CRC

BIOTREATMENT SYSTEMS

Volume II

Donald L. Wise

CRC PRESS

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Editor

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PREFACE

Biotreatment Systems is a uniquely valuable reference text consisting of contributed chapters in which are described the most insightful research and development programs around the world. The authors of these contributed chapters are those very conscientious and thoughtful technologists who are investigating pragmatic solutions to environmental problems. This important text has as the major theme the biotreatment of organic residues. This major theme primarily encompasses the field of anaerobic methane fermentation, with an emphasis on treatment of complex wastes. The text is intended to present a comprehensive overview of the most practical research programs that are being carried out in this emerging field of international significance. Due to the fact that both research and development have been carried out at major centers around the world, great care has been taken to include chapters from an international perspective. Further, as a perusal of the chapter titles will indicate, a special emphasis has been made to address both the important research aspects and the practical aspects of the work on biotreatment systems. It is to be noted that each chapter included in this text is the work of a particular individual or group. There are no multiple chapters by more than one author or group. Thus, each of the included chapters most often reflects the dedicated career efforts of these workers. Further, each contributed chapter is presented on a stand-alone basis so that the reader will find it helpful to consider only the theme of each chapter. On the other hand, there is the unifying theme with all chapters of addressing biotreatment systems research and development. A reader of this text, just entering the field, will find this text provides an excellent state-of-the-art presentation of the international import of work on biotreatment systems, with an emphasis on methane fermentation. A reader of this text, who has experience in this field, will find the text to be essential for assessment and referral of this increasingly valuable area of technology.

THE EDITOR

Donald L. Wise, Ph.D., P.E., is Founder and President of Cambridge Scientific, Inc., Belmont, Massachusetts. Dr. Wise also holds the Cabot Chair of Chemical Engineering at Northeastern University, Boston, Massachusetts. Dr. Wise received his B.S. (magna cum laude), M.S., and Ph.D. degrees in chemical engineering at the University of Pittsburgh. Dr. Wise is a specialist in process and biochemical engineering as well as advanced biomaterials development. During his career he has managed a series of programs to develop processes for production of fuel gas, liquid fuels, and organic chemicals from municipal solid waste, an array of agricultural residues, and a wide variety of crop-grown biomass, especially aquatic biomass. Dr. Wise has also been primarily responsible for the initiation of development work on fossil fuels such as peat and lignite to gaseous fuel, liquid fuels, and organic chemicals, and he also originated work on the bioconversion of coal gasifier product gases to these products. Dr. Wise initiated a program to establish the engineering feasibility of converting large-scale combined agricultural residues to fuel gas by the action of microorganisms, a project ultimately involving joint effort with research workers in fifteen countries around the world.

Dr. Wise has worked in the area of biotechnology research and development for 2 decades, has approximately 50 publications in the field, and has edited a number of reference texts. As Associate Editor of *Solar Energy*, the journal of the International Solar Energy Society, he is responsible for the review of manuscripts in the biomass/bioconversion area. Dr. Wise is also on the Editorial Board of *Resources and Conservation*, an international journal published by Elsevier, Amsterdam. He has served as an international consultant in bioconversion for the United Nations and for the U.S. Agency for International Development (AID).

A meaningful portion of these programs that Dr. Wise initiated, and has been carrying out, is his meeting with experts across the U.S. and around the world, to become familiar with both current and practical aspects of bioconversion systems.

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Chapter 1

TOXICITY OF HEAVY METALS TO THERMOPHILIC ANAEROBIC DIGESTION

Birgitte K. Ahring and Peter Westermann

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I. INTRODUCTION

Anaerobic digestion of organic materials has been known for decades as a means of reducing the biological oxygen demand (BOD) and simultaneously recovering chemically bound energy as a combustible gas, mainly consisting of methane and carbon dioxide.

Municipal and industrial waste waters containing high organic loads have primarily been treated in aerobic activated sludge processes, which involves several disadvantages such as (1) large aeration costs to ensure optimal oxygen penetration throughout the sludge, (2) odor problems in connection with sludge treatment in open basins, (3) low organic loadings, generally below 2 kg BOD per cubic meter sludge, and (4) production of large quantities of unstabilized sludge difficult to dewater.

Because of rapidly increasing energy prices, anaerobic biological treatment of sewage sludge and waste from farm animals has gained interest during the recent years. The major disadvantage of the anaerobic treatment process, however, is the prolonged retention time (typically 15 to 35 days) required to attain a satisfactory BOD removal, gas production, and sludge stabilization. This is primarily due to the slow metabolic growth rates of the bacteria involved in the degradation process in contrast to the corresponding aerobic metabolism of organic matter. By increasing the temperature to the thermophilic range (50 to 60°C), a higher rate of microbial metabolism and, hence, a decrease in retention time to less than 10 days may be obtained.[1] This fact, in combination with other advantages, which will be discussed later, has increased the interest in thermophilic anaerobic digestion as a means of biological treatment of various organic wastes.

The organic matter subjected to anaerobic digestion is generally very heterogeneous and might contain compounds toxic to the anaerobic microorganisms in the digester. Heavy metals such as copper, cadmium, chromium, mercury, nickel, and zinc are often present in combined municipal and industrial waste water, and originate predominantly from industrial discharges. Wastes from farm animals, however, also may contain toxic amounts of heavy metals added to the cereal diets of the growing animals.

Historically, the interest in the presence of heavy metals in waste water has been moderate as long as the concentrations were at subtoxic levels. Early investigations of cation toxicity to anaerobic digestion showed that virtually all cations were toxic at some level, but heavy metals were found more toxic than light metal ions.[2] Interestingly, stimulatory effects of subtoxic concentrations of heavy metals also have been observed. Hotchkiss,[3] for instance, found that very low concentrations of lead and mercury stimulated growth of various bacterial cultures.

The increasing consciousness concerning environmental protection, the evidence of toxicity of heavy metals even at low concentrations, and the fact that heavy metals concentrate through the food chain terminally reaching man have led to increased requirements for investigations of the fate and effects of heavy metals in various biological systems.

The present chapter discusses the impact of heavy metals as environmental pollutants to anaerobic digestion. As thermophilic digestion is an expanding technology within the treatment of organic wastes and, as very few investigations concerning toxicity of heavy metals have been carried out within this area, this chapter will focus on the thermophilic aspects of heavy metal toxicity. Toxicity to mesophilic digestion, however, will form a large part of the referred investigations, as the majority of experiments and observations have taken place within this field. Furthermore, many parallels can be drawn between mesophilic and thermophilic digestion, as will be discussed later.

A. Microbiology of Anaerobic Digestion
The main components of the dry matter of sewage sludge and manure are polymeric compounds such as proteins, celluloses, hemicelluloses, and lignins. Oligomeric and mon-
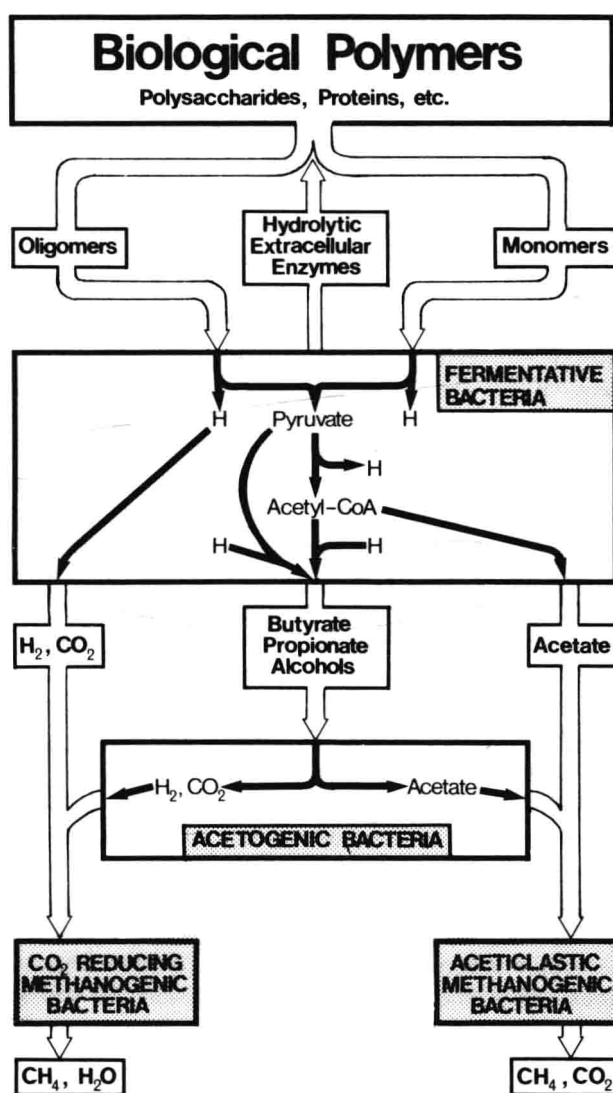
FIGURE 1. Main pathways in the anaerobic digestion process.

omeric compounds also occur in the waste as a result of the partial degradation of the polymers. Furthermore, lipids and hydrocarbons are present in various amounts in the waste. Figure 1 shows the main features of anaerobic metabolism and the microbial groups responsible for the degradation of the organic material supplied to a digester. At least three entirely different groups of organisms are active in the conversion of complex organic molecules into the main terminal compounds: methane, carbon dioxide, and water. The fermentative bacteria hydrolyze the polymers to mono- and oligomers by means of extracellular enzymes such as proteases, cellulases, and amylases. The soluble products are then absorbed by the same group of bacteria and fermented to an array of products such as alcohols, short-chained fatty acids, hydrogen, and carbon dioxide. The alcohols and the short-chained fatty acids are then oxidized by the hydrogen-producing acetogenic bacteria leading to the formation of hydrogen, carbon dioxide, and acetate. The acetogenic bacteria are only able to metabolize the intermediates if the partial pressure of hydrogen is kept low, primarily due to the activity of the carbon dioxide-reducing methanogenic bacteria. Acetate

is split into methane and carbon dioxide by aceticlastic methanogens and perhaps into hydrogen and carbon dioxide by a recently discovered syntrophic bacterium.[4] The hydrogen and some of the carbon dioxide are finally converted into methane by carbon dioxide-reducing methane bacteria. Significant amounts of acetate are also produced by the fermentative bacteria and may be directly converted into methane and carbon dioxide. The further thermodynamic principles of anaerobic digestion have been extensively discussed by Bryant.[5]

Although many studies have been carried out on thermophilic anaerobic digestion, only few isolations of the bacterial species involved in the metabolism have been published compared with mesophilic conditions. Among the fermentative bacteria, thermophilic *Clostridia* with various fermentation patterns[6-8] and acetate- and ethanol-producing bacteria[9-11] belonging to several known or new genera have been isolated. Varel[12] characterized 64 randomly picked fermentative anaerobic bacteria isolated from a thermophilic bench scale digester and found that species diversity among the isolated bacteria was low. Besides the acetate-oxidizing rod described by Zinder and Koch,[4] only few thermophilic obligately proton-reducing bacteria have been described until now. Henson and Smith[13] recently described a butyrate-oxidizing thermophilic bacterium in coculture with a hydrogen-utilizing methanogen. Another butyrate-oxidizing thermophilic acetogen has been isolated in coculture with both acetate decarboxylating and hydrogen-utilizing methanogens.[14,15] Only a few methanogens have been characterized from thermophilic anaerobic digesters. *Methanosarcina thermophila*[16,17] is an obligate acetate-decarboxylating methanogen with a temperature optimum near 50°C and a maximum growth temperature of 55°C. *Methanobacterium thermoautotrophicum*[18] is a carbon dioxide-reducing methanogen with a temperature optimum at 65 to 70°C and a maximum growth temperature of 75°C. The thermophilic acetate-decarboxylating methanogen (TAM) has a temperature optimum at 60°C, and a maximum growth temperature of 70°C.[19,20]

Recently, two new thermophilic *Methanobacterium* sp. and a *Methanogenium* sp. have been isolated from sewage sludge or biogas plants.[21-23]

B. Mesophilic and Thermophilic Digestion

Compared to mesophilic anaerobic digestion, thermophilic digestion offers several advantages. As previously mentioned, the retention time is reduced under thermophilic conditions as the growth rates and, hence, the substrate utilization rates are higher at the elevated temperature. This may reduce the capital investments necessary for the construction of the digester, as a smaller volume is required to treat the same amount of waste compared to a mesophilic digester. Decreasing the retention time without decreasing the efficiency by shifting the operation from mesophilic to thermophilic temperatures also may save the capital investments of constructing a new digester if an already existing mesophilic unit is overburdened.[24]

The high temperature maintained during thermophilic digestion leads to an increased destruction of pathogenic organisms found in the sludge. In the disposal of digested sludge upon land, minimization of pathogenic organisms is very important to avoid public health hazards. In the digestion of animal wastes, destruction of parasites is also important, as the disposal of digested sludge on pastures might lead to a reinfection of grazing animals. Popova and Bolotina[25] found that mesophilic-digested sludge retained up to 20% viable helminth eggs, while thermophilic-digested sludge was free of viable eggs. Cooney and Wise[26] calculated that a residence time of 1 hr was sufficient to reduce a coliform population by eight orders of magnitude at 55°C.

Improved solids-liquid separation is important if digested sludge is to be dewatered prior to further processing or ultimate disposal. Vacuum filter yields were found to increase several times when thermophilic-digested sludge was compared with mesophilic-digested sludge.[27] Several reasons may account for the improved separation. Garber[27] considered that the higher

filter yields were a result of larger particles and different morphology of the biomass in thermophilc digested sludge. Cooney and Wise[26] also assigned the improved separation to a decrease in water viscosity which is diminished by 50% at a temperature increase from 20 to 55°C.

The degradation of long-chained fatty acids proceeds at least twice as fast at thermophilic temperatures than under mesophilic temperatures. This implies better decomposition of fatty acids such as stearate and palmitate usually present in domestic waste water.[28]

As the energy required for cell maintainance is higher at thermophilic temperatures, smaller amounts of substrate are consumed for biomass production, and a larger part is transformed into terminal products of the energy metabolism.[26]

As a consequence of the extreme conditions maintained during thermophilic anerobic digestion, and the fewer species involved in the anaerobic metabolism, one would expect that process instability might be the most significant disadvantage of the process, since instability is one of the major problems in mesophilic anaerobic digestion. In considering the application of thermophilic anaerobic digestion to wastes containing toxic agents such as heavy metals, process instability becomes a central and very important problem. The experiences attained regarding stability problems in thermophilic anaerobic digestion are contradictory.[1] Generally, temperature fluctuations have been considered to be a major destabilizing factor, as very few investigations have dealt with other potential causes of destabilization. Reports concerning specific observations of heavy-metal toxicity will be discussed in a later section.

Higher energy amounts are required to bring the sludge to the operating temperature and to maintain the thermophilic conditions, when compared to mesophilic digestion. The higher metabolic rate and the shorter retention time, however, should counter-balance this requirement, resulting in a net energy surplus if the heating system is conveniently constructed and heat exchangers are used to preheat the incoming feed sludge.[1]

II. HEAVY METALS IN WASTES

A. Sources of Heavy Metals

The heavy metals found in an anaerobic digester may originate from either natural or artificial sources. Primary natural sources include weathering of rocks and sediments and release from hydrothermal and volcanic activity. The heavy metals released from these sources may be transported to other sites, either suspended as airborne particles or as solutes in streams and rivers, finally reaching the biosphere. The artificial sources are usually associated with man-made activities and constitute the predominating part of the heavy metals present in wastes reaching waste treatment plants, and will be the only sources discussed in the following.

The main source of cadmium in waste water is industrial manufacturing and utilization processes. In the European Economic Communities, the electroplating industry is responsible for more than 50% of the cadmium discharged to sewage treatment plants.[29] The pigment manufacturing industry is responsible for approximately 25% of the cadmium released to the waste waters, while about 20% is discarded from the manufacturing of stabilizers and nickel-cadmium batteries. Minor contributions of cadmium reaching waste treatment plants are due to alloy manufacturing and airborne releases associated with iron and steel industry and mining.

Chromium is primarily used in the plating industry and as an alloy metal and most of the metal discarded to waste waters is derived from these industrial sources. Smaller amounts are derived from the chrome-tanning and wood-impregnation industries.

Copper is released in large amounts from the electroplating industry, and has been shown to cause several failures in waste water treatment plants.[30] Smaller amounts of copper

originate from copper tubes used as water pipes in sanitary installations. Piggery wastes might contain significant amounts of copper, as cereal diets of fattening pigs often contain up to 200 mg/ℓ copper to increase food conversion efficiency.[31] As the copper accumulates in the waste, farm digesters might be subjected to continuous or shock loadings of large concentrations of copper.

Most of the mercury released as a consequence of man-made activities originates from evaporation due to the volatile character of this metal. The major industrial sources responsible for mercury discarded to the waste water are phosphoric acid, chlorine-alkali, and cellulose-manufacturing industries. Smaller amounts are released in laboratory wastes and from instrument production.

Nickel, like chromium, is primarily used as an alloy metal and in the electroplating industry. The most important sources of the metal in the waste water are industries engaged in this manufacturing. The production of accumulators is responsible for minor contributions to waste waters.

Elevated concentrations of lead in waste waters might originate from surface runoffs containing residues from combustion of leaded gasoline, coal burning, and metal-smelting activities. Furthermore, lead may originate from corrosion of solderings and lead tubes used in sanitary installations. The major industrial sources of lead pollution include the manufacturing of antiknock compounds, brass production, and the production of accumulators.

Industrial wastes containing heavy metals may be discharged continuously in, for instance, drainage and rinse water from metal-plating operations, or over short periods from plating bath dumps or spills. The particular manner of discharge is important for the acclimational ability of the anaerobic digester to withstand increased amounts of heavy metals without serious failure. Hayes and Theis[32] found that mesophilic anaerobic bench-scale digesters shock loaded with various heavy metals produced a lower toxic limit than when the corresponding metals were step fed to the digesters at increasing concentrations.

B. Distribution of Heavy Metals in Sludge

The toxicity of heavy metals to anaerobic digestion depends upon the various chemical forms which the metals may assume under anaerobic conditions at the temperature and pH value in the digester. Furthermore, the binding of the heavy metals to environmental constituents may determine the toxicity to the bacteria involved in the digestion process. If a metal is partially or totally removed from solution by precipitation or binding, a decrease or complete disappearance of toxicity may be the result. Several compounds present in waste water and farmyard manure may bind or precipitate heavy metals, thereby reducing the acessibility and, hence, the toxicity to the bacteria in the anaerobic digesters.

The chemical forms of the precipitated metals also may be of importance, as the possible removal of precipitated metals from digested sludge prior to dewatering depends upon the potential solubilization of the precipitated metals. If all metals present in the sludge are precipitated as sulfides, carbonates, and hydroxides, metal removal can be achieved through a mild acidification, followed by a dewatering of the sludge. Organically bound metals, however, require prolonged treatment in strong acid to release the metals, and thus to enable a separation.

Activated sludge has been shown to remove and accumulate heavy metal ions from the waste water solution. Cheng et al.[33] found that heavy metals in low concentrations bind to the biofloc through the formation of metallo-organic complexes, while higher metal concentrations resulted in the precipitation of inorganic metal compounds in addition to sludge uptake. The accumulation of heavy metals in primary and secondary sludge was found to result in concentrations of 5 to 38 times the metal concentrations in the original waste water.[34] As the anaerobic digesters in sewage treatment plants normally are fed primary and secondary sludge, heavy metals may reach considerable concentrations in the digesters compared with the concentration in the waste water.

Clay particles have been found to reduce the toxic effects of zinc and cadmiun due to sorption of the metals to the clay lattices by cation exchange.[35] The sorption of heavy metals to humic matter such as fulvic and humic acid by cation exchange also may reduce the free metal concentration and, hence, the toxicity to the bacteria.[36] Several organic compounds such as amino acids, simple aliphatic acids, sugar acids, and phenols present in waste water and farmyard manure may act as complexing and chelating agents reducing the availability of the heavy metals.[36]

Inorganic precipitation of heavy metals also contributes as an important toxicity-reducing factor in anaerobic digestion. At the low redox potentials found in a digester, microbial conversions of sulfate to sulfide result in the formation of extremely insoluble metal sulfides as long as the metal concentrations are below the total sulfide level. At very low sulfide concentrations and moderate metal concentrations, immobilization of the heavy metals is primarily due to the formation of metallo-organic complexes with the sludge as found under aerobic conditions.[37] At higher metal concentrations the metals also precipitate as hydroxides, carbonates, and phosphates.[33] The significance of sulfide as a precipitant in anaerobic digestion has been extensively studied by Lawrence and McCarthy.[30] They added several hundred milligrams per liter of different heavy metal sulfates to mesophilic anaerobic bench-scale digesters, and found a satisfactory performance due to the precipitation of the metals as sulfides upon reduction of the sulfate to sulfide. However, gas production immediately decreased to almost zero.

The low redox potential in an anaerobic digester also affects the valences of the heavy metals present, resulting in primarily reduced forms of the metal ions. The differently charged form of the same metal may cause differences in toxicity to the bacteria exposed. Forsberg[38] found that hexavalent chromium decreased the fermentation rate of rumen bacteria by 50% at a concentration of 70 mg/ℓ, while divalent chromium had no effect even at a concentration of 640 mg/ℓ. Mosey and Hughes,[39] however, found that the toxicity of hexavalent chromiun to mesophilic anaerobic digestion was similar to the toxicity of trivalent chromium, as a consequence of an immediate reduction of chromium VI to III.

Next to the redox potential, the pH value in the digester is a very important parameter to the solubility of heavy metals. Slightly alkaline conditions promote the formation of insoluble carbonates and hydroxides of several heavy metals, while low pH values generally increase the solubility of heavy metals. The effect of pH on the toxicity of heavy metals is equivocal, however. In this way, the toxicity of lead to *Tricoderma viridae* was found to decrease when pH was raised to a slightly alkaline level, while the toxicity of cadmium to various bacteria and fungi was increased when pH was raised to 9.[36]

In toxicity studies performed with pure and mixed cultures in more or less artificial media, organic compounds such as cysteine, ethylenediaminetetraacetic acid (EDTA), and nitrilo-triacetate included in the mediun have been found to affect the toxicity of the added heavy metals. Rumen fluid, commonly added to anaerobic culture media, was reported to reduce the toxicity of heavy metals to anaerobic bacteria.[38] However, Hallas et al.[40] found that the chelating reducing amino acid, cysteine, had no effect upon the toxicity of tin to estuarine microorganisms.[40] Sodium citrate was found to increase the toxicity of cadmium to pure and mixed populations of aerobic microorganisms.[41] Walker and Houston.[42] even found that the toxicity of cadmium to *Pseudomonas aeruginosa and Aeromonas* sp. was higher, when grown in a complex medium instead of a mineral medium.

Hayes and Theis[32] investigated the distribution of nickel, cadmium, zinc, copper, and chromium in mesophilic anaerobic digester and divided the sludge into four metal-containing fractions: (1) the soluble fraction, (2) the precipitated fraction, (3) the intracellular fraction, and (4) the extracellular fraction. Negligible amounts of the investigated metals were found in the soluble phase, except for nickel, were 1 to 3% were found to be in solution. Less than 1% was found in the extracellular (EDTA-extractable) fraction while 20 to 30% of the

added metals were found in the intracellular fraction. Generally, the major part of the heavy metals (60 to 70%) was precipitated, except for chromium where 60% was found in the intracellular fraction, while only 35% was precipitated. In investigations of heavy-metal distribution in thermophilic anaerobic digesters, considerably higher amounts of cadmium, copper and nickel were found to remain in solution.[43] After the addition of 10 mg and 300 mg/ℓ cadmium, respectively, 0.1 and 1.6% were found in the soluble phase. The corresponding values for copper were 2.2 and 2.7% while 7.4 and 7.5% nickel were found in the soluble phase of the digested sludge.

III. TOXICITY OF HEAVY METALS TO MICROORGANISMS

The effects of heavy metals on the metabolism of microorganisms have been shown to be concentration dependent. In this way, very low metal concentrations may be stimulatory or even required for growth. For instance, copper, zinc, nickel, and iron may act as components or activators of enzymes in the microorganisms. Normally, heavy metals present in the waste water are regarded as toxic to the bacteria involved in the digestion process, as even low concentrations cause inhibition of the metabolism of the involved bacteria.

A. Microbial Uptake of Heavy Metals

Uptake of heavy metals by microorganisms has been shown to occur in mainly two ways: (1) either the heavy metals may be involved in a nonspecific binding to the cell surfaces, slime layers, extracellular matrices, or (2) they may be involved in a metabolically dependent intracellular uptake. For instance, uranium was found to accumulate extracellularly as needle-like fibrils in a layer approximately 2 μm on the surface of *Saccharomyces cerevisiae*, but formed dense intracellular deposits in *P. aeruginosa*.[44] In contrast to the intracellular uptake of metal cations by microorganisms which involves an energy-dependent transport system, metal adsorption by negatively charged groups on the bacterial surfaces is reversible, and occurs independently of the microbial energy metabolism.[45] Several factors influence the microbial removal of heavy metals from aqueous solutions, such as the specific surface properties of the organism, the cell metabolism, and the physiochemical parameters of the environment.[46] Surface binding is very important to slime-producing bacteria, having an extracellular matrix acting as a barrier, where metals may be adsorbed and precipitated. For instance, bacterial extracellular polysaccharides in activated sludge maintain an extensive complexing capacity for heavy metals. Biomass, mainly consisting of an acidic polysaccharide produced by *Zoogloea ramigera*, has been used as an adsorbing agent in a continuous process for the recovery of heavy metals.[47] Also the immobilized cells of *Citrobacter* sp. removed heavy metals such as cadmium, copper, and lead with high efficiency from challenge flows containing the metals.[48] The mechanism of metal uptake is mediated by a cell-bound metal phosphatase which liberates inorganic from organic phosphate to precipitate the heavy metals as cell-bound metal phosphates. Dead cells and isolated cell walls have been shown to be effective in binding heavy metals to the surfaces of these cells. Hoyle and Beveridge[49] investigated the binding of metal ions to the outer membrane of *Escherichia coli*. They found a selective trapping of metal cations on the hydrophilic surface of the cell membrane, which also was observed in investigations of cell walls from Gram-positive bacteria.[50] Beveridge[50] observed a nonstoichiometric accumulation of metals on isolated cell walls of *Bacillus subtilis*. He suggested that metal molecules complex with existing reactive sites, and that additional metal ions "crystallize" on these bound molecules. Kurek et al[51] investigated the ability of different soil constituents to remove cadmium from a liquid medium, and reached the conclusion that microbial sorption, in particular by dead cells, may be an important immobilizing factor of cadmium in soil environments. When the same amounts (on a dry-weight basis) of living and dead bacterial cells, sand, and clay were compared

the dead cells were the most efficient in sorption of cadmium. No attempt, however, was performed to elucidate the mechanism of cadmium sorption or to determine whether the heavy metals were bound to the cell surfaces or taken up intracellularly. In most of the organisms studied, the amount of metals bound by the cell surfaces is relatively small when compared to the amounts that can be taken up intracellularly by energy-requiring processes.[37] *Methanosarcina barkeri* was shown to incorporate lead or cadmium into all cell compartments, whereas zinc and copper were only incorporated to a minor degree.[52] At high metal concentrations, intracellular precipitation of the metals may occur after uptake. This might be a means of detoxification, since the metals in this way are compartmentated and may be converted to a more innocuous form.

B. Organic Binding of Heavy Metals

The toxic effects of heavy metals to microorganisms are primarily due to the potent inactivation of enzymes through reactions with, for instance, sulfhydryl groups. The binding of metals to organic compounds depends upon the nature of the compounds and the size of the metals. Carboxylic, hydroxylic, phosphate, and amino groups preferentially complex with small, highly electropositive metal ions such as aluminium, chromium, cobalt, iron, titanium, zinc, and tin.[53] Sulfhydryl groups preferentially form covalent and covalent-type bindings with large metal ions such as copper, silver, gold, mercury, and cadmium.[53]

Proteins contain positively and negatively charged groups such as NH, S, and CO that may attract as well as repulse the approaching metal ions, even though they are not necessarily involved in the possible binding of the metals. Possible sites for binding, however, may be buried inside the proteins, and thus be inaccessible to the metals.

Other mechanisms whereby heavy metals affect microorganisms include production of stable precipitates or chelates with essential metabolites, catalyzation of the decomposition of essential metabolites, and replacement of structurally or electrochemically important elements, thereby interfering with enzymatic or cellular functions.[54]

C. Sensitivity of Microorganisms to Heavy Metals

The response of a microorganism to heavy metals is influenced by numerous biotic and abiotic factors. The biotic factors of importance include the physiological state of the organism in question (nutritional level, acclimation/adaption to the toxic substance, genetic adaptions such as plasmid-conferred resistance) while the abiotic factors include the physiochemical characteristics of the environment (precipitation and complexation of the metals, pH, temperature, and redox potential).

Very few investigations have been executed concerning the sensitivity of pure cultures of anaerobic bacteria to heavy metals, although data on the inhibitory concentrations might provide a means to better understand the responses of the metabolically important bacterial groups active during anaerobic digestion of waste. Forsberg[38] studied the inhibitory effects of heavy metals to the fermentation of the rumen microflora and to pure cultures of eight rumen bacteria. No uniform pattern of toxicity of the heavy metals tested (mercury, copper, chromium, cadmium, lead, cobalt, nickel, tin, selen, methylmercury, aluminium — all as chlorides) was observed, except that copper and mercury generally were the most toxic elements. The most sensitive bacteria included *Bacteroides succinogenes, B. amylophilus, Ruminococcus albus,* and *Eubacterium ruminantium.* Bacteria showing intermediate sensitivity to the metals were *Butyrivibrio fibrisolvens, Selenomonas ruminantium,* and *Megasphaera elsdenii,* while *Streptococcus bovis* exibited the lowest sensitivity. The lowest metal concentrations causing growth inhibition of the tested rumen bacteria were in the ranges of 10 to 326 mg/ℓ mercury, 10 to 250 mg/ℓ copper, 200 mg/ℓ chromium, 20 to 200 mg/ℓ cadmium, and 20 to 200 mg/ℓ selen. The highest concentrations of lead (150 mg/ℓ) tin (200mg/ℓ), and aluminum (200 gm/ℓ) tested had no effect on the growth of the bacteria.