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Cyanide Degradation under Alkaline Conditions by a Strain of *Fusarium solani* Isolated from Contaminated Soils

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Several cyanide-tolerant microorganisms have been selected from alkaline wastes and soils contaminated with cyanide. Among them, a fungus identified as *Fusarium solani* IHEM 8026 shows a good potential for cyanide biodegradation under alkaline conditions (pH 9.2 to 10.7). Results of $K^{14}CN$ biodegradation studies show that fungal metabolism seems to proceed by a two-step hydrolytic mechanism: (i) the first reaction involves the conversion of cyanide to formamide by a cyanide-hydrolyzing enzyme, cyanide hydratase (EC 4.2.1.66); and (ii) the second reaction consists of the conversion of formamide to formate, which is associated with fungal growth. No growth occurred during the first step of cyanide degradation, suggesting that cyanide is toxic to some degree even in cyanide-degrading microorganisms, such as *F. solani*. The presence of organic nutrients in the medium has a major influence on the occurrence of the second step. Addition of small amounts of yeast extract led to fungal growth, whereas no growth was observed in media containing cyanide as the sole source of carbon and nitrogen. The simple hydrolytic detoxification pathway identified in the present study could be used for the treatment of many industrial alkaline effluents and wastes containing free cyanide without a prior acidification step, thus limiting the risk of cyanhydric acid volatilization; this should be of great interest from an environmental and health point of view.

Large amounts of cyanides are released in solid wastes and wastewaters of different industrial activities related to metal plating, aluminum electrolysis, coal gasification, coal coking, ore leaching, and the production of pharmaceuticals, synthetic fibers, and plastics. Since cyanide is a toxic compound well-known as a metabolic inhibitor, cyanide-containing effluents cannot be discharged without being subjected to treatment to reduce their cyanide contents to very low levels (<0.1 mg of CN^- per liter) (38). Current chemical treatment methods are not always well adapted and efficient with regard to technological, cost, and disposal considerations. For example, the main handicap of alkaline chlorination, a widely used process, is the need for chlorine, a hazardous reagent which has the potential to create toxic residues requiring a sequel treatment.

Despite cyanide's toxicity to living organisms (13, 38), biological treatments are feasible alternatives to chemical methods, because a wide range of microorganisms are known to metabolize such chemicals (6, 11, 15). A number of microorganisms have thus developed metabolic cyanide detoxification pathways, which have been utilized in industry for the last 40 years (18, 31). The majority of biological processes have been aerobic: e.g., activated sludge (26, 33), trickling filters, aerobic lagoons, and rotating biological contactors (28, 42). Carbon dioxide and ammonia have often been found as products of cyanide degradation (2). However, in most of these cases, either the microorganisms responsible for cyanide detoxification or the primary metabolites released were not identified or studied in detail.

More recently, microorganisms were isolated and cyanide degradation pathways were studied with pure cultures (21–23). For instance, strains of the bacterium *Pseudomonas fluorescens* were shown to utilize cyanide as a source of nitrogen for growth (14, 36). They can convert cyanide to carbon dioxide and ammonia via an NADH-linked cyanide oxygenase system (8, 23). Finally, the enzymatic reactions of cyanide transformation and biodegradation were loosely categorized into four types: substitution/addition, hydrolysis, oxidation, and reduction (32).

Of these enzymatic reactions, hydrolytic pathways seem to be among the most promising for cyanide detoxification (15, 32). They show good activity at high cyanide concentrations (200 to 10,000 mg · $liter^{-1}$ [34]), produce either formamide or formic acid, and require no additional cofactors (32). Several fungi that are pathogenic for cyanogenic plants, such as *Stremphylium loti* and *Gloeocercospora sorghi*, utilize the enzyme cyanide hydratase to convert cyanide into formamide and remove the toxic HCN released by plants (11, 12). Such fungi can be immobilized and used in packed-cell columns to continuously degrade cyanide (29, 30). Furthermore, ICI Biological Products marketed a spray preparation of dried mycelia for detoxification of industrial wastes (34, 35). Novo Industries has also introduced a cyanidase preparation, from a strain of *Alcaligenes denitrificans*, that converts cyanide directly into formate and ammonia (4, 19, 21).

However, those hydrolytic systems have some limitations and gaps: (i) high K_m values for proteins result in reduced efficiency at low cyanide concentrations; (ii) the neutral optimum pH for enzyme activity is not adapted to cyanide-containing wastes, such as aluminum residue produced by electrolysis, which normally have a higher (alkaline) pH; and (iii) the volatility of free cyanide as toxic HCN at neutral or a slightly alkaline pH (pK_a of HCN/pKa of CN^- [ratio] = 9.2 at 25°C) strongly and dangerously increases the disappearance of cyanide by volatilization and competes with biodegradation. Consequently, caution must be used in the interpretation of

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the results. As scanty information is available on the ability of microbes to degrade and remove cyanide under highly alkaline conditions, the present work was undertaken to isolate cyanide-metabolizing microorganisms from contaminated alkaline wastes and soils and investigate enzymatic cyanide degradation under extreme-pH conditions.

MATERIALS AND METHODS

Isolation of microorganisms growing in the presence of cyanide. Microbial isolation procedures were conducted on contaminated soil samples collected from an industrial site, following the dilution plate method (43) with nutrient agar plates supplemented with different concentrations of potassium cyanide (0, 20, or 50 mg · liter^{-1}). Before sterilization, the pH of the medium was adjusted to 11 by addition of sodium hydroxide. Potassium cyanide from a filter-sterilized (0.2-μm-pore-size filter) solution was added to the agar medium just before it solidified. Growth of colonies on cyanide-containing media was considered evidence of cyanide resistance, attributable either to their ability to biodegrade cyanide or to their development of a respiratory system resistant to this inhibitor, referred to as an alternative oxidase (16, 24).

Selection of cyanide-degrading microorganisms. Selection of cyanide-degrading microorganisms was conducted in minimal liquid media. These media were prepared from an autoclave-sterilized basal solution (BS) consisting of 0.001 N sodium hydroxide (pH 11) amended with KH_2PO_4 (10 mg · liter^{-1}) and 10 ml of a trace salts solution per ml. This solution contained, per 1,000 ml of deionized water, 100 mg of $FeCl_3 \cdot 6H_2O$, 100 mg of $MgSO_4 \cdot 7H_2O$, 100 mg of $CaCl_2 \cdot 2H_2O$, 50 mg of $MnSO_4 \cdot 4H_2O$, 50 mg of $ZnSO_4 \cdot 7H_2O$, 10 mg of $CoCl_2 \cdot 2H_2O$, and 10 mg of $Na_2MoO_4 \cdot 2H_2O$. Three media were then prepared by addition of KCN (50 mg · liter^{-1}) as the sole source of carbon and nitrogen, KCN (50 mg · liter^{-1}) supplemented with yeast extract (20 mg · liter^{-1}), or KCN (50 mg · liter^{-1}) supplemented with filter-sterilized glucose (20 mg · liter^{-1}). The growth responses of individual isolates and the degradation of cyanide were determined in 250-ml cotton-plugged plasma flasks, each containing 100 ml of one of the three minimal media. The cultures were incubated at 30°C and stirred at 120 rpm for 1 week. The pH of each culture was adjusted to about 10 once a day with sterile 1 N NaOH. Isolates selected for their ability to degrade cyanide were reisolated and stored on nutrient agar slants at 4°C for a maximum of 2 weeks.

Characterization and identification of microorganisms. Microorganisms were identified on the basis of cell and colony morphologies. Bacteria were tested for their Gram reactions, and either other physiological and biochemical tests, such as catalase and oxidase, were performed or the Biolog identification system (Biolog Inc., Hayward, Calif.) was used. Identification of fungal isolates was based on criteria established by Burgess and Liddell (5).

Preparation of the fungal inoculum. The fungal inoculum was prepared by suspending mycelia, grown on agar slants, in 10 ml of sterile physiological water. Incubations were done aerobically in 250-ml cotton-plugged flasks containing 100 ml of nutrient broth medium, adjusted to pH 8 with 1 N NaOH. The cultures were incubated at 30°C in a rotary shaker (120 rpm) for 3 days. Then, in accordance with the method of Nazly et al. (29), the fungal cyanide-degrading enzymes were induced by addition of 0.5 mM KCN to the medium 12 h before harvesting. Mycelia were then harvested by centrifugation (14,000 × *g* for 30 min at 4°C), washed twice in physiological water, and fragmented in a manual handheld homogenizer. The final inoculum, consisting of mixtures of mycelium fragments and spores, was used in KCN biodegradation assays.

KCN biodegradation assays. Biodegradation assays were conducted with labeled (^{14}C) and unlabeled cyanide in two types of media: one medium contained cyanide as the sole source of carbon and nitrogen (medium B), and the other was supplemented with yeast extract (medium A). These four types of assays, as presented in Table 1, were performed in a 1.5-liter Pyrex reactor (Biolafitte fermentor) containing 1,200 ml of medium, maintained at 30°C by a water jacket, having a top-drive stirrer (120 rpm), and supplied with CO_2-free filtered-sterilized air at 50 ml · min^{-1}. The pH was maintained automatically at 10.1 ± 0.05, with sterilized 1 N NaOH or 1 N HCl, via a regulation device (Biocontroller Biolafitte) controlling two peristaltic pumps. Two gas scrubbers filled with concentrated sodium hydroxide (4 N) were placed at the exit of the airstream to trap cyanhydric acid (HCN) and carbon dioxide. At various intervals, samples were collected from the reaction mixture with a hypodermic needle and syringe to follow cyanide degradation, fungal growth, and the fate of the ^{14}C.

Analytical methods. Radioactivity measurements were obtained by procedures previously described by Volkel et al. (39). Radiolabeled carbon was separated into three fractions: (i) microbial cells and macromolecular compounds of microbial origin collected on a 0.2-μm-pore-size filter and dried at 100°C, (ii) filtered solution, and (iii) volatile compounds collected at the reactor exit in 4 N NaOH scrubbers. Fermentor filtered solutions were collected and separated into two fractions. One (2 ml) was placed in a Pankhurst tube (a two-compartment tube) to be acidified in one compartment with 1 N HCl. The volatilized compounds (HCN and CO_2) were trapped in the second compartment, which was filled with 2 ml of 1 N NaOH. The equilibrium and exchange between the two solutions were guaranteed by gentle agitation overnight. Both fractions, acidified and alkaline trapped, were then counted by a current scintillation method.

TABLE 1. Experimental design for KCN biodegradation assays

Medium[a]	Concn of KCN (mM)	$K^{14}CN$ activity (MBq)[b]	Concn of yeast extract (mg · liter^{-1})	*F. solani* inoculum (mg [dry wt] · liter^{-1})
A (KCN + yeast extract)				
Unlabeled	2.05	0	125	0.022
Labeled (A*)	2.05	25	125	0.022
B (KCN only)				
Unlabeled	1.73	0	0	0.4
Labeled (B*)	1.73	18	0	0.4

[a] The pH of each of the media was 10.1 ± 0.05. Experiments performed with unlabeled KCN involved two replicates; those performed with labeled KCN were single assays.

[b] The $K^{14}CN$ used (Sigma Aldrich Chimie) had a specific activity of 673.4 MBq · mmol^{-1}.

Cyanide degradation products, such as formamide and formic acid, were analyzed by thin-layer chromatography and high-pressure liquid chromatography (HPLC) as described by Dumestre (9). Free cyanides in solution were determined colorimetrically by the French Association for Normalization method (1) or with the Aquaquant 14429 test kit (Merck) used in association with a Philips PU8620 spectrophotometer. Biomass determinations were based on optical density measurements at 600 nm (Philips PU8620 spectrophotometer), direct microscopic cell counts, colony counts (CFU) on nutrient agar, and, especially for fungal biomass, cell dry weights after desiccation at 100°C.

Chemicals. [^{14}C]potassium cyanide, [^{14}C]glucose, and [^{14}C]formic acid were obtained from Sigma Aldrich Chimie. Other chemicals and reagents used were purchased from Merck or Sigma and were of the highest grade commercially available. Yeast extract, nutrient broth, and nutrient agar were obtained from Difco.

RESULTS

Isolation of microorganisms growing in the presence of cyanide. Three bacteria and one fungus were successfully and repeatedly isolated from contaminated soil samples by their ability to grow in media that had been supplemented with cyanide. Those isolates showed significant growth with a lag phase of 2 days on nutrient agar adjusted to pH 11 and were thus considered alkalinotolerant organisms. Each isolated microorganism was able to grow aerobically on cyanide-containing nutrient agar plates with a lag phase of 5 to 7 days. However, the colonies obtained were smaller and sparser than those grown on media without cyanide. The three purified bacteria, whose cells were gram positive and oxidase negative, were tentatively identified (by the Biolog system) as strains of *Corynebacterium* sp., *Rhodococcus* sp., and *Bacillus* sp. The fungal isolate that seemed to be well adapted to cyanide-containing media was identified both in our laboratory, by using the keys proposed by Burgess and Liddell (5), and by the Institute of Hygiene and Epidemiology Mycology (IHEM) of Brussels, Belgium. It was identified as *Fusarium solani* and deposited at the IHEM under the reference *F. solani* IHEM 8026.

Selection of a cyanide-degrading fungus. The four microorganisms isolated on nutrient agar plates (as described above) were assayed in batch culture for their ability to biodegrade cyanide under alkaline conditions. The three bacterial isolates were not able to biodegrade cyanide even in media supplemented with nutrients, such as glucose or yeast extract. Their growth started in those cyanide-containing media after a lag phase corresponding to the removal of cyanide by abiotic HCN volatilization.

The most interesting results were undoubtedly observed with the isolate of *F. solani*. This fungus was able to biodegrade

cyanide under highly alkaline conditions (pH up to 10.7 [9]) even in minimal medium containing cyanide as the sole source of carbon and nitrogen (see below). Cyanide biodegradation was specifically observed in batch cultures in which cyanide was successively pulsed (0.5 mM and, later, 0.8 mM) into a diluted yeast extract basal medium. The cyanide disappeared in an exponential manner in the presence of *F. solani*, whereas the rate of loss in uninoculated sterile medium was very low, due to weak HCN volatilization when the pH was always maintained above 9.2. Fungal growth was observed in yeast extract medium during the initial period of 40 h preceding the cyanide pulse but stopped immediately after cyanide addition.

Assays conducted in glucose minimal medium showed a rapid loss of cyanide; this was also observed in uninoculated sterile medium. Complementary experiments showed that this loss also occurred in galactose, lactose, and maltose minimal media and in potato dextrose broth but not in saccharose minimal medium, yeast extract basal medium, or nutrient broth under abiotic alkaline conditions. This can be explained by the reaction between cyanide and sugars (17), which is catalyzed at a highly alkaline pH and is well documented as the Kiliani reaction (27, 37). This underscores the necessity to observe and discuss carefully the protocols and the results of cyanide biodegradation studies. The pH and medium composition must be carefully chosen, taking into account both the volatilization of free cyanide and its high degree of reactivity with many compounds (such as metals or sugars).

K^{14}CN biodegradation and fungal growth. Considering the results of selection assays, a pH value of 10.1 was chosen because it seemed to be the most interesting experimental condition relating to both the limitation of HCN volatilization and the ability of fungi to biodegrade cyanide. Living cells of *F. solani* were able to decrease the cyanide concentration by about 2 mM in BS medium, adjusted to pH 10.1 ± 0.05, with or without the addition of nutrients (Fig. 1). In both cases, initial rates of cyanide removal were very low—the means ± standard deviations were 1.176 ± 0.098 mmol of $CN \cdot h^{-1} \cdot (mg\ [dry\ weight]\ of\ cells)^{-1}$ in the presence of yeast extract (Fig. 1a) and 0.863 ± 0.041 mmol of $CN \cdot h^{-1} \cdot (mg\ [dry\ weight]\ of\ cells)^{-1}$ in KCN media lacking yeast extract (Fig. 1b)—but were quite similar. Furthermore, throughout the experiment, no residual cyanide was measured in gas scrubbers, showing that no loss of HCN occurred by volatilization and that cyanide disappearance is of biological origin.

Even though the addition of nutrients had little effect on the first step of cyanide metabolism, it seemed to play a major role in the second step starting after almost 3 days of incubation. Actually, growth of *F. solani* did not resume until the cyanide concentration had been reduced to 40 to 80 μM, and only in yeast extract-containing media (Fig. 1a). Furthermore, the automatic addition of sodium hydroxide to the medium for pH regulation could correspond to the release of acidic metabolites associated with growth and CO_2 production (data not shown). On the other hand, in the absence of nutrients (Fig. 1b), no growth occurred even after the complete detoxification of the medium, thus showing that *F. solani* is unable to grow on cyanide as the sole source of carbon and nitrogen.

Fate of ^{14}C from labeled KCN. At the end of the assay, about 90% of the radioactivity remained in both cultures (with and without yeast extract) in which adapted fungal inoculum had been added (Tables 2 and 3). Less than 0.5% was trapped in the gas scrubber, confirming that no cyanide was lost by gas circulation and volatilization. The distribution of radioactivity was as follows: while it was initially present in solution in the alkaline fraction (in the form of $^{14}CN^-$), radioactivity was transferred to the acidic fraction (obtained by medium acidification in Pankhurst tubes) after 3 days of incubation. This showed that radioactivity was not associated with either HCN, which had been almost completely degraded (Fig. 1), or the CO_2 released into the medium. The increase of radioactivity in the acidic fraction of the solution could be related to biomass growth and activity. The fungal biomass concentrated only very small amounts of radioactivity, since at the end of the experiment only 5.1% of the radioactivity in the medium supplemented with yeast extract was cell associated. Although it was low, this assimilation of carbon compounds by *F. solani* should be considered significant, in comparison with the amount of radioactivity assimilated in medium B (without yeast extract), in which no growth occurred. However, in both media, the ^{14}C remaining in solution can be attributed to soluble metabolic compounds that have been identified (see below).

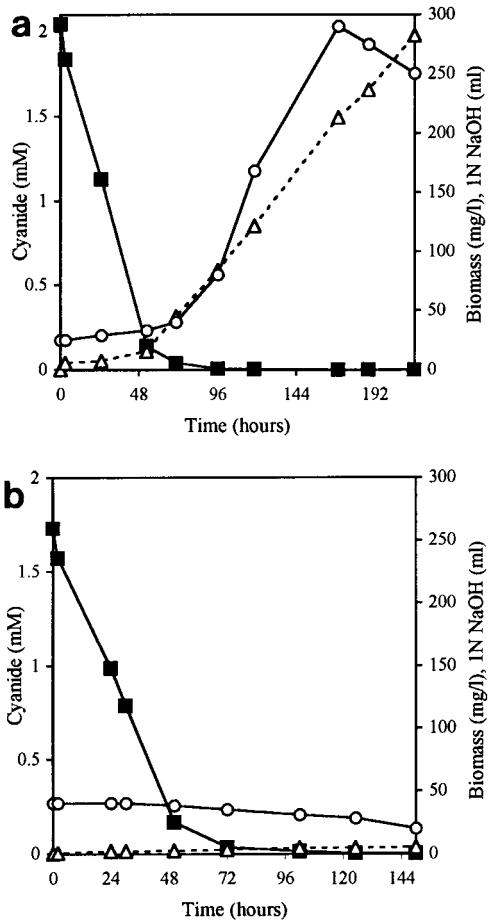

FIG. 1. KCN biodegradation at pH 10.1: cyanide disappearance (average of three assays conducted with labeled and unlabeled cyanide) and *F. solani* growth in two different media (with and without yeast extract). (a) Medium A (BS medium plus 2 mM KCN and 125 mg of yeast extract per liter; (b) medium B (BS medium plus 1.73 mM KCN). Symbols: ■, KCN concentration; ○, fungal biomass; △, NaOH addition for pH regulation.

Identification of the cyanide degradation products. Two labeled metabolites were detected in acidified samples collected from the culture medium at different times and identified essentially by HPLC. The first labeled compound was detected both spectrophotometrically and with a radioactivity detector after 31 min of elution. This retention time can be related to the formamide standard, $HCONH_2$. The second metabolite was detected after 18 min of elution with a Flo-one radioac-

TABLE 2. Balance and fate of ^{14}C from [^{14}C]cyanide during culture of *F. solani* in medium A* at pH 10.1[a]

Day	Activity (MBq) in fraction[b]:				
	Filtered solution			Biomass[c]	Gas trap[d]
	Total	Alkaline fraction[e]	Acidic fraction[f]		
0	25.02 (99.8%)	21.86 (87.4%)	1.5 (6%)	0	0
3	24.93 (99.7%)	1.27 (5.1%)	22.06 (88.2%)	0.06 (0.25%)	0.075 (0.3%)
9[g]	21.71 (86.8%)	1.0 (4.0%)	19.58 (78.3%)	1.3 (5.1%)	0.06 (0.25%)

[a] Medium A* consists of BS medium plus 125 mg of yeast extract per liter plus 2 mM $K^{14}CN$.
[b] Numbers in parentheses are percentages of initial radioactivity.
[c] Activity recalculated from biomass density (milligrams [dry weight] per liter) and biomass specific activity (becquerels per milligram [dry weight]).
[d] Activity measured in scrubbers containing 4 N NaOH.
[e] Activity remaining in solution in the alkaline fraction after separation in Pankhurst tubes.
[f] Activity remaining in solution in the acidic fraction after separation in Pankhurst tubes.
[g] End of experiment.

tivity detector but was below the limit of detection of the spectrophotometric method. It was identified as formic acid, by comparison with an $H^{14}COOH$ standard (Sigma Aldrich Chimie).

The distributions of these two compounds in the culture varied as a function of medium composition. In the presence of yeast extract (medium A), formamide and formic acid appeared successively in the medium, as shown in Fig. 2. In that case, KCN biodegradation seemed to follow a two-step mechanism. On the other hand, in minimal medium (without yeast extract), the results were more difficult to interpret. In two assays, formamide was the only compound released and its concentration remained stable in the medium. In the third one, formic acid was detected as the major metabolite present in the medium. However, in that case, samples were stored under strongly acidic conditions for 2 weeks prior to analysis, during which time a chemical hydrolysis of formamide in formic acid could have occurred (25). Furthermore, this production of formic acid was not in accordance with the results presented in Fig. 1b, which showed that there was no acidification of the medium during the entire experiment.

Further assays were also conducted to study the metabolism of formamide by *F. solani* and to confirm the biological origin of its conversion in the presence of yeast extract under alkaline conditions. Table 4 presents the protocol used and the results obtained in these assays. It shows that even at pH 10, and under abiotic conditions, formamide was slowly chemically degraded. No biological conversion occurred in BS medium containing formamide as the sole source of carbon and nitrogen. However, in the presence of yeast extract, a slow biodegradation by *F. solani* was observed, confirming the results obtained in the previous KCN biodegradation assays (Fig. 2).

DISCUSSION

Selection of microorganisms from the autochthonous microflora of cyanide-contaminated soils led to the isolation of several gram-positive bacteria and one fungus. All isolates of bacteria were tolerant to cyanide and grew under alkaline conditions (to pH 11) on cyanide-supplemented nutrient agar medium. However, their tolerance wasn't due to cyanide degradation ability but most probably was attributable to the development of alternative oxidases permitting bacterial respiration in the presence of cyanide (16, 24). On the other hand, the fungus identified as *F. solani* (IHEM 8026) shows an efficient cyanide-degrading ability. It belongs to a well-known family of fungal pathogens of cyanogenic plants (11). The tolerance of these fungi to HCN is associated with their ability to metabolize cyanide with a hydrolytic enzyme. This cyanide hydratase (EC 4.2.1.66), which was first found by Fry and Millar (12) in *Stemphylium loti*, catalyzes the hydration of cyanide to formamide with a 1:1 stoichiometry. More recently, the enzyme was purified from *Gloeocercospora sorghi* (40) and *Fusarium lateritium* (7) and characterized, and it was also found for the first time in a strain of *P. fluorescens* (23). Considering this previous knowledge, it was both amazing and particularly interesting to find in this work that this type of fungus is well adapted to cyanide-contaminated soils and to alkaline environments and that it exhibits cyanide-degrading activity to pH 10.7 (9).

The present study demonstrates that *F. solani* is able to

TABLE 3. Balance and fate of ^{14}C from [^{14}C]cyanide during culture of *F. solani* in medium B* at pH 10.1[a]

Day	Activity (MBq) in fraction[b]:				
	Filtered solution			Biomass[c]	Gas trap[d]
	Total	Alkaline fraction[e]	Acidic fraction[f]		
0	18.0 (99.7%)	16.7 (92.6%)	0.87 (4.8%)	0	0
3	16.33 (90.5%)	1.58 (8.75%)	14.0 (77.6%)	ND[g]	0.32 (1.8%)
9[h]	16.5 (91.5%)	0.83 (4.6%)	14.83 (82.2%)	0.01 (0.05%)	0.045 (0.25%)

[a] Medium B* consists of BS medium plus 1.73 mM $K^{14}CN$.
[b] Numbers in parentheses are percentages of initial radioactivity.
[c] Activity recalculated from biomass density (milligrams [dry weight] per liter) and biomass specific activity (becquerels per milligram [dry weight]).
[d] Activity measured in scrubbers containing 4 N NaOH.
[e] Activity remaining in solution in the alkaline fraction after separation in Pankhurst tubes.
[f] Activity remaining in solution in the acidic fraction after separation in Pankhurst tubes.
[g] ND, not determined.
[h] End of experiment.

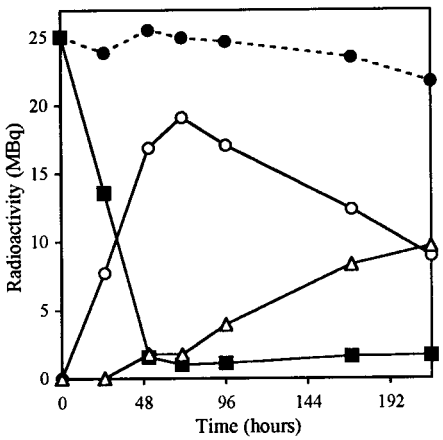

FIG. 2. $K^{14}CN$ biodegradation in medium A* (with 125 mg of yeast extract per liter): distribution of radioactivity versus time after analysis by HPLC coupled detection of radioactivity by a Packard Flo-one radioactivity detector. Symbols: ●, total radioactivity in solution; ■, radiolabeled cyanide; ○, radiolabeled formamide; △, radiolabeled formic acid.

degrade cyanide without an additional cofactor under alkaline conditions. The rate of cyanide biodegradation, although low—about 1 mmol of $CN \cdot h^{-1} \cdot (mg\ [dry\ weight]\ of\ cells)^{-1}$—was slightly affected by the complete absence of organic nutrients such as yeast extract. pH plays a major role, since cyanide hydrolysis is significantly slower at pH 9 and above. Calculation of the specific constants k_{HCN} and k_{CN^-}, corresponding to HCN and CN^- degradation, respectively ($k_{HCN} = 0.0714\ h^{-1}$; $k_{CN^-} = 0.0017 h^{-1}$), suggested that cyanhydric acid is preferentially degraded by *F. solani* compared CN^-, as presented by Dumestre et al. (10). Thus, the slower kinetics at high pH values seems to be attributed more to substrate unavailability (i.e., the inability of the fungus to biodegrade cyanide present in the form of CN^- [$pK_a = 9.2$]) than to a decrease in enzyme activity under alkaline conditions. Our results underscore the fact that no fungal growth occurred during this initial detoxification step, suggesting a high toxicity of cyanide even for cyanide-degrading microorganisms (14, 36). There seems to exist a threshold value of about 40 to 80 μM, corresponding to the minimum cyanide concentration inhibiting growth; such a threshold had already been observed for a *Pseudomonas* strain but at around 100 to 180 μM (41).

Other important results are related to general considerations of cyanide metabolism. Many authors have made a direct analogy between cyanide hydrolytic pathways (including cyanide hydratase) and nitrile metabolism (15, 20). However, until recently, only a few of them gave arguments to support this hypothesis (3, 23). The utilization of labeled cyanide and

TABLE 4. Formamide degradation by *F. solani*: experimental conditions and results of assays[a]

Medium	Concn of:			% Formamide degraded[b]
	Formamide ($mg \cdot liter^{-1}$)	Yeast extract ($mg \cdot liter^{-1}$)	*F. solani* inoculum ($mg\ [dry\ wt] \cdot liter^{-1}$)	
F_A	100	50	0.03	59.2 ± 10.5
F_B	100	0	0.03	36.0 ± 8.7
Control	100	0	0	37.2 ± 11

[a] Assays were conducted for 8 days in 250-ml aerated flasks with carbonate-buffered BS media at pH 10.1.
[b] Results are means ± standard deviations.

First step :

$$H^*CN + H_2O \xrightarrow{\text{Cyanide Hydratase}} H^*CONH_2 \qquad \textit{(reaction 1)}$$

Second step :

$$H^*CONH_2 + H_2O \xrightarrow{\text{Formamidase}} H^*COOH + NH_3 \qquad \textit{(reaction 2)}$$

$$\text{Inoculum} + \text{Yeast Extract} + O_2 \rightarrow \text{Biomass} + CO_2 + \text{Metabolites} \qquad \textit{(reaction 3)}$$

FIG. 3. Proposed two-step cyanide metabolism in *F. solani* IHEM 8026 under alkaline conditions (pH 10.1). See text for details. The asterisks indicate labeled carbon.

highly alkaline conditions in the present study provides new information on the subject. Formamide and formic acid are confirmed as the two main products of cyanide biodegradation in *F. solani*. This fungus is not able to grow on these compounds as sole sources of carbon and nitrogen, even if it can assimilate a small amount of them (about 5% of total labeled carbon) in the presence of supplementary nutrients (Table 2). Formamide conversion seems to be dependent also on the presence of nutrients such as yeast extract. It could be attributed to a formamidase, such as had already been described in cyanide-degrading microorganisms such as *Alcaligenes xylosoxidans* (19, 20) and *Pseudomonas putida* (3). The rate of formamide conversion by *F. solani* observed in our experiments under alkaline conditions is about 25% of the rate of cyanide conversion by cyanide hydratase, showing good agreement with the value of 30% measured by Ingvorsen et al. (21) at neutral pH. These results allow us to propose a two-step hydrolytic mechanism for cyanide biodegradation under alkaline conditions, presented in Fig. 3. Nevertheless, further studies on the specific activities of cyanide-degrading enzymes—particularly formamidase activity—are needed to confirm this hypothesis and to verify metabolite assimilation by *F. solani.*

From an environmental and health point of view, the degradation of cyanide by a strain of *F. solani* under highly alkaline conditions at an even slower rate than at neutral pH is of great interest since it provides insight into the involvement and occurrence of such processes and organisms in extreme environments. Under such alkaline conditions, biodegradation is a more effective means of removing cyanide than volatilization, occurring strongly at a slightly alkaline or neutral pH (pK_a of HCN/pKa of CN^- [ratio] = 9.2 at 25°C). This application is also promising as this simple hydrolytic pathway of detoxification could be used for the treatment of many industrial alkaline effluents and wastes containing free cyanide, without prior acidification step, thus limiting risks of cyanhydric acid volatilization.

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