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# Project Summary

# Biological Degradation of Cyanide by Nitrogen-Fixing Cyanobacteria

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**This study examined the ability of nitrogen-fixing *Anabaena* to biodegrade cyanide in batch reactors. Mixed second-order rate constants were obtained that described the biologically mediated decrease in cyanide for reactors containing initial cyanide concentrations of 3 ppm. For *Anabaena* cultures not previously exposed to cyanide, the rate constants were a function of pH. Faster rates of cyanide biodegradation were observed at higher pH values. *Anabaena* cultures acclimated to the presence of cyanide had rate constants that were at least 20 times faster than rate constants for unacclimated cultures.**

**Mixed second-order rate constants were also obtained for the ability of nitrogenase, the enzyme normally responsible for nitrogen-fixation, to reduce hydrogen cyanide to methane and ammonia. Based on literature values for nitrogen fixation, the rate constants for methane production were at least 10 times faster than expected in batch reactors with initial cyanide concentrations of 30 ppb. This suggests that nitrogenase will preferentially use hydrogen cyanide rather than molecular nitrogen as a substrate. Also, the rate constants for methane production were of the same order of magnitude as the rate constants for total cyanide removal. This indicates nitrogenase is an important mechanism for the biodegradation of trace concentrations of cyanide.**

**The magnitude of the cyanide biodegradation rate constants suggests that the utilization of nitrogen-fixing cyanobacteria in the treatment of cyanide wastes can be a feasible process in some applications, i.e., secondary or tertiary treatment at larger treatment facilities.**

***This Project Summary was developed by EPA's Risk Reduction Engineering Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).***

## Introduction

The basic premise of the study summarized here was that the use of nitrogen-fixing cyanobacteria (blue-green algae) in the biological treatment of small concentrations of free cyanides (HCN and $CN^-$) can be a cost-effective alternative to existing treatment processes. A potential application of a cyanobacteria-based process would be in secondary treatment of cyanides that escape alkaline chlorination. The steady and small concentrations of cyanide in the effluent of an alkaline-chlorination process would be conducive to maintaining cyanobacteria in a secondary treatment process, i.e., alkaline chlorination would protect the cyanobacteria from cyanide concentration fluctuations.

Because the extent of cyanide oxidation in alkaline chlorination is an equilibrium-driven phenomena, use of a microbial process to detoxify the last fraction of cyanide should result in lower alkaline-chlorination operating costs.

Several potential advantages are associated with the use of nitrogen-fixing cyanobacteria in the biological treatment of small concentrations of cyanide. First, because cyanobacteria are photosynthetic, they do not require aeration to obtain oxygen and do not require the presence of organic substrates to maintain biomass. Thus, the use of cyanobacteria in the biological treatment of small amounts of cyanide should have lower operating costs than the use of heterotrophic bacteria. Second, because of the presence of cyanide-resistant respiration and cyanide detoxification pathways, nitrogen-fixing cyanobacteria have the ability to survive in low to moderate concentrations of hydrogen cyanide. Third, the nitrogen-fixing cyanobacteria can destroy hydrogen cyanide with the enzyme nitrogenase. Although normally responsible for reducing molecular nitrogen (dinitrogen) to ammonia, nitrogenase can also reduce hydrogen cyanide to methane and ammonia instead of its normal substrate dinitrogen.

Despite the considerable amount of information indicating the ability of cyanobacteria to survive in the presence of cyanide and to detoxify cyanide, the kinetic data required to assess the feasibility of using cyanobacteria in the treatment of cyanide wastes does not exist. This study provides an initial assessment of the rate at which nitrogen-fixing cyanobacteria are able to degrade free cyanide.

## Procedure

The objectives of the study were (1) to determine the rates at which nitrogen-fixing *Anabaena* cultures decreased total cyanide concentrations and (2) to determine the rate at which HCN was reduced to methane by nitrogenase activity. Rates of total cyanide biodegradation were determined in a 1.3-L reactor under batch conditions with initial cyanide concentrations of 3,000 µg CN/L. The extent of cyanide volatilization was measured for each batch test, so that the reported rate constants represented biodegradation and not the sum of biodegradation and volatilization. The methane production experiments were conducted in small 0.037-L, gas-tight vials under batch conditions with initial cyanide concentrations of 30 to 400 µg CN/L.

## Results and Discussion

### Total Cyanide Biodegradation Rates

The rate of cyanide biodegradation was assumed to follow mixed second-order kinetics,

$$\frac{ds}{dt} = -K_b \times S$$

in which S is the total cyanide concentration (µg CN/L), t is time (hr), $K_b$ is the mixed second-order rate constant (L/[µg chl hr]), and X is the concentration of *Anabaena* biomass in terms of chlorophyll-a (µg chl/L).

For *Anabaena* cultures not previously exposed to cyanide, the values of $K_b$ ranged from $5.0 \cdot 10^{-6}$ to $2.2 \cdot 10^{-4}$ L/(µg chl/hr). This observed 44-fold variation in $K_b$ appeared to be a function of pH. That is, as the time-averaged pH value for each batch test increased, the observed $K_b$ value increased.

Because $K_b$ increased as pH increased, the $K_b$ values were probably responding to ALPHA,

$$\text{ALPHA} = \frac{[HCN]}{[HCN] + [CN^-]},$$

the fraction of the total free cyanide existing as HCN. As pH values increase to levels above the $pK_a$ (acid dissociation constant) for HCN, the ALPHA values decrease. Thus, the rate at which unacclimated *Anabaena* cultures decreased total cyanide concentrations was optimized by reducing the fraction of cyanide that HCN is more toxic and inhibitory than is $CN^-$.

One set of experiments was performed to assess the effect that previous exposures to cyanide had on biodegradation rates. For batch tests conducted at the same pH, an *Anabaena* culture previously exposed to cyanide had a mixed second-order rate constant ($K_b$) 12 times greater than the $K_b$ value for a culture not previously exposed to cyanide. Thus, when *Anabaena* had time for enzyme induction, the rate constants for cyanide biodegradation increased.

### Methane Production Rates

The rate of methane production due to nitrogenase activity was assumed to follow mixed second-order kinetics,

$$\frac{dP}{dt} = K_n \times S$$

in which P is the water-phase concentration of cyanide corresponding to the mass of methane produced (µg CN/L), t is time (hr), $K_n$ is the mixed second-order rate constant for methane production by nitrogenase (L/µg chl/hr]), X is the concentration of *Anabaena* (µg chl/hr), and S is the total cyanide concentration (µg CN/L). Because nitrogenase was not the only biological mechanism responsible for reducing cyanide concentrations (S), in the vials, determination of $K_n$ involved fitting the following equation to the methane production data:

$$\frac{dP}{dt} = K_n \times S_O \exp(-K_b \times t)$$

in which $S_o$ is the cyanide concentration at the start of the batch experiment (µg CN/L) and $K_b$ is the mixed second-order rate constant for total cyanide biodegradation by *Anabaena* cultures (L/[µg chl/hr]), as determined in the previous batch experiments.

The values of $K_n$ obtained from the batch experiments appeared to be an inverse function of initial cyanide concentrations. For example, for $S_o$ values of 30 and 400 µg CN/L, the observed $K_n$ values were $2.6 \cdot 10^{-4}$ and $2.6 \cdot 10^{-6}$ L/(µg chl/hr), respectively. The decrease in $K_n$ with increasing $S_o$ was probably due to HCN inhibition of the ATP (a form of biochemical energy) generating pathways in the heterocysts.

The observed $K_n$ value when $S_o$ was 30 µg CN/L was almost 30 times larger than a mixed second-order rate constant for hydrogen cyanide reduction by nitrogenase obtained from combining existing *in vivo* nitrogen fixation data and *in vitro* hydrogen cyanide reduction data. This suggests that nitrogen-fixing *Anabaena* cultures convert cyanide to methane at a faster-than-expected rate. Thus, if nitrogenase's requirements for ATP and electrons can be continuously satisfied, the enzymatic apparatus in *Anabaena* normally responsible for nitrogen fixing may play an important role in determining the rate at which low concentrations of cyanide are biodegraded in a treatment process.

Based on methane production data, unacclimated *Anabaena* cultures have the capacity to decrease total cyanide concentrations from 30 µg CN/L to less than 20 µg CN/L in 1.75 hr. The ability of nitrogen-fixing cyanobacteria to reduce cyanide concentrations below 20 µg CN/L may be significant, because the literature reports that no cyanide destruction process has demonstrated the ability to reduce total cyanide concentrations to levels less than 25 µg CN/L. Thus, nitrogen-fixing cyanobacteria may be well suited for use in the secondary or tertiary treatment of cyanide wastes.

## Conclusion and Recommendations

In batch reactors with initial cyanide concentrations of 3 mg CN/L, the mixed second-order rate constants ($K_b$) for the removal of cyanide by unacclimated *Anabaena* cultures were a function of pH. Faster rate constants were observed at higher pH values. Because HCN is much more toxic and inhibitory than $CN^-$ and because an increase in pH would reduce HCN concentrations, the faster biodegradation rates observed at higher pH values were probably due to a reduction in inhibition.

When previously exposed to cyanide, *Anabaena* biodegraded cyanide at a faster rate. The $K_b$ value for an acclimated *Anabaena* culture was 10 times faster than that for an unacclimated *Anabaena* culture. Thus, reactors operating under steady-state conditions should have more rapid cyanide removal rates than those observed in the batch experiments.

In batch experiments with initial cyanide concentrations of 30 µg CN/L, the mixed second-order rate constant ($K_n$) for the reduction of hydrogen cyanide to methane by nitrogenase was at least 10 times faster than expected based on existing nitrogen-fixation kinetic data. This supported the *in vitro* observation that nitrogenase will preferentially reduce hydrogen cyanide rather than its normal substrate of molecular nitrogen. Based on the amount of methane produced during the batch tests, nitrogenase activity decreased cyanide concentrations from 30 to 20 µg CN/L. Because few cyanide destruction processes are able to attack cyanide at such low concentrations, the use of nitrogen-fixing cyanobacteria to treat trace levels of cyanide is worth further examination.

This study demonstrated the ability of nitrogen-fixing cyanobacteria to biodegrade small concentrations of free cyanides in batch reactors. Future studies need to examine the ability of the cyanobacteria to degrade cyanide under steady-state reactors to slight perturbations in influent cyanide concentrations. If such laboratory-scale experiments continue to demonstrate the attractiveness of using nitrogen-fixing cyanobacteria to treat small and trace cyanide concentrations, then a pilot-scale study should be done to determine the economic and technical feasibility of the process.

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*The complete report, entitled "Biological Degradation of Cyanide by Nitrogen-Fixing Cyanobacteria," (Order No. PB89-222 509/AS; Cost: $17.00, subject to change) will be available only from:*
*National Technical Information Service*
*5285 Port Royal Road*
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