



**Environmental Chemistry and
Biological Degradation of Metallocyanide
Complexes in Gold Mines**

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**‘Whatever befalls the earth befalls the sons of the earth. Man
did not weave the web of life; he is merely a strand in it.
Whatever he does to the web, he does to himself.’**

**(Quote from a speech attributed to Chief Seattle, Chief of the Suquamish,
in 1854)**

SUMMARY

Gold from ancient times to the present, has been prized. The inert quality, bright yellow colour, lustre and scarcity of gold has ensured its lasting value. Few chemicals react with this metal, but with sodium cyanide, auro complexes are formed.

Mining gold results in some toxic cyanide wastes which are usually stored in impoundment areas. This can be problematic since huge volumes of tailings need to be held in storage for many years and monitored to eliminate spillage and leakage. Environmental pollution by toxic compounds has occurred since the development of many modern industrial processes. One such process being the inclusion of sodium cyanide as a lixiviant for gold in the mining industry.

A possible alternative to long-term tailings storage is biodegradation of the cyanide compounds and this has often been a subject for research.

Investigative research into the possible utilization of the cyanide (CN^-) as a food source for microorganism has been carried out for many years, usually concentrating on *Pseudomonas* species, with a few studies favouring the *Bacillus* group. Also investigations have centred around the simpler cyanides e.g. hydrogen cyanide and sodium or potassium cyanides.

The subject of this thesis is the comparison of three systems for the degradation of three heavy metal cyanide compounds, copper(I) cyanide, sodium tetracyanonickelate and potassium hexacyanoferrate(III). It was concluded that the three heavy metal cyanides could be degraded by microorganisms, with

varying degrees of efficiency in the following three systems; in shake flask cultures, bioreactors and using individual bacterial species in test tube cultures. The advantages of biodegradation include self generation and perpetuation, simple designs for equipment and the use of innocuous chemicals.

Copper(I) cyanide degradation research

Copper(I) cyanide is generally regarded as a simple cyanide compound with a relative low solubility in water. However, over a period of 7 days, the solubility was found to increase from 2.26 to 41.98 mg l⁻¹, measured by using Reverse Phase Ion-interaction High Performance Liquid Chromatography. The cyanide ligands can be removed from the copper(I) cyanide complex by treatment with weak acids. Thermodynamic and kinetic reasons cause the cyanide ligands to be labile in this complex. As the cyanide anion concentration increased in solution, the bacteria were able to use the carbon and nitrogen for metabolic processes thereby, removing the cyanide from the system.

In the shake flask cultures, the consortium of bacteria utilized the cyanide more efficiently when provided with additional organic material e.g. peptone. A build-up of the by-product cyanate was measured at pH 8 but no such accumulation was found in the larger bioreactor system, where the cyanate was in turn hydrolyzed to ammonia and carbon dioxide. Moreover, an acceleration of the degradation process after a pretreatment with peptone was confirmed in the bioreactor experiments.

The main bacterial species identified both in the shake flask cultures and the bioreactors were *Pseudomonas stutzeri* and *Bacillus pumilus*. Although, when two

strains of *P. stutzeri* and three strains of *B. pumilus* were inoculated as pure cultures into the copper(I) cyanide mineral salts medium, either very low or no degradation was noted. The best species for degradation was found to be *Bacillus sphaericus* followed by *Sphingomonas paucimobilis*.

The *B. sphaericus* and *S. paucimobilis* strains appeared to be neither efficient cyanate producers nor degraders, which inferred that the enzymes cyanide monooxygenase and cyanase were inactive during the pure culture tests. This was contrary to what was seen with the bacterial consortium active in the bioreactors. Therefore, the biomass in the bioreactors contained some bacterial species that degraded the cyanide to cyanate which was further hydrolysed to ammonium-nitrogen and carbon dioxide by other strains. Some bacteria were able to convert the cyanide directly to ammonium-nitrogen through the action of the two enzymes cyanide dioxygenase and cyanidase.

The consortium of bacteria active in the biomass of the shake flask cultures and bioreactors was more effective than when using the pure cultures.

Sodium tetracyanonickelate degradation research

Sodium tetracyanonickelate is a moderately stable complex which is soluble in water. Utilization of the cyanide anion occurred at pH 8 and pH 10 in the shake flasks when an organic supplement, peptone, was added. Furthermore, degradation also proceeded in the absence of peptone but only at pH 8. The independence of the degradation process in regards to peptone was more clearly apparent in the bioreactors.

The by-product cyanate accumulated at pH 8 and reached a higher level when

peptone was added. This was confirmed in the bioreactor experiments where cyanate was detected for a longer period in the bioreactor which had the peptone pretreatment.

A faster conversion of cyanate to ammonium-nitrogen occurred in the bioreactor where no pretreatment with peptone was carried out. This indicated that the sodium tetracyanonickelate was degraded by the enzyme cyanide monooxygenase, followed by the action of cyanase.

Bacterial isolations were only successful from the peptone treatments with *Pseudomonas stutzeri*, *Bacillus firmus*, *B. sphaericus*, *B. filicolonicus* and *Sphingomonas paucimobilis* being identified from the shake flask cultures. Some of these species were also isolated from the bioreactor experiments, in addition to *B. cereus*, and *B. pumilus*.

In the pure cultures trials, two unnamed strains nos. 101 and 94 performed the best, followed by *S. paucimobilis*, *Bacillus globisporus* and the unnamed strain no.157. Cyanate was not detected in 4 of the 5 cultures with only *B. globisporus* producing a small amount.

The bacterial strains, no.101 and no. 94 were able to use sodium cyanate showing that the enzyme cyanase was induced. Other bacterial species isolated during the degradation experiments in the shake flasks and bioreactors were *P. stutzeri*, *B. filicolonicus* and *B. firmus* which also utilized cyanate as a substrate to form ammonium-nitrogen.

The formation of ammonium-nitrogen by the *S. paucimobilis* culture may have been due to the enzymes cyanide dioxygenase and/or cyanidase since no cyanate was produced. Bacterial species isolated from the shake flask and bioreactor

experiments, were predominantly different to those that best degraded sodium tetracyanonickelate in the pure cultures. Only *S. paucimobilis* was regularly isolated from the biomass in both the shake flask cultures and the bioreactors and also performed well as a pure culture.

Potassium hexacyanoferrate(III) degradation research

The potassium hexacyanoferrate(III) complex is very soluble in water. This complex has been regarded as a stable metalocyanide where decomposition required extreme conditions and UV irradiation. However, in the research, potassium hexacyanoferrate was found to be very reactive.

The better conditions for degradation in the shake flask cultures were at pH 8 and in the presence of peptone, but a high level of cyanate accumulated under these conditions. In the bioreactors, however, degradation proceeded efficiently regardless of any pretreatment with peptone and with no build-up of cyanate. From the shake flask cultures many bacterial species were isolated including, *Cellulomonas cellulans*, *Bacillus cereus*, *B. filicolonicus*, *B. pumilus*, *B. sphaericus* and *Pseudomonas stutzeri*. Similar species were present in the bioreactors, including *Sphingomonas paucimobilis*, *B. firmus* and *B. thuringiensis*.

As pure cultures, 30 out of the 31 species tested were effective in the degradation of potassium hexacyanoferrate(III) with *B. sphaericus* being the best. Some species formed cyanate during the degradation process with *S. paucimobilis* producing the greatest amount. This signified that the enzyme cyanide monooxygenase was activated in these species.

In addition, high levels of ammonium-nitrogen but no cyanate was detected during the pure bacterial species tests, indicating that the degradation of potassium hexacyanoferrate was facilitated by the enzymes cyanide dioxygenase and/or cyanidase.

Ammonium-nitrogen values were low when peptone was absent in the shake flasks. This confirmed that the enzyme cyanase was present at a low level.

The degradation of potassium hexacyanoferrate was most efficient when the bacteria were used as pure cultures, followed by degradation involving the biomass in the bioreactor and shake flask systems.

Sodium cyanate utilization

When sodium cyanate was introduced as the substrate, it was best utilized by *P. stutzeri*, with only 9 out of the 31 species being able to hydrolyse the sodium cyanate. The production of ammonium-nitrogen in the 20 to 38 mg l⁻¹ range, indicating that the enzyme cyanase was present but only in a few of the bacterial species tested.

The nine bacterial strains included three *Bacillus* spp., three *Pseudomonas* spp. and three of the four unnamed strains. Furthermore, each time when bacterial isolations were carried out during the shake flask culture and bioreactor experiments, some of the sodium cyanide utilizing strains were found to be present.

This therefore explained the disappearance of cyanate that was measured during the degradation experiments of the three metal cyanides in the shake flask cultures and the bioreactors.

Declaration of Candidate

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference has been made in the text.

Rita Fedel-Moen

NAME: Rita Fedel-Moen COURSE: PH.D.

I give consent to this copy of my thesis, when deposited in the University Libraries, being available for photocopying and loan.

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