Biological Treatment of Mercury Waste

Conly L Hansen and Gour S. Choudhury
Department of Nutrition and Food Sciences and Department of Civil and Environmental Engineering, Utah State University

INTRODUCTION

Mercury is one of the most significant examples of toxic heavy metal pollution. Anthropogenic sources of mercury include those associated with its use in chlor-alkali, paint, agriculture, pharmaceutical, and paper and pulp industries.

A widely used method for removing mercury from polluted water is addition of sodium sulfide. Insoluble mercuric sulfide (HgS), which forms is removed as a sludge (Price et al. 1972) that is then landfilled. It has been assumed that HgS remains permanently insoluble, thus preventing movement of mercury into the surrounding environment. Mercury waste has been treated and buried in this manner for many years.

However chemical methods of mercury detoxification are far from adequate. It has become evident that mercury can be solubilized from HgS under conditions that could be present in a landfill. We have demonstrated chemical solubilization followed by volatilization with $Fe_2(SO_4)_3$, a product of oxidation of $FeSO_4$ (pyrite) by *Thiobacillus ferrooxidans* (data not shown). Other researchers have indicated that *T. ferrooxidans* can facilitate solubilization and volatilization of Hg^0 from HgS. Growth of *T. ferrooxidans* in the presence of cinnabar (mercury ore-contains HgS and some impurities) by Silver and Torma (1984) resulted in dissolved mercury concentration in the bioreactor of 64 mg/L (the form of Hg was not given). In similar experiments with *T. ferrooxidans* and cinnabar, Baldi and Olson (1987) did not

report high concentrations of soluble Hg, but did find volatilization of Hg^0 from cinnabar (0.26 mg/L Hg^0 in the headspace of cultures grown overnight).

Using the biological approach for mercury detoxification presented below, mercury is removed from polluted water and may be recovered in the elemental form, thereby avoiding the sludge disposal problem and recycling the mercury. Even if mercury vapor is released to the air from this process due to equipment malfunction or for some other reason, it must be kept in mind that the amount released, even from severely mercury polluted waste is relatively low. The National Emission Standards for Hazardous Air Pollutants (NESHAP) permits 2300 g of mercury per 24 hours from mercury ore processing facilities and mercury cell chlor alkali plants. Emissions from sludge incinerators or dryers shall not exceed 3200 g/24 hr (40 CFR, 1984). This means that the mercury from 20,000 - 25,000 kg (44,000 - 55,000 lbs) of sludge containing 100 mg/L mercury can be released per day depending on which regulation applies, assuming nearly 100% removal of mercury from the sludge. However, our research efforts are directed towards removal and recovery of elemental mercury from contaminated water and sludges.

Biological removal of mercury involves enzymic reduction of Hg^{2+} to Hg^0 and subsequent volatilization of this hazardous metal from contaminated water and sediments. The detoxification of organomercury compounds takes place by initial cleavage of C-Hg bond followed by reduction and volatilization. Many groups of bacteria, including heterotropic microorganisms harmless to man are resistant to mercuric ion and capable of converting Hg^{2+} to the elemental form, Hg^0. For the mercurial resistance determinants, enzymes mercuric reductase and organomercurial lyase detoxify inorganic and organic mercury, respectively (Silver et al., 1986). Mercuric reductase can convert both Hg^+ and Hg^{2+} to Hg^0 (Ijaki, 1981). This form is readily eliminated from the growth medium resulting in detoxification of the mercury polluted aquatic environment (Summers and Silver, 1972). Mercury reduction requires an intact *mer* operon, which consists of 6 genetic elements (Williams and Silver, 1984). The merA (mercuric reductase) and merB (organomercurial lyase) genes from the mercurial resistance determinants of gram negative bacteria have been identified (Laddaga et al., 1987). The merB gene that determines the enzyme organomercurial lyase which cleaves the C-Hg bond of phenylmercury has also been identified (Griffin et al., 1987).

BACTERIAL STRAIN AND GROWTH MEDIA

Several groups of bacteria in a wide variety of Gram-negative and Gram-positive species have been reported to be resistant to mercury and can convert Hg^{2+} to the elemental form, Hg^0. Bacterial strains capable of detoxifying mercury are *Escherichia Coli* (Komura and Izaki, 1971; Komura et. al., 1971; Summers and Silver, 1972; Summers and Lewis, 1973), *Staphyloccus aureus* (Summers and Lewis, 1973; Weiss et. al., 1977), *Pseudomonas aeruginosa* (Summers and Lewis, 1973; Clark et. al., 1977), *Pseudomonas* K-62 (Furukawa et. al., 1969; Furukawa and Tonomura, 1972; Tezuka and Tonomura, 1976, 1978), *Bacillus cereus* (Izaki, 1980), and *Thiobacillus ferrooxidans* (Olson et. al., 1982). Most of these strains were isolated from mercury contaminated sites. Bacterial strains isolated from sediments were found to be intrinsically more resistant to mercury than those isolated from water (Nelson et. al., 1972). Mercury volatilizing activities have also been reported in *Streptomyces* species and group B *Streptococcus* from clinical sources (Nakahara et. al., 1985).

Growth of bacterial cultures in media containing mercury was found to enhance resistance of these cultures. A decrease in resistance to mercury was observed by Colwell et. al. (1975) after several transfers of the cultures in mercury-free media.

Initially, we examined the growth and mercury removal characteristics of shake-flask cultures of *Escherichia coli* KP245 harboring the cloned plasmid pRR130 which contains the *mer* resistance genes. The bacteria were grown and maintained on minimal salt media supplemented with 0.5 g/L yeast extract, 6 g/L sucrose, and 5 mg/L $HgCl_2$. It was observed that mercury increases the lag phase, but does not effect the growth rate of these bacteria. It was also demonstrated that the growth rate was minimal during a phase of rapid mercury removal, after which growth resumed. It was found that the mercury resistant cultures can also be grown and maintained in domestic sewage and whey permeate, a cheese industry waste (Hansen et. al., 1984; Xu et. al., 1987).

Considerable progress have been made in elucidating mechanisms of microbial resistance and detoxification of mercury (Robinson and Touvinen, 1984; Silver et. al., 1986). With recent developments in genetic engineering, it is possible to improve these strains and to engineer the bacteria to induce resistances to potentially growth-inhibitory compounds present in a complex waste stream. It is also possible to produce purified enzymes on an industrial scale. The enzymes responsible for biotransformation of mercury can be modified or altered by genetic engineering techniques to improve their capabilities and robustness (Holmes, 1988). However, more research efforts are needed in this area.

THE BIOLOGICAL PROCESS

In order for the process to be useful to industry and field application, a continuous biological process for mercury removal was developed (Hansen et. al. 1984). The process is shown schematically in Figure 1.

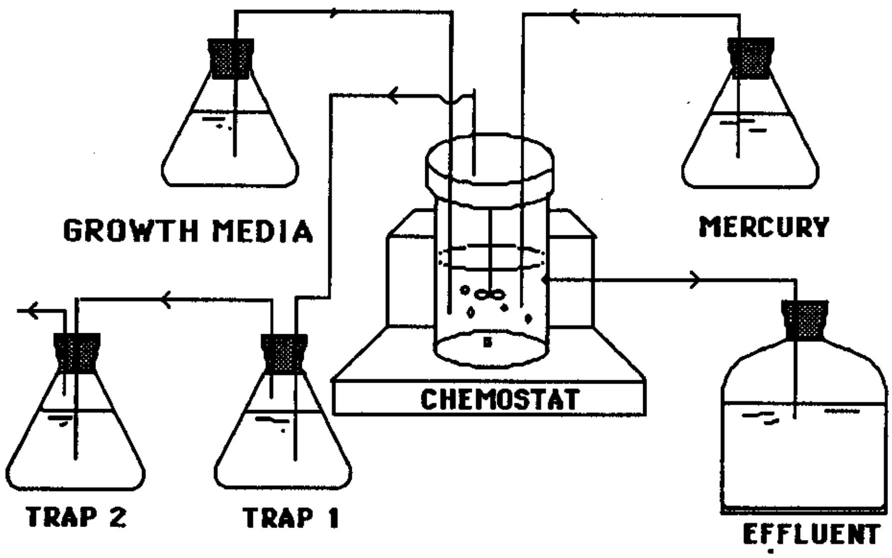

Figure 1. Schematic Diagram of the Bench Scale Biological Process.

The process consists of feeding mercury contaminated waste under nonsterile conditions to a continuous mixed culture of resistant organism maintained on raw sewage in a New Brunswick Scientific Microferm MF 115, 14 L fermenter equipped with variable mixing and aeration. The bioreactor has automatic temperature and liquid level control. The temperature of the reactor was maintained at 37 ^{o}C and was aerated at 2 LPM. The working volume of the reactor was 5L and the agitator speed was 120 rpm. Continuous mercury removal was accomplished by a relatively stable mixed population of mercury resistant bacteria after the addition of the starter culture under non-sterile condition. The bioreactor was able to remove Hg^{2+} from sewage containing 70 mg/L mercury at the rate of 2.5 mg/L h with an efficiency of 88%. The process was used to treat an industrial waste sample from a chlor-alkali plant in Northern Ohio. In a similar process utilizing whey permeate as growth media, Xu et al. (1987) reported removal of mercury from influent (100 mg/L) with 90% efficiency using a mixed culture from a secondary wastewater treatment aeration tank acclimated to mercury.

The efficacy of the biological mercury removal process has been adequately demonstrated from a microbiological and biochemical viewpoint. The kinetics of the biological process, bioreactor design and the the effects of process parameters such as aeration rate, SRT and HRT variations on removal of mercury are presently being studied. A full-scale bioprocess being investigated in our laboratory is schematically shown in Figure 2. Whey permeate will be used as the carbon/energy source since it is well distributed in the U.S., inexpensive compared to other sources, and due to its high COD (78,000 mg/L), the volume of permeate required will be small. The process will therefore co-treat Hg-laden waste and whey permeate, a cheese industry waste. When fully developed and characterized, the process will represent a substantial advancement in the state of the art of mercury pollution abatement.

RECOVERY OF ELEMENTAL MERCURY

An important engineering consideration in the design and operation of the biological process for mercury removal is the fate of the reduced mercury during treatment. A significant portion of the mercury that is reduced in the biological process will exit the bioreactor as a vapor along with other effluent gases (mainly N_2, O_2 and CO_2). When air is used as the oxygen source for the biological process, the partial pressure of the elemental mercury vapor in the effluent gas stream is very low. This makes it difficult to condense the majority of mercury from the effluent gas because condenser temperatures must be very low (theoretically, -40 ^oC or lower under worst case conditions).

One other method of recovering mercury from the vapor phase is to extract mercury using a suitable solvent (e.g. toluene or chloroform) in a scrubber, e.g. a packed tower. The mercury in the solvent can be reprocessed commercially. But, the poor solubility of mercury in such solvents warrants consumption of huge quantities of solvent thus limiting the use of a packed tower process for mercury recovery. It is therefore apparent that a preconcentration step must be used to facilitate the removal and recovery of mercury from the air phase.

In our laboratory research efforts are directed towards development of a mercury recovery process based on adsorption/desorption mechanisms. The schematic diagram of the process is shown in Figures 3 and 4.

Mercury laden air from the bioreactor will be passed through an adsorbent bed where mercury from the air phase will be extracted and concentrated on the surface of the adsorbent. Two types of adsorbent being investigated are activated carbon type 'AC' and type 'Mersorb 245'. Type 'AC' is an unimpregnated activated carbon which adsorbs by physical forces, whereas type 'Mersorb 245' is impregnated with organic iodide, and chemisorption also takes place. The lean air exiting from the adsorbent bed will be passed through two acidified $K_2Cr_2O_7$ baths in series to eliminate residual mercury, if any.

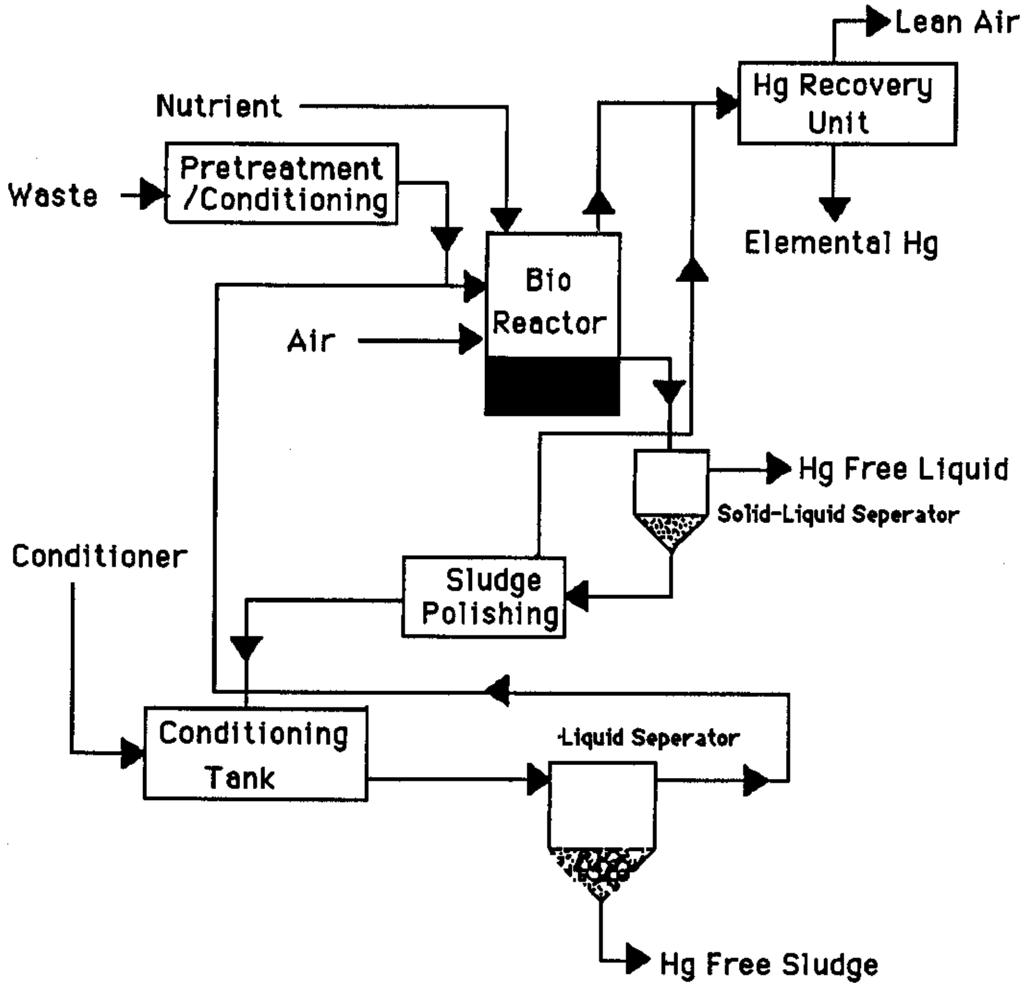

Figure 2. Flow Schematic of the Full Scale Biological Process

The saturated adsorbent column containing Hg will be desorbed under heat and a purge medium to elute the adsorbate. The eluting gases would be condensed to recover the Hg vapor in the liquid form. The carrier gas from the condenser will also be passed through two acidified $K_2Cr_2O_7$ baths in series to eliminate any uncondensed residual mercury.

Preliminary experiments have shown promise for this process. However, research is incomplete at this point to present conclusive data.

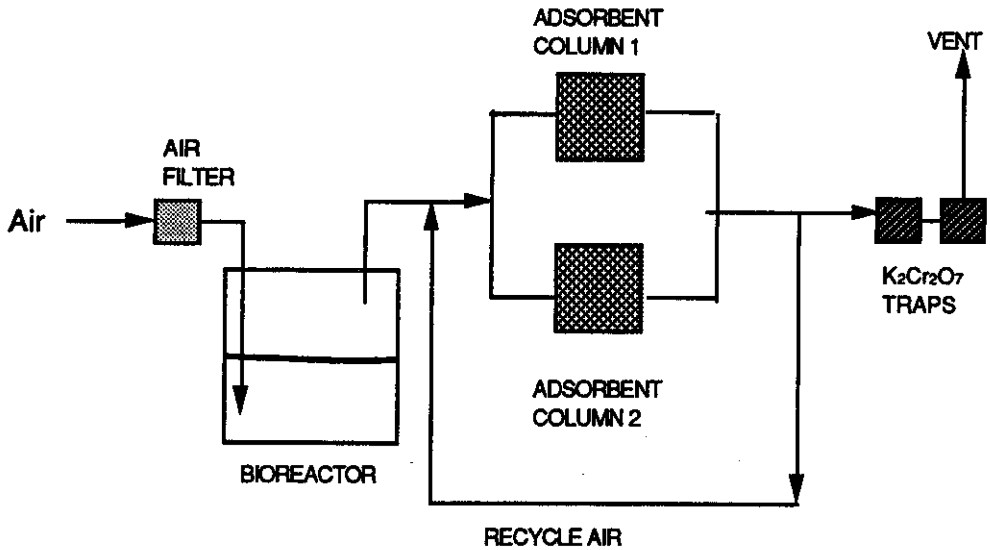

Figure 3. Adsorption of Mercury Vapor Emanating from the Bioreactor

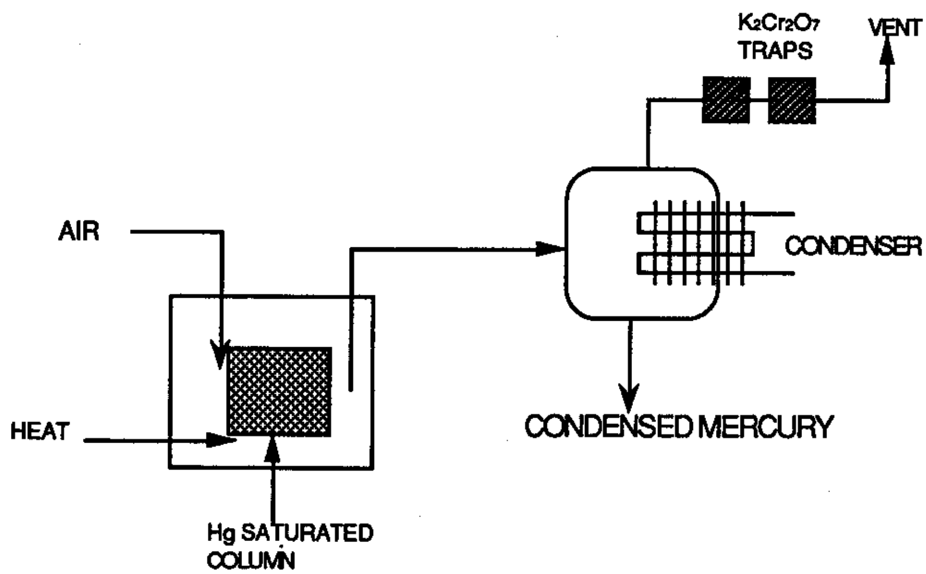

Figure 4. Desorption and Condensation of Elemental Mercury

CONCLUSION

A biological process for detoxification of mercury in polluted water and sludges has been developed. Recovery of elemental mercury from the vapor phase for reuse is being studied and preliminary results show promise for the process. A full-scale process is under investigation for field/commercial application.

REFERENCES

Baldi, F. and G. J. Olson. 1987. Effect of cinnabar pyrite oxidation by *Thiobacillus ferrooxidans* and cinnabar mobilization by a mercury-resistant strain. Appl. Environ. Microbiol. **53**:772-776.

Clark, D. L., A. A. Weiss, and S. Silver. 1977. Mercury and organomercurial resistance determined by plasmids in *Pseudomonas*. J. Bacteriol. **132**:186-196.

Colwell, R. R., S G. Berk, G. S. Sayler, J. D. Nelson, Jr., and J. M. Esser. 1975. Mobilization of mercury by aquatic microorganisms. Sym. Proc. of Int. Conf. on Heavy Metals in the Environment.

Federal Register Office, 1984. Code of Federal Regulation Title 40.

Furukawa, K., T. Suzuki, and K. Tonumura, 1969. Decomposition of organic mercurial compounds by mercury resistant bacteria. Agric. Biol. Chem. **33**:128-130.

Furukawa, K., and K. Tonumura, 1972. Metallic mercury-releasing enzyme in mercury resistant *Pseudomonas*. Agric. Biol. Chem. **36**:217-226.

Griffin, H. G., T. J. Foster, S. Silver, and T. K. Misra. 1987. Cloning and DNA sequence of the mercuric- and organomercurial-resistance determinants of the plasmid pDU1358. Biochem. **84**:3112-3116

Hansen, C. L., G. Zwolinski, D. Martin, and J. W. Williams. 1984. Bacterial removal of mercury from sewage. Biotech. Bioeng. **26**:1330-1333.

Holmes, D. S. 1988. Biotechnology in the mining and metal processing industries: challenges and opportunities. Minerals and Metallurgical Processing. May, pp 49-56.

Ijaki, K. 1981. Enzymatic reduction of mercurous and mercuric ions in *Bacillus cereus*. Can. J. Microbiol. **27**:192-197.

Komura, I., and K. Izaki. 1971. Mechanism of mercuric chloride resistance in microorganisms. I. Vaporization of a mercury compound from mercuric chloride by multiple drug resistance strains of *Escherichia coli*. J. Biochem. (Tokyo) **70**:885-893.

Komura, I., T. Funaba, and K. Izaki. 1971. Mechanism of mercuric chloride resistance in microorganisms. II. NADPH-dependent reduction of mercuric chloride and vaporization of mercury from mercuric chloride by a multiple drug-resistant strain of *Escherichia coli*. J. Biochem. (Tokyo) **70**:895-901.

Laddaga, R. A., L. Chu, T. K. Misra, and S. Silver. 1987. Nucleotide sequence and expression of the mercurial-resistance operon from *Staphylococcus aureus* plasmid pI258. Biochem. **84**:5106-5110.

Nakahara, H., J. L. Schottel, T. Yamada, Y. Miyakawa, M. Asakawa, J. Harville, and S. Silver. 1985. Mercuric reductase enzymes from *Streptomyces* species and group B *Streptococcus*. J. Gen. Microbiol. **131**: 1053-1059.

Nelson, J. D., Jr., H. L. McClam, and R. R. Colwell. 1972. The ecoligy of mercury resistant bacteria in Chesapeake Bay. Proc. Eighth Ann. Conf., Marine Technol. Soc., Washington D.C. pp 303-312.

Olson, G. J., F. D. Porter, J. Rubinstein, and S. Silver. 1982. Mercuric reductase from a mercury-volatilizing strain of *Thiobacillus ferrooxidans*. J. Bacteriol. **151**(3):1230-1236.

Price, F., S. Ross and R. L.Davidson. 1972. Business and the Environment (McGraw-Hill, New York), pp 14-18.

Robinson, J. B., and O. H. Touvinen. 1984. Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biochemical, and genetic analyses. Microbiol. Rev. **48**(2): 95-124.

Silver, S., B. P. Rosen, and T. K. Misra. 1986. DNA sequencing analysis of mercuric and arsenic operons of plasmids from gram negative and gram positive bacteria. In 5th International Symposium on the Genetics of Industrial Microorganisms. Alacevic, M., D. Hranueli, and Z. Toman (eds). pp 357-371.

Silver, M. and A. E. Torma. 1974. Oxidation of metal sulfides by *Thiobacillus ferrooxidans* grown on different substrates Can. J. Microbiol. **20**:141-147.

Summers, A. O., and S. Silver. 1972. Mercury resistance in a plasmid-bearing strain of *Escherichia coli*. J. Bacteriol. **112**:1228-1236.

Tezuka, T., and K. Tonomura. 1976. Purification and properties of an enzyme catalyzing the splitting of carbon-mercury linkages from mercury-resistant *Pseudomonas* K-62. J. Biochem. (Tokyo) **80**:79-87.

Tezuka, T.,and K. Tonomura. 1978. Purification and properties of an enzyme catalyzing the splitting of carbon-mercury linkages from mercury-resistant *Pseudomonas* K-62. J. Bacteriol. **135**:138-143.

Weiss, A. A., S. D. Murphy, and S. Silver. 1977. Mercury and organomercurial resistance determined by plasmids in *Staphylococcus aureus*. J. Bacteriol. **132**:197-208.

Williams, J. W. and S. Silver. 1984. Bacterial resistance and detoxification of heavy metals. Enzyme Microb. Technol. **6**:530-537.

Xu, H. J., C. L. Hansen, and G. S. Choudhury. 1987. Using dairy byproduct for mercury detoxification. Presented at Food Processing Waste Conference, Atlanta, Ga.

Copper Mine Drainage Treatment Plant Driven by Water Wheel

Frank H. Pearson and John L. Potter
Richmond Field Station, University of California at Berkeley

ABSTRACT

A innovative wastewater treatment plant was designed, constructed and operated to remove toxic heavy metals from copper mine drainage at an isolated, subalpine site in Northern California. Though on pilot scale, up to 60% of wastewater from the mine was treated to remove an average of 72% of copper at high loadings, or 93% average copper removal at lower loadings. Treatment involved neutralization first with crushed limestone, then with alkaline reagent solution to approximately *pH* 10, followed by settlement in a basin. Metals removal efficiency was limited by solids separation in the basin, which was consistent with fourth-order removal kinetics. A rudimentary filter intended to remove residual flocculated metal was found ineffectual, conventional filtration being impractical.

Lacking electricity at the site, a water wheel was used to drive the three mechanized neutralization processes used, two processes using crushed limestone for economical preneutralization, and the third dispensing an alkaline reagent solution to attain the required precipitation *pH*. In one limestone process, called the tumbling drum, a cylindrical drum packed with crushed stone rotates at several revolutions per minute to tumble and abrade the stone to fines which dissolve into the acidic water. The other limestone process, called the autogenous mill, is a drum rotating a few revolutions per day for attrition of coatings from crushed stone tumbling inside, which neutralizes water passing through the drum. Also connected to the water wheel is a metering pump to dispense a neutralizing solution of sodium carbonate and\or sodium hydroxide to attain the *pH* necessary for precipitation of toxic heavy metals, prior to removal of metals by settlement in a basin.

As the site is snowbound for about six months of each year, the treatment plant was designed to operate unattended for months on end. Unattended operation over short periods was demonstrated, though after the final inspection before winter the wastewater supply from the mine to the treatment plant was interrupted by rupture of the feed pipe under snow loading, which also crushed two sheds at the site. However, a flume packed with crushed limestone to neutralize mine drainage operated continuously through the winter under snowpack.

This paper presents design procedures and calculations, and construction details for this mine wastewater treatment plant. ©