10^{-3} mol·l^{-1} or higher resulted in a decrease of the concentrations of both bacteria whatever the cyanide used. Therefore, the MIC for KCN and $K_2Ni(CN)_4$ is higher than 10×10^{-3} mol·l^{-1} (0.651 and 2.409 g·l^{-1} respectively) but lower than 20×10^{-3} mol·l^{-1} (1.302 and 4.819 g·l^{-1} respectively).

For both strains, the cell density was lower using $K_2Ni(CN)_4$ than KCN. This

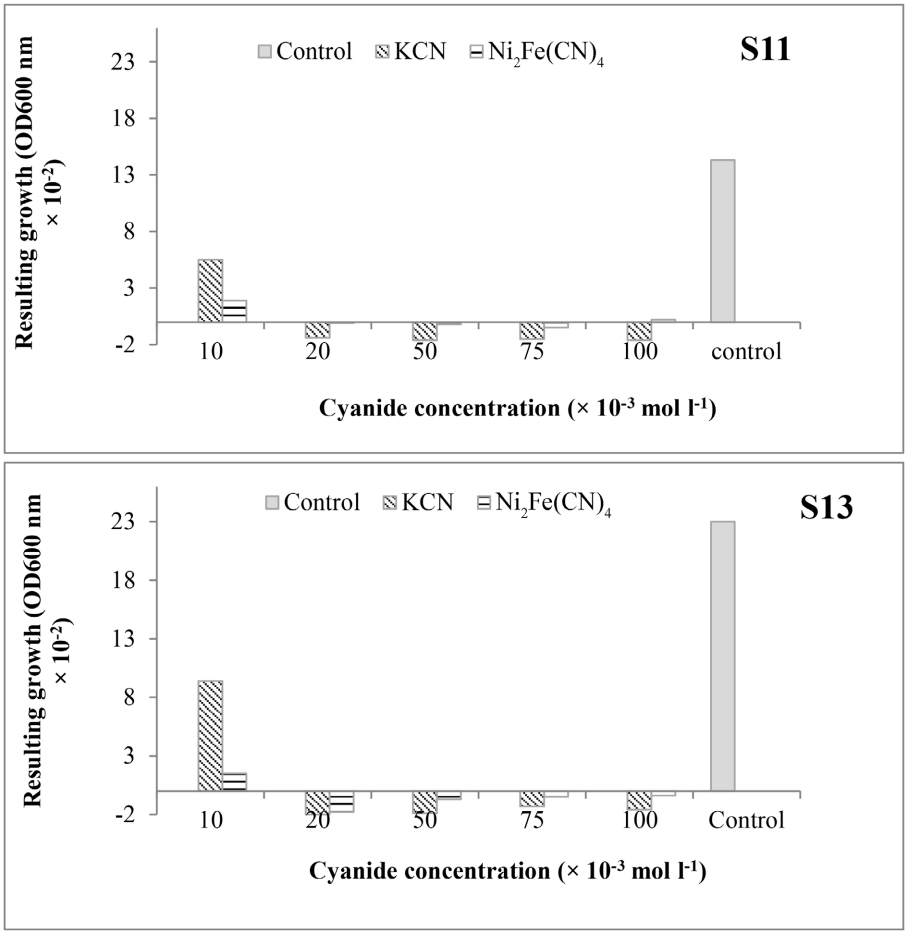

Figure 1. Minimum inhibitory concentrations (MIC) for strains S11 and S13.

finding could be explained by the cumulative toxicity of CN and nickel, both of which can be toxic. Indeed, when comparing the ability of bacterial isolates to grow on $K_2Ni(CN)_4$, the toxicity of nickel was also tested with $NiCl_2$. This compound which ionizes to Ni^{2+} was found to be significantly more toxic than was $K_2Ni(CN)_4$ for all isolates tested [23]. Furthermore, using *Pseudomonas resinovorans* cultivated with potassium ferrocyanide ($K_4Fe(CN)_4$), Bouari *et al.*, [24] obtained an increase of 0.051 in OD600 nm at a concentration of 12.5×10^{-3} mol·l^{-1} while a decrease was noticed at a concentration of 25×10^{-3} mol·l^{-1}.

3.3. Effect of Initial pH on the Biodegradation Potential

Figure 2 shows that both strains (S11 and S13) were capable to degrade KCN at a concentration of 10^{-3} mol·l^{-1} (by measuring generation of N-NH_3) when the initial pH of the media were set at 7.0 or 9.0. For either S11 or S13, the production

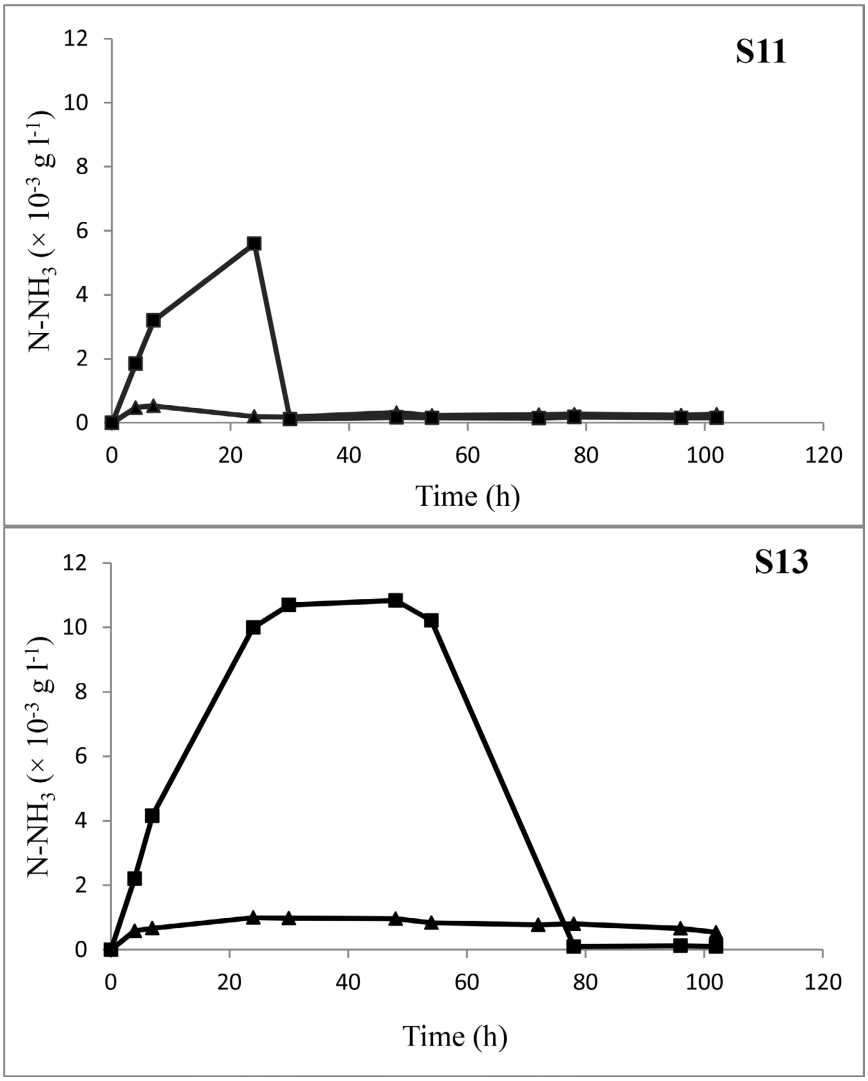

Figure 2. Effect of initial pH on the biodegradation of KCN (measured as ammonia generated) by strains S11 and S13. Ammonia concentration reported as the mean of duplicate samples.

of N-NH_3 started just before the fourth hour of incubation, increased to a maximum and declined the following hours. However, at both pH, S13 generated the highest amount of ammonia than S11 (**Figure 2**). In addition, the pH of 9.0 was more suitable to the biodegradation of KCN by S11 and S13 based on the amount of N-NH_3 generated. Indeed, for pH 7.0, the maximum of N-NH_3 generated was 0.53×10^{-3} g·l^{-1} (reached after 7 hours) and 10^{-3} g·l^{-1} (reached after 24 h) by S11 and S13 respectively. While the initial pH was increased to 9.0, the maximum amount of N-NH_3 generated increased to 5.6×10^{-3} g·l^{-1} (reached after 24 hours) for S11 and 10.7×10^{-3} g·l^{-1} (reached after 30 hours) for S13 (**Figure 2**). Under pH 9.0 conditions, after reaching the maximum production of N-NH_3, S13 maintained this optimal activity for an additional 24 h before it decreased.

Besides the small amount of N-NH_3 produced by S11 under pH 9.0 conditions (more adapted to the biodegradation), this strain is also the first that was observed to stop the biodegradation process after it started. In fact, the production of N-NH_3 stopped after 30 h while that of S13 continued until the 78th hour.

Previous studies have reported the potential of bacterial strains to degrade cyanide compounds at slightly neutral pHs. For example, the optimum pH for the biodegradation of silver cyanide by a bacterial consortium was found to be 6.5 at 35˚C [25]. A strain of *Burkholderia phytofirmans* with the capability to degrade thiocyanate at pH < 9.0 was successfully obtained from contaminated soils at a gold mine tailings facility [26].

Furthermore, Nwokoro and Dibua [27] observed cyanide degradation using *Pseudomonas stutzeri* and *Bacillus subtilis* cultivated in a minimal medium containing 1.5 g·l^{-1} KCN. Cyanide was not detected after 80 minutes for *Pseudomonas stutzeri*, and 100 minutes for *Bacillus subtilis*.

The biodegradation of KCN by microorganisms requires the production of enzymes that can catalyze the process. Globally, the degradation of KCN with ammonia generation is performed via two pathways: hydrolytic and oxidative. Cyanide dihydratase (cyanidase) and cyanide hydratase have been identified as the main enzymes involved in the hydrolytic conversion of cyanide compounds like KCN, to carbon dioxide and ammonia, with formamide being an intermediate in the reaction [28] [29]. Cyanide dihydratase is mainly of bacterial origin [30]. Several studies have demonstrated that some bacterial strains (*Bacillus pumilus* C1, *Pseudomonas fluorescens* NCIMB 11764, *Pseudomonas stutzeri* AK61) can biotransform cyanide to ammonia and formate using a cyanide dihydratase [31] [32] [33]. With respect to cyanide hydratase, it is mainly of fungal origin [30]. For example, it has been reported that *Fusarium solani*, a plant pathogenic fungus, converts cyanide to ammonia through its hydrolysis by cyanide hydratase to formamide, which in turn, is hydrolyzed to ammonia and formic acid by an amidase. The ammonia generated is utilized for the growth and the formate presumably is converted to carbon dioxide by a formate dehydrogenase [34].

Two enzymes are involved in the oxidative pathway of cyanide degradation [30]: 1) a cyanase that converts cyanide to cyanate, and then cyanate is converted

to ammonia and carbon dioxide, which is bicarbonate dependent; 2) the direct conversion of cyanide to ammonia, and carbon dioxide is catalyzed by a cyanide dioxygenase which is pterin dependent. Cyanases have been identified in numerous microorganisms, bacteria, as well as fungi [1] [35].

The presence of ammonia in our biodegradation medium as a product of cyanide degradation can assume the synthesis of cyanide degrading enzymes (releasing ammonia) by the bacteria that could be cyanide dihydratase, cyanase or cyanide dioxygenase. The optimum pH of cyanide dihydratase was reported to be 8.0 [36]. However, Meyers *et al.* [31] showed that the optimal enzymatic activity of cyanide dihydratase isolated from *Bacillus pumilus* C1 occurred between 7.8 and 8.0 with no activity above pH 8.4. The authors subsequently demonstrated that this drastic effect of increased pH can be avoided by the presence of trivalent metal ions (20×10^{-3} mol·l^{-1} of Cr^{3+} or Tb^{3+}) in the culture medium. Therefore, the highest ammonia produced at pH 9.0 in our study, could be explained by either (i) the presence of enzymes with maximum activity around pH 9.0, suggesting that the effect of pH on cyanide hydratases will vary with the nature of the bacterial strain or (ii) the presence of components in the biodegradation medium protecting the enzymes from the drastic effect of high pH.

The maximum activity of cyanase from *Pseudomonas pseudoalcaligenes* [18] and cyanide dioxygenase from *Pseudomonas* species [37] were reported to be 8.5, which is close to pH 9.0; this could explain the maximum activity recorded at pH 9.0 in our study.

3.4. Effect of Initial Cyanide Concentration on Biodegradation Potential

The results of the effect of initial pH on the biodegradation capability of the strains showed that 1) pH 9.0 was more suitable for the biodegradation of KCN diluted in M9 minimal medium at 10^{-3} mol·l^{-1} and 2) the strain S13 was the most effective bacteria (**Figure 2**). Therefore, the strains were cultivated in 2 different concentrations of KCN (10^{-3} and 5×10^{-3} mol·l^{-1}) at pH 9.0 in order to determine the effect of initial cyanide concentration on the biodegradation potential. The results showed that strains S11 and S13 were both able to transform up to 5×10^{-3} mol·l^{-1} KCN into ammonia when the initial pH was fixed at 9.0 (**Figure 3**). Ammonia was generated from the early hours of contact of the bacteria with the KCN. The amount produced increased rapidly to reach the maximum after 24 h, whatever the initial concentration of KCN (10^{-3} or 5×10^{-3} mol·l^{-1}) used for both bacteria. As mentioned above, at 10^{-3} mol·l^{-1} KCN, the production of N-NH_3 by S11 dropped drastically and ended at the 30th hour while S13 maintained a constant maximal production until the 54th hour before it dropped to lower values. This decline could be related to a possible depletion of KCN because of its low initial concentration. At 5×10^{-3} mol·l^{-1} KCN, the production of N-NH_3 was maximal and maintained almost constant from the 24th hour for both bacteria. The highest N-NH_3 values (59×10^{-3} and 60×10^{-3} g·l^{-1} for S11

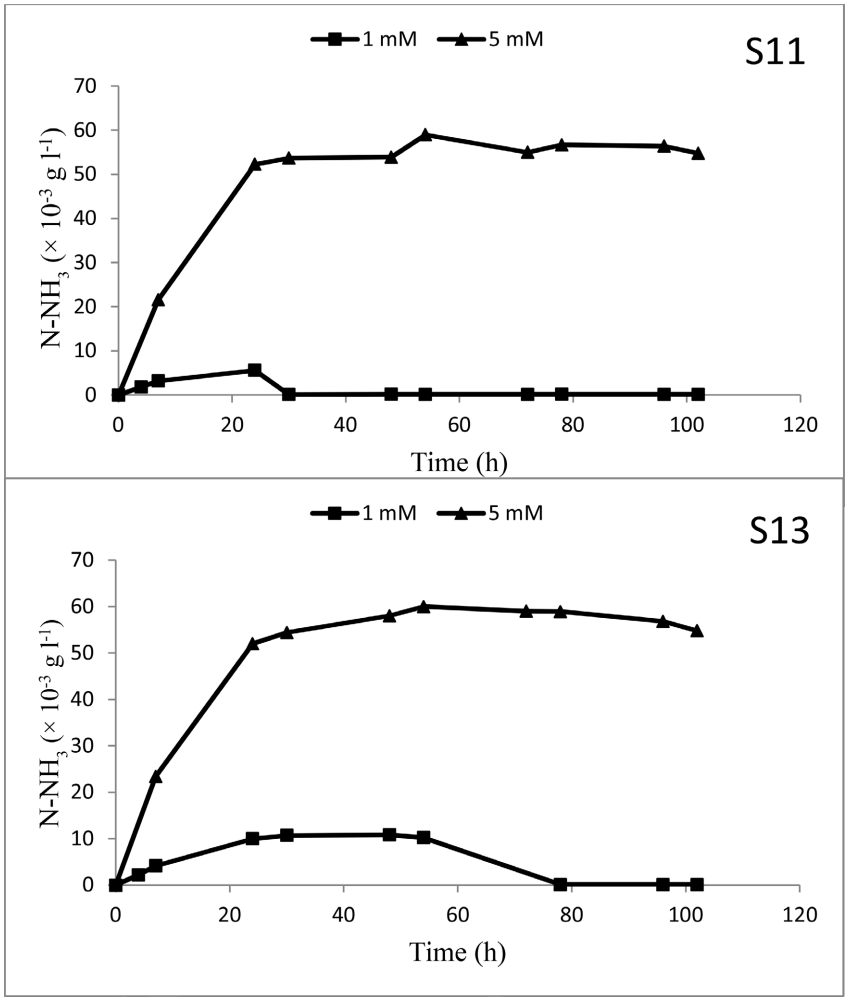

Figure 3. Effect of initial KCN concentration on its biodegradation at the optimum initial pH 9.0 (measured as ammonia generated) by strains S11 and S13. Ammonia concentration reported as the mean of duplicate samples

and S13 respectively) were produced at 54 hours. Mirizadeh *et al.* [15] obtained concentrations of 1.7×10^{-3} g·l^{-1} NH3 and 3.6×10^{-3} g·l^{-1} NO_3^- after 2 days growth of a bacterium on 0.2 g·l^{-1} (*i.e.* 3.07×10^{-3} mol·l^{-1}) KCN at an initial pH of 10.2. After 3 days, these concentrations increased to 2.3×10^{-3} and 4.1×10^{-3} g·l^{-1} respectively. These low concentrations of ammonia compared to our results could be explained by many reasons including the nature of the strain involved, the concentration of KCN and the subsequent transformation of ammonia to nitrate.

3.5. Statistical Comparison of the Effects of pH, Cyanide Concentration and Type of Strain on the Ammonia Generation

An ANOVA was used to compare the production of ammonia between time 0 to 102 h. When the data obtained from the experiments with strains S11 and S12 were compared (ANOVA, $P < 0.05$), the following results were obtained. First, the type of strain did not significantly impact the cyanide degradation since the

amounts of ammonia generated by S11 and S13 were not significantly different (P = 0.572). Second, whatever the strain used, varying the initial pH from 7.0 to 9.0 was shown to significantly enhance the production of ammonia from KCN degradation (P < 0.0001); this variation had a significant effect on the degradation of KCN at pH 7.0 and pH 9.0. This finding of the bacterial strains being more efficient in cyanide biodegradation under alkaline conditions is particularly important because it will prevent cyanide volatilization during the biodegradation process. Indeed, low pH promotes HCN volatilization which is highly toxic.

In addition, the increase of KCN concentration from 10^{-3} to 5×10^{-3} mol·l^{-1} was observed to significantly improve the production of ammonia. Indeed, the amount of ammonia generated at 5×10^{-3} mol·l^{-1} of KCN was significantly higher than that of 10^{-3} mol·l^{-1} (P < 0.0001).

Furthermore, when the combined effect of strain-pH was considered, the Tukey (HSD) test highlighted the formation of 2 distinct groups A and B (Table 2(a)).

The amounts of ammonia produced when the strains were grown at pH 9.0 (group A) were significantly higher than the ammonia generated when they were cultivated at pH 7.0 (group B); specifically, S13 cultivated at pH 9.0 produced the highest amount of ammonia from KCN followed by S11 cultivated at pH 9.0. S11 cultivated at pH 7.0 was the less adapted to KCN degradation.

The comparison of the combined effect of strain type and KCN concentration allowed the formation of 2 groups A and B (Table 2(b)). The strain S13 cultivated at 5×10^{-3} mol·l^{-1} KCN generated the highest content of ammonia while the lowest amount of ammonia was produced by strain S11 grown at 10^{-3}

Table 2. Comparison of the combined effects of (a) strain type and pH and (b) strain type and KCN concentration on the biodegradation potential.

(a)

Strain-pH	Mean N-NH_3^* ($\times 10^{-3}$ g·l^{-1})
S13-pH 9	25.808^A
S11-pH 9	23.231^A
S13-pH 7	0.716^B
S11-pH 7	0.249^B

(b)

Strain-KCN concentration	Mean N-NH_3^* ($\times 10^{-3}$ g·l^{-1})
S13-KCN 5×10^{-3} mol·l^{-1}	46.478^A
S11-KCN 5×10^{-3} mol·l^{-1}	45.378^A
S13-KCN 10^{-3} mol·l^{-1}	2.927^B
S11-KCN 10^{-3} mol·l^{-1}	0.667^B

*Mean N-NH3 with different capital letters are significantly different.

mol·l^{-1}. Globally, when the strains were grown with a KCN concentration of 5 × 10^{-3} mol·l^{-1}, the ammonia production (*i.e.* KCN degradation) was significantly higher than the quantity generated at 10^{-3} mol·l^{-1} concentration. This could be related to a rapid depletion of KCN from the culture medium given its low initial concentration of 10^{-3} mol·l^{-1}.

This study demonstrated that the bacterial strains isolated from contaminated soil were able to grow using KCN and $K_2Ni(CN)_4$ as a nitrogen source with MIC values between 10 × 10^{-3} mol·l^{-1} and 20 × 10^{-3} mol·l^{-1}. The bacteria degraded KCN at 10^{-3} mol·l^{-1} (65.11 × 10^{-3} g·l^{-1}) and 5 × 10^{-3} mol·l^{-1} (325.55 × 10^{-3} g·l^{-1}) with highest ammonia released at pH 9.0 and high KCN concentration. This ability opens prospects for the application of these bacteria for the remediation of cyanide contaminated soil and wastewater at alkaline pH, which will prevent HCN volatilization.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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