Microbiology, Vol. 69, No. 2, 2000, pp. 167–173. Translated from Mikrobiologiya, Vol. 69, No. 2, 2000, pp. 209–216.

EXPERIMENTAL ARTICLES

Microbial Degradation of Cyanide and Thiocyanate

G. I. Karavaiko*, T. F. Kondrat'eva*, E. E. Savari, N. V. Grigor'eva*, and Z. A. Avakyan*†**

**Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117811 Russia*

***Central Research Institute for Geological Exploration of Nonferrous and Precious Metals, Varshavskoe sh. 129b, Moscow, 113545 Russia*

Received April 13, 1999

Abstract—The role played by a bacterial community composed of *Pseudomonas putida*, strain 21; *Pseudomonas stutzeri*, strain 18; and *Pseudomonas* sp., strain 5, and by physical and chemical factors in the degradation of CN^- and SCN^- was studied. It was shown that the degradation of CN^- is determined both by the action of bacteria and by abiotic physical and chemical factors (pH, O_2, temperature, the medium agitation rate, etc.). The contribution of chemical degradation was found to increase drastically at pH below 9.0; when air was blown through the medium (irrespective of the pH value); under active agitation of the medium; and when the medium surface interfacing air was increased. Even at elevated pH values (9.0–9.2), suboptimal for bacterial growth, the microbial degradation could account for at most 20–25 mg/l of CN^-, regardless of its initial concentration. When CN^- and SCN^- were concurrently present in the medium, the former compound was the first to be degraded by microorganisms. The rate of bacterial degradation of SCN^- under continuous cultivation in a chain of reactors was found to depend on its concentration, the medium flow rate, agitation rate, and the pattern of carbon source supply and could exceed 1 g/(l day). CN^- and SCN^- are utilized by bacteria solely as nitrogen sources. The mechanism of CN^- and SCN^- degradation by the microbial community is discussed.

Key words: cyanide, thiocyanate, degradation, *Pseudomonas putida*, *Pseudomonas stutzeri*

The most significant sources of cyanide pollution are galvanic wastes and the gold mining industry. Several techniques to detoxify cyanide-containing wastewaters are currently employed; the most important one is chlorination. This method, however, is itself environmentally hazardous and fails to bring wastewaters to permissible concentrations of CN^-, SCN^-, and their complexes with metals.

The role of microorganisms and microbial enzymes in the degradation of cyanide has been studied for a long time. Extensive literature on the subject has been generalized in several surveys and books [1–3]. However, the impact of microorganisms on this process is difficult to evaluate due to a lack of knowledge of the action of the chemical factors [4–8]. Without taking into account the possibility of HCN volatilization, the approaches taken to the study of the bacterial degradation of cyanide included aeration, active agitation, the addition of reducing sugars, the maintenance of pH at relatively low values, etc. No tests for the chemical decomposition of CN^- were ordinarily performed. However, microorganisms are currently used on the industrial scale to decontaminate cyanide-containing wastes at the Homestake gold mining plant, the United States [2, 9]. These are tailing wastewaters with very low concentrations of CN^- (0.5–11.5 mg/l), SCN^- (up to 110 mg/l), and their bound forms with precious metals. There were so far virtually no studies on the role of microorganisms in the degradation of CN^-, SCN_-, and their metal complexes at elevated and high concentrations of these pollutants.

The purpose of this work was to study the role played by the bacterial community and the impact of chemical and physical factors in the degradation of CN^- and SCN^- for a broad range of their concentrations.

MATERIALS AND METHODS

Microorganisms and cultivation conditions. An experimentally created bacterial community composed of *Pseudomonas putida*, strain 21; *Pseudomonas stutzeri*, strain 18; and *Pseudomonas* sp., strain 5, was used in tests. These strains were characterized elsewhere by Grigor'eva *et al.* [10].

The bacteria were cultured and CN^- and SCN^- degradation was studied using the same medium composed of (g/l) $K_2HPO_4 \cdot 3H_2O$, 3.0; Na_2CO_3, 0.5; $MgSO_4 \cdot 7H_2O$, 0.3; NaCl, 0.1; potassium lactate, 1.2, or sucrose, 1.0; tap water; the pH was 9.0–9.2. KCN and NaSCN were added depending on the objective of the experiment. No nitrogen sources other than CN^- and

†Deceased.

0026-2617/00/6902-0167$25.00 © 2000 MAIK "Nauka/Interperiodica"

SCN^- were supplied because, otherwise, the bacteria under study failed to degrade cyanide and thiocyanate.

Analytical methods. The concentration of CN^- in solutions was determined by titration with silver nitrate [11] or by using an I-130 ionometer with a KRITUR type 06-27 crystalline ion-selective electrode probe and a standard curve of electron motive force voltage as a function of CN^- concentration. The probe was able to detect CN^- concentrations ranging from 260 to 0.13 mg/l (10^{-2} to 5×10^{-6} mol/l).

SCN^- was determined by titration with potassium permanganate [12] or using an ANION-410D apparatus (Infraspak-Analit, Russia) equipped with a KRI-TUR type 58-27 crystalline ion-selective probe. The apparatus was calibrated by storing the parameters of standard solutions in its memory. The probe was able to detect SCN^- concentrations in the range of 5800 to 0.058 mg/l (10^{-1} to 10^{-6} mol/l).

The concentration of NH_4^+ in the medium was determined with an EKOM-NH_4^+ ion-selective probe, similarly to measurements of the SCN^- concentration with the ANION-410D apparatus. The probe was able to detect NH_4^+ concentrations from 1800 to 0.3 mg/l (10^{-1} to 5×0^{-5} mol/l).

SO_4^{2-} was determined using an Aquaquant test kit (Merck, Germany). Lactate utilization was estimated from lactate dehydrogenase activity, determined with a B10-LA test kit (Lachema, Czechia). The pH value was determined with an I-130.2M.I ionometer. The biomass accumulated was measured as dry cell weight.

RESULTS AND DISCUSSION

Quantitative analysis of the chemical degradation of CN^-. Tests to determine the rate of the chemical degradation of CN^- were conducted at room temperature (20–22°C) in 100-ml Erlenmeyer flasks containing 50 ml of the solution either under static conditions or with agitation by a magnetic mixer (800 rpm) or with air blown through the medium.

The assay results are shown in Figs. 1a–1c. The initial concentrations of CN^- in the solutions were different because of the difference in the initial pH values. Chemical degradation of CN^- was most vigorous when air was blown through the medium (Fig. 1a), decreased in the case of agitation by a magnetic mixer (Fig. 1b), and was least active under static conditions (Fig. 1c). The rate of degradation increased at lower initial and final pH values of the medium, independent of other conditions. The final pH level of the medium (Figs. 1a–1c) is determined by how actively the medium interacts with air, mostly by the following well-known reactions:

$$H^+ + OH^- + CO_2 \longrightarrow HCO_3^-, \quad (1)$$

$$HCO_3^- \longrightarrow H_2O + CO_2. \quad (2)$$

This conclusion is also confirmed by additional experiments conducted under similar conditions except for the presence of CN^-. In these tests, the pH of the solutions decreased to about the same level as in tests with CN^- (data not shown). Another control experiment without bacteria was performed in a reactor with mechanical agitation under conditions identical to those applied in experiments on the bacterial degradation of CN^-.

The experiments were carried out in a reactor with a volume of 3.0 l containing 2 l of the medium; the agitation rate was 200 rpm, and the temperature was 28–30°C. The initial pH value was 9.2, and the initial CN^- concentration was 34 mg/l. As follows from the experiments described above, the CN^- concentrations close to this one are relatively stable at pH 8.7–9.3.

As one can see from Fig. 1d, at pH 9.2, the concentration of CN^- in the medium dropped from 34 to 24–25 mg/l over a period of 1.5 h; however, when the medium acidity was adjusted to pH 8.8, the concentration of CN^- decreased over the next 2 h to 18 mg/l and then stabilized at that level.

With an agitation rate of 136 rpm, an initial CN^- concentration of 39 mg/l, a temperature of 28–30°C, and pH 9.3, the concentration of cyanide did not change for 18 h, while at 300 rpm, it decreased over 24 h by about 50% of its initial level of 50 mg/l. All other conditions being equal, the rate of chemical degradation of CN^- decreased when the temperature was lowered to 24°C. Thus, over a period of 24 h, the concentration of CN^- decreased from its initial value of 40 mg/l to 24.4 mg/l, that is, by 39%. When the agitation intensity was reduced to 200 rpm without changing the temperature, the concentration of CN^- dropped over the same period of time from 40 to 30 mg/l, which means that only 25% of CN^- was chemically degraded.

Tests to determine the effect of the medium volume in the reactor were carried out at an agitation intensity of 300 rpm, 29–30°C, initial pH 9.3, and with an initial CN^- concentration of 50 mg/l. In a 3-l reactor containing 2 l of the medium, the concentration of CN^- decreased over 24 h from 50 to 25 mg/l (50%), whereas, with the medium volume of 1.3 l, it lowered from 54 to 18 mg/l (a decrease of 66.7%).

In a closed reactor with no access to air, no chemical degradation of CN^- took place when its initial concentration was 71 mg/l, the pH was 9.2–9.4, the temperature was 24°C, and the agitation rate was 300 rpm. However, when the medium pH was adjusted to 8.4, the concentration of CN^- dropped over 7 days from 71 mg/l to zero. After that, the medium was alkalized to pH 9.4, and the CN^- concentration in the medium returned to 71 mg/l. This indicates that, in an airtight reactor, a more acidic pH caused CN^- to pass into the gas phase in the form of HCN by the hydrolysis reaction,

$$CN^- + H_2O = HCN + OH^-. \quad (3)$$

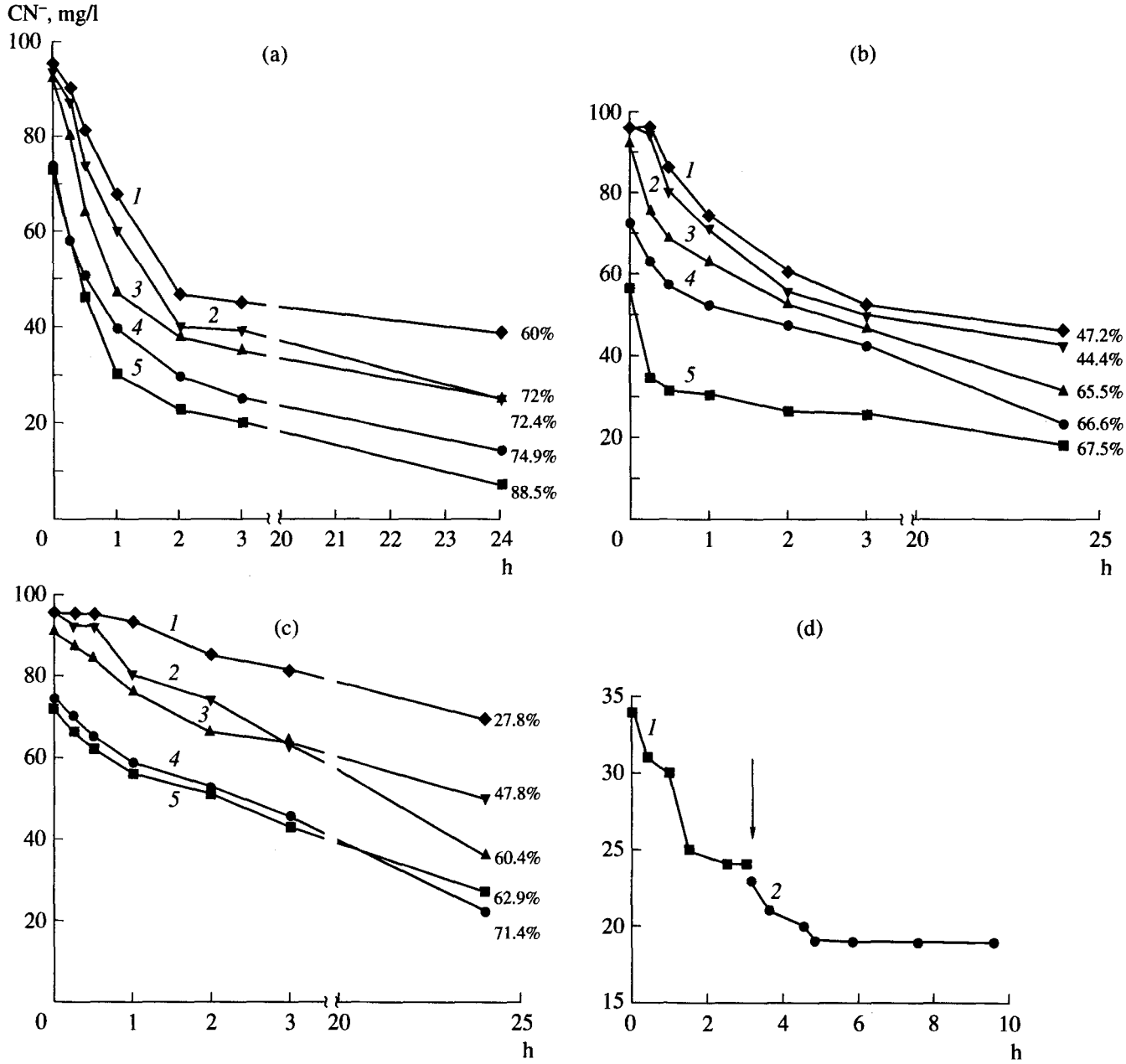

Fig. 1. Chemical degradation of CN^- at different pH values of the medium under (a) aeration with air ((*1*) pH 10.5–8.4; (*2*) pH 9.7–8.26; (*3*) pH 9.25–8.15; (*4*) pH 9.0–7.9; and (*5*) pH 8.7–7.5); (b) medium agitation with a magnetic mixer ((*1*) pH 10.5–8.9; (*2*) pH 9.7–8.95; (*3*) pH 9.25–8.17; (*4*) pH 9.0–8.15; and (*5*) pH 8.6–8.1); (c) static conditions ((*1*) pH 10.5–9.8; (*2*) pH 9.7–9.4; (*3*) pH 9.25–9.0; (*4*) pH 9.0–8.9; and (*5*) pH 8.7–7.5); and (d) in a reactor with mechanical stirring (200 rpm); the point of pH adjustment with H_2SO_4 to pH 8.8 is indicated by an arrow ((*1*) pH 9.2–9.1; and (*2*) pH 8.8–8.6). The initial and final pH values are given. The figures to the right of the curves give the percent of CN^- degraded.

It seems that, when the medium was alkalized, HCN was partly converted to CN^- and partly present in the medium in the form of HCN as a weak acid. The term *free cyanides* refers both to cyanide ions (CN^-) and hydrogen cyanide (HCN). The ratio between the concentrations of HCN and CN^- strongly depends on the pH of the medium (Table 1) [2].

As one can see from Table 1, in the range of pH values 7.0–9.0 which are optimal or suboptimal for the majority of organotrophic microorganisms, free cyanides predominantly occur as volatile HCN, which has its boiling point at 25.7°C.

In the presence of air, CN^- is decomposed under the action of carbon dioxide by the well-known reaction

$$2KCN + H_2O + CO_2 = K_2CO_3 + 2HCN. \quad (4)$$

It is worth mentioning that cyanides are also decomposed by reducing sugars [13, 14]. In the presence of

Table 1. Ratio of CN^- ions and hydrogen cyanide as a function of the medium pH

pH	HCN, %	CN^-, %
7	99	1
8	96	4
9	70	30
9.36	50	50
10	12	88
11	1	99

Table 2. Microbial degradation of CN^- in medium with potassium lactate

Parameters	Time, h			
	0	2	17	25
Content of CN^-, mg/l	30	24	7	0
pH	9.2	9.2	8.6	8.3
Bacterial biomass, g/l	0.075	0.075	0.57	0.72

1% glucose, CN^-, at a concentration of 100 mg/l, was completely degraded at pH 7.0 to 9.0 in 50 h [13].

There are also other chemical reactions involving cyanides, for example, their oxidation, which produces cyanates:

$$HCN + 1/2O_2 = HCNO. \quad (5)$$

However, under natural environmental conditions, when no chemical and enzymatic catalysts are present, this reaction can scarcely proceed [2], and, apparently, it failed to do so in our control experiments. It follows that the chemical degradation of CN^- is determined, among other things, by such factors as pH, temperature, aeration, and carbon sources. All these factors should be taken into account when assessing the role of microorganisms in the degradation of CN^- and when solving engineering problems.

Degradation of CN^- by a microbial population. Tests to study the microbial degradation of CN^- were conducted using a batch culture in a 3-l reactor open to atmospheric air and filled with 2 l of the medium containing 30 mg/l CN^- and 1.2 g/l potassium lactate. The initial pH of the medium was 9.2. The temperature was maintained at 28–30°C, and the agitation rate was 200 rpm. The inoculum (a bacterial community consisting of *P. putida, P. stutzeri*, and *Pseudomonas* sp.) was added in an amount of 10% of the medium volume. About 20% of CN^- under such conditions is degraded chemically. The results of assays are given in Table 2. During the first 2 h after inoculation, the concentration of CN^- in the medium declined to 24 mg/l as a result of HCN volatilization. During the next 23 h, CN^- was degraded completely, mainly by the action of bacteria utilizing potassium lactate. In the course of lactate metabolism, the biomass of bacteria increased from 0.075 to 0.72 g of dry cell weight per liter. Some part of CN^- was evidently removed through HCN volatilization, since, in the course of bacterial growth, the pH decreased to 8.3, and, as one can see from Fig. 1d, the stable concentration of CN^- in the medium at pH 8.8 under the given conditions is 18 mg/l.

When potassium lactate as the source of carbon was replaced with sucrose (1 g/l), the rate of bacterial degradation of CN^- remained close to that in the presence of lactate. With the initial CN^- concentration of 39 mg/l, its chemical and bacterial degradation over a period of 23 h amounted to about 54 and 46%, respectively.

Degradation of concurrently present CN^- and SCN^-. In addition to cyanides, industrial wastewaters also contain thiocyanates at concentrations exceeding those of CN^- by one or two orders of magnitude.

Tests to study bacterial degradation of concurrently present CN^- and SCN^- were run under the conditions analogous to those used for studying CN^- degradation. Sucrose at a concentration of 1 g/l was added as the source of carbon. To decrease the volatilization of HCN, the degradation of CN^- was initially carried out at 24°C; later, for SCN^- degradation, the temperature was raised to 28–30°C.

As can be seen from Fig. 2, with 40 or 53 mg/l of CN^- and 820 or 480 mg/l of SCN^- added to the medium, the bacterial community first proceeded with the degradation of CN^-. After that, when CN^- was almost totally degraded, the degradation of SCN^- got under way. A two-phase pattern of CN^- and SCN^- degradation in the case of their combined presence was previously demonstrated by Grigor'eva *et al.* [10].

Degradation of SCN^-. Thiocyanates are known to originate under natural conditions and in industrial processes from the reactions of CN^- with reduced sulfur compounds [2]. The two forms of sulfur most actively reacting with cyanide are polysulfide and thiosulfate:

$$S_x^{2-} + CN^- = [S_{(x-1)}]^{2-} + SCN^-, \quad (6)$$

$$CN^- + S_2O_3^{2-} = SO_3^{2-} + SCN^-. \quad (7)$$

Thiocyanate concentration in wastewaters of gold-extracting factories can be as high as several grams per liter. Preliminary tests carried out in a reactor with a batch culture showed weak bacterial degradation of SCN^- (8.0–9.4 mg/(l h) at an initial concentration of 170–600 mg/l).

Experiments on the degradation of SCN^- under continuous cultivation conditions were conducted at 29–30°C, an agitation rate of 300 rpm, and an initial pH of 9.2–9.4 for different medium flow rates in a chain of five to six sequentially connected reactors. Their volumes were 1.5 l (no. 1) and 1.0 l (nos. 2 to 6); the medium was fed to reactor no. 1 from the 10-l reactor no. 0. The medium used in these experiments was composed of (g/l) potassium lactate, 1.2 (added in several portions

into each reactor depending on the flow rate); Na_2CO_3, 0.2; $MgSO_4 \cdot 7H_2O$, 0.1; $K_2HPO_4 \cdot 3H_2O$, 0.5; and NaSCN, 290–1000 mg/l. Before the start of the experiment, all reactors were filled with the medium containing no sources of carbon or nitrogen. Potassium lactate as the source of carbon and energy was added separately to each reactor (once, for a flow rate of 1.4 l/day; twice, by 0.6 g, for flow rates of 1.8 and 2.2 l/day; and four times, by 0.3 g, for a flow rate of 3.7 l/day). SCN^-, as the source of nitrogen, was supplied only to reactor no. 0. This made it possible to attain complete degradation of SCN^- with no limitation of bacterial growth by carbon in any of the reactors. No bacterial degradation of SCN^- was observed in the absence of the carbon source.

As can be seen from Fig. 3, at a flow rate of 1.4 l/day, 1088 mg/l of SCN^- was degraded over a period of 24 h in the first four reactors. With the medium flow rate increased to 1.8 l/day and the initial SCN^- concentration equal to 918 mg/l, complete degradation of SCN^- was attained in reactor no. 5, the dry weight of the accumulated biomass being 0.7 g/l. When the initial SCN^- concentration was decreased to 609 mg/l, its total degradation was already accomplished in reactor no. 4, although the flow rate was increased to 2.2 l/day, and the biomass accumulated was 0.4 g/l. When the flow rate of the medium was brought up to 3.7 l/day and SCN^- was added at an initial concentration of 604 mg/l, its degradation was accomplished only in reactor no. 6, containing 0.7 g/l of biomass. The increase in the amount of biomass (0.7 g/l) at a higher flow rate (3.7 l/day) as compared to the biomass (0.4 g/l) grown with the same initial concentration of SCN^- but at a lower flow rate (2.2 l/day) can be explained by the adopted stepwise lactate supply procedure. At a flow rate of 2.2 l/day and with lactate added in two portions, the shortage of carbon experienced by bacteria was stronger than when lactate was added in four portions and the flow rate was 3.7 l/day. It is evident that both increasing the concentration of SCN^- at a flow rate of 3.7 l/day and raising the flow rate at a given thiocyanate concentration would require the chain of reactors to be extended. At an initial concentration of SCN^- of 290 mg/l and a flow rate of 3.7 l/day, thiocyanate was completely degraded already in rector no. 3, containing a relatively small bacterial biomass (0.12 g/l). It is clear, therefore, that the degree of SCN^- degradation in a flow-through chain of reactors depended on the thiocyanate concentration, the flow rate, and the supply of the carbon source to bacteria.

It is seen from Fig. 4 that the accumulated biomass of the bacterial community and the utilization efficiency of SCN^- nitrogen decreased with the increase of the medium flow rate. Further studies of the process of accumulation of biomass, exometabolites, and mineral nitrogen forms in the medium will make it possible to determine the efficiency of utilization of organic compounds as the carbon sources and of SCN^- as the nitrogen source under various conditions of continuous cultivation.

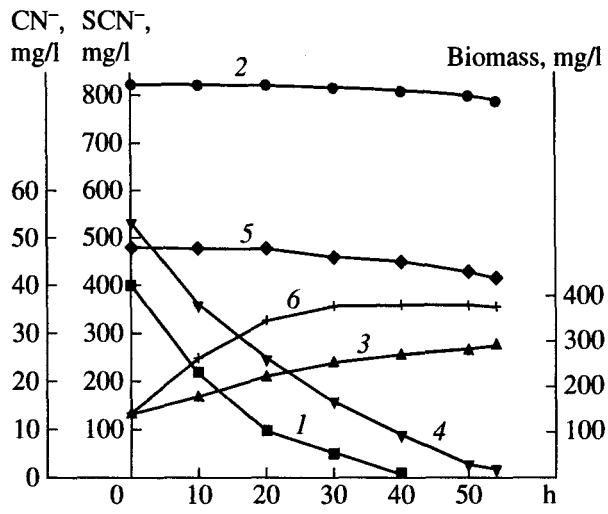

Fig. 2. Dynamics of CN^- and SCN^- degradation by a bacterial community (mg/l): (*1*, *4*) CN^-; (*2*, *5*) SCN^-; (*3*, *6*) biomass.

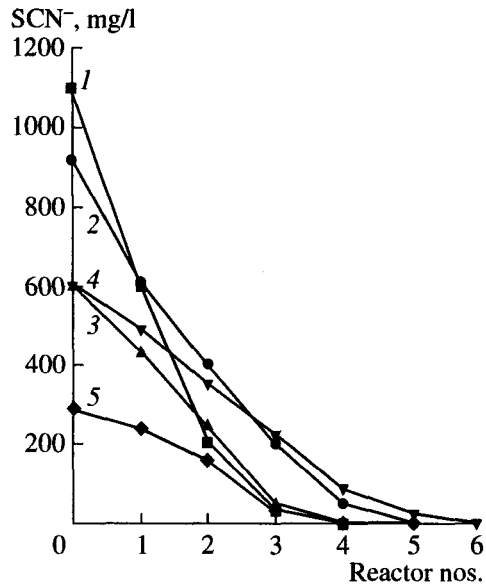

Fig. 3. Degradation of SCN^- in a chain of reactors in a period of 24 h for different initial concentrations of SCN^- and flow rates of the medium of (*1*) 1.4, (*2*) 1.8, (*3*) 2.2, and (*4*, *5*) 3.7 l/day.

Metabolic pathways of CN^- degradation in many microorganisms were considered in several publications [1, 3, 15–18]. Different microorganisms have their specific enzymes and cyanide degradation pathways. The metabolic pathways of CN^- degradation most pertinent to the case in hand are likely to be those established in the close bacterial species *Pseudomonas fluorescens* NCIMB 11764 and *Pseudomonas stutzeri* AK61 [15–17]. In *P. fluorescens*, one of the pathways

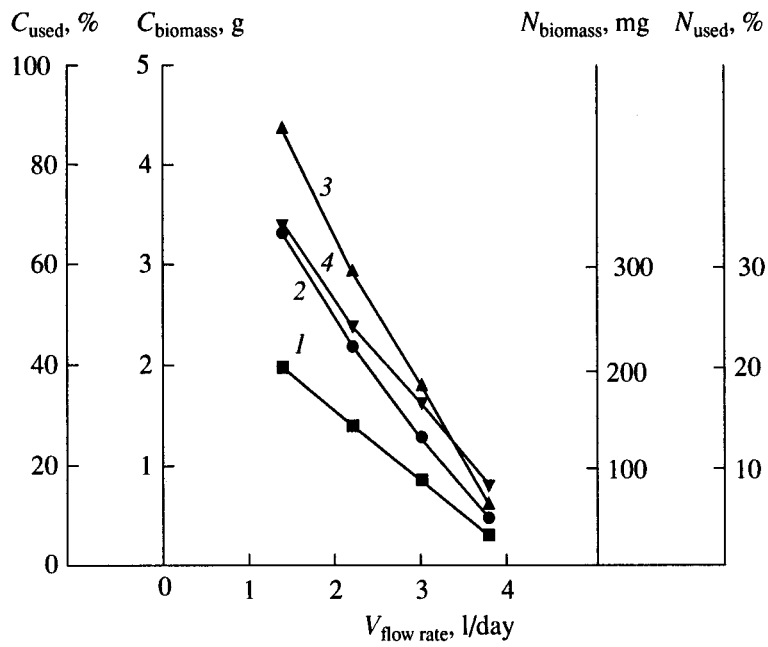

Fig. 4. Efficiency of potassium lactate utilization as the carbon source and of SCN^- as the nitrogen source as a function of medium flow rates: (*1*) biomass carbon; (*2*) biomass nitrogen; (*3*) the percent of lactate carbon utilized in biomass production; (*4*) the percent of SCN^- nitrogen utilized in biomass production.

involves an oxygen-dependent conversion of CN^- to CO_2 and NH_3:

$$HCN + O_2 + 2H^+ + NADPH \longrightarrow CO_2 + NH_4^+ + NADP^+. \quad (8)$$

The two other pathways effective in the presence and absence of oxygen have their own reactions producing formamide of formate and NH_3. The utilization of CN^- as a source of nitrogen is believed to proceed through NH_3.

P. stutzeri also degraded CN^- with the formation of NH_3. The isolated cyanide-degrading enzyme turned out to be a protein composed of polypeptide subunits of 38 kDa in size. Similarly to cyanidase (cyanide dehydratase), catalyzing the hydrolysis of CN^- to NH_3 and formate in *Alcaligenes xylosoxidans* subsp. *denitrificans* D1-3 [18] and *Bacillus pumilis* C1 [19], the enzyme of *P. stutzeri* could also catalyze the hydrolysis of CN^- to NH_3 and formate [17].

The degradation of SCN^- by *Thiobacillus thiocyanoxidans* (reclassified as *Thiobacillus thioparus* [20]) was shown to start with enzymatic hydrolysis leading to the formation of HS^-, NH_3, and CO_2 [21]:

$$SCN^- + H_2O \longrightarrow HCNO + HS^-, \quad (9)$$

$$HCNO + H_2O \longrightarrow CO_2 + NH_3. \quad (10)$$

The HS^- ion was oxidized to SO_4^{2-} with no intermediate products formed. Other pathways of SCN^- degradation are also possible. As shown by Wood *et al.* [22], *Methylobacterium thiocyanatum* degrades SCN^- to $S_2O_3^{2-}$ as the major final product.

The analysis of the mineral forms of nitrogen and sulfur occurring in our experiments showed that NH_3 was formed in the degradation of CN^-, and NH_3, SO_4^{2-}, and HS^- were formed in the degradation of SCN^-. It can be concluded, therefore, that the degradation of CN^- and SCN^- by the given community of pseudomonads proceeded according to the above well-known reactions (8)–(10).

It follows from our study that the bacterial degradation of CN^- is possible only under conditions that prevent its chemical decomposition and volatilization as HCN. In particular, medium aeration with air, a decreasing pH, and the use of reducing sugars as the carbon source are to be definitely avoided. The part played by bacteria in this process cannot be evaluated unless control tests are run to determine the extent of the chemical degradation of CN^- in each particular case. Bacterial degradation of SCN^- has a considerable potential. Under continuous cultivation, the bacterial community was able to degrade more than 1 g/l of SCN^- over 24 h. By optimizing the process parameters, the rate of bacterial degradation of SCN^- can be obviously increased. Therefore, a feasible approach to tackling the problem of the high content of cyanides in liquid wastes would be to convert them to SCN^- and then to degrade the latter compound with the aid of a bacterial community. This technology, however, is still under development. As was shown by our study, when CN^-

and SCN^- are both present in a chain of reactors, their degradation proceeds in two steps. The first step is the bacterial degradation of residual cyanide, which should proceed under strictly determined and controlled conditions. The second step is the very active bacterial degradation of SCN^-, which requires a notable adjustment of mass-exchange conditions and other medium parameters.

ACKNOWLEDGMENTS

This study was supported by the State Research and Development Program "Novel Bioengineering Methods" and by AO Turn.

REFERENCES

1. Knowles, C.J. and Bunch, A.W., Microbial Cyanide Metabolism, *Adv. Microb. Physiol.*, 1986, vol. 27, pp. 73–111.
2. Smith, A. and Mudder, T., *Chemistry and Treatment of Cyanidation Wastes*, London: Mining J. Books, 1991.
3. Dubey, S.K. and Holmes, D.S., Biological Cyanide Destruction by Microorganisms, *Wld. J. Microbiol. Biotechnol.*, 1995, vol. 11, pp. 257–265.
4. Wong-Chong, G.M., Biological Degradation of Cyanide in Complex Industrial Wastewaters, *Biohydrometallurgy-89*, Salley, J. *et al.*, Eds., CANMET8p89-10, 1989, pp. 289–300.
5. Gomez, N.C.M., Camardos, E.R.S., Dras, J.C.T., Figureira, M.M., and Linardi, V.R., Preliminary Studies on Metal and Cyanide Removal by *Aspergillus niger, Biohydrometallurgical Processing*, Jerez, C.A. *et al.*, Eds., Chile, 1995, vol. 2, pp. 401–405.
6. Ul'berg, Z.R., Podol'skaya, V.I., Sanakulov, K.S., Vember, V.E., Grishchenko, N.I., and Garbara, S.V., Cyanide Degradation by a Culture of *Pseudomonas fluorescens* VKM B-5040, *Prikl. Biokhim. Mikrobiol.*, 1994, vol. 30, no. 2, pp. 260–264.
7. Podol'skaya, V.I., Ul'berg, Z.R., Shpak, V.E., Grishchenko, N.I., and Yakubenko, L.N., Decomposition of a Silver Cyanide Complex by a Culture of *Pseudomonas fluorescens* VKM B-5040, *Prikl. Biokhim. Mikrobiol.*, 1994, vol. 30, no. 1, pp. 73–77.
8. Babu, G.R.V., Wolfram, J.H., and Chapatuala, K.D., Degradation of Inorganic Cyanides by Immobilized *Pseudomonas putida* Cells, *Biohydrometallurgical Technologies*, Torma, A.E. *et al.*, Eds., Jackson Hole, 1993, vol. II, pp. 159–165.
9. Whitlock, J.L., Biological Detoxification of Precious Metal Processing Wastewaters, *Geomicrobiol. J.*, 1990, vol. 8, no. 3/4, pp. 241–249.
10. Grigor'eva, N.V., Avakyan, Z.A., Turova, T.P., Kondrat'eva, T.F., and Karavaiko, G.I., The Search for and Study of Microorganisms That Degrade Cyanides and Thiocyanates, *Mikrobiologiya*, 1999, vol. 68, no. 4, pp. 453–460.
11. Zelenov, V.I., *Metodika issledovaniya rud* (Methods for Investigation of Ores), Moscow: Nauka, 1989, pp. 124–126.
12. Gamil'ton, E.M., *Rukovodstvo po tsianirovaniyu zolotykh i serebryanykh rud* (A Manual for Cyanidation of Gold and Silver Ores), Moscow: Tsvetmetizdat, 1932, pp. 16–30.
13. Andrede, M.C., Figueira, M.M., and Linardi, V.R., Short Communication: Utilization of Ammonia, Generated from Abiotic Cyanide Degradation, by *Rhodotorula rubra, Wld. J. Microbiol. Biotechnol.*, 1995, vol. 11, pp. 343–344.
14. Hope, K.M. and Knowles, C.J., The Anaerobic Utilization of Cyanide in the Presence of Sugars by Microbial Cultures Can Involve an Abiotic Process, *FEMS Microbiol. Lett.*, 1991, vol. 88, pp. 217–220.
15. Harris, R. and Knowles, C.J., The Conversion of Cyanide to Ammonia by Extracts of a Strain of *Pseudomonas fluorescens* That Utilises Cyanide as a Source of Nitrogen, *FEMS Microbiol. Lett.*, 1983, vol. 20, pp. 337–341.
16. Kunz, D.A., Nagappan, O., Avalos, J.S., and Delong, G.T., Utilization of Cyanide as a Nitrogenous Substrate by *Pseudomonas fluorescens* NCIMB 11764: Evidence for Multiple Pathway of Metabolic Conversion, *Appl. Environ. Microbiol.*, 1992, vol. 58, pp. 2022–2029.
17. Watanabe, A., Yano, K., Ikebuturo, K., and Karube, I., Cyanide Hydrolysis in a Cyanide-degrading Bacterium, *Pseudomonas stutzeri* AK61, by Cyanidase, *Microbiology* (Reading, UK), 1998, vol. 144, pp. 1677–1682.
18. Ingvorsen, K., Hojer-Pedersen, B., and Godtfredsen, S.E., Novel Cyanide-hydrolyzing Enzyme from *Alcaligenes xylosoxidans* subsp. *denitrificans, Appl. Environ. Microbiol.*, 1991, vol. 57, pp. 1783–1789.
19. Meyers, P.R., Rawlings, D.E., Woods, D.R., and Lindsey, G.G., Isolation and Characterization of a Cyanide Dehydratase from *Bacillus pumilis* C1, *J. Bacteriol.*, 1993, vol. 175, pp. 6105–6112.
20. *Bergey's Manual of Determinative Bacteriology*, 8th ed., Buchanan, R.E. and Gibbons, N.E, Eds., 1974, p. 458.
21. Youatt, I.B., Studies on the Metabolism of *Thiobacillus thiocyanoxidans, J. Gen. Microbiol.*, 1954, vol. 11, pp. 139–149.
22. Wood, A.P., Kelly, D.P., McDonald, I.R., Jordan, S.I., *Arch. Microbial.* Morgan, T.D., Khan, S., Murrell, J.C., and Borodina, E., A Novel Pink-pigmented Facultative Methylotroph, *Methylobacterium thiocyanatum* sp. nov., Capable of Growth on Thiocyanate or Cyanate as Sole Nitrogen Sources, SCN^- 1998, vol. 169, no. 2, pp. 148–158.