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ABSTRACT

Water samples containing elevated concentrations of cyanide and thiocyanate were investigated for the presence of cyanide degrading bacteria. Several bacterial colonial types were found that could degrade cyanide and this degradation was enhanced by presence of carbon and nitrogen co-metabolites.

INTRODUCTION

It is of great importance to dispose of cyanide and thiocyanate containing wastes from gold mining operations in such a manner as to cause the least impact to the terrestrial and aquatic environment. One of the more conventional forms of disposal is to store the wastes in tailings ponds and allow natural microbial, chemical and biochemical degradation to occur. To this end a study was initiated to establish whether or not bacteria capable of degrading cyanide or thiocyanate could be found in a Northern Canadian tailings pond at Dome Mines, South Porcupine, Ontario.

METHODS

Organism Isolation

Barren bleed holding pond samples were streaked onto the following four media:

1. membrane filter sterilized pond water + 12 g agar + 10 mg $Zn(CN)_2$ + 15 mg KCN/litre.
2. membrane filter sterilized pond water + 12 g agar + 10 mg $Zn(CN)_2$ + 15 mg KCN + 2 g glucose (co-metabolite)/litre.

3. membrane filter sterilized pond water + 12 g agar + 10 mg $Zn(CN)_2$ + 15 mg KCN + 5 g peptone/litre.
4. membrane filter sterilized pond water + 12 g agar + 10 mg $Zn(CN)_2$ + 15 mg KCN + 2 g glucose + 5 g peptone/litre.

The inocculated media were incubated up to 21 days at 20°C. After incubation, single colony picks from the most dominant species on each medium (up to 5) were transferred to and incubated on medium #5 for 3 weeks at 20°C and those organisms which grew well were then transferred to media #6 and #7 for 21 days at 20°C:

5. carbon free medium (Liu and Dutka, 1972) + 12 g agar + 15 mg $Zn(CN)_2$ + 25 mg KCN/litre.
6. carbon free medium + 12 g agar + 25 mg $Zn(CN)_2$ + 40 mg KCN/litre.
7. carbon free medium + 12 g agar + 25 mg $Zn(CN)_2$ + 40 mg KCN + 2 g glucose + 5 g peptone/litre.

The organisms surviving and growing well on media #6 and #7 (two colony types #6 and three colony types #7) were used to evaluate the degradation of CN found in the tailings pond.

Degradation Studies

In the first study, a May sample of tailings pond water was membrane filter sterilized, pH adjusted to 7.5 and split into three 1.5 L samples in wide bottom erlenmyer flasks. One sample was the control, another sample had 1.0 g glucose and 1.0 g peptone/litre added and the third sample had 5.0 g glucose + 5.0 g peptone added per litre as co-metabolites. Each flask was then inocculated with 1 mL of each culture of the five surviving organisms to give a final total inocculum of $\approx 10^6$ bacteria per mL. The flasks were loosely capped with foil, placed on a shaker at 20°C and incubated for six weeks, with subsamples being collected 2 hr after bacterial inocculation, and after 2, 4 and 6 weeks. The subsamples were tested for cyanide and thiocyanate and bacterial growth was confirmed.

In the second study, procedures similar to the above were followed with the following exceptions:

a) tailings pond water samples were collected in August,
b) a sterile control sample containing no co-metabolites was added to the study. This control was a duplicate of the normal control, except that no bacteria were added to the flask, and

c) the flasks to which the co-metabolites, glucose and peptone were added as carbon and nitrogen sources were duplicated at each concentration level.

Results and Discussion

The results of the biodegradation processes with the May and August tailings pond samples are presented in Table 1. The May samples originally contained 52.5 mg/L CN and 41 mg/L thiocyanate (CNS). Over the six week period it can be seen that the CN concentration was decreased in all three samples, with the greatest reduction 99.1%, occurring in the sample containing 1.0 g of each co-metabolite (Table 2). The least CN reduction, 69.5%, was in the control flask to which no co-metabolites were added. All three flasks indicated that CNS levels increased during the six week incubation process, with the control flask having the lowest increase in CNS levels (7.6%) and the flask with 1.0 g additions of co-metabolite had the greatest increase (19%) in CNS levels.

In the sterile control flask of the August study (Table 1) there appears to be a non-biological decrease in CN (41%) and an insignificant decrease in CNS level after six weeks incubation at 20°C on a mechanical shaker. The control flask (bacteria added) was also found to have a decrease in CN concentration, 57.6% versus 69.5% in the May study and a slight increase in CNS level, 3.3% versus a

7.6% increase in May. In the other August study flasks containing co-metabolites, the basic pattern observed at the end of the six week May study, CN decrease with CNS increase, was found again, with minor variations. Maximum CN reduction (92.4%) appears to have occurred in the flasks containing 1.0 g/L of each co-metabolite, and it is doubtful that these reductions are significantly different from those observed in the flasks containing 5.0 g/L of each co-metabolite (90.6%). However, the difference in CNS increase between the flasks containing 1.0 g/L co-metabolite (26.8%) and 5.0 g/L co-metabolites (40.6%) can be accepted as real. These findings are the reverse of the May study and suggest that there likely is an ideal level of co-metabolite concentration which may lie somewhere between the 1 to 5 g/L level, and that the ideal relationship may not necessarily be on a 1 gram peptone (nitrogen) to 1 gram glucose (carbon) basis. These results do support the concept that co-metabolites aid in the biodegradation of CN and confirm the earlier literature referenced in Howe 1965 that glucose or dextrose aid in microbial metabolism of cyanide.

CONCLUSIONS

In this study it was found:

1. that cyanide degrading organisms are part of the normal holding pond flora,
2. that some non-biological breakdown of CN can occur without a concomitant increase in CNS,
3. that similar to the findings of Ilyalemdinov *et al.* (1977) the study confirmed that biological breakdown of

CN can occur without the introduction of extraneous sources of organic carbon,

4. that contrary to the findings of Ilyalemdinov et al. (1977) the study indicated that by the introduction of extraneous organic carbon and nitrogen the reduction of CN could be enhanced,
5. that it may be assumed that one of the products of CN degradation was CNS which increased with increased incubation and decreasing CN (Ilwanoff in Howe 1965).

This study specifically used organisms able to survive on and/or utilize CN as a nutrient source. It is known that in nature there are organisms which can degrade and thus reduce the thiocyanate (CNS) concentration (Howe 1965) and this probably occurs in tailings ponds with elevated thiocyanate levels.

It may be worthwhile to extend this study by investigating the effect of introducing HCNS degrading organisms into the system after 4 to 6 weeks of incubation or even at the beginning of the incubation period. Another factor which could be explored is to extend the incubation period (with and without co-metabolites) and establish whether or not the organisms will start to utilize the CNS after all the CN is degraded.

The last factor which could be investigated is to find the optimal mix of co-metabolites which would maximize CN or HCNS degradation.

REFERENCES

Ilyalemdinov, A.N., P.B. Enker and Z.G. Vlasova, 1977. Role of microorganisms in removing cyanides from effluents. Mikrobiologiya 46:358-362.

Liu, D.L.S. and B.J. Dutka, 1972. Bacterial seeding techniques: novel approach to oil spill problems. Can. Research 5:17-20.

Howe, R.H.L., 1965. Bio-destruction of cyanide wastes - advantages and disadvantages. Int. J. Air Wat. Poll. 9:463-478.

Table 1. Effect of bacteria, incubation duration and co-metabolites on the breakdown of CN and SCN in natural samples.

Sample	Initial Concentration*		Incubated at 20°C on shaker for					
			2 weeks		4 weeks		6 weeks	
	CN	SCN	CN	CNS	CN	CNS	CN	CNS
Shallow Pond Water, May								
Control pH adj. 7.5**	52.2	41.0	29.1	40.6	21.2	44.0	15.9	44.1
1.0 g glucose and 1.0 g peptone per litre, pH 7.5	52.2	41.0	5.3	48.2	0.52	47.5	0.46	48.8
5.0 g glucose and 5.0 g peptone per litre, pH 7.5	52.2	41.0	25.3	40.2	18.1	44.3	12.6	44.5
Shallow Pond Water - August								
Sterile control pH 7.5	87.1	66.2	71.0	62.4	57.4	66.9	51.0	65.8
Control pH 7.5	87.1	66.2	64.2	63.8	48.4	68.1	37.0	68.4
1.0 g glucose + 1.0 g peptone pH 7.5	87.1	66.2	25.6	69.5	10.4	76.5	7.4	83.1
1.0 g glucose + 1.0 g peptone pH 7.5	87.1	66.2	26.5	66.9	7.4	79.9	5.9	84.8
5.0 g glucose + 5.0 g peptone pH 7.5	87.1	66.2	20.9	88.0	12.2	94.5	8.2	96.9
5.0 g glucose + 5.0 g peptone pH 7.5	87.1	66.2	24.3	75.2	6.3	89.6	8.2	89.2

* ppm; ** all flasks inoculated with $\approx 10^6$ bacteria per mL except sterile control.

Table 2. Percentage variations in CN and SCN concentrations from control samples at time zero.

Sample	Initial Concentration* CN	SCN	Percentage Reduction after incubation for 2 weeks CN	CNS	4 weeks CN	CNS	6 weeks CN	CNS
Shallow Pond Water, May								
Control pH adj. 7.5	52.2	41.0	44.7	10.0	59.4	+ 7.3	69.5	+ 7.6
1.0 g glucose and 1.0 g peptone per litre, pH 7.5	52.2	41.0	89.8	+17.6	99.0	+15.9	99.1	+19.0
5.0 g glucose and 5.0 g peptone per litre, pH 7.5	52.2	41.0	51.5	2.0	65.3	+ 8.0	75.9	+ 8.5
Shallow Pond Water - August								
Sterile control pH 7.5	87.1	66.2	18.5	6.0	34.1	+ 1.1	41.4	0.6
Control pH 7.5	87.1	66.2	26.3	3.6	44.4	+ 2.9	57.5	+ 3.3
1.0 g glucose + 1.0 g peptone pH 7.5*	87.1	66.2	70.1	+ 3.0	89.8	+18.1	92.4	+26.8
5.0 g glucose + 5.0 g peptone pH 7.5*	87.1	66.2	74.1	+23.3	89.4	+39.0	90.6	+40.9

* average of duplicated tests