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Chromium Toxicity Effects on Microorganisms and Treatment Processes

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Abstract

Chromium is one of the heavy metals that has adverse effects on microbes and treatment processes. The toxicity and potential carcinogenicity of chromium has led to the concern about its effects in the environment. Even though chromium occurs in various oxidation states, hexavalent and trivalent forms are the most significant compounds. Hexavalent chromium is mobile in soil, more toxic, and penetrates more readily into the cell membranes than the trivalent form.

Many factors like the biotic and abiotic factors in the environment, and characteristics of the pollutant influence the toxicity of chromium on microorganisms. The adverse effects of chromium can be seen in many microbe-mediated processes including carbon mineralization, nitrogen transformation and mineralization of phosphorous and sulfur. The presence of chromium decreases microbial populations and also affects microbial respiration. Chromium is found to be both toxic and mutagenic to various microorganisms.

Chromium also affects growth rate, Monod constant, and microbial population in the treatment processes. Various factors like acclimation, type of injection (stepwise or shock injection), and temperature influence the effects of chromium on the treatment processes.

This paper reviews the toxic effects of chromium on microorganisms and on some of the treatment processes like activated sludge, anaerobic digestion, and denitrification.

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Chapter 1

Introduction

The activities, ecologies, and population of microorganisms in many habitats are adversely influenced by increased deposition of harmful pollutants into the environment. These pollutants may be derived from both industrial and domestic sources. Microbes are the key components in the biogeochemical cycling of various chemical elements, in the incorporation of energy, in the processes necessary for the maintenance of the fertility of aquatic and terrestrial habitats, and for waste reduction. Pollutants affect complex microbial interactions and can cause elimination, diminution, or enhancement of specific microbial populations and hence affect the ecology of the biosphere.

Chromium (Cr) is a toxic, heavy metal contaminant and can have adverse effects on microbes and microbe-mediated ecological processes. Improper disposal of chromium has led to the contamination of soil and groundwater. The toxicity and potential carcinogenicity of chromium has led to the concern about its fate in the environment and the potential for human exposure. This paper deals with the toxic effects of chromium on the microorganisms and how it affects the treatment processes.

1.1 Background

Chromium, unlike many of the other metals, was not known to ancient people. There is no record that they knew or used chromium and its compounds. This could have been due to the unattractive appearance and refractory nature of the mineral chromite, and the scarcity of the brilliant orange colored lead chromate (Udy, 1956).

Chromium was discovered (Udy, 1956) around 1797 by a French chemist, Louis Nicolaus Vauquelin, in ore from Siberia. Chromite was first used to make chromium chemicals about 1800-1816 and was first employed as a refractory in France around 1879 (Udy, 1956). Intensified use of chromium metal in steel started about 1908-1912 (Udy, 1956).

Chromium is one of the rock forming elements of the earth's crust and occurs in simple silicates, alumino silicates, and complex salts. Chromium is ubiquitous in the universe (Udy, 1956), ranking sixth on the earth's crust, fifteenth in sea water and fifteenth in the body of man. In crust and sea water its average concentration is 200ppm and 1 to 2.5ppb, respectively (Burrows, 1983). Chromium combines with iron and oxygen to form chromite ore. The Soviet Union, South Africa, Albania, and Zimbabwe together account for 75% of the world's chromite production (Kapil, 1990).

Chromite ore has not been mined in the United States since 1961. In 1985, the U.S. became completely dependent on importation for its primary chromium supply (Kapil,1990).

Chromium metal is obtained from chrome ore, chromite (FeCr_2O_4), by electrolysis of chromium solutions by direct reduction (ferro chrome) and by reducing the oxide with finely divided carbon or aluminium (MSDS, 1989).

1.2 Physical and Chemical Properties

Chromium with atomic number of 24 and a mass of 52.01 belongs to the first series of the transition elements. Its position in the periodic table is under VI B. Chromium can occur in everyone of the oxidation states from -2 to +6, but only 0, +2, +3, and +6 states are common (Burrows,1983).

Chromium in chromite ore is in its trivalent state whereas industrial processes produce elemental metal and hexavalent chromium. The health effects of chromium are at least partially related to the valence state of the metal at the time of exposure (Kapil,1990). Hexavalent (Cr VI) and trivalent (Cr III) compounds are the most biologically significant compounds (Kapil, 1990). Cr (III) is an essential dietary mineral in low doses, whereas certain compounds of Cr (VI) appear to be carcinogenic (Kapil, 1990).

Hexavalent chromium has been considered to be a toxic metal for many years because of the poisonous effects of high exposures to industrial bichromates. Trivalent chromium is believed to be less toxic (Burrows, 1983). Table 1.1 gives the physical properties of chromium (MSDS, 1989).

Empirical formula	Cr
CAS Number	7440-47-3
Atomic Weight	52 g/mol
Specific Gravity	7.2 @ 68°F
Boiling Point	4788°F
Melting Point	3452°F
Vapor Pressure	1 mm Hg @ 2941°F
Vapor Density	1.79
Appearance	Steel gray, lustrous
Odor	No odor
Water Solubility	Insoluble

Table 1.1 Physical properties of chromium (MSDS, 1989)

Chromium is a hard, steel gray metal, highly resistant to oxidation even at high temperatures (MSDS,1989). Chromium reacts readily with dilute (not nitric) acids to form chromous salts. It is soluble in acids (not nitric) and strong alkalis. Its powder is incompatible with strong oxidizing agents, including high oxygen concentration (MSDS, 1989). Evaporation of mercury from chromium amalgam leaves pyrophoric chromium. Finely divided chromium attains incandescence with nitrogen oxide, potassium

chlorate, and sulfur dioxide (MSDS,1989). Molten lithium at 18°C severely attacks chromium. Thermal oxidative decomposition of chromium can produce toxic chromium oxide fumes (MSDS,1989).

1.3 Uses

Chromium is used in three basic industries- metallurgical, chemical, and refractory (heat resistance applications). In the metallurgical industry, chromium is an important component of stainless steels and various metal alloys. Metal joint prostheses made of chromium alloys are widely employed in clinical orthopedics (Kapil, 1990). In the chemical industry, chromium is used primarily in paint pigments (chromium compounds can be red, yellow, orange, and green), chrome plating, leather tanning, and wood treatment. Smaller amounts are used in drilling muds, water treatment, catalysts, safety matches, copy machine toners, corrosion inhibitors, photographic chemicals and magnetic tapes (Kapil, 1990). Refractory uses of chromium include magnesite-chrome fire bricks for metallurgical furnace linings and granular chromite for various other heat- resistant applications (Kapil, 1990). Other uses of chromium are as follows (MSDS,1989)

- * Chromeplating other metals
- * For greatly increasing metal resistance and durability
- * In manufacturing chrome-steel or chrome-nickel steel

alloys (stainless steel)

- * As a constituent of inorganic pigments
- * As protective coating for automotive and equipment accessories
- * In nuclear and high temperature research

1.4 Health Hazards

Cr (III) and Cr (VI) are released to the environment primarily from stationary point sources resulting from human activities. Of the total atmospheric chromium emissions in the United States, approximately 64% is due to Cr (III) from fuel combustion (residential, commercial, and industrial) and steel production (Kapil, 1990); while 32% is due to Cr (VI) from chemical manufacture, chrome plating, and industrial cooling towers using chromate chemicals as rust inhibitors (Kapil, 1990). A U.S. E.P.A. report estimates that in the United States about 2,840 metric tons of total chromium are emitted annually into the atmosphere, compared to approximately 110,000 tons of chromium metal produced each year (Kapil, 1990).

Electroplating, leather tanning, and textile industries release relatively large amounts of chromium. Less significant environmental sources of chromium include road dust contaminated by emissions of chromium-based

catalytic converters or erosion products of asbestos brake lining, cement dust, tobacco smoke, and food stuffs (Kapil, 1990).

Table 1.2 gives the acceptable concentrations of chromium (risk based concentrations) in the environment.

MCL	0.1 mg/L
Tap water	18 µg/L
Ambient air	0.00015 µg/m ³
Fish	0.68 mg/Kg
Industrial soil	510 mg/Kg
Residential soil	39 mg/Kg

Table 1.2 E.P.A. Region III Risk-based Screening Table (EPA, 1994)

Environmental sources of chromium exposure includes air borne emissions from chemical plants and incineration facilities, effluents from chemical plants, contaminated landfills, cement dust, road dust from catalytic converter erosion and asbestos brake lining erosion, and tobacco smoke (Kapil, 1990). Occupational sources of chromium exposure are welding of alloys and steel, leather tanning (soluble Cr III), chrome plating (soluble Cr VI), chrome alloy production, textile manufacturing, paints and pigments (insoluble Cr VI), photo engraving, and copier serving.

Workers in industries using chromium, especially stainless steel welding, chromate production, chrome plating, and chrome pigment

industries, where exposure is primarily due to Cr (VI), are at increased risk of chromium's effects (Kapil, 1990).

Entry routes of chromium into the human body include inhalation, ingestion, and dermal absorption. Rates of chromium uptake from the gastrointestinal tract (Kapil, 1990) are relatively low and depend on a number of factors including :

- * Valance state (Cr VI more readily absorbed than Cr III)
- * Chemical form (organic chromium more readily absorbed than inorganic chromium)
- * Water solubility of the compound
- * Gastrointestinal transit time

In humans and animals, less than 1% of inorganic Cr (III) and about 10% of inorganic Cr (VI) is absorbed from the gut. Cr (VI) is better absorbed from the gut, lungs, and skin than Cr (III) (Kapil, 1990). After entering the body, Cr (III) does not readily cross cell membranes, but binds directly to transferrin, an iron-transporting protein in the plasma (Kapil, 1990).

However, in the case of Cr (VI), after absorption, it is rapidly taken up by erythrocytes and reduced to Cr (III) inside the cell (Kapil, 1990). Regardless of the source, Cr (III) is widely distributed in the body and accounts for most of the chromium in the plasma or tissues (Kapil, 1990). Excretion of chromium occurs primarily through the urine and small amounts are excreted through hair, nails, and sweat (Kapil, 1990).

Chromium (III) is an essential trace metal in human nutrition and plays a role in maintaining normal metabolism of glucose, fat, and cholesterol (Kapil, 1990). The estimated safe and adequate daily intake of chromium for adults is in the range of 50 - 200 μ g/day (Kapil, 1990). Chromium deficiency is characterized by glucose intolerance, glycosuria, hyper cholesterolemia, decreased longevity, decreased sperm counts, and impaired fertility (Kapil, 1990). Major factors governing the toxicity of chromium compounds are the oxidation state and solubility (Kapil, 1990). Chromium (VI) compounds are powerful oxidizing agents and tend to be irritating, corrosive, and appear to be much more toxic than chromium (III) compounds, given similar amounts and solubilities (Kapil, 1990). This differing toxicity can be related to the ease with which Cr (VI) can pass the cell membrane and its subsequent intracellular reduction to reactive intermediates (Kapil, 1990).

Chromic acid, dichromates and other Cr (VI) compounds are not only corrosive but also powerful skin irritants. Severe dermatitis and skin ulcers can result from contact with Cr (VI) salts (Kapil, 1990). When inhaled, Cr (VI) is a respiratory tract irritant resulting in airway irritation, airway obstruction, and possibly lung cancer (Kapil, 1990). It may also cause

pulmonary sensitization which results in an asthmatic response, which is more common from Cr (VI) than from Cr (III) (Kapil,1990). Pulmonary irritant effects after prolonged inhalation of chromate (VI) dust includes chronic irritation, congestion and hypermia, polyps of the upper respiratory tract, and tracheobronchitis (Kapil, 1990). Nasal mucosa injury (inflamed mucosa, ulcerated septum, perforated septum) is common in workers exposed to Cr (VI) in chrome-plating plants and tanneries (Kapil, 1990). Acute exposure to chromium dust may cause headache, coughing, shortness of breath, fever, weight loss, nasal irritation, inflammation of the conjuctiva and dermatitis (MSDS,1989).

AGENCY	FOCUS	LEVEL	COMMENTS
ACGIH	Air- Workplace	50 $\mu\text{g}/\text{m}^3$	Advisory; TWA to avoid carcinogenic risk from certain insoluble chromium compounds
NIOSH	Air- Workplace	1 $\mu\text{g}/\text{m}^3$	Advisory; TWA (10 hours) for carcinogenic Cr (VI) salts
		25 $\mu\text{g}/\text{m}^3$	TWA (10 hours) for noncarcinogenic Cr (VI) salts, including chromic acid
		50 $\mu\text{g}/\text{m}^3$	15-minute ceiling limit for noncarcinogenic Cr (VI) salts
OSHA	Air - Workplace	100 $\mu\text{g}/\text{m}^3$	Regulation; PEL for chromic acid and chromates (ceiling)
		500 $\mu\text{g}/\text{m}^3$	PEL for soluble chromic salts (8-hour TWA)
		1000 $\mu\text{g}/\text{m}^3$	PEL for chromium metal and insoluble salts (8-hour TWA)
EPA	Air - Environment	N/A	Under review
	Drinking Water	50 $\mu\text{g}/\text{L}$	Regulation; current MCL for total chromium; proposed MCL is 100 $\mu\text{g}/\text{L}$

Table 1.3 The U.S. Standards and Regulations for chromium (Kapil, 1990)

ACGIH	American Conference of Governmental Industrial Hygienists
NIOSH	National Institute for Occupational Safety and Health
OSHA	Occupational Safety and Health Administration
EPA	Environmental Protection Agency
TWA	Time Weighted Average concentration for a normal workday and 40 hr. workweek to which nearly all workers may be repeatedly exposed
PEL	Permissible Exposure Limit- An allowable exposure level in workplace air
MCL	Maximum Contaminant Level- Enforceable standard for drinking water

CHAPTER 2

Chromium Toxicity Effects on Microorganisms

Microorganisms are sensitive to the effects of heavy metal pollution like other components of the biota. Soil microflora may play a key role in the modification of heavy metals added to the soil as a result of man's industrial and agricultural activity. For such changes to occur, soils must contain populations of microbes resistant to the given toxic metal and capable of altering the chemical form of the added metal (Drucker, 1978)

2.1 Environmental Factors Influencing the Toxicity of Heavy Metals to Microorganisms

The response of a microbe to a pollutant *in situ* is dependent on numerous biotic and abiotic factors. The potential effects of these factors must be studied to estimate the influence of pollutants on the microbes as well as on the other components of the biosphere (Babich et al.,1980).

The biotic factors that influence the sensitivity of microbes to pollutants include (Babich et al.,1980):

- * Pigmentation (e.g. pigmented fungal spores are more resistant to ozone than non-pigmented spores)
- * Slime production (e.g. capsulated strains of *Klebsiella aerogenes* are resistant to cadmium than are non-capsulated strains)

- * Physiological age
- * Morphological state
- * Nutritional state (e.g. non-starved cells of *Saccharomyces ellipsoideus* are more resistant to cadmium than are carbon- or nitrogen-starved cell)
- * Physiological adaptations
- * Generic adaptations (e.g. strains of *Pseudomonas aeruginosa* carry plasmids conferring resistance to hexavalent chromium compounds)

Apart from the biotic factors which greatly affect the sensitivity of microbes to pollutants, the characteristics of the pollutant that influence the toxicity of heavy metals to microbes are (Babich et al., 1980):

- * Pollutant form
- * Pollutant concentration
- * Interactions between pollutants

The chemical form of the pollutant that is emitted from an industrial or domestic activity determines its mobility and toxicity to the indigenous microbes in the recipient environment (Babich et al., 1980). It has been found that Cr (VI) is more toxic (Bartlett et al., 1979) than Cr (III) to plants and microorganisms. The amount of pollutant that is deposited in the environment also influences the response of the indigenous microbial population to that pollutant. When the concentration of the pollutant is increased above tolerable levels, the initial response is an inhibition of

microbial activity, and as the concentration of the pollutant increases, death ensues (Babich et al., 1980). The adverse responses of the microbes to pollutants include (Babich et al., 1980):

- * Prolongation of the generation time of bacteria
- * Decreased spore germination, mycelial proliferation, and spore formation by fungi
- * Decreased nitrogen-fixation of blue green algae and lichens
- * Decreased photosynthesis activity of green algae and lichens
- * Inactivation of viral infectivity

Although in many cases the microbes show a negative response to the presence of the pollutants, at times, low concentrations of pollutants may also have a stimulatory effect on the microbiota (Babich et al., 1980).

Interactions between the pollutants, whether synergistic or antagonistic, may also evoke responses from the indigenous microbiota that are different from those evoked by the exposure to each pollutant individually (Babich et al., 1980). It has been found that toxicity of cadmium to the growth of *E.coli* is reduced by the addition of lead, tin, chromium, and iron and the inhibition of the growth of *E.coli* by mercury is affected by the addition of manganese, copper, cobalt, nickel, lead, tin, iron, and chromium (Ohta et al., 1977).

The physicochemical characteristics of an ecosystem into which metals are deposited determine the chemical speciation forms and hence the bioavailability and toxicity of the metals to the indigenous microbiota (Babich et al., 1985). Such abiotic environmental factors include (Babich et al., 1980):

- * pH
- * Oxidation-Reduction potential (E_h)
- * Inorganic anions and cations
- * Clay minerals
- * Hydrous metal oxides
- * Organic matter
- * Temperature
- * Hydrostatic pressure

The pH of the environment into which a pollutant is deposited may influence the chemical form, solubility, mobility, and toxicity of the pollutant to the indigenous microbiota (Babich et al., 1980). Also, E_h of the environment, which is a measure of availability of electrons, influences the valency of many heavy metals. Positive values of E_h indicate an oxidative environment and negative E_h values indicate a reducing environment.

Differentially charged forms of the same element exert different effects on the microbiota (Babich et al., 1980). Trivalent chromium (Cr^{3+}) was neither toxic nor mutagenic to *Salmonella typhimurium*, but hexavalent chromium (Cr^{6+}) was both toxic and mutagenic causing frameshift mutations and

basepair substitutions of the bacterial DNA (Petrilli, 1977). A concentration of 70 $\mu\text{g Cr}^{6+}/\text{mL}$ decreased the rates of fermentation of a mixed rumen microbiota by 50%, whereas divalent chromium (Cr^{2+}), even at a concentration of 640 $\mu\text{g}/\text{mL}$, was not inhibitory (Forsberg, 1978).

The toxicity of the metals to the microbiota is also influenced by the inorganic anionic and cationic components of the environment. For example, the degree of hardness as CaCO_3 , influenced the toxicity of several metals to fungi in freshwater. Further, the concentration of sulfide (S^{2-}) and phosphate (PO_4^{3-}) both in the aquatic and terrestrial environments influenced the bioavailability and toxicity of metals, as metal- S^{2-} or metal- PO_4^{3-} precipitates are relatively unavailable for uptake by microbes (Babich et al., 1985).

Clay minerals possess surfaces that are mainly negatively charged and to which charge-compensating cations (like H^+ , K^+ , NH_4^+ , Na^+ , Mg^{2+} , Ca^{2+}) are adsorbed (Babich et al., 1980). These cations are not permanent components of the clays and are constantly exchanged by other cations in the environment. Heavy metals introduced into the environment may be exchanged for cations on the exchange complex of clays, and the metal toxicants are removed, at least temporarily, from the solution and their

uptake by the microbiota is reduced (Babich et al., 1980). Hydrous metal oxides, organic matter, temperature, and hydrostatic pressure are other factors that affect the toxicity of heavy metals to microbes (Babich et al., 1980).

2.2 Chromium and Microbe-mediated Ecologic Processes

Microbial activities including primary productivity; mineralization of C, N, S, and P; N fixation; and litter decomposition in aquatic and terrestrial environments are sensitive to heavy metal pollution (Babich et al., 1985). Adverse effects by metals on any of these microbe-mediated ecologic processes will in turn adversely affect the homeostatic functioning of the biosphere. For example, decomposition of plant litter and other organic debris form an important part in the cycling of the nutrients and is needed for the continuous productivity of the ecosystem. About 80% - 90% of the net primary productivity in terrestrial ecosystem is acted upon by decomposer organisms. Thus any interruption or reduction in organic matter decomposition may result in soil nutrient deficiency (Babich et al., 1985). Toxicity of heavy metals can affect

* Microbial numbers and species diversity

- * Cycling of carbon, nitrogen, sulfur, and phosphorous
- * Litter decomposition

Microorganisms are sensitive to heavy metals. Effects on microbes due to the environment contaminated by heavy metals include (Babich et al., 1985):

- * Reduction in the number and species diversity of the biota
- * Development of metal- resistant microbial populations

Soil obtained at a site 15 km from a secondary lead smelter and containing 28,000 ppm Pb, 972 ppm As, 599 ppm Cu, and 151 ppm Cd had lower numbers of bacteria including actinomycetes and fungi than control soils obtained 1000 km from the smelter which contained lower concentrations of the pollutants (Babich et al., 1985). Heavy metals also affect the number and species diversity of the microbiota in the aquatic ecosystem (Babich et al., 1985) . In freshwater amended with up to 49.5 ppm Cr (III), diatoms dominated over cyanobacteria and green algae, whereas with 376.5 ppm Cr (III), cyanobacteria were dominant, followed by green algae and greatly reduced numbers of diatoms (Patrick, 1978). Development of heavy metal resistant microbial population has also been noted in heavy-metal contaminated soils and aquatic systems (Babich et al., 1985). For example,

gram negative bacteria which were isolated from a river contaminated with metals was found to be resistant to Cr (VI) (Simon-Pujol et al., 1978).

2.2.1 Carbon Mineralization

The rate and amount of evolution of carbon-dioxide (CO₂) from soils is often used as an indicator of the effects of heavy metals and other pollutants on the overall heterotrophic activity of microorganisms involved in the decomposition of organic matter (Babich et al., 1985). These studies have evaluated the adverse effects of heavy metals on endogenous (i.e., soils not amended with organics) respiration and on the degradation of added substrates like glucose, starch, animal excrements, and plant litter. During a 3-week incubation, CO₂ evolution was decreased in two acidic soils amended with either 10 or 100 ppm Cr (VI) or 100 ppm Cr (III) (Ross et al., 1981). The rate of decomposition of added starch and cellulose, but not of glucose and protein, was decreased in an organic soil obtained from a coniferous forest surrounding a brass mill and contaminated by zinc and copper (Babich et al., 1985).

2.2.2 Nitrogen Transformation

The rates of ammonification, nitrification, and net N mineralization (i.e., the accumulation of NH_4^+ and NO_3^-) in soils may be stimulated or inhibited by the application of metals (Babich et al., 1985). Premi et al. (1969) showed that under anaerobic conditions (so as to retard nitrification), the rate of ammonification in an alkaline sandy loam soil could be stimulated by 100 ppm Cr (III), but unaffected by 1000 ppm Cr (III). When maintained aerobically, net N mineralization was inhibited by 1000 ppm Cr (III), but not affected by 100 ppm Cr (III). In another experiment by Chang et al. (1982), the net N mineralization and nitrification in a slightly acidic silt loam soil amended with a combination of 100 ppm $\text{NH}_4^+\text{-N}$, 1% sewage sludge and 1% ground alfalfa was inhibited after 12 weeks of incubation by the addition of 400 ppm of various metals, with the sequence of toxicity being Cr (III) > Cd > Cu > Zn > Mn > Pb. Liang et al. (1978) found the comparative toxicity of metals, added at 5 $\mu\text{M/g}$ soil, to nitrification in three diverse soil types in the sequence of Hg > Cr (III) > Cd > Ni > Fe (III) > Co > Cu > Sn > Fe (II) > Zn > V > Mn > Pb. Studies showed that nitrification

in surface waters collected from the Chesapeake Bay was completely inhibited by 100 ppm Cr (III) (Mills et al., 1977).

2.2.3 Mineralization of Phosphorous and Sulfur

Enzyme acid phosphatase activity was (Babich, 1985) reduced by 27% in soils amended with 25 $\mu\text{M/g}$ soil of Cr (III). In the case of alkaline phosphatase, its activity was reduced by 33% due to the presence of 25 $\mu\text{M/g}$ soil of Cr (III) in soils (Babich, 1985).

Arylsulfatases are involved in the cycling of sulfur (Babich et al., 1985), as they catalyze the hydrolysis of an arylsulfate anion by the fission of the O- S bond ($\text{ROSO}_3 + \text{H}_2\text{O} \rightarrow \text{ROH} + \text{H}^+ + \text{SO}_4^{2-}$). Presence of Cr (III) resulted in a 41% inhibition in arylsulfatase activity in soils amended with 1 $\mu\text{M/g}$ soil of Cr (III) (Babich, 1985). Amendments to an anaerobically maintained salt marsh sediment of 100 ppm Cr (VI) inhibited SO_4^{2-} reduction to H_2S (Capone et al., 1983).

2.2.4 Enzyme Inhibition and Synthesis

Extra cellular enzymes, derived from microbial and plant cells are responsible for many biochemical processes in the soils and sediments (Babich et al., 1985) . The activity of many of these “ environmental” enzymes is inhibited by metals. The inhibition can be (Babich et al., 1985) due to the following

- * Masking of catalytically active groups
- * Protein denaturation
- * Effects on enzyme conformation
- * Competition with activating cations involved in the formation of enzyme-substrate complexes.

By exerting an inhibitory or lethal effect on the microbiota which is indigenous to a specific environment, heavy metals can also cause a decrease in the synthesis of enzymes as well as in the ATP content. (Babich et al., 1985). Experiments conducted by Zibliski et al. (1982) on bacterial proliferation in soil amended with anaerobically digested sewage sludge to which various metals were added was studied during 80 days of incubation. The ATP content of soils treated with Cr (III) increased during the first 2 weeks and then decreased after the second week when compared with control soil. Inhibition of enzyme activity in soil due to the presence of Cr (III) is as shown in Table 2.1 (Babich, 1985). It can be seen from the

table that the activity of enzyme arylsulfatase is reduced by 41% due to 1 $\mu\text{M/g}$ soil of Cr (III). However the activity of enzyme urease is reduced by only 3% when 50 $\mu\text{g/g}$ soil of Cr (III) was present and that of enzyme amidase is decreased by 4% when 5 $\mu\text{M/g}$ soil of Cr (III) was present. The table shows that the activity of enzyme arylsulfatase is highly inhibited due to the presence of Cr (III) when compared with other enzymes in the table.

<----- Average % Inhibition----->					
Metal	Arylsulfatase $\mu\text{M/g}$ soil	Amidase 5 $\mu\text{M/g}$ soil	Acid Phosphatase 25 $\mu\text{M/g}$ soil	Alkaline Phosphatase 25 $\mu\text{M/g}$ soil	Urease 50 $\mu\text{g/g}$ soil
Cr III	41	4	27	33	3

Table 2.1 Inhibition of enzymes activity in soil (Babich, 1985)

2.3 Chromium effects on soil microorganisms

Increasing use of soil as a repository for human waste products has brought about increased concern regarding the effects of toxic substances on the soil (Ross et al.,1981) . Chromium is often found in waste sludges from tanning, metallurgic and other industries. It appears that Cr (III) is less toxic than Cr (VI) (Ross et al.,1981). Inorganic Cr (III) is not mobile in soils and is not readily taken up by cells, probably because of its low solubility and tendency to form large hydroxy polymers at neutral pH levels. However,

Cr (VI) is mobile in soils and it penetrates cell membranes readily. Once inside the cells, it is reduced; its toxicity probably results from oxidation of cell components (National Research Council, 1974). After reduction to Cr (III) inside the cell, it may interfere with protein function. Mutations have been observed in bacteria grown in the presence of Cr (VI), indicating DNA susceptibility (Petrilli et al., 1977).

2.3.1 Soil Population Concentration

Drucker et al. (1978) studied the toxic effects of chromium in soil as a function of soil population concentrations. A Ritzville surface soil, 15 cm, taken on the Hanford Reservation, was utilized for the microbial studies. Chromium was added to the soil as K_2CrO_4 . To determine the effect of chromium on the number and types of microorganisms, they measured the viable organisms using plate counts. Microbial counts were performed on selected classes of organisms at 0, 2, and 6 days of incubation with 0, 1, 10, and 100 ppm Cr present. Table 2.2 gives the results of their experiment.

Their results can be summarized as follows:

Total aerobic bacteria: At 1 and 10 ppm there was a slower growth rate for the interval 0 to 2 days. But at 2 to 6 days, number was almost equivalent to a no-metal added control

	<p>At 100 ppm Cr, the number decreased by almost an order of magnitude when compared with no-metal added control, both at 2 and 6 days of incubation</p> <p>At 100 ppm, number of organisms decreased markedly from day 0 to 2; the metal was exerting a killing effect</p>
Aerobic spore forming bacteria	<p>Not affected by chromium at 1 ppm</p> <p>Pronounced bacteriostatic effect at 100 ppm Cr</p> <p>Apparent bactericidal effect at 100 ppm Cr, with little or no recovery of organisms over the interval of incubation</p>
Spore forming anaerobic bacteria	<p>At 1 ppm Cr, growth rate was not grossly affected between 0 and 6 days of incubation</p> <p>At 10 and 100 ppm Cr, numbers decreased by an order of magnitude relative to numbers at 0 day of incubation</p> <p>At 10 and 100 ppm Cr, numbers decreased by two orders of magnitude relative to no-metal added control at 2 and 6 days</p> <p>Little apparent recovery from the bactericidal effect of the metal at 100 ppm Cr</p>
Actinomycetes	<p>At 1 and 10 ppm Cr, numbers decreased relative to control throughout the 6 day incubation period</p> <p>At 100 ppm Cr, bactericidal effects were observed at 2 days of incubation</p> <p>Growth resumed in the interval from 2 to 6 days of incubation</p>
Fungi	<p>Appeared to be least sensitive to chromate toxicity</p> <p>Absolute numbers of these organisms decreased only at 100 ppm Cr</p>

Metal concentration	Total aerobes —0 time	Organisms/g soil $\times 10^6$		Total anaerobe	Organisms/g soil $\times 10^6$	
		2 days	6 days		2 days	6 days
0	160	840	1,600	0.84	7.2	7.1
1	150	400	1,300	0.78	3.9	5.5
10	140	450	1,100	1.1	0.78	1.2
100	130	35	190	0.77	0.13	0.28
Metal concentration	Aerobe spore-forming	Organisms/g soil $\times 10^6$		Anaerobe spore-forming	Organisms/g soil $\times 10^6$	
		2 days	6 days		2 days	6 days
ϕ	0.44	4.1	5.2	0.53	5.7	5.5
1	0.56	3.5	4.9	0.50	4.8	3.4
10	0.66	0.46	1.4	0.51	0.053	0.37
100	0.79	0.42	0.43	0.55	0.058	0.082
Metal concentration	Actinomycetes	Organisms/g soil $\times 10^6$		Fungi	Organisms/g soil $\times 10^6$	
		2 days	6 days		2 days	6 days
0	25	200	290	0	0.23	0.28
1	21	160	200	1	0.18	0.10
10	25	150	180	10	0.32	0.30
100	14	7.7	59	100	0.20	0.13

Table 2.2 Microbial populations in Cr-stressed soils (Drucker et al.)

In another experiment by Zibilski et al. (1982), bacterial proliferation in soil amended with anaerobically digested sewage sludge to which various metals were added was studied during 80 days of incubation. Growth was stimulated initially (during first week of incubation) in soil amended with sludge containing 1 ppm Cr, but with time, growth decreased when compared to that in the control soil. Sludge containing 44 and 556 ppm Cr (III) reduced growth throughout the 80 days. Differences in the distribution of the major fungal genera (i.e., *Penicillium*, *Paecilomyces*, *Trichoderma*, *Rhizopus*, and *Aspergillus*) did not occur in soils amended with sludge containing Cr (III).

Thus chromium has both bactericidal (capable of killing bacteria) and bacteriostatic (no recovery of microorganisms) effects on soil microflora. These effects are a function of chromium concentration, duration of exposure to the metal, and class of microorganisms (Drucker et al., 1978).

2.3.2 Soil Microbe Respiration

The rate and amount of evolution of carbon-dioxide (CO₂) from soils has been used as an indicator of the effects of heavy metals and other

pollutants on the overall heterotrophic activity of microorganisms involved in the decomposition of organic matter (Babich, 1985). According to EPA, (1979) since soil respiration can "rebound from an initial insult", such as that caused by the addition of heavy metals, CO₂ evolution must be monitored for at least 30 days to allow for soil reequilibration before the irreversible toxic effects of metals can be identified.

Drucker et al. (1978) examined the effects of chromium on soil respiration. They measured CO₂ evolution from a starch carbon source. CO₂ evolution was measured from soil cultures amended with 0,1,10 or 100 ppm Cr over a period of 13 days. As it can be seen from Fig 2.1, the lower chromium levels (1 and 10 ppm Cr) do not seem to affect CO₂ production until day 3 of incubation. The 1 ppm Cr culture appeared to show a greater degree of CO₂ evolution than 10 ppm-amended culture. Both are lower than control culture in CO₂ production. CO₂ evolved from the soil cultures amended with 100ppm Cr is qualitatively different from the other three soil cultures. It can be seen that 0,1, and 10 ppm amended cultures produce CO₂ at an initial rapid rate, followed by a slower rate. Respiration in the 100 ppm culture resulted in a slow rate throughout the incubation period. Fig 2.2 shows the CO₂ evolution rate as a function of incubation time. For 0, 1,

Fig. 2.1 Cumulative respiration (CO_2 evolved) as a function of incubation time (Drucker et al.)

Fig. 2.2 Rate of soil respiration (CO_2 evolved) as a function of incubation time (Drucker et al.)

10 ppm Cr amended cultures, there is a clearly defined optima in respiration rates at 1.5 days of incubation. The rates then fall and become more or less constant for the remaining period. In the case of 100 ppm Cr culture, there is no clearly defined maximum for respiration and the rate remains approximately constant throughout the incubation period. They suggested that the effect on respiration at lower Cr concentrations could be due to the slower rates of carbon metabolism (the nature of this metabolism not changing from the soil containing no added Cr). They also suggested that the effect on respiration at 100 ppm Cr could be due to a radical change in carbon metabolism. They said that chromium added would have inhibited the enzymes responsible for glucose release from carbon source (starch) resulting in less available glucose. CO₂ evolution is thus limited in rate by the availability of the sugar. These results showed that the 100 ppm culture used less glucose and evolved less CO₂. As seen in Table 2.3, the ratio mM CO₂/mM glucose utilized is about 4.5:1 for 1 and 10 ppm Cr. It drops to 2.4:1 at 100 ppm Cr. Their result suggest that there is a change in carbon metabolism for soil cultures grown at the higher Cr dose. While the cultures at lower concentrations of chromium appear to metabolize glucose in an oxidative fashion (ratios CO₂/ glucose utilized is similar to sugars

metabolized through tricarboxylic acid cycle), the soil cultures at

Level of chromium addition $\mu\text{g/g}$	Total glucose utilized mM (a,b)	Total CO_2 evolved from soil mM (a,b)	mM CO_2 / mM glucose
0	9.2	41.5	4.5
1	9.1	39.5	4.3
10	9.0	40.3	4.5
100	5.5	13.1	2.4

Table 2.3 Influence of chromium on microbial utilization of glucose relative to soil respiration (CO_2 evolution). (Drucker et al.)

a Soil weight 220 g

b Calculated for the incubation period 3-11 days except at the 100 $\mu\text{g/g}$ addition level which was calculated for the period 11-22 days

100 ppm Cr appear to be metabolizing the sugar in a fermentative manner (ratio CO_2 / glucose utilized is similar to metabolism through acetate or ethanol formation). They were also able to find the ability of mixed microbial cultures to alter the chemical form of chromium (added as chromate). Based on gel permeation chromatography, and thin layer electrophoresis and chromatography, they concluded that metabolites produced as a result of growth of chromium-resistant cultures appear capable of modifying the form of chromium added to the soil (Drucker et al., 1978).

The toxicity of Cr to soil bacterial isolates was also studied by Ross et

al. (1981), by measuring the turbidity of liquid cultures supplemented with Cr (VI) or Cr (III). Ten to 12 ppm Cr (VI) was found inhibitory to most isolates growing in either a soil-extract medium or in a semisynthetic medium. Gram negative bacteria were more affected than gram positive bacteria by Cr (VI), both at 10 to 12 ppm and at 1 ppm. Toxicity due to Cr (III) was not observed at similar levels. Two acidic soils were amended with 10 and 100 ppm Cr (VI) and 100 ppm Cr (III). All three treatments showed significant decrease in CO₂ evolution for a 3 week incubation, when compared with the control. Extractable Cr (VI) in these soils decreased rapidly during this time, indicating that the reduction of added Cr (VI) was taking place. The toxicity of low levels of Cr (VI) to gram negative bacteria in aqueous culture indicates that soil microbial transformations, like nitrification, may be affected. The fact that both Cr (III) and Cr (VI) inhibit microbial activity in soil shows that more caution may be needed in handling Cr containing wastes, regardless of the form of chromium (Ross et al., 1981).

2.3.3 Toxicity and Mutagenicity

Chromium, particularly in the trivalent form, is considered to have a low order of toxicity. Clinical studies indicate that individuals exposed for long periods of time to the Cr (VI) ion can develop tissue necrosis (Royle, 1975). Chromium exhibits properties most consistent with a mutagenic initiation mechanism. Cr (VI) induces chromosomal aberrations, promotes transformation of animal cells in culture, and increases copying errors by DNA polymerases *in vitro* (Flessel, 1978). In addition, the mutagenic activity of Cr (VI) is much greater than Cr (III) and is strikingly decreased by microsomal metabolism. Cell free investigations suggest that the ultimate intracellular mutagen is probably Cr (III) which may act by forming strong ligand complexes with DNA, thereby altering the coding properties (Flessel, 1978). Evidence from genetic assays (Flessel, 1978) consistently suggest that carcinogenesis may involve mutagenic initiation. Intracellular reduction and binding to DNA best explain chromium's genetic toxicity (Flessel, 1978). Chromium (VI) is mutagenic in *Salmonella* (Petrilli et al., 1977), *E.coli* (Nishioka, 1975), and yeast (Bonatti et al., 1976).

Four hexavalent (sodium dichromate, chromic acid, calcium chromate, potassium chromate) and two trivalent (chromium potassium sulfate,

chromic chloride) chromium compounds were tested by Petrilli et al. (1977) for toxicity and mutagenicity by means of the *Salmonella typhimurium*/mammalian-microsome test. All hexavalent compounds yielded complete inhibition of bacterial growth at doses of 400 to 800 µg/plate, a significant increase of his⁺ revertant colonies at doses ranging from 10 to 200 µg, and no effect at doses of less than 10 µg. The distinctive sensitivity of the four *Salmonella* strains tested (TA1535, TA1537, TA98, TA100) suggested that Cr (VI) directly interacts with bacterial DNA by causing both frameshift mutations and basepair substitutions. The latter mutations, which are prevalent, are amplified by an error-prone recombinational repair of the damaged DNA (Petrilli et al., 1977). On an average, 1 µM of hexavalent chromium yielded approximately 500 revertants of the TA100 strain, irrespective of the compound tested. The mutagenic potency of Cr (VI) was not enhanced by adding microsomal fraction of rat hepatocytes induced either with sodium barbital or with Aroclor 1254. The two Cr (III) compounds, with or without the microsomal fraction, were neither toxic nor mutagenic for the bacterial tester strains (Petrilli et al., 1977).

Chromium inhibits DNA synthesis (Flessel, 1978). Chromium in several of its oxidation states form a variety of ligand complexes. Favored

ligand binding sites are nitrogen and oxygen. Trivalent chromium forms coordination complexes with biological ligands, including nucleic acids. Direct binding of chromium to DNA was suggested by the observation that DNA isolated from cells treated with $K_2Cr_2O_7$ contained as much as 1% chromium (Flessel, 1978). Chromium (III) forms highly insoluble ligand complexes with nucleic acids but soluble complexes with proteins (Flessel, 1978).

Studies by Eisinger et al. (1962), on DNA binding of metal ions in solution showed that Cr (III) tightly binds to phosphate groups on the outside of the DNA. Binding reached saturation at mole ratios of about 2.5 metal ions per phosphate. Addition of Cr (III) in excess of this saturation density resulted in the precipitation of DNA from solution. X-ray diffraction measurements of chromium-treated DNA fibres indicated that Cr (III) binding to DNA was not regular.

A possible mechanism for Cr mutagenesis (Flessel, 1978) is that Cr (VI) enters the cell readily. Once inside, a portion of the Cr is reduced to Cr (III), in the process of which Cr (III) ions may become bound to the sugar-phosphate backbone of DNA. Binding may induce configurational changes in DNA, like chain distortion or base puckering, which lead to errors during gene replication, recombination, or repair (Flessel, 1978).

Petrilli et al. (1978 a) found that addition of a strong oxidizing agent (potassium permanganate) can oxidize chromium from the trivalent to the active, mutagenic hexavalent state. Toxic effects were observed in plates incorporating more than 40 - 80 μg of trivalent chromium compounds and elicited a dose- effect mutagenic response at 10 - 40 μg per plate.

Oxidation of trivalent chromium to the hexavalent form has also been observed in the presence of oxidized manganese (Mn) in the soil, a condition which is prevalent in many soils (Bartlett et al., 1979). Oxidized Mn acts as electron acceptor in the reaction. Adsorption of Cr (III) by Mn oxides is the first step in the oxidation of Cr (III) by Mn. When the trivalent form is oxidized to the hexavalent form, chromium is no longer adsorbed by the Mn mineral and is leached from the system (Bartlett et al., 1979). Since solubility of Cr (III) is low above pH 5.5, Cr oxidation is favored at low pH. It was seen that oxidation of Cr (III) was (Bartlett et al., 1979):

- * Not shown in soils very low in Mn
- * Accompanied by an increase in reduced Mn extractable from the soil
- * Less in the soil in which much of the oxidized Mn has been converted to the reduced form by drying and storage

Addition of either microsomal fractions from rat liver microsomal preparations or of human erythrocyte lysates can result in a complete loss of mutagenicity (Petrilli et al., 1978 b). Reversal of mutagenicity could be due to reduction of the metal to the inactive trivalent form through a simple oxido-reductive reaction. Reducing agents (ascorbic acid and sodium sulfite) and metabolites (GSH, DPNH, and TPNH) prevent Cr (VI) mutagenicity (Petrilli et al., 1978 b), whereas an oxidizing agent (potassium permanganate) totally inhibits reversal of mutagenicity by liver and erythrocyte preparations. Enzymic conversion appears to be involved in the deactivation process through a large production of TPNH via the hexose monophosphate oxidative pathway or other ancillary systems (Petrilli et al., 1978 b).

Chapter 3

Chromium Toxicity Effects on Treatment Processes

Trace amounts of metals are often beneficial to microbes to reach optimum growth rates. According to McCarty (1964), metals actually stimulate biological reaction for up to a certain metal concentration, and, at higher concentration the effect is reversed. However, heavy metals like hexavalent chromium are usually regarded as toxic to most organisms even at low concentrations (Barth et al., 1965)

Chromium compounds can have a negative effect on the efficiency of waste water treatment. The toxicity mechanisms of chromium compounds depends on their valency. The trivalent compounds usually accumulate in the cell membrane while the hexavalent compounds are transported into the cells. Inside the cell, the hexavalent ions are reduced to trivalent form and react with intracellular material (Mazierski, 1995). Several parameters like the respiration rate, and the rate of substrate utilization are used to determine the toxic effect of hexavalent chromium.

3.1 Effects of Chromium on Activated Sludge Process

The effect of hexavalent chromium on the growth rate of activated sludge bacteria was found using N-propyl alcohol as organic source and

ammonium chloride as nitrogen source. The activated sludge bacteria showed a decrease in the maximum growth rate μ_m when Cr (VI) was present in the range of 11 mg/l (Mazierski, 1995). An increase in the Monod constant K_s was also observed. Table 3.1 gives the steady state parameters of the Monod equation calculated using the Lineweaver-Burk method.

Cr(VI) conc. mg/l	$\mu_{\max i}$	K_{si}
0	0.5352	1.2071
0.1	0.5372	1.5942
2.0	0.5256	6.7083
4.0	0.4981	14.6026
7.5	0.4565	34.9445
9.0	0.4717	67.3021
10.0	0.3626	71.9824
11.0	0.3328	139.6646

Table 3.1 Effect of Cr (VI) on $\mu_{\max i}$ and K_s on activated sludge bacteria (Mazierski, 1995)

$\mu_{\max i}$ Inhibited growth rate (T^{-1})

K_{si} Monod constant in the presence of inhibitor (ML^{-3})

It can be observed that the maximum growth rate decreases and the Monod constant increases, when the chromium concentration is increased (Mazierski, 1995). It was also found that Cr (VI) acts as a non-competitive inhibitor (Mazierski, 1995).

Tolerance of microorganisms to toxic metals is also possible after a proper acclimation period is allowed. During acclimation either resistant organisms are selected and/or microorganisms are adapted to the metal

environment metabolically. It has been shown that an acclimated activated sludge culture is fully capable of coping with Cr (VI) containing wastes and is significantly stimulated by up to 25mg/l Cr(VI) (Gokcay et al., 1991). All concentrations of this metal tested (1,5,10,25 mg/l Cr(VI)) significantly stimulated the maximum growth rate μ_m of the culture (Table 3.2). The summary of their results are given in Table 3.2. The biomass yield of the activated sludge culture markedly increased in the presence of Cr (VI).

Fig.3.1 shows the exit substrate concentration (S_e) and the suspended solids concentration (X) at different dilution rates with the synthetic wastewater without chromium.

Fig. 3.1 Dilute-out curve for the baseline study with synthetic wastewater devoid of metal (Gokcay et al., 1991)

Cr (VI) conc.mg/l	μ_m (h ⁻¹) 1	D_c (h ⁻¹) 2	μ_m (h ⁻¹) 3	K_s mg/l COD	Conclusions
Baseline	0.31	0.32	0.41	85	Fig.3.1
1.0	0.37	0.36	-	126	Figs. 3.2 and 3.3 Caused a significant increase in the biomass yield, maximum at 0.24/h dilution rate. MLSS concentration at this dilution rate was 1400 mg/l. Substrate removal was at a steady level. Excellent settlability of the sludge. Biological flocs were large in size and settled well
5.0	0.39	0.38	0.37	23	Figs. 3.4 and 3.5 Two distinct dilute-out curves due to the heterogeneous nature of the activated sludge. Presence of dual plateau suggests two different microbial communities. Improved settlability of the flocs
10.0	0.56	0.52	0.52	74	Figs. 3.6 and 3.7 Growth rate was stimulated. There was a minor drop in the COD removal efficiency at the lower dilution rates with respect to the baseline study which became marked after 0.14/h and up to 0.26/h
25.0	0.64	0.53	0.52	145	Figs. 3.8 and 3.9 Biomass concentration dropped sharply at dilution rates below 0.12/h while substrate remained at a steady low level

Table 3.2 Effect of Cr (VI) on acclimated activated sludge (Gokcay et al., 1991)

- 1 From Lineweaver-Burk plot
- 2 From dilute-out curve
- 3 From non-steady state dilution experiment
- μ_m Maximum specific growth rate

D_c Critical dilution rate
 K_s Half saturation substrate concentration

Fig. 3.2 is the dilute-out curve (X , S_e , Dilution rate (D)) and Fig. 3.3 is the Lineweaver-Burk plot (substrate vs dilution rate) for the wastewater containing 1.0 mg/l Cr (VI). It can be seen that there is an unusual change in the shape of the dilute-out curve (Fig 3.2) when compared with that obtained from baseline study in Fig. 3.1. The dilute-out curve and Lineweaver-Burk plot in the case of wastewater containing 5.0mg/l Cr (VI) is shown in Figs. 3.4 and 3.5 respectively. Two distinct dilute-out curves for the biomass are obtained. The culture represented by the lower curve was apparently not affected by the 5.0 mg/l Cr (VI) and took over when the conditions favoured. Fig. 3.6 represent the dilute-out curve for wastewater containing 10.0 mg/l Cr (VI). It can be seen that there are two dilute-out patterns throughout the dilution range. The dilution range 0.18-0.32 h^{-1} appeared as a transition zone. Complete wash-out of the culture was seen at 0.52/h dilution rate. Fig. 3.7 which is the Lineweaver-Burk plot at 10.0 mg/l Cr (VI) shows two different straight lines. This could be due to the existence of two different species of microorganisms with different biokinetic properties. Figs. 3.8 and 3.9 represent the dilute-out curve and the Lineweaver-Burk plot for the wastewater containing 25.0 mg/l Cr(VI).

Fig. 3.2 Dilute-out curve for synthetic wastewater containing 1.0 mg/l Cr (VI) (Gokcay et al., 1991)

Fig. 3.3 Lineweaver-Burt plot of the substrate vs dilution rate data obtained with synthetic wastewater containing 1.0 mg/l Cr (VI) (Gokcay et al., 1991)

Fig. 3.4 Dilute-out curve for synthetic wastewater containing 5.0 mg/l Cr (VI) (Gokcay et al., 1991)

Fig. 3.5 Lineweaver-Burt plot of the substrate vs dilution rate data obtained with synthetic wastewater containing 5.0 mg/l Cr (VI) (Gokcay et al., 1991)

Fig. 3.6 Dilute-out curve for synthetic wastewater containing 10.0 mg/l Cr (VI) (Gokcay et al., 1991)

Fig. 3.7 Lineweaver-Burt plot of the substrate vs dilution rate data obtained with synthetic wastewater containing 10.0 mg/l Cr (VI) (Gokcay et al., 1991)

Fig. 3.8 Dilute-out curve for synthetic wastewater containing 25.0 mg/l Cr (VI) (Gokcay et al., 1991)

Fig. 3.9 Lineweaver-Burt plot of the substrate vs dilution rate data obtained with synthetic wastewater containing 25.0 mg/l Cr (VI) (Gokcay et al., 1991)

3.2 Effects of Chromium on Anaerobic Digestion Process

The distribution and adverse effects of heavy metals in anaerobic digestion processes have been the topic of many investigators since this process appeared to be particularly vulnerable to heavy metal toxicity compared to other biological treatment systems (Alkan et al., 1996).

Chromium is one of the heavy metals that causes toxicity in anaerobic digestion, and has often been blamed for unsatisfactory operation or failure of anaerobic digesters. Chromium toxicity in the anaerobic digestion process is brought about by an accumulation of the metal in the intracellular fraction of the biomass (Alkan et al., 1996). The affinity of biomass for metal ions is much greater than that of organics in the supernatant (Alkan et al., 1996). Very little difference is found between the toxicities of Cr (III) and Cr (VI), probably because of the rapid reduction of Cr (VI) to Cr (III) in the anaerobic environment of the digesters. Toxicity level particularly depends on operational conditions like temperature, pH, hydraulic retention time, solid retention time, and mixed liquor volatile suspended solids (MLVSS) in anaerobic digestion (Alkan et al., 1996).

The toxicity studies of Cr (III) to anaerobic digestion system were studied using completely mixed anaerobic digesters (Alkan et al., 1996). One of the digesters was operated as a control, one was dosed in a stepwise

manner with increasing concentrations of chromium (Digester II), allowing acclimation of the bacterial population, and two digesters were used for shock injections of chromium with no acclimation (Digester III, Digester IV). A total concentration of 1,140 mg/l Cr (III) was toxic to the anaerobic process when injected in a stepwise manner (Digester II). Presence of Cr (III) resulted in a significant decrease in total gas production rate, a fall in pH, decrease in COD removal efficiency, decrease in the methane % in the gas produced, and increase in volatile fatty acids. The loss in the gas production rate was accompanied by an increase in CO₂ content of the gas that caused the production of methane to be lower than the expected values (Fig. 3.10). The presence of Cr (III) in the digester had a direct effect on the methanogenic bacteria, whereas acidogenic bacteria was not adversely affected. Each case of a sharp decrease in gas production rate was accompanied by a noticeable increase in volatile fatty acids as seen in Fig. 3.11 and a decrease in COD removal efficiency (Fig. 3.12). A shock injection of 400 mg/l of Cr (III) (Digester III) caused a short term inhibition of the process with recovery taking place within a week. When a shock injection of 500 mg/l of Cr (III) (Digester IV) was applied, it resulted in process failure. Thus a shock injection of Cr (III) seems to have more adverse effects on the anaerobic digestion process than the stepwise

Fig 3.10 Effect of Cr (III) on methane proportion (Alkan et al., 1996)

Fig. 3.11 Effect of Cr (III) on gas production and total volatile fatty acids in Digester II (Alkan et al., 1996)

Fig. 3.12 Effect of Cr (III) on COD removal efficiency
(Alkan et al., 1996)

injection.

The production of volatile fatty acids (VFA) in the anaerobic digestion is also affected by the presence of heavy metals (Lin, 1993). The relative toxicity of heavy metals to the production of acetic acid (HAc) and n-butyric acid (n-HBu) was found to be $\text{Cu} > \text{Zn} > \text{Cr} > \text{Cd} > \text{Pb} > \text{Ni}$ and $\text{Cu} > \text{Zn} > \text{Cr} > \text{Cd} > \text{Ni} > \text{Pb}$, respectively (Lin, 1993). The acidogens responsible for acidogenesis are generally considered to be less sensitive to environmental conditions, such as toxicant concentrations, than methanogens (Lin, 1993). The VFA production activity (A_v), defined as the fraction of VFA production in 30h for metal dosed seed sludge and control, decreases as the metal concentration increases. The activities of HAc and HBu production also decreases as Cr dosage increases (Lin, 1993).

Toxic effects of chromium were also observed on VFA degradation in anaerobic digestion (Lin, 1992). The relative toxicity of heavy metals to degradation of HAc, propionic acid (HPr), and n-HBu was found to be $\text{Cd} > \text{Cu} > \text{Cr} > \text{Zn} > \text{Pb} > \text{Ni}$, $\text{Cd} > \text{Cu} \geq \text{Zn} = \text{Cr} > \text{Pb} > \text{Ni}$, and $\text{Cd} > \text{Cu} > \text{Cr} > \text{Zn} > \text{Pb} > \text{Ni}$ respectively (Lin, 1992). The A_v values of HAc, HPr, and HBu decreased dramatically when Cr dosage was increased from 5 to 10 mg/l. The Cr concentration causing 50% inhibition of the

degradation of HAc, HPr, and HBU were 9.6, 7.5, and 9.7 mg/l. These values indicate that the degree of inhibition of VAF degradation is $HPr > HAc = HBU$ (Lin, 1992).

3.3 Effects of Chromium on Denitrification Process

Under oxygen deficient conditions many bacteria use nitrates and nitrites contained in waste waters for respiration. Nitrates and nitrites act as the final electron acceptor. Thus denitrification process is therefore a convenient method for the removal of nitrates and nitrites from waste waters. The rate of denitrification process depends on various factors like temperature, type of carbon source, presence of toxic substances (Mazierski, 1994).

The long term influence of Cr on bacterial denitrification was observed in packed bed reactors (PBR) using methanol, ethanol, n-propanol, n-butanol, sec-butanol, tert-butanol, iso-butanol, and n-pentanol as the carbon and energy sources for denitrification (Lewandowski, 1985). The short term influence was investigated by determining the inhibition coefficient K_i within the fresh activated sludge under anoxic conditions. K_i is defined as the concentration of the inhibitor which causes a decrease in the reaction rate to half of the maximum reaction rate. The measured

inhibition constant K_i was 84.2 mg/l Cr (VI) (mean value) and independent of the kind of organic compound utilized as the electron donor for bacterial system.

The effect of Cr in the case of PBR was observed as a relation between the chromium concentration and the nitrate removal rate. The chromium concentration, Cr (VI), added as $K_2Cr_2O_7$, was increased from 1 mg/l Cr (VI) until progressive deterioration of the results in terms of denitrification efficiency was obtained (Lewandowski, 1985).

Denitrification efficiency decreased with increase in Cr concentration.

While the reactor supplied with propanol operated with full efficiency with the Cr concentration in the influent equal to 8 mg/l Cr (VI), in the case of reactors supplied with methanol, sec-butanol, and tert-butanol, the start-up procedures failed with the Cr concentration in the influent equal to 1 mg/l Cr (VI). Decreasing the Cr concentration to 0.5 mg/l Cr (VI) improved the results in the case of methanol, and sec-butanol. In the case of tert-butanol, even the removal of Cr from the influent did not allow the reactor to operate with an efficiency close to 100% denitrification. Thus it was evident that the tolerance limit of individual reactors to the presence of Cr depends on the kind of carbon substrate oxidized. The concentration of the Cr at which the denitrification efficiency decreases to 95% is called the reactor resistance to

inhibition (RRI). The concentration of Cr that caused a decrease in denitrification efficiency and which was used as the inhibition starting point depended on the kind of compound used as electron donor and was different for the individual reactors (Table 3.3).

Electron donor	Cr (VI) mg/l
Methanol	1.0
Ethanol	3.0
n-Propanol	8.0
n-Butanol	5.0
sec-Butanol	0.5
tert-Butanol	0.0
iso-Butanol	3.5
n-Pentanol	4.0

Table 3.3 Concentration of Cr which causes a decrease in denitrification efficiency to 95% (Lewandowski, 1985)

In the presence of chromium the bacterial system is inhibited in two ways:

- * Inhibition of the reaction rate
- * Inhibition of growth

Further, no adaptation was seen to the presence of Cr during the operating period (Lewandowski, 1985).

Experimental results in the PBR operated for denitrification in the presence of Cr (VI) with iso-butanol as the sole carbon and energy source for the denitrifiers gave a value of $RRI=2.9$ mg/l Cr (VI) and $K_i=1.2$ mg/l Cr (VI) (Lewandowski, 1987). However in the case of fresh activated sludge, the value of K_i was found to be 84.4 mg/l Cr (VI) for iso-butanol

(Lewandowski, 1985). Thus there is a significant difference in K_i values for Cr (VI) inhibition for denitrification in the PBR and in the fresh activated sludge. This indicates that the denitrification process in the fresh activated sludge is much less sensitive to the presence of Cr than the same process to the PBR (Lewandowski, 1987). The large differences can be caused by different microbial composition of the two systems.

The effect of Cr (VI) on the growth rate of denitrifying bacteria under chemostatic culture showed that Cr (VI) in the range to 0.6 mg/l decreases the maximum growth rate $\mu_{\max}$ and the Monod constant K_s (Mazierski, 1994). It was observed that $\mu_{\max}$ and K_s decreased with an increase in Cr (VI) concentration (Table 3.4). On the basis of the obtained experimental data (Mazierski, 1994), it was found that chromium acts as non-competitive inhibitor on the growth rate of denitrifying bacteria.

Cr (VI) conc mg/l	$\mu_{\max i}$	K_{si}
0	0.3929	20.9916
0.2	0.3162	15.0006
0.4	0.2689	9.9273
0.6	0.2281	6.6971

Table 3.4 Effect of Cr (VI) on the biomass growth rate in the denitrification of nitrate-nitrogen (Mazierski, 1994)

$\mu_{\max i}$ Inhibited maximum growth rate (T^{-1})

K_{si} Monod constant in the presence of inhibitor (ML^{-3})

Chapter 4

Conclusions

Chromium is a toxic, heavy metal pollutant that has adverse effects on the microbes and microbe-mediated ecological processes. The health effects of chromium mainly depends on its valence state. Hexavalent and trivalent forms are the most significant forms of chromium.

The hexavalent form is more mobile in soil and more toxic than the trivalent form. Cr (VI) penetrates readily into the cell membranes than the trivalent form. Chromium is considered as a harmful health hazard for human and animals when its concentration is above acceptable limits.

Although chromium has harmful effects on microorganisms, it has been used in metallurgical, chemical, and refractory industries. Chromeplating and manufacturing of stainless steel are some of the common uses of chromium.

Many factors like biotic (temperature, organic matter, pH, oxidation-reduction potential, clay minerals, inorganic anions and cations, organic matter) and abiotic (physiological age, nutritional state, pigmentation, generic adaptations, slime production, physiological adaptations) factors,

and characteristics of the pollutant (pollutant form, pollutant concentration, interaction between pollutants) influence the toxicity of chromium on microorganisms.

Adverse effects of chromium have been observed in microbe-mediated ecological processes. Chromium affects carbon mineralization, nitrogen transformation, and mineralization of phosphorous and sulfur. It also causes a decrease in enzyme synthesis and ATP content. Presence of elevated levels of chromium in soil decreases soil microbial population concentration and affects microbial respiration. Fungi have been found to be the least sensitive microorganisms to chromium toxicity.

Chromium (VI) can be considered to be toxic and mutagenic to microorganisms. Cr (VI) directly reacts with bacterial DNA causing mutations and basepair substitution. Chromium also inhibits DNA synthesis. Presence of strong oxidizing agents can oxidize trivalent form to the active, mutagenic hexavalent form. Complete loss of mutagenicity has been observed by the addition of either microsomal fractions from rat liver microsomal preparations or of human erythrocyte lysates.

Chromium compounds have a negative effect on the efficiency of wastewater treatment processes. Chromium decreases the maximum growth rate of activated sludge bacteria. However, it has been found that proper acclimation period improves the tolerance of microorganisms to toxic metals. As a result of acclimation, activated sludge microorganisms are adapted to the metal environment metabolically. Acclimation can stimulate the growth rate of activated sludge bacteria.

Chromium is also considered to be toxic in anaerobic treatment processes. Toxicity of chromium in anaerobic digestion is due to the accumulation of the metal in the intracellular fraction of the biomass. A shock injection of chromium has a more severe adverse effects on the anaerobic digestion process than the step-wise injection of the metal. Acidogens responsible for acidogenesis are less sensitive to chromium than methanogens. Chromium also affects the production and degradation of volatile fatty acids.

The harmful effects of chromium in the denitrification process includes decrease in the efficiency of the process with increase in chromium concentration, and a decrease in the growth rate of denitrifying bacteria. Tolerance limit of the process to the presence of chromium depends on the

kind of carbon substrate oxidized. It has been found that the denitrification process in fresh activated sludge is much less sensitive to chromium than the same process in a packed bed reactor.

The toxic effects of chromium to microorganisms and treatment processes makes it a metal of concern in the environment.

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