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Anaerobic Nitrogen Transformations in a Gold-Cyanide Leach Residue

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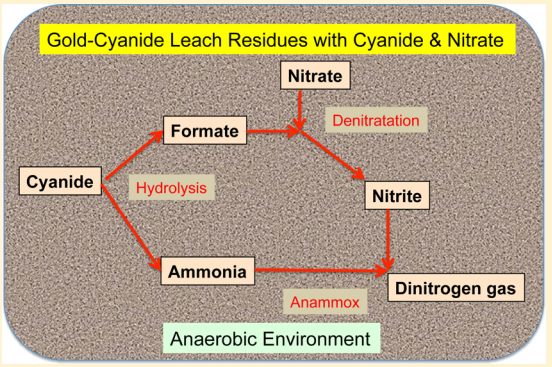
ABSTRACT: Laboratory incubations of sediment collected from gold cyanidation heap leach residues revealed cyanide and nitrogen transformations that have not been previously documented in the absence of added organic carbon and under anaerobic conditions. Heap leach residues were incubated anaerobically with process water spiked with free cyanide and minimal nutrients at 4 and 22 °C in the dark. Three important nitrogen transformations were observed in the microcosms. The native bacteria converted cyanide to formate and ammonia and subsequently consumed the formate. Nitrate reduction was consistent with denitratation (reduction of nitrate to nitrite) coupled with formate as the electron donor. The changes in ammonia and nitrite concentrations and the loss of total aqueous nitrogen were consistent with the anammox pathway. Harnessing these anaerobic nitrogen transformations has the potential to significantly reduce the treatment cost for the detoxification of cyanide-containing water, residue, and sediments because no added organic carbon is required.



INTRODUCTION

Cyanide is an acutely toxic compound used in a wide range of industrial processes[1] and has been reported at >28% of National Priority List sites. Cyanide may be released to the environment from manufacturing of cyanide compounds, organic chemical production, plastics manufacturing, iron and steel plants, electroplating operations, and the application of cyanide-containing pesticides or road salts. In 2003 alone, the release of 2.7 and 6.3 million pounds of hydrogen cyanide and cyanide compounds, respectively, was reported to the Toxic Release Inventory database.[2] A substantial fraction of the annual cyanide production is used to extract metals, primarily gold, from the parent ore.[3,4] Cyanide, ammonia, and nitrate are often found in spent ore heap leach pads and mill leached tailings storage facilities. The removal of cyanide as well as ammonia and nitrate is sometimes required prior to any discharge of the water off-site. Cyanide removal is typically accomplished by expensive chemical methods.[5] However, biodegradation is a potentially low-cost and environmentally friendly way to concurrently reduce all three nitrogen species to acceptable concentrations. Cyanide-containing wastewaters from other industrial applications may also contain a mixture of nitrogen species and thus have similar treatment issues.

An in situ microbial treatment process that could (1) degrade cyanide, (2) reduce total nitrogen, ammonia, nitrite, and nitrate levels to acceptable levels, and (3) accomplish the first two goals without adding organic carbon reagents would reduce treatment costs by avoiding pumping, infrastructure, and carbon reagent costs. A variety of organisms have been shown to degrade cyanide, often utilizing cyanide as a sole nitrogen source;[6−8] however, the vast majority of studies have been conducted with added organic carbon.[9−11] Only Babu et al.[12] have convincingly demonstrated growth with cyanide as the only carbon source under aerobic conditions. Mudder and Whitlock[13] demonstrated aerobic growth on a mixture of cyanide and thiocyanate. However, no total nitrogen reduction can be achieved under aerobic conditions.

Under anaerobic conditions, the degradation of cyanide may proceed via hydrolytic or reductive reactions.[11] The only known reductive reaction is catalyzed by nitrogenase,[14,15] an enzyme used by nitrogen-fixing bacteria in the metabolically expensive reduction of nitrogen gas. Nitrogenase has also been found to act on the triply bonded and isoelectronically and isostructually similar cyanide, resulting in the production of either methenamine, methylamine, formaldehyde and ammonia, or methane and ammonia.[15] Hardy and Knight[14] found this reaction occurred only when the nitrogen-fixing bacteria that were investigated were raised in a nitrogen gas-containing environment that lacked urea or ammonia. It is unlikely that bacteria in a nitrate and/or ammonia rich environment such as heap leaches would expend the energy associated with nitrogen fixation. Thus, the degradation of cyanide under anaerobic conditions is most likely hydrolytic. Bacterial hydrolysis of

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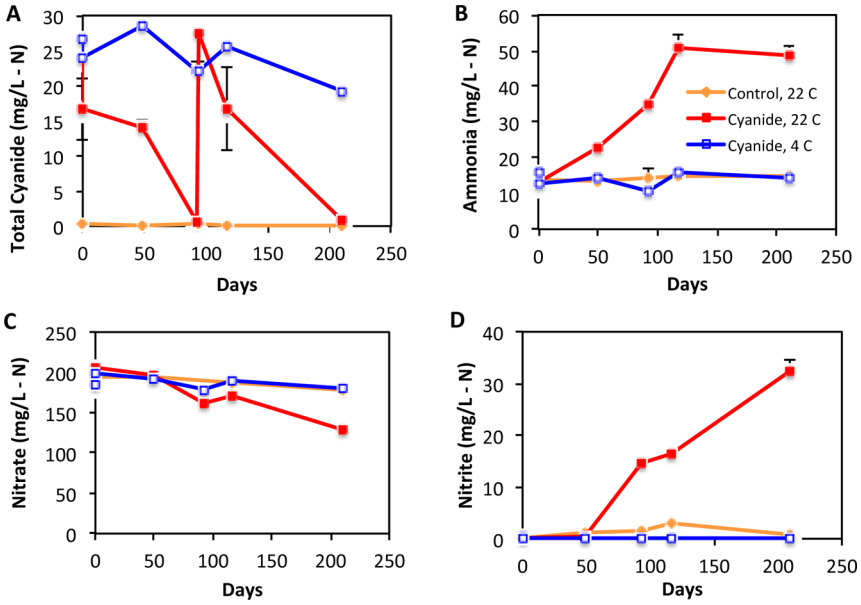

Figure 1. Average (A) cyanide, (B) ammonia, (C) nitrate, and (D) nitrite concentrations in anaerobic microcosms incubated at room temperature and 4 °C. No cyanide was added to the 22 °C control microcosms that contained leach water and residue. The initial cyanide concentration was calculated (first data point) to be 27 mg/L, and the first measured data point was approximately 1.5 h after assembly. Error bars represent the standard deviation between replicate microcosms (if the error bars are smaller than the data symbol, they are not visible). The 22 °C microcosms were redosed with cyanide (27 mg/L as N) on day 94. The concentration immediately after redosing was calculated from the amounts remaining before addition and the amount added and was not measured.

cyanide has been associated with the cyanidase enzyme.[16] In anaerobic systems, hydrolytic degradation of cyanide has been catalyzed by bacteria previously or concurrently grown with organic carbon.[16] We could find no studies to support anaerobic degradation of cyanide without the use of organic carbon to promote growth of bacteria capable of hydrolysis.

Currently employed engineered biodegradation strategies for cyanide attenuation for mining-related applications generally involve ex situ facilities operating aerobically, resulting in the oxidation of cyanide to nitrate and carbon dioxide;[17] again, there is no net removal of nitrogen. An additional denitrification step is sometimes incorporated, but this is relatively expensive because an organic carbon such as methanol must be added.

Hydrolysis of cyanide to formate and ammonia by the enzyme cyanidase[11] is energetically favorable (eq 1). Thus, the potential exists for nitrate to be removed following cyanide hydrolysis by heterotrophic denitrification. The degradation of formate is energetically favorable using nitrate as an electron acceptor (eq 2).

Free cyanide hydrolysis:

$$HCN + 2H_2O \rightarrow HCOO^- + NH_4^+$$

$$\Delta G_r^\circ = -81\ \text{kJ/mol of HCN} \quad (1)$$

Denitrification:

$$0.5HCOO^- + 0.2NO_3^- + 0.2H^+ \rightarrow 0.5HCO_3^- + 0.1N_2 + 0.1H_2O$$

$$\Delta G_r^\circ = -111\ \text{kJ/e}^- \quad (2)$$

Nitrogen transformation mechanisms were explored in microcosms containing a leaching solution and residue collected from a gold cyanide heap leach operation. The cyanide in these experiments was added as free cyanide (HCN or CN^-). The cyanide speciation was evaluated by the measurement of total, free, and weak acid dissociable (WAD) cyanide, cyanate, and thiocyanate. The results are presented as total cyanide, which includes free, WAD (e.g., complexed with Zn and Cu), and strongly complexed (e.g., complexed with Fe and Co) cyanide.

METHODS

Sample Collection and Storage. Heap leach residue (sediment) and heap leach (process) water were collected from an active heap leach operation. Two residue samples were collected from a zone about 2−4 in. deep in an area that had standing water and transferred to zip-lock bags. The water samples came from pregnant solution ponds at the same site and were stored in polyethylene bottles. Samples were packed in a cooler with ice packs, shipped overnight, and then stored in a refrigerator until use.

Microcosm Assembly. The pregnant heap leach water samples were combined and diluted [4 parts deionized (DI) water + 1 part leach water] with DI water containing the same approximate sulfate concentration. ICP and IC analysis of the resulting water showed NO_3^- (186 mg as N/L) and SO_4^{2-} were 98% of the anions and cyanide-complexing metal concentrations were all <1 mg/L. The total cyanide concentration in the diluted leach water was 0.16 mg/L and the ammonia was 1.2 mg as N/L. The residue samples were combined and homogenized. X-ray powder diffraction (XRD) analysis of the residue indicated 59% quartz, 14% illite/sericite, 23% calcite, 3% dolomite, 0.9% goethite, and 0.5% pyrite by weight. To assemble the microcosms, 100 g of sieved (<2 cm) moist (11.5%) residue and 800 mL of diluted heap leach water were added to 1 L glass media bottles. NH_4Cl was added at a concentration of 16 mg/L as N. A vitamin mixture was added (0.5 mL of Sigma-Aldrich RPMI 1640-100X, in each 1 L bottle). Controls were set up using 0.5 L bottles, half the

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quantity of residue, and water, with the same concentration of ammonia and vitamins.

The bottles were sealed with butyl rubber stoppers and aluminum crimps, purged with argon for 1.5 h, and wrapped in aluminum foil to prevent cyanide photolysis. Cyanide was added in concentrated form (prepared from anhydrous NaCN) to achieve a final concentration of 27 mg/L as N using a syringe needle, and the pH was adjusted to 7.5−8.0 at this time (the initial pH was between 8.5 and 9.5). However, the pH rebounded to ∼8.7, and no more pH adjustments were performed. The bottles were shaken vigorously (sediment was suspended and well-mixed) three times a week for the first week, twice a week during the second week, and once a week thereafter. The cyanide-containing microcosms were incubated anaerobically at 22 °C (room temperature) and 4 °C, and a no cyanide control was incubated at 22 °C; all conditions were run in duplicate. A second dose of 27 mg/L as N cyanide was added to the 22 °C cyanide microcosms on day 94.

No autoclaved controls were included because autoclaving was ineffective at inactivating bacteria in similar previous experiments. In the previous experiments, triple-autoclaved controls were included, but the presence of motile bacteria and chemical changes indicated active microbial metabolism.

Sampling and Analysis. Microcosms were shaken and settled for 1 h. A 60 mL plastic syringe was purged and filled with 60 mL of argon. A 6 in. syringe needle was used to first inject the argon and then withdraw 55 mL of the settled liquid sample. The samples were filtered (0.45 μm), and the pH was measured. After 2 mL had been subsampled for high-performance liquid chromatography (HPLC), the pH was adjusted to ≥11 for cyanide preservation. The samples were then sealed and shipped overnight on ice to Newmont, where free, WAD, and total cyanide, cyanate, thiocyanate, nitrite, nitrate, and ammonia were measured.[18−22] Nitrite, nitrate, and ammonia were also measured using HACH TNT kits for select samples. The pH 11 sample preservation was demonstrated to have a negligible effect on ammonia measurement during previous experiments. Ammonia was measured at CSM with HACH TNT tests (product number 2606945) before pH adjustment, and pH-adjusted samples were shipped to Newmont; 10 samples on two separate occasions were evaluated. Newmont ammonia results were, on average, 97% of the value obtained with the HACH measurement.

RESULTS AND DISCUSSION

The pH in the 4 and 22 °C microcosms rebounded to 8.7 ± 0.2 and was stable over the 210 days. Formic acid was detected by HPLC as a degradation product of cyanide in near-stoichiometric amounts and was subsequently consumed (data not shown). The temporal changes of total cyanide, ammonia, nitrate, and nitrite concentrations are presented in Figure 1. Cyanide was removed (>98% of total added) from the anaerobic microcosms incubated at 22 °C (Figure 1A). Ammonia and nitrite production and nitrate removal were also observed in these microcosms. In the no cyanide control, the nitrate concentration decreased by 8 ± 4 mg/L as N and limited nitrite production was observed. The electron donor for the nitrate reduction in the no cyanide control may be organic matter from the residue or leach water or ferrous iron[23,24] from the 0.5% pyrite in the residue. The majority of the cyanide was present as free cyanide (80−100%), and at the end of the experiment, total, free, and WAD (weak acid dissociable) forms of cyanide were within 0.5 mg/L of each other. Low concentrations of cyanate (<1 mg/L) were detected in the 22 °C microcosms early in the experiment, but the values decrease to below detection limits by the end. Approximately 1 mg/L cyanate persisted in the 4 °C microcosms. Similar results were obtained for thiocyanate; levels were below detection limits in all microcosms by the end of the experiment. Light microscope examination of liquid phase samples collected on day 99 revealed a much higher ratio of motile to nonmotile bacteria in the 22 °C microcosms amended with cyanide relative to the no cyanide controls. The higher proportion of motile bacteria at 22 °C with cyanide and the limited activity at 4 °C support the biological nature of the observed transformations. We believe this is the first reported instance of anaerobic cyanide degradation in the absence of added organic carbon.

Nitrogen Transformations. The data in Figure 1 suggest that a series of nitrogen transformations more complex than the originally postulated cyanide hydrolysis and denitrification are occurring. In the 22 °C microcosms amended with cyanide, ammonia initially appeared concurrently with cyanide degradation in roughly stoichiometric amounts, but this was not true during the final phase of the experiment. By day 210, the average cyanide removed was 53 mg/L as N but ammonia increased by only 33 mg/L as N. This leaves 20 mg/L as N of ammonia unaccounted for. The formate produced from cyanide hydrolysis was sufficient to reduce a maximum of 21 mg/L as N nitrate to N_2 via denitrification (eq 2). However, the nitrate concentration in the 22 °C microcosms dosed with cyanide decreased by 50 mg/L as N beyond that observed in the no cyanide controls, and the nitrite production was not anticipated.

Additional reactions were postulated for denitratation and anammox as shown in eqs 3 and 4, to account for the observed changes in nitrite, ammonia, and nitrate. The stoichiometric equations presented neglect cell growth and therefore represent the maximal change in nitrogen species for postulated reactions.

Denitratation:

$$0.5HCOO^- + 0.5NO_3^- \rightarrow 0.5HCO_3^- + 0.5NO_2^-$$

$$\Delta G_r^\circ = -81\ \text{kJ/e}^- \quad (3)$$

Anammox:

$$0.33NH_4^+ + 0.33NO_2^- \rightarrow 0.33N_2 + 0.66H_2O$$

$$\Delta G_r^\circ = -119\ \text{kJ/e}^- \quad (4)$$

Three scenarios were examined assuming complete hydrolysis of the added cyanide and different combinations of the nitrogen transformation (eqs 2−4): denitrification only, denitratation only, or a combination of denitratation and anammox. The measured and projected changes in nitrogen species based on combinations of postulated reactions are listed in Table 1.

The observed cyanide disappearance would be sufficient to reduce 21 mg/L as N of nitrate to nitrogen gas (eqs 1 and 2). However, if the formate was used for denitratation, 53 mg/L as N of nitrate could be reduced to nitrite, which is within the range of the measured decrease in nitrate (50 ± 5 mg/L as N), yet only 32 mg/L as N of net nitrite production was observed. If the unaccounted for nitrite (21 mg/L as N) was consumed by anammox, 21 mg/L as N ammonia would have been consumed, resulting in the conversion of 42 mg/L nitrogen to nitrogen gas. This is within the range of the observed decrease in total nitrogen species (−38 ± 8 mg/L as N). Anammox

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Table 1. Measured and Projected Changes in Nitrogen Species (milligrams per liter as N) in the 22 °C Microcosms Spiked with Cyanide Using the Specified Combinations of eqs 1−4[a]

	CN^-	NH_4^+	NO_2^-	NO_3^-	TN
measured	−53	+33	+32	−50	−38
projected					
eqs 1 and 2	−53	+53	0	−21	−21
eqs 1 and 3	−53	+53	+53	−53	0
eqs 1, 3, and 4	−53	0	0	−53	−106
eqs 1, 3, and 4[b]	−53	+32	+32	−53	−42

[a]The change in nitrate concentration was reduced by the amount of nitrate reduction observed in the no cyanide controls. [b]Incomplete anammox based on the observed partial consumption of nitrite as described in the following paragraph.

organisms reproduce slowly even under optimal conditions, with reported ingrowth rates of 14−52 weeks.[25−28] Slow ingrowth of anammox organisms could explain the residual nitrite and ammonia in the microcosms.

Broader Implications. Our data demonstrate that anaerobic biological degradation of cyanide is occurring in the absence of added organic carbon, the first documented occurrence. In addition, our data suggest that the formate resulting from cyanide hydrolysis is driving denitratation and indirectly supporting anammox. This is also the first report of cyanide ultimately acting as the electron donor for the reduction of nitrate. These findings suggest that the coupled processes of anaerobic cyanide biodegradation, denitratation, and anammox have the potential to concurrently reduce cyanide and total nitrogen in a very cost-effective manner. Further study is needed to determine if strongly complexed cyanide species (e.g., ferrocyanide or ferricyanide) are amenable to degradation under these conditions. It would also be desirable to determine if the degradation processes would occur in the absence of added vitamins and nutrients (ammonia and phosphorus). In addition, DNA sequencing would be the next step to identifying the organisms responsible for the transformations. The original funding for this research did not include sequencing.

New management strategies that depend on the relative amount of cyanide, ammonia, and nitrate in a waste stream are envisioned. Nearly equimolar amounts of cyanide and nitrate may allow for complete nitrogen removal under anaerobic conditions. If nitrate is in excess of the cyanide, carbon addition would be required to drive denitrification after the denitratration/anammox phase. If cyanide only or cyanide and ammonia are present, then a reaction sequence of addition of a low concentration of dissolved oxygen followed by anammox could be implemented. Manipulation of dissolved oxygen and pH has been used to promote concurrent nitrification and anammox.[29,30] Nitrogen removal processes in domestic wastewater applications have implemented oxygen control and limited carbon addition to promote nitritation (partial oxidation of ammonia to nitrite), denitratation, and anammox.[31] A similar strategy could be developed for waste streams containing cyanide, ammonia, and nitrate.

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Notes

The authors declare no competing financial interest.

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