Toxicity and degradation of metal-complexed cyanide by a bacterial consortium under sulfate-reducing conditions

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Abstract

Free cyanide at 1 mM decreased the initial sulfate reduction rate of a batch culture of granular sludge from 0.3 to 0.14 mmol d^{-1} g^{-1} SS (suspended solid), whereas 0.5 mM cyanide had a minimal effect (0.25 mmol d^{-1} g^{-1} SS). The order of toxicity of metal-complexed cyanides to the sludge was as follows: zinc-complexed cyanide (most toxic) > free cyanide = nickel-complexed cyanide > copper-complexed cyanide (least toxic), which also corresponds well with the order of the stability (dissociation) constants of the metal-cyanide complexes. A consortium degrading cyanide was enriched using nickel cyanide as the sole nitrogen source. This consortium completely removed 0.5 mM of nickel-complexed cyanide under sulfate-reducing conditions in 11 d. Analysis of clone library of 16S rRNA genes shows that the consortium was composed of three major phylotypes including *Desulfovibrio*.

Introduction

Under anaerobic conditions, sulfate-reducing bacteria (SRB) oxidize simple organic compounds using sulfate as an electron acceptor. The H_2S that is produced effectively precipitates heavy metals as metal sulfides which are the most stable form of metal precipitates (Dean 1985). As such, bacterial sulfate reduction is a potentially cost-effective process for removing metals from industrial wastewaters (Tuttle *et al.* 1969). Metal-containing wastewaters, arising from metal extraction (mainly gold), electroplating, metal finishing, metal hardening and printed circuit board manufacturing, contain up to 300 mg cyanide l^{-1} (Akcil & Mudder 2003). In the wastewaters, cyanide complexes with heavy metals (e.g. copper, nickel, zinc, cadmium, iron, silver, gold, etc.) with varying stabilities and toxicities (Patil & Paknikar 1999). Therefore, in order to apply the sulfate reduction for treating a wide range of heavy metal-containing wastewaters, the effect of cyanide on sulfate reduction must first be determined.

The effect and degradation of cyanide have already been studied in methanogenic conditions (Gijzen *et al.* 2000, Fallon *et al.* 1991, Annachhatre & Amornkaew 2001) and sulfate-reducing conditions (Song *et al.* 1998). However, these previous researches only described the effect and degradation of free cyanide, whereas there has been no report on the metal-complexed cyanide. In this study, we present the inhibitory effect of metal-complexed cyanides on sulfate reduction and the characteristics of a cyanide-degrading consortium that efficiently removed nickel-complexed cyanide under sulfate-reducing conditions.

Materials and methods

Granular sludge and enrichment sources

The granular sludge was taken from an upflow anaerobic sludge blanket (UASB) reactor at a beer brewery wastewater treatment plant in Kwangju, Korea. To enrich the microorganisms degrading metal-cyanide complex under sulfate-reducing conditions, other

sludges from different (electroplating, petrochemical, municipal industrial) wastewater treatment plants were also used as microbial sources.

Inhibition of sulfate reduction by free and metal-complexed cyanides

The basal medium used in the anaerobic sulfate reduction was as follows (g l^{-1}): 0.85 Na_2SO_4, 0.5 KH_2PO_4, 0.06 $CaCl_2 \cdot 2H_2O$, 0.8 $MgCl_2$, and 0.9 sodium lactate. The pH of the medium was adjusted to 7–7.2 using 2 M HCl. Resazurin was added as a redox indicator. Serum bottles, 165 ml, containing 135 ml medium were used to assess the toxicity of free and metal-complexed cyanide to sulfate reduction of the granular sludge. The metal cyanide complexes were made as described by Rollinson *et al.* (1987). Granular sludge, 15 g wet wt, 1.5 g suspended solids, from the wastewater treatment UASB reactor was added as inoculum. Thereafter, O_2-free He was flushed into the bottles containing the medium and granular sludge, which were then tightly closed with a butyl rubber stopper and aluminum seal. All experiments were performed at 37 °C in duplicate.

Enrichment of cyanide-degrading consortia

Cyanide-degrading consortia were enriched under sulfate-reducing conditions by repeated subcultures in the basal medium supplemented with 20 mM sodium sulfate and 0.5 mM nickel-complexed cyanide, as described above.

16S rRNA gene cloning and phylogenetic analysis

Mixed liquor was sampled from the enrichment bottle for DNA extraction. Genomic DNA was extracted using DNeasy Tissue Kit (QIAGEN, Canada) and the 16S rRNA was amplified by PCR using 9F and 1512R as primers. Cloning and sequencing of 16S rRNA genes and the construction of phylogenetic tree were carried out as described by Yoon *et al.* (2000).

Analysis of sulfate and nickel-complexed cyanide

Sulfate concentrations were analyzed using an ion chromatograph equipped with a conductivity detector and anion exchange column (Anion SUPP4). Residual nickel-cyanide complex was analyzed spectrophotometrically as described previously (Rollinson *et al.* 1987) after precipitating the sulfide with 10 mM
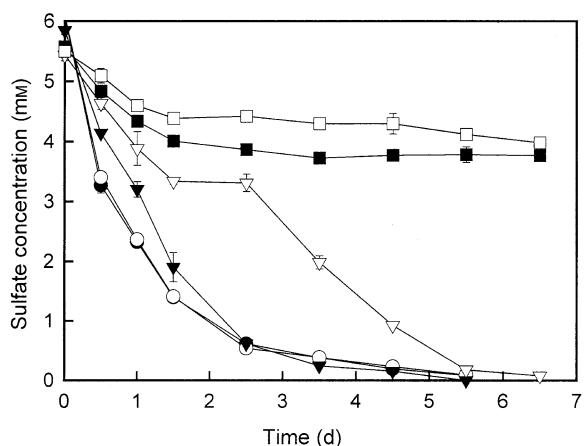

Fig. 1. Effect of free cyanide on sulfate reduction of an anaerobic granular sludge. Sludges were incubated under anaerobic conditions in the presence of various amounts of free cyanide. ●, Control; ○, 0.2 mM; ▼, 0.5 mM; ▽, 1 mM; ■, 2 mM; □, 5 mM.
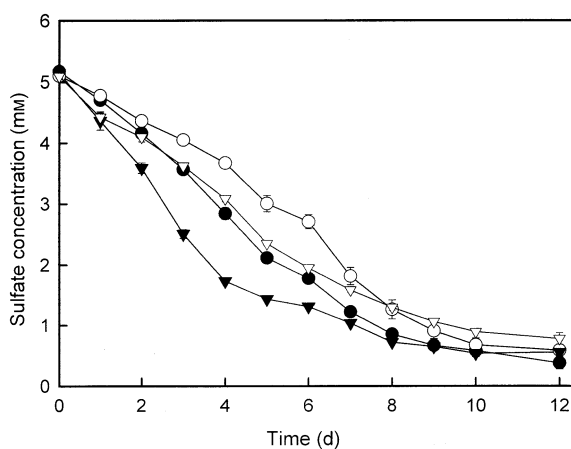

Fig. 2. Effect of metal-complexed cyanide on sulfate reduction of an anaerobic granular sludge. In the presence of various form of metal-complexed cyanide at 1 mM (● free cyanide, ○ zinc cyanide, ▼ copper cyanide, ▽ nickel cyanide), the sulfate reduction was monitored during incubation.

$Pb(NO_3)_2$ and filtrating through a SEP-PAK C18 cartridge.

Results and discussion

Inhibition of sulfate reduction with various concentrations of free cyanide

The effect of free cyanide was tested in a batch culture of granular sludge under sulfate-reducing conditions (Figure 1). The initial (up to 1.5 d) sulfate reduction rate decreased from 0.3 ± 0.003 to 0.141 ± 0.001 mmol d^{-1} g^{-1} suspended solids (SS) with 1 mM

cyanide, whereas 0.5 mM cyanide had less effect (to 0.255 ± 0.013 mmol d^{-1} g^{-1} SS) on sulfate reduction. However, with more than 2 mM cyanide, there was no sulfate reduction over 30 d. Similar results were reported by Song *et al.* (1998), where 1 mM cyanide inhibited sulfate reduction by 50% compared to the control. Gijzen *et al.* (2000) reported that 0.2 mM cyanide sharply reduced the methane production rate to 30% of that observed in the absence of cyanide in a UASB reactor. This result indicates that sulphate reducing bacteria (SRB) might be less sensitive to cyanide toxicity than methangen.

Inhibition of sulfate reduction by metal-complexed cyanides

The toxicity of 1 mM cyanide was tested in different metal complex forms. As shown in Figure 2, the inhibitory effect of each cyanide complex on sulfate reduction was in the following order: zinc-complexed cyanide (most toxic) > nickel-complexed cyanide $\cong$ free cyanide > copper-complexed cyanide (least toxic). When using 2 mM cyanide as metal complexes, the reduction of sulfate was completely inhibited by each metal-cyanide complex (data not shown). Copper is more toxic than zinc to sulfate reduction (Utgikar *et al.* 2001). Therefore, the current results mean that the inhibition order of the metal-complexed cyanides was unrelated to the order of heavy metal toxicity. According to the speciation diagram involving copper cyanide and zinc cyanide constructed by Bose *et al.* (2002), at pH 7, more than 50% of zinc is present as free ions. However, most of copper (more than 99%) is present in a cyanide-complexed form. This is also supported by the dissociation constant of the metal-complexed cyanide. The order of the metal-complexed cyanide toxicity corresponded well to the order of the metal-complexed cyanide stability (pK$_{dissociation}$) (Zn(CN)$_4^{2-}$: 18.9, Ni(CN)$_4^{2-}$: 22, Cu(CN)$_4^{2-}$: 30.7; quoted from Gerhartz (1987)). Gijzen *et al.* (2000) and Rollinson *et al.* (1987) previously suggested that metal-complexed cyanide is less toxic than free cyanide. However, in the current study zinc-complexed cyanide was more toxic than free cyanide. With 1 mM zinc-complexed cyanide, the concentration of zinc is 0.25 mM, in which 50% decrease in sulfate reduction was observed by Utgikar *et al.* (2001). As such, the inhibition by the zinc-complexed cyanide was likely to be the result of an accumulative effect of free cyanide and zinc ions. Therefore, the stability of the metal-complexed cyanide and toxicity of free metal itself
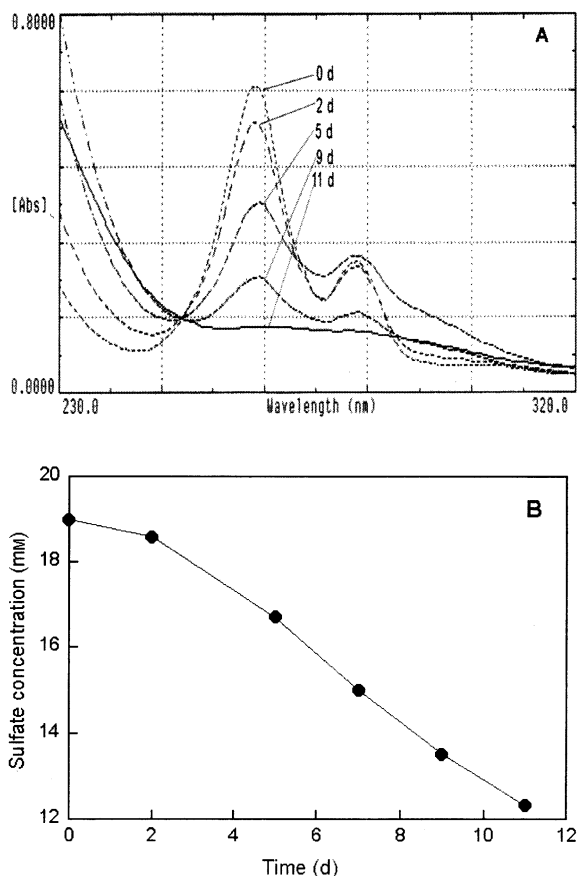

Fig. 3. Degradation of nickel-complexed cyanide (A) during sulfate reduction (B) by the consortium. The initial concentration of nickel-complexed cyanide was 0.5 mM. (A) Change of absorbance spectrum was monitored during 11 d of incubation. The absorbance peak at 267 nm was proportional to the concentration of nickel cyanide complex. Simultaneously, (B) sulfate reduction was observed during nickel cyanide degradation.

can be used to predict the inhibitory effect of various metal-complexed cyanides on wastewater treatment.

Degradation of nickel-complexed cyanide under sulfate-reducing conditions

Sulfate reduction is an efficient method for treating metal-containing wastewater. Yet, according to cyanide toxicity tests, only 10% of cyanide was removed during sulfate reduction, even with 0.5 mM cyanide. For the improvement of cyanide degradation capabilities in sulfate-reduction, the characterization of cyanide-degrading bacteria would be a prerequisite. As a model compound, we used nickel-cyanide complex difficult compound to treat with most commonly adopted cyanide treatment methods, such as alkaline
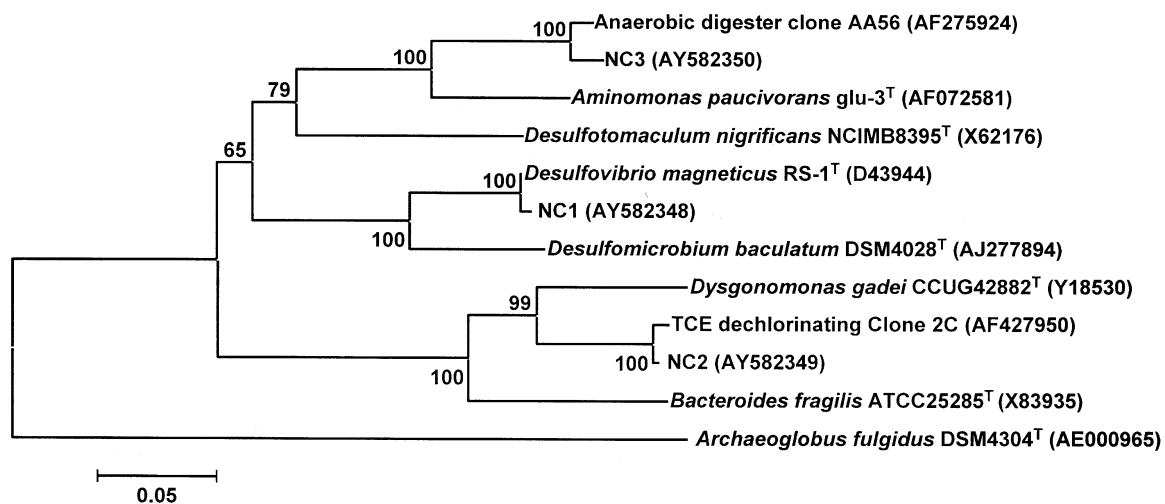
Fig. 4. Phylogenetic position of the three phylotypes identified in the consortium. The tree was constructed using neighbor-joining algorithm with 1000 bootstrappings. *Archaeoglobus fulgidus* was selected as the out-group species. The horizontal bar corresponds to 5 nucleotide substitution per 100 sequence positions. Bootstrap values are shown at the branch points. The number in parenthesis of each sequence indicates the accession number of GenBank. [T] means type strain.

chlorination oxidation, due to slow reaction rates (Eckenfelder 1989). Among the various sludge enrichments tested, a consortium enriched from the sludge of an electroplating wastewater treatment plant in Daegu, Korea exhibited the highest cyanide-degrading activity under sulfate-reducing conditions. Figure 3A shows the decrease in the peaks at 267 nm and 283 nm, which are specific to nickel-complexed cyanide, plus 0.5 mM nickel-cyanide complex was completely removed after 11 d (Figure 3A) with a simultaneous decrease in the sulfate concentration (Figure 3B). The cyanide-complexed nickel did not react with the sulfide due to the high stability of the nickel-cyanide complex. Yet, when cyanide degradation occurred, the free nickel released from the complex reacted quickly with the sulfide to make a nickel sulfide precipitate. Therefore, decrease of the nickel-cyanide complex (Figure 3A) and precipitation of nickel sulfide represented the degradation of cyanide. Nickel dicyanide is normal byproduct in aerobic nickel-cyanide degradation. However, in our preliminary experiment, we determined that the apple-green color of nickel dicyanide immediately changes to the black nickel sulfide in the presence of sulfide, indicating that nickel sulfide is more stable than nickel dicyanide. The consortium degraded 0.2 mM free cyanide to 0.05 mM under sulfate-reducing conditions after 17 d, indicating that the degradation rate of free cyanide was only 20% of that of nickel-complexed cyanide.

The attempt to isolate the cyanide-degrading bacteria from the enriched consortium failed. We made 16S rRNA gene library of the consortium to analyze microbial community. Sequence analysis of 41 clones revealed that the consortium which degrading nickel-complexed cyanide was composed of three major phylotypes, named as NC1 (16 clones), NC2 (18 clones) and NC3 (7 clones). Figure 4 illustrates the phylogenetic positions of the three phylotypes identified in this study and their respective close relatives based on 16S rRNA sequence homology. The group NC1 was a member of sulfate reducing bacteria of *Desulfovibrio*, resembling *Desulfovibrio magneticus* with 99.4% similarity. NC2 was closely related to an uncultured bacterium 2C (Richardson *et al.* 2002) (97.7%) which was from anaerobic TCE dechlorinating enrichment. NC3 was close to clone AA56 (96.3% similarity) which became dominant in an anaerobic digester degrading lactate (Delbès *et al.* 2000). This result shows that the consortium is highly enriched. More work is needed to elucidate the function of each phylotype in the degradation of cyanide.

Conclusion

The toxicity and degradation of metal-cyanide complexes were investigated under sulfate-reducing conditions. The toxicity of the metal-cyanide complexes was found to be correlated with the stability of the

complex, which thus can be used to predict the effect of various metal-complexed cyanides in wastewater treatment. The consortium enriched for cyanide degradation effectively removed nickel-complexed cyanide under sulfate-reducing conditions. This consortium was composed of three major phylotypes including a SRB, *Desulfovibrio*. Accordingly, since both cyanide and nickel could be removed simultaneously, sulfate reduction would appear to be a plausible treatment method for heavy metal- and cyanide-containing wastewater.

Acknowledgement

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