CHAPTER 2

LITERATURE SURVEY

2.1 CHROMIUM

2.1.1 SOURCES

The amounts of chromium in natural environments are widely varied. In rocks, the Cr concentration varies from an average of 5 mg/kg to 1800 mg/kg. The earth's most important deposits are either in the elemental or the trivalent oxidation state. In most soils, chromium occurs in low concentrations (2 – 60 mg/kg), but values of up to 4 g/kg have been reported in some uncontaminated soils. Only a fraction of this chromium is available to plants. It is not known whether chromium is an essential nutrient for plants, but all plants contain the element up to 0.19 mg/kg on a wet weight basis (WHO, 1988).

Almost all the hexavalent chromium in the environment arises from human activities. It is derived from the industrial oxidation of mined chromium deposits and possibly from the combustion of fossil fuels, wood, paper, etc. In this oxidation state, chromium is relatively stable in air and pure water, but it is reduced to the trivalent state, when it comes into contact with organic matter in air, biota, soils and water. Chromium compounds are used in ferrochrome production, electroplating, pigment production and tanning. Those industries, the burning of fossil fuels and waste incineration are sources of chromium in air and water. Most of the liquid effluent from the chromium industries is trapped and disposed of in landfills and sewage sludges, the chromium being in the form of the insoluble trivalent hydroxide.

2.1.2 PHYSICAL AND CHEMICAL PROPERTIES

Chromium (atomic number 24, relative atomic mass 51.996) occurs in each of the oxidation states from –2 to +6, but only the 0 (elemental), +2, +3 and +6 states are common. Only the trivalent and hexavalent oxidation states are important for human health. It is of great importance to realize that these two oxidation states have very different properties and biological effects on living organisms, including man. This relation between the hexavalent and trivalent states of chromium is described by the equation:

$$Cr_2O_7^{2-} + 14H^+ + 6e \rightarrow 2Cr(III) + 7H_2O + 1.33v.$$

The difference electric potential between these 2 states reflects the strong oxidizing properties of hexavalent chromium and the substantial energy needed to oxidize the trivalent to the hexavalent form. It can be stated that this oxidation never occurs in biological systems. The reduction of hexavalent chromium occurs spontaneously in the organism, unless present in an insoluble form. A gradual reduction of hexavalent chromium to the trivalent state is demonstrated by the color change of the conventional chromate cleaning solution in the laboratory from bright orange to green, in the presence of organic matter. Although a compound CrF_6 is well known, the stable forms of hexavalent chromium are almost always bound to oxygen (e. g., CrO_4^{2-}, $Cr_2O_7^{2-}$). The trivalent form exists in coordination compounds, but never as the free ion. As a rule, its coordination number is 6, the complexes being generally octahedral.

A large number of complexes and chelates of chromium have been investigated, ranging from simple hexa- or tetra complexes to those with organic acids, vitamins, amino acids and others. The rate of ligand

exchange of chromium complexes is slow in comparison with other transition elements, most of the Cr(III)-complexes are kinetically stable in solutions. This property adds to the relative inertness of trivalent compounds, in addition to the electrochemical stability of the trivalent state. However, at near neutral or alkaline pH, the simple chromium compounds to which the organism is exposed in the environment or through supplementation, rapidly become insoluble, because hydroxyl ions replace the coordinated water molecules from the metal and form bridges, linking the chromium atoms into very large, insoluble complexes. Coordination of trivalent chromium to biological ligands is the prerequisite for its solubility at physiological pH and therefore for its biological function and for its availability for intestinal absorption.

2.1.3 USES

The principal uses of chromium are in the metallurgical processing of ferrochromium and other metallurgical products, stainless steel, the refractory processing of chrome bricks and chemical processing to make chromic acid and chromate. Chromate is used for the oxidation of various organic and inorganic materials in the purification of chemicals and the production of pigments. A large percentage of chromic acid is used for plating. Dichromate is converted to chromic sulfate for tanning. Fungicides and wood preservatives consume an estimated 1.3 million kg of chromium annually. Chromate is used as rust and corrosion inhibitors, for example, in diesel engines. Because chromate has a high melting point and is chemically inert, it is used in the manufacture of bricks for lining metallurgical furnaces.

2.1.4 TOXIC EFFECTS OF CHROMIUM ON LIVING THINGS

2.1.4.1 Toxic Effects on Man

Studies on man have established the essential role of trivalent chromium for the maintenance of normal glucose metabolism. Chromium deficiency has been demonstrated in children and two patients on total parental nutrition, the basic disturbance being an impairment of the action of circulating insulin. In adult human, the lethal oral dose is 50-70 mg soluble chromate/kg body weight. The most important clinical features produced following this route of entry are liver and kidney necrosis and poisoning of blood-forming organs.

Hexavalent chromium causes marked irritation of the respiratory tract. Frequently, ulceration and perforation of the nasal septum have occurred in workers employed in the chromate producing and hexavalent chromium-using industries. In addition to inhalation, direct contact of the nasal septum with contaminated hands contributes to nasal exposure. Cancer of the septum has not been reported. Rhinitis, bronchospasm, and pneumonia may result from exposure to hexavalent compounds together with impairment of pneumodynamics during respiration.

Chromate compounds, mainly sodium and potassium chromate and dichromate, cause irritation of the skin and ulcers may develop at the site of skin damage. Exposure to trivalent chromium does not produce such effects. Certain persons manifest allergic skin reactions to hexavalent and possibly trivalent chromium. Skin reactions through dermal exposure to chromium are often described, chromate being the most common contact allergen. However, cancer of the skin dues to chromium exposure has not been reported.

Chronic effects of exposure to chromium occur in the lung, liver, kidney, gastrointestinal tract and circulatory system. Teratogenic risks from chromium exposure have not been reported for human; a mutagenic potency is shown for potassium dichromate and, therefore, can not be excluded for chromate in the chromate-using industries. The few data available indicate that, before the danger of cancer was recognized, the exposure levels in such plants were very high. Some epidemiological data suggest that an excess of lung cancer has also occurred in the chromate-pigment industry. A few cases of cancer involving the upper respiratory tract have been reported, but cancer has not been convincingly demonstrated in other body tissues. This is based on the theory that these compounds remain in contact with the tissues for long periods of time (depot effect).

Trivalent chromium is not considered to be carcinogenic for the following reasons: (a) there was no evidence of excess cancer in studies in two industries where only trivalent compounds were present; (b) results of animals and mutagenicity studies with trivalent chromium, were negative; and (c) because of the chemical and biological characteristics of the trivalent state, i. e., non-oxidizing, non-irritating and probably unable to penetrate cell membranes.

2.1.4.2 Toxic Effects on Animals

Dose of hexavalent chromium greater than 10 mg/kg diet affect mainly the gastrointestinal tract, kidneys and probably the haematopoetic system. Toxic effects from trivalent chromium have been reported only following parental administration. Dietary toxicity has not been reported, even in studies on cat administered amounts of up to 1 g/day for 1 – 3 months. When intravenously injected in mice, the LD_{50} of chromium carbonyl was 30 mg/kg body weight; this represents a 10,000-

fold excess over the therapeutic dose required to cure signs of chromium deficiency. Many studies on experimental animals have been conducted with chromium compounds in efforts to reproduce cancer similar to that found in man, when exposed to chromium.

Hexavalent chromium compounds have been administered to rats by intrabronchial implantation or intratracheal instillation. Compounds, i. e., calcium chromate, strontium chromate and certain forms of zinc chromate produced bronchogenic carcinomas; lead chromate and barium chromate produced weak responses. Sodium dichromate and calcium chromate produced bronchogenic tumors. Lead chromate, lead chromate oxide and cobalt-chromium alloy resulted in the production of local sarcomas. Thus, there is sufficient evidence that hexavalent chromium compounds are carcinogenic for animals. No increased tumor incidence was observed when trivalent compounds were given orally; however, the doses administered were low. Hexavalent chromium has been reported to cause various forms of genetic damage in short-term mutagenicity tests, including damage to DNA, misincoporation of nucleotides in DNA transcription and also mutagenic in mammalian cells *in vitro* and *in vivo*. Hexavalent chromium caused chromosomal aberrations and sister chromatid exchange in mammalian cells *in vitro* only with very high doses and could be explained by nonspecific toxic effects. It induced formation of micronuclei in mice *in vivo* and potassium dichromate also induced dominant lethal mutations.

Trivalent chromium is genetically active only in *in vitro* tests, where it can have a direct interaction with DNA, e. g., in experiments using purified DNA or tests to measure decreased DNA synthesis *in vitro*. Reduction of chromium(VI) within the cell nucleus and the formation of chromium(III) complexes suggests that chromium(III) would be the ultimate mutagenic form of chromium. Trivalent chromium was present

in RNA from all sources examined and probably contributes to the stability of the structure. Injected chromium trichloride ($CrCl_3$) accumulated in the cell nucleus (up to 20% of cellular chromium content), it enhanced RNA synthesis in mice and in regenerating rat liver, suggesting that chromium(III) is involved directly in RNA synthesis. On the other hand, chromium(VI) inhibited RNA synthesis and DNA replication in several systems.

2.1.4.3 Toxic Effects on Plants

Although chromium is present in all plants, it has not been proved to be an essential element for plants. Most substances, including chromium, can be absorbed through either the root or the leaf surface. Several factors affect the availability of chromium for the plant including the pH of the soil, interaction with other minerals or organic chelating compounds, and carbon dioxide and oxygen concentrations.

Little chromium is translocated from the site of absorption, the chelated form is transported throughout the plant. Chromium in high concentrations can be toxic for plants, but there was no specific pattern of chromium intoxication. When combined with a high pH, it showed an inhibition of germination and growth in white mustard plants (***Sinapis alba***) growing on waste heaps. The main feature of chromium intoxication is chlorosis, which is similar to iron deficiency. Soybeans treated in nutrient culture containing 0 – 5 mg hexavalent chromium/liter showed decreased uptake of calcium, potassium, phosphorus, iron and manganese. Death of plants occurred within 3 days of treatment with 30 or 60 mg chromium/L (WHO, 1988).

Chromium affects the carbohydrate metabolism, and the leaf chlorophyll concentration decreased with increasing hexavalent chromium concentration (0.01 – 1 mg hexavalent chromium/L).

Hexavalent appears to be more toxic than trivalent chromium (Stanley, 1974). At present, no data are available concerning the mechanism action or the dose-dependent pattern of chromium intoxication.

2.1.4.4 Toxic Effects on Microorganisms

Most microorganisms (protozoa, protophyta, fungi, algae and bacteria) are able to absorb chromium. The active uptake of chromate by the sulfate transport system has been shown in ***Neurospora crassa*** (Robert and Marzluf, 1971). In general, toxicity for most microorganisms occurs in the range of 0.05 – 5 mg chromium/kg of medium. The internal concentration of chromium depends on the species. Trivalent chromium is less toxic than hexavalent. The main features are inhibition of growth (at concentrations greater than 0.5 mg/L in ***Chlorella*** cultures) and inhibition of various metabolic processes, such as photosynthesis or protein synthesis (US EPA, 1978). The toxicity of chromium for soil bacterial isolates was studied by measuring the turbidity of liquid cultures supplemented with hexavalent chromium and trivalent chromium. Gram-negative bacteria were more affected by hexavalent chromium (1 – 12 mg/kg) than gram-positive bacteria. Toxicity due to trivalent chromium was not observed at similar levels. The toxicity at low levels of hexavalent chromium (1 mg/kg) indicates that soil microbial transformations, such as nitrification, may be affected (WHO, 1988).

2.2 PHENOL

2.2.1 SOURCES

Phenol is a constituent of coal tar and is formed during the natural decomposition of organic materials. Phenol has been detected among the volatile components from liquid manure at concentrations of 7-55 μg/kg dry weight. The major part of phenol present in the

environment, is of anthropogenic origin. The most commonly used production method for phenol, on a worldwide scale, is from cumene (isopropylbenzene). Phenol is also produced from chlorobenzene and toluene. A small but steady supply of phenol is recovered as a by-product of metallurgical coke manufacture. Production and use of phenol and its products is the basic feedstock from which a number of commercially important materials, especially phenolic resins, bisphenol A (2,2-bis-1-hydroxyphenylpropane), caprolactam, alkyl phenols, as well as chlorophenols such as pentachlorophenol (WHO, 1994).

Phenolic resins are used as a binding materials, for example, insulation materials, chipboard and triplex, paints and casting sand foundries. In addition, phenol may be released as a result of thermal decomposition of the resins. Phenol has been detected in the exhaust gases of private cars at concentrations of 0.3 ppm (approximately 1.2 mg/m^3) to 1.4-2.0 ppm (5.4-7.7 mg/m^3). It has also been identified in cigarette smoke. Emission gases from all material incinerators and home fires, especially, wood-burning, may contain substantial quantities of phenol. Another potential source of phenol is the atmospheric degradation of benzene under the influence of light. An important additional source of human phenol exposure may be the *in vivo* formation from various xenobiotics, e. g., benzene.

2.2.2 PHYSICAL AND CHEMICAL PROPERTIES

Phenol is a white crystalline solid, which melts at 43°C and liquefies upon contact with water. It has a characteristic acrid odor and a sharp burning taste. It is soluble in most organic solvents (aromatic hydrocarbons, alcohol, ketone, ethers, acids, halogenated hydrocarbons), its solubility in water is limited at room temperature, above 68°C, it is entirely water-soluble. The solubility is limited in aliphatic solvents. In

the molten state, it is a clear, colorless liquid with a low viscosity. A solution with approximately 10% water is called phenolum liquefactum, as this mixture is liquid at room temperature. Phenol is moderately volatile at room temperature. It is a weak acid, and its ionized form very sensitive to electrophile substitution reactions, such as halogenation and sulfonation, and oxidation.

The chemical properties of phenol are affected by the resonance stabilization possibilities of phenol and, in particular, the phenolate ion. Phenol is sensitive to oxidizing agents. Splitting of the hydrogen atom from the phenolic hydroxyl group is followed by resonance stabilization of the resulting phenyloxy radical. The radical formed can easily be further oxidized. Depending on the oxidizing agent applied and the reaction conditions, various products, such as dihydroxy- and trihydroxybenzenes and quinones are formed. These properties make phenol suitable as an antioxidant, functioning as a radical trapping agent. It also reacts with carbonyl compounds in both acidic and alkaline media. In the presence of formaldehyde, phenol is readily hydroxymethylated with subsequent condensation to resins.

2.2.3 USES

The largest single use of phenol is the production of phenolic resins and use in the production of caprolactam, an intermediate in the production of nylon 6, and 2,2-bis-1-hydroxyphenylpropane (bisphenol A), which is mainly used in the production of phenolic resins. Phenol was widely used for wound treatment and as an antiseptic and local anaesthetic. The medical uses of phenol today include incorporation into disinfectants, antiseptics, lotions, salves and ointments. Another medical application of phenol is its use as a neurolytic agent, applied in order to relieve spasm and chronic pain. In addition to the applications, phenol is

used in the manufacture of paint and varnish removers, lacquers, paints robber, ink, illuminating gases, tanning dyes, perfumes, soaps and toys.

2.2.4 TOXIC EFFECTS OF PHENOL ON LIVING THINGS

2.2.4.1 Toxic Effects on Man

A wide range of adverse effects has been reported following human exposure to phenol by the dermal, oral or intravenous routes. Gastrointestinal irritation has been reported following ingestion. Local effects following dermal exposure range from painless blanching or erythema to corrosion and deep necrosis. Systemic effects include cardiac dysrhythmias, metabolic acidosis, hyperventilation, respiratory distress, acute renal failure, renal damage, dark urine, methaemoglobinaemia, neurological effects (including convulsions), cardiovascular shock, coma and dealth. The lowest reported dose resulting in a human death was 4.8 g by ingestion, death occurred within 10 min (WHO, 1994).

The potential for poisoning through inhalation of phenol vapours has long been recognized, but no cases of death following this route of exposure have been reported. Symptoms associated with inhalation of phenol included anorexia, weight loss, headache, vertigo, salivation and dark urine. Phenol is not a sensitizing agent. The human odor threshold for phenol in water has been reported to range from 7.9 mg/L and the taste threshold 0.3 mg/L. Adequate human data on the carcinogenicity of phenol are not available (WHO, 1994).

2.2.4.2 Toxic Effects on Animals

Phenol has moderate acute toxicity for mammals. Oral LD_{50} values in rodent range from 300 to 600 mg phenol/kg body weight. Dermal LD_{50} values for rats and rabbits range from 670 to 1400 mg/kg

body weight, respectively, and the 8-hr LC_{50} for rats by inhalation is more than 900 mg phenol/m^3. Clinical symptoms after acute exposure are neuromuscular hyperexcitability and severe convulsions, necrosis of skin and mucous membranes of the throat and effects on lungs, nerve fibers, kidneys, liver and the pupil response to light. Solutions of phenol are corrosive to skin and eyes. Phenol vapors can irritate the respiratory tract. There is evidence that phenol is not a skin sensitizer. The most important effects reported in short-term animal studies were neurotoxicity, liver and kidney damage, respiratory effects and growth retardation. There are no adequate studies on the reproductive toxicity of phenol. Phenol has been identified as a developmental toxicant in studies with rats and mice. Mutations, chromosomal damage and DNA effects have been observed in mammalian cells *in vitro*. Phenol has no effect on intercellular communication in cultured mammalian cells. Induction of micronuclei in bone narrow cells of mice has been observed, no micronuclei were observed in mice studies at lower doses. Two carcinogenicity studies have been conducted with male and female rats and mice receiving phenol in water, malignancies were only seen in low-dose male rats (WHO, 1994).

2.2.4.3 Toxic Effects on Microorganisms

In microorganisms, growth inhibition is usually observed after phenol exposure. In studies on single bacterial species, the EC_{50} values (EC_{50}= calculated concentration affecting 50% of test population) found for growth inhibition varied from 244 mg phenol/L in a newly developed, 6-hr test with ***P. putida*** to 1600 mg phenol/L after 18-hr of exposure in a more conventional test with ***Aeromonas hydrophila***. Bringmann and Kuhn (1977) reported a toxicity threshold of 64 mg/L after 16-hr. EC_{50} values for reduced photoluminescence in ***Photobacterium phosphoreum***

of 28-34 mg phenol/L and 40 mg phenol/L have been reported. In activated sludge, the EC_{50} for a reduced oxygen uptake was reported to be 520-1500 mg phenol/L, whereas a lower value was found for substrate consumption inhibition (104 mg phenol/L). The lowest reported concentration affecting activated sludge was 10 mg phenol/L; 1 mg phenol/L has no effect (WHO, 1994).

Reported toxicity thresholds for protozoa were of the same order of magnitude as for bacteria: 33-144 mg phenol/L. For algae, values were lower, but were observed after a longer exposure period: 6 mg phenol/L for cyanobacteria (blue-green algae) and 8 mg phenol/L for green algae, after 7-8 days of exposure. The IC_{50} values (concentration causing 50% growth inhibition) reported for various fungi were of the same order of magnitude as the above EC_{50} values for bacterial growth inhibition: 460-1000 mg phenol/L. These values are also within the range of concentrations observed to cause initial or complete growth inhibition in various fungi (100-1000 mg phenol/L and 750- > 1000 mg phenol/L, respectively). An increase in salinity (0-30%) increased the toxicity of phenol to fungi.

2.3 METHODS OF METAL REMOVAL

2.3.1 PHYSICO-CHEMICAL METHODS

2.3.1.1 Adsorption

Activated carbon adsorption is a popular method in both water and wastewater treatment to remove soluble heavy metal ions, for example, the removal of copper and chromium from electroplating wastewaters (Smithson, 1971).

Activated carbon, amorphous form of carbon, is made from different raw materials, i. e., coal, wood, coconut shells, pulp mill residues, petroleum base residues and char from sewage sludge pyrolysis.

It is sequential produced by controlled dehydration, carbonation and oxidation. Activated carbon is not a pure carbon and has high affinity to adsorb heavy metals and other compounds. It is able to be used in either granular or powder forms, depending on the application and economic (Wentz, 1995).

Adsorption on activated carbon occurs when it contacts to solution and will be stopped at the rate of adsorption and desorption is equal. The capacity of adsorption per unit weight of carbon is as increase as the concentration of solute is increased. Adsorption equilibrium is controlled by two types of interaction, i. e., solute-adsorbent and solute-solvent. There is competition between the forces of dissolution and adsorption, any charge in a system which tends to decrease the dissolution force or increase the adsorption forces will shift equilibrium toward higher adsorption per unit weight of carbon. Anyway, adsorption is depended on various factors, i. e., surface area, pore sizes, solubility of solute, pH and temperature.

2.3.1.2 Evaporation

Evaporation of liquid is applied in heavy metals management. This method is depended on a vapor pressure. At high vapor pressure, evaporation will be readily, but at low vapor pressure, evaporation is more slowly. The boiling temperature of a pure liquid has been reached when the vapor pressure of liquid equals the atmospheric pressure. Soluble salts and other compounds elevate the boiling point. So, physical separation techniques should be used before evaporation process.

Evaporation are used in single and multi-effect evaporation. To conserve energy and enhance separation of volatile wastes from liquids, a multi-effect evaporator may be used.

2.3.1.3 Ion Exchange

Ion exchange is a reversible exchange of ions between liquid and solid phases. Ions held by electrostatic forces to charge functional groups on the surface of an insoluble solid are replaced by similar ions charge in a solution (Wentz, 1995). Ion exchange materials should have ion active site in whole structure, high capacity, selectivity for ionic species, capability of regeneration, chemical and physical stability and low solubility.

Synthetic ion exchange material is widely used in commercial application, its has both cation and anion exchanges, cation exchange resin has a high ion exchange capacity and has reactive groups, such as sulfonic, phenolic and carboxylic group and anion exchange resin has reactive groups, such as quaternary ammonium or amine groups.

2.3.1.4 Precipitation

The usual method for removal of heavy metals is chemical precipitation. Precipitation have been used to removal of metal from wastewater, including Cr, Cu, Zn, Pb, Cd (cadmium), Mg (magnesium) and Hg (mercury). Precipitation of heavy metal is depended on pH. Due to the type of metal ion has resulting in the formation of an insoluble form.

The hydroxide of heavy metals are usual insoluble, generally, lime is used to precipitate them. Precipitation of heavy metal is depended on pH. The optimum pH is unique for each metal. Furthermore, carbonates and sulfides are used to precipitate heavy metal. Sodium sulfide (Na_2S) and sodium bisulfide (NaSH), popular sulfide chemicals, are widely added to precipitate heavy metals. However, addition of sulfide compounds must be controlled to minimize odor and potential

toxicity, because precipitation by sulfide is able to generate another unattractive by-product, hydrogen sulfide (H_2S) gas.

2.3.1.5 Reverse Osmosis

Osmosis is a process which a solvents flows through a semipermeable membrane from a dilute to a more concentrated solution (Hess, 1988). In general, the solvent flows in the direction that will reduce the concentration of solution. The osmotic pressure of the solution is applied to the solution will just prevent the passage of the solvent through the semipermeable membrane. In reverse osmosis, a differential pressure that exceeds the osmotic pressure is applied to the membrane, causing the solvent to flow from the stronger to the weaker solution. In application, reverse osmosis is used to removal heavy metal include Cu from electroplating rinse water and desalination of sea or blackish water.

2.3.2 BIOLOGICAL METHODS

2.3.2.1 Detoxification

The most important role of microorganisms in the transformation of pollutants is their ability to bring about detoxification (sometimes called detoxication), refers to the change in a molecule that presents it less harmful to one or more susceptible species, such as humans, animals, plants, other microorganisms, or the detoxifying population itself. Special approach is given to detoxification that make organic compounds less harmful to humans, but an abundant body of information also exists on detoxification reactions that alter the toxicity to animals and plants. In studies of environmental pollution, detoxification that reduce the harm to microorganisms have received little investigation (Alexander, 1994).

A simple way of demonstrating detoxification is to measure the effect of environmental samples on the behavior, growth, or viability of susceptible species. Detoxification is advantageous to the microorganisms carrying out the transformation if the concentration of the chemical is in the range that suppresses these species. If the reaction is the first step in a process by which organisms use the molecule as a carbon source, the reaction is also beneficial, not because it inactivates the molecule but by virtue of its helping the cell to acquire carbon. However, the transformations are important in public health, agriculture, or natural biological communities but not for the microorganisms responsible for the conversion.

The enzymatic step or sequence that results in the conversion of the active molecule into the innocuous product usually occurs within the cell. The product may then undergo one of three fates: (i) it may be excreted; (ii) after one or more additional enzymatic steps, it may be changed to a compound that enters the normal metabolic pathways within the cell and ultimately the carbon is excreted as an organic waste; or (iii) it may be modified to a new molecule that becomes subject to these normal reaction sequences, and finally the carbon is released as CO_2. The product in the first case is structurally similar to the toxin, but it is harmless at the prevailing concentration. The last fate is mineralization, the mineralization of inhibitors being detoxification, but the actual detoxification step occurs at some early step in the catabolic sequence that finally yields CO_2. Cometabolic processes often are detoxification, but the products of the transformations are structurally similar to the original substrate.

A particular microorganism or a microbial community may detoxify a single toxicant in several ways. Such multiple pathways are initiated by entirely different enzymes. Other pesticides are also acted on

by several dissimilar enzymes, which may thus yield several inactive products. Indeed, several such reactions may yield products far more toxic than the original substrates. Furthermore, a reaction or a sequence that yields a product not injurious to one species may not result in detoxification for a second species.

2.3.2.2 Cr(VI) Reduction

Chromium contamination of the environment is extensive. The reduction of highly toxic and mobile Cr(VI) to the less toxic, less mobile Cr(III) is likely to be a useful process for the remediation of contaminated waters and soils. This problem has stimulated interest in microorganisms that can use Cr(VI) as an electron acceptor (Lovley, 1993).

(a) Cr(VI)-Reducing Microorganisms

Early investigations demonstrated that facultative anaerobes such as ***Pseudomonas dechromaticans***, ***P. chromatophila*** and ***Aeromonas dechromatica*** remove Cr(VI) from solution by the formation of a Cr(III) precipitate, presumably $Cr(OH)_3$. Subsequent studies have demonstrated that the capacity for Cr(VI) reduction is widespread and found in such organisms as ***Salmonella typhimurium*** (Petrilli and Flora, 1977), ***Bacillus cereus***, ***B. subtilis*** and ***P. aeruginosa*** (Summers and Jacoby, 1978; and Cervantes et.al., 1990), ***P. fluorescens*** (Bopp et.al., 1983; Ohtake et.al., 1987; and Appanna et.al., 1996), ***P. ambigua*** (Horitsu et.al., 1987; and Suzuki et.al., 1992), ***P. putida*** (Ishibashi et.al., 1990), ***Streptomyces*** **sp.** (Das and Chandra, 1990) and ***Micrococcus roseus***, as well as ***Enterobacter clocae*** (Hardoyo et.al., 1991), ***E. coli*** (Shen and Wang, 1992, 1993, 1995a and 1995b), ***Achromobacter eurydice*** and ***Agrobacterium radiobacter*** (Llovera et.al., 1993),

Desulfovibrio desulfuricans and ***D. vulgaris*** (Lovley and Phillips, 1994) and ***Alcaligenes eutrophus*** (Peitzsch et.al., 1998).

Many of these organisms reduce Cr(VI) better under aerobic conditions than under anaerobic conditions and the physiological role of this aerobic Cr(VI) reduction has not been well defined. Cr(VI) reduction is not a Cr(VI)-resistance mechanism and Cr(VI) reduction in ***Pseudomonas putida*** and possible other Cr(VI) reducers is a side activity for enzymes that have other, as yet unidentified, natural substrates.

Although some microorganisms reduce Cr(VI) during anaerobic growth in media in which Cr(VI) is provided as the sole electron acceptor, in no instance has Cr(VI) reduction definitely been shown to yield energy to support anaerobic growth. For example, ***P. chromatophila*** purportedly uses Cr(VI) as an electron acceptor to support growth under anaerobic conditions with a variety of electron acceptors, including the nonfermentable substrate, acetate. However, in the only data shown, most of the Cr(VI) reduction took place after growth stopped. No evident demonstrating that anaerobic growth depended upon the presence of Cr(VI) was presented.

The catalytic mechanism of Cr(VI) reductase by ***P. ambigua*** **G-1**, the reduction of Cr(VI) to Cr(III) by two (or more) steps. First, Cr(VI) accepts one electron from one NADH molecule and generates Cr(V) as an intermediate. Second, the Cr(V) intermediate accepts two electrons from two molecules of the same coenzyme. The first step proceeds more rapidly than the second one (Suzuki et.el., 1992).

The catalytic mechanisms of Cr(VI) reduction in ***E. coli*** **ATCC33456**, the activity of the reductase, predominant activity of soluble reductase and a minor activity associated with the respiratory chain involved in Cr(VI) reduction were observed (Shen and Wang, 1994a). The respiratory chain-linked activity depends on the physical

presence of the soluble reductase, which may be used to mediate the electron transfer from cytochrome to Cr(VI), one reductase is probably responsible for passing the electron to Cr(VI). Stimulation of the overall rate of Cr(VI) reduction, two or more active sites were present that only one reductase is responsible for electron transfer to Cr(VI), as shown in **Figure 2.1.**

(b) Enzymatic Mechanisms for Cr(VI) Reduction

Cr(VI) reduction is an enzymatically catalyzed reaction in the Cr(VI) reducers that have been studied in detail. For example, spent medium from cultures of ***P. fluorescens*** **LB300** does not reduce Cr(VI), and washed cell suspensions reduce Cr(VI) only if glucose or another suitable electron donor is provided and the Cr(VI)-reducing capacity is lost when the membrane fraction is removed.

Evidence for an enzymatic role in Cr(VI) reduction in ***Enterobacter cloacae*** is that: Cr(VI) is reduced faster with higher cell densities; no Cr(VI) reduction occurs in cell-free controls. Cell-free filtrates of cultures do not reduce Cr(VI). Cr(VI) also inhibits Cr(VI) reduction, i. e. the rate of reduction declines as the concentration rises above 1 mM. Temperature and pH optima for Cr(VI) reduction are characteristics of an enzymatically catalyzed reaction (Wang et. al., 1990).

The Cr(VI) reductase activity in ***E. cloacae*** is located in the membrane fraction. When membrane vesicles are reduced with NADH and then exposed to Cr(VI), the c- and b-type cytochromes are oxidized. Further analyses have suggested that c_{548} of the identifiable cytochromes in the membrane vesicles (c_{548}, c_{549}, c_{550}, c_{555}, c_{556}, and c_{558}), might be specifically involved in Cr(VI) reduction, serving as a branch point between Cr(VI) and O_2 reduction (Lovley D. R., 1993).

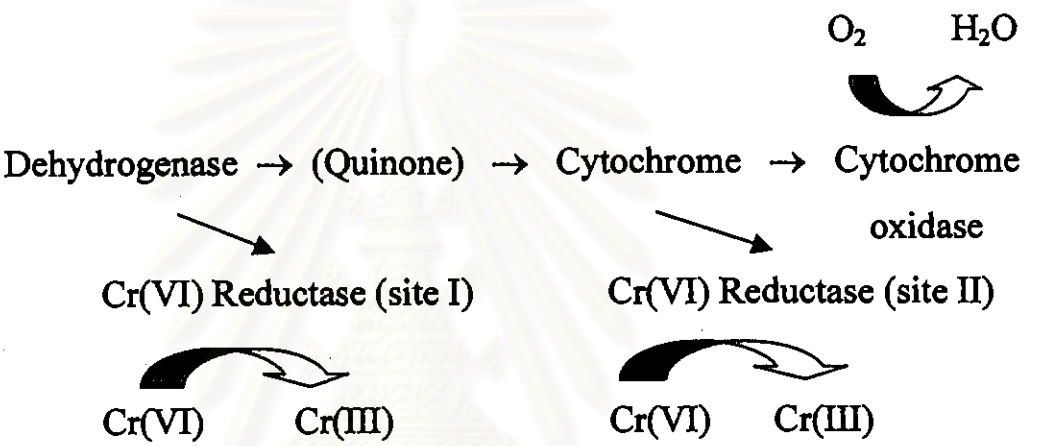

Figure 2.1 Schematic representation of electron transport to Cr(VI) in ***E. coli*** for two pathways of electron transport to Cr(VI)

Source : Shen and Wang (1994a)

In contrast to ***Pseudomonas fluorescens*** and ***E. cloacae***, the Cr(VI)-reducing activity in ***P. ambigua*** and ***P. putida*** is in the soluble fraction of the cell. A 65-kDa protein has been purified from ***P. ambigua*** that can reduce Cr(VI) with NADH or NADPH serving as the electron donor. Initially, Cr(VI) was released to Cr(V), an intermediate, in the absence of the enzyme, but the rate of nonenzymatic reduction was slower.

Washed cell suspension of ***Desulfovibrio desulfuricans*** and ***D. vulgaris*** rapidly reduce Cr(VI) to Cr(III) under anaerobic conditions with H_2 as the electron donor. H_2-dependent Cr(VI) reductase activity in the soluble, cell-free fraction of ***D. vulgaris*** is lost when the soluble fraction is passed over a cation-exchange column that removes cytochrome c_3, a periplasmic protein. The capacity for Cr(VI) reduction is restored when cytochrome c_3 is added back. In the presence of H_2 and an excess of hydrogenase, cytochrome c_3 reduces Cr(VI) at a rate 50-fold faster than the maximum rate for Cr(VI) reduction by the Cr(VI) reductase purified from ***P. ambigua***.

(c) Genetic for Cr(VI) Reduction

Resistance to chromate governed by bacterial plasmids appears to have nothing to do with chromate reduction. Furthermore, it is not clear whether the chromate reduction ability found with several bacterial isolates confers resistance to CrO_4^{2-}. Plasmid-determined chromate resistance results from reduced uptake of CrO_4^{2-} by the resistant cells, but we have been unable to determine whether there is chromate efflux or a direct block on uptake. The DNA sequences of the ***P. aeruginosa*** and ***Alcaligenes eutrophus*** chromate resistance systems share homologous ***chr*A** genes, which encode membrane proteins. The ***A. eutrophus*** chromate resistance determinant contains as additional

upstream gene, ***chr*B**, that was proposed to be responsible for the inducibility of the resistance. The ***P. aeruginosa*** determinant as cloned lacks both the *chr*B gene and inducibility.

Recently, two chromate resistance related sequences have been recognized in sequence libraries. Nicholson and Laudenbach (GenBank accession U20224; locus SSU20224) sequenced a DNA open reading frame from the large plasmid of the cyanobacterium ***Synechococcus* PCC7942** that encodes a protein closer to ***chr*A** of ***A. eutrophus*** (67% amino acid identities) than original ***Pseudomonas chr*A** sequence (only 28% identical amino acids). This sequence occurs in a region involved in sulfate metabolism, suggesting a functional role in divalent oxyanion transport. The other sequence (GenBank protein S41228) is of a fragment that encodes only 74 amino acids, but with a high similarity (39% amino acid identities) to the N-terminal region of the cyanobacterial ***chr*A** (Silver and Phung, 1996).

2.3.2.3 Biodegradation

There are two general types of biodegradation, i. e., mineralization and biotransformation. Mineralization occurs when organic compounds are converted by living organisms to mineral (nonorganic) end products:

$$C_aH_bO_cN_dP_eS_fCl_g \rightarrow iCO_2 + jH_2O + kNH_4^+ + lPO_4^{3-} + mSO_4^{2-} + nCl^-$$

(organic)

Energy is produced during mineralization. Biotransformation occurs when parent organic compounds are not completely mineralized, a portion is converted into other organic. Fermentation represents all biotransformations. Energy is often obtained from fermentation, but may not be with all biotransformations.

Biotransformation of halogenated organic is somewhat more complicated. It had been felt that as the number of chlorine atoms in a molecule increases, it becomes more toxic and more difficult to degrade. However, recent evidence indicates that such behavior is organism and environment specific. The following is a list of transformation reactions that can be mediated by bacteria:

(i) Oxidation : release of electrons during transformation

(ii) Reduction : addition of electrons during transformation

(iii) Hydrolysis : addition of water

(iv) Substitution : exchange of one group for another (e. g., OH^- or HS^- for Cl^-)

(v) Elimination : removal of atoms from adjacent carbons, leaving a double bond between them

(vi) Dealkylation : removal of an alkyl group

(vii) Deamination : removal of an NH_2 group

(viii) Condensation : production of a larger molecule from smaller molecules

(ix) Isomerization : conversion of one isomer into another (e. g., conversion of 1,2-cis-dichloroethylene to 1,2-trans-dichloroethylene)

(x) Ring cleavage : opening of an organic ring structure, generally for the purpose of further biotransformation

(a) Phenol Degradation

These biotransformations are important to environmental engineers, particularly for halogenated, xenobiotic compounds, but are novel in the sense that the organic compounds are not being used as electron donors or primary substrates. Xenobiotic chemicals are those that are foreign to natural biota. Anthropogenic compounds are those that

are synthetic. Examples included in both categories are pesticides, PCBs, and chlorinated solvents. These compounds are considered hazardous and toxic. Some toxic organic chemicals are naturally occurring such as BTEXs, phenol, and PAHs.

Organic compounds in general can be biotransformed by microorganism in several ways, and this applied to xenobiotics and other toxic organic compounds as well. The best known and understood is when the organic compound serves as a primary substrate, or a source of energy and carbon for growth. Primary substrates can typically be mineralized to inorganic constituents. Organic compounds can also be degraded via secondary utilization. Here, the microorganisms do not derive sufficient energy or carbon for net growth from compound oxidation. There are several types of secondary utilization. Microorganisms may not derive energy or carbon because the concentration of the target organic is too low. Organic compounds that cannot serve as carbon or energy sources for bacteria may also be degraded via cometabolism, which occurs only when a primary substrate is present to support growth. Finally, organic compounds can be transformed while serving as electron acceptors and are reduced in the process. Such reactions may or may not be cometabolic as well. This is an especially important reaction for some halogenated organic compounds.

Transformation reactions important in the fate and removal of toxic compounds were mediated by microorganisms (biotic) and some can occur chemically (abiotic). In general, biotic transformation reactions are faster than abiotic reactions, but this is not always the case (Vogel et. al., 1987). Microbial phenol degradation is also occurred in; ***P. putida*** (Feist and Hegeman, 1969; and Bayly and Wigmore, 1973),

P. acidovoran (Schmidt and Alexander, 1985) and *A. eutrophus* (Kim et.al., 1996).

The aromatic compounds that support the growth of fluorescent pseudomonads (*P. aeruginosa*, *P. putida* and *P. fluorescens*) are catabolized via the common diphenolic intermediate, catechol (Feist and Hegeman, 1969). The growth of a fluorescent pseudomonad at the expense of a catechol precursor elicits the induction of catechol 1,2-oxygenase and associated enzymes of the β-ketoadipate pathway, as shown in **Figure 2.2.** However, those *Pseudomonas* strains of the fluorescent group can decompose catechol by an alternative inducible pathway (**Figure 2.3**) involving ring cleavage by a catechol 2,3-oxygenase. We shall term the former pathway the ortho cleavage pathway, and the latter, the meta cleavage pathway. *P. putida* that posses the genetic capability to compose catechol through both the ortho and meta cleavage pathways is determined by the nature of the primary aromatic substrate. The enzymes and functional inducers of the ortho and meta pathways in *P. putida* are shown in **Figure 2.4.**

(b) Genetic for Phenol Degradation

The *xyl* genes of *P. putida* TOL plasmid that specify catabolism of toluene and xylenes are organized in four transcriptional units: the upper-operon *xylUWCAMBN* for conversion of toluene/xylenes into benzoate/alkylbenzoates; the meta-operon *xylXYZLTEGFJQKIH*, which encodes the enzymes for further conversion of these compounds into Krebs cycle intermediates; and *xylS* and *xylR*, which are involved in transcriptional control. The XylS and XylR proteins are members of the XylS/AraC and NtrC families, respectively, of transcriptional regulators. The *xylS* gene is constitutively expressed at a low level from the Ps2 promoter. The XylS protein is activated by interaction with

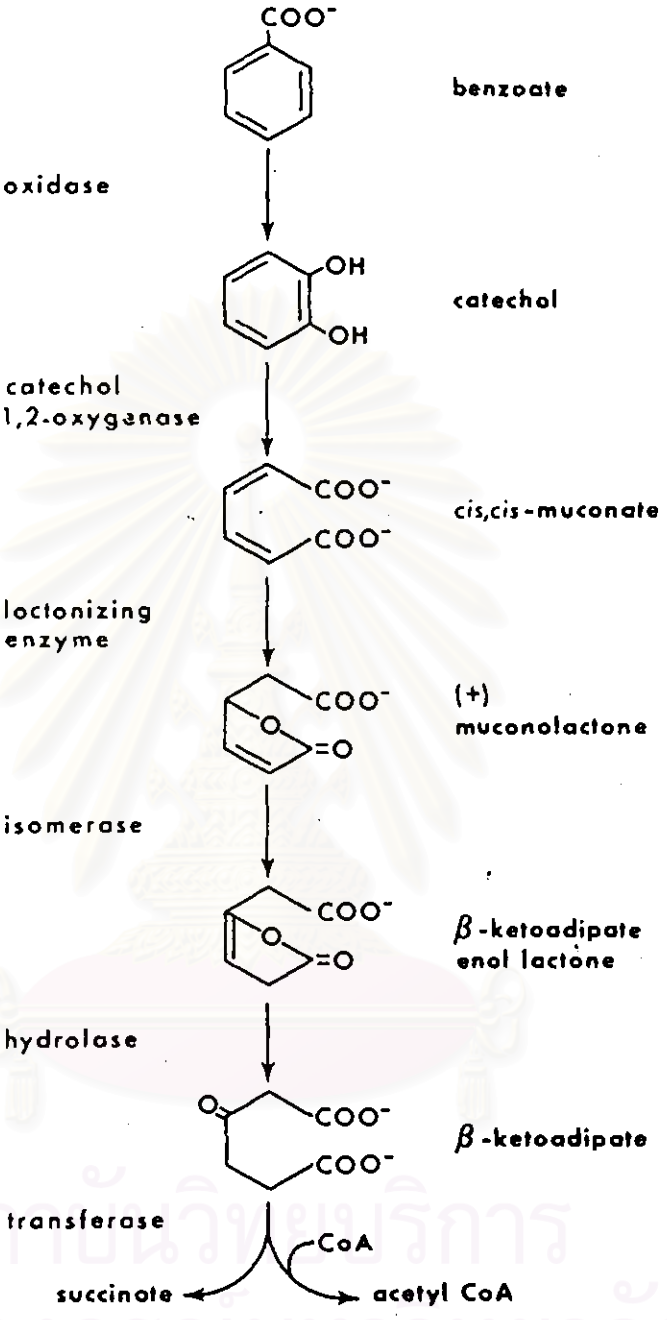

Figure 2.2 The ortho cleavage pathway (β-ketoadipate pathway) for oxidation of benzoate.

Source : Feist and Hegeman (1969)

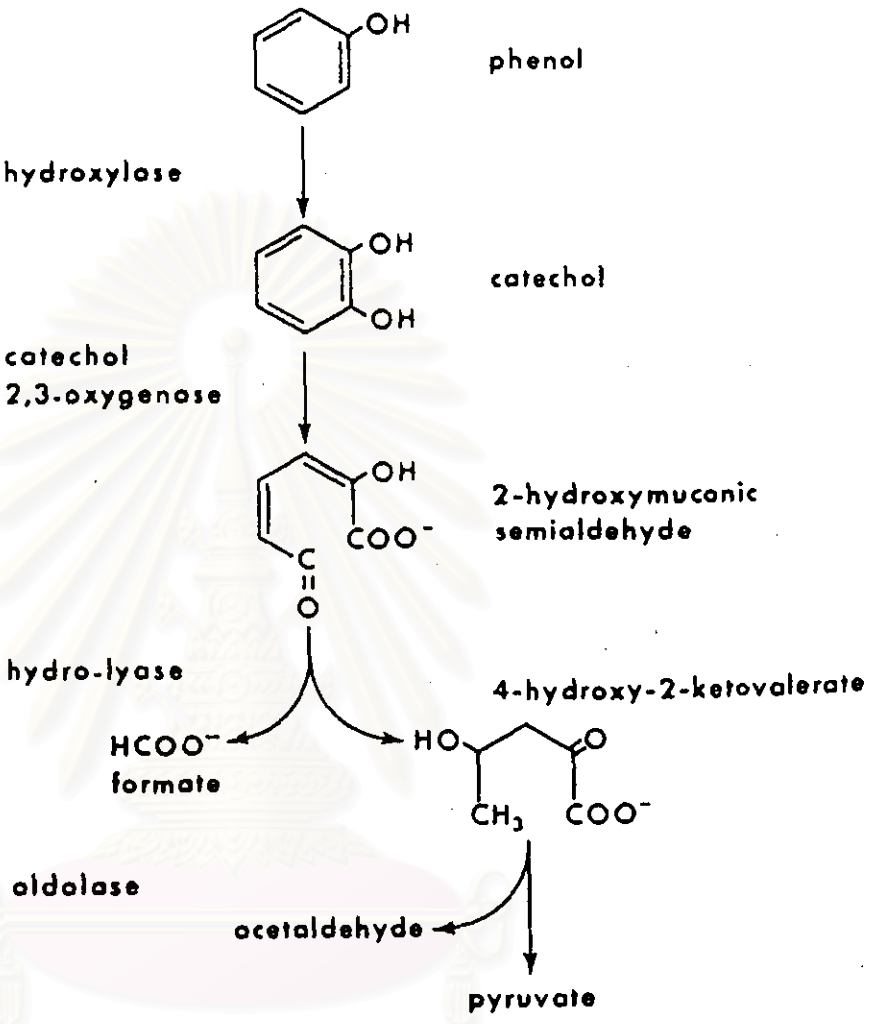

Figure 2.3 The meta cleavage pathway for oxidation of phenol

Source : Feist and Hegeman (1969)

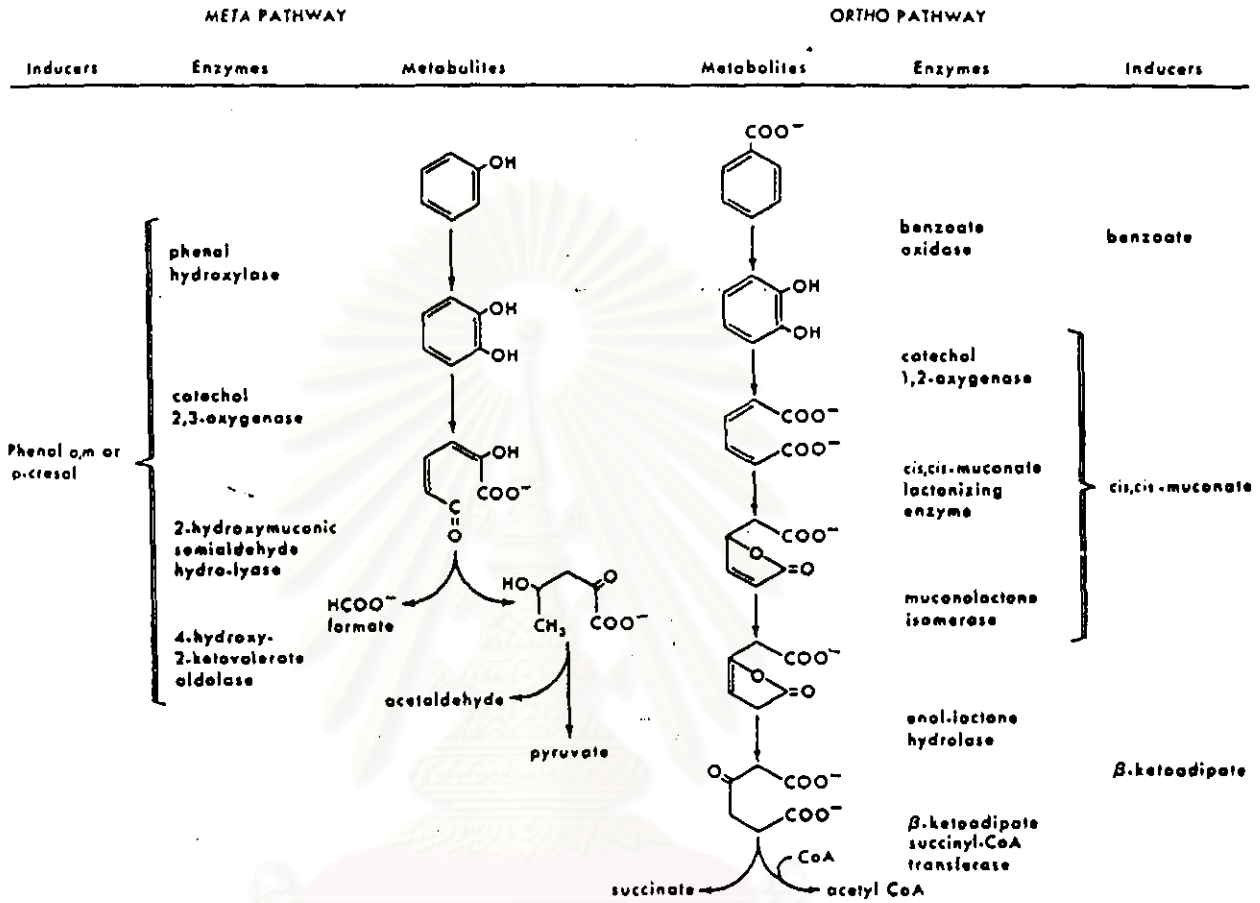

Figure 2.4 Metabolites, enzymes and inducers of the ortho and meta pathways in ***P. putida***.

Source : Feist and Hegeman (1969)

alkylbenzoates and this active form stimulates transcription from Pm by σ^{70}- or σ^{S}-containing RNA polymerase (the meta loop). The ***xylR*** gene is also expressed constitutively. The XylR protein, which in the absence of effectors binds in a nonactive form to target DNA sequences, is activated by aromatic hydrocarbons and ATP; it subsequently undergoes multi mineralization and structural changes that result in stimulation of transcription from Pu of the upper operon. This latter process is assisted by the integration host factor (IHF) protein and mediated by σ^{54}-containing RNA polymerase. Once activated, the XylR protein also stimulates transcription from the Ps1 promoter of ***xylS*** without interfering with expression from Ps2. This process is assisted by the HU protein and is mediated by σ^{54}-containing RNA polymerase. As a consequence of hyperexpression of the ***xylS*** gene, the XylS protein is hyperproduced and stimulates transcription from Pm even in the absence of effectors (the cascade loop). The two σ^{54}-dependent promoters are additionally subject to global (catabolite repression) control (Ramos et.al., 1997).

In ***P. putida*** **PaW85**, the 11.4 kb ***Xho*****I** fragment cloned from phenol degradation plasmid pEST1226 into pKT240 (recombinant plasmid pAT1140) contains the inducible ***phe*****BA** operon that encodes catechol 1,2-dioxygenase (gene ***phe*****B**) and phenol monooxygenase (gene ***phe*****A**), the first two enzymes for the phenol degradation pathway. The promoter of the ***phe*****BA** operon is mapped 1.5kb upstream of the ***phe*****B** gene. The plasmid pAT1140, when introduced into ***P. putida*** **PaW85**, enables the bacteria to use the hybrid plasmid-chromosome-encoded pathway for phenol degradation. The synthesis of the plasmid-encoded phenol monooxygenase and catechol 1,2-dioxygenase is induced by cis,cis-muconate. The expression studies of the deletion subclones derived from pAT1140 revealed that the transcription of the ***phe*****BA** operon is positively controlled by a regulatory protein that is

chromosomally encoded in ***P. putida***. cis,cis-Muconate in cooperation with positive transcription factor CatR activates the transcription of the chromosomal ortho-pathway genes ***cat*A** and ***cat*BC** in ***P. putida*** (Rothmel et.al., 1990). The inability to express the ***phe*BA** operon in a ***P. putida*** CatR$^-$ background and activation of transcription of the ***phe*BA** operon in ***E. coli*** in the presence of the ***cat*R**-expressing plasmid demonstrated that the transcription of the ***phe*BA** operon in ***P. putida*** **PaW85** carrying pEST1226 is controlled by the chromosomally encoded CatR (Kasak et.al., 1993). Expression of the gene ***phe*A**, which encodes phenol monooxygenase and is linked to the ***phe*BA** operon, allows pseudomonads to use phenol as a growth substrate (Peters et.al., 1997).

2.4 METAL-METAL TOXIC INTERACTIONS

2.4.1 Cr-Zn INTERACTIONS

Although intestinal absorption of Cr(III) appears to be a process of passive diffusion, the intestinal absorption of Cr(III) and Zn is increased in the Zn-deficient rat. In the presense of Zn, the absorption of Cr(III) is inhibited and vice versa. This shows that, at least under conditions of Zn deficiency, Cr(III) may interact with Zn-binding ligands in the small intestine (Corn, 1993).

2.4.2 Cr-Fe INTERACTIONS

Transport of Cr(III) in the blood occurs mainly in the plasma by binding to transferrin. For this transport protein, a competitive binding of Fe(III) and Cr(III) ions is established. Since Cr(III) may play a role in insulin metabolism, its displacement from transferrin in iron overhead conditions, and thus its reduced body retention, suggest a contribution in the mechanism of hemochromatotic diabetes.

2.5 PHENOL AS CARBON AND ENERGY SOURCES FOR GROWTH

Microorganisms use natural occurring and many synthetic chemicals for their growth. They use these molecules as a source of carbon, energy, nitrogen (N), phosphorus (P), sulfur (S) or another element needed by the cells. Most attention has been focused on the gain of carbon and energy to support the growth of bacteria and fungi. For the synthetic substrates that are extensively degraded, the molecule is simply another organic substrate from which the population can obtain the needed elements or the energy required for biosynthetic reactions (Alexander, 1994).

A common research procedure that relies on the ability of microorganisms to use organic compounds as sources of carbon and energy for growth is known as the enrichment-culture technique. The method is based on the selective advantage gained by an organism that is able to use a particular test compound as a carbon and energy sources in a medium containing inorganic nutrients but no other sources of carbon and energy. Under these conditions, a population of the species that is able to grow by utilizing that chemical will increase.

The enrichment culture technique has been the basis for the isolation of pure cultures of bacteria and fungi that are able to use a large number of organic molecules as carbon and energy sources. However, attempts to obtain microorganisms that are able to grow on a variety of other organic compounds have met with failure from misuse of the technique or errors in the approach of the investigator. Example, sometimes the concentration of the organic nutrient may be low to give detectable turbidity in the enrichment solution or too high so that the microorganisms fail to develop because of the toxicity.

Members of a large number of bacteria and fungi have been isolated that grow on one or more synthetic compounds. Much of the early literature relates with sugars, amino acids, other organic acids and other cellular or tissue constituents of living organisms, but a variety of pesticides have also been shown to support the growth. Under these conditions, bacteria increase in numbers and fungi increase in biomass in culture media, and at the same time, the chemical disappears.

Mineralization of organic compounds is characteristic of growth-linked biodegradation, in which the organism converts the substrate to CO_2, cell components and products similar to the usual catabolic pathways. However, that mineralization in nature occasionally may not be linked to growth but instead results from decreasing populations. Conversely, some species growing at the expense of a carbon compound may still not mineralize and produce CO_2 from the substrate. However, if O_2 is present, the organic products excreted by one species probably will be converted to CO_2 by another species, so that even if the initial population does not produce CO_2, the second species will. The net effect is still one of mineralization.

Compound, such as many environmental pollutants, that represents a novel carbon and energy sources for a particular population still is transformed by the metabolic pathways that are characteristic of heterotrophic microorganisms. For the organism to grow on the compound, it must be converted to the intermediates that characterize these major metabolic sequences. If the compound cannot be modified enzymatically to yield such intermediates, it will not serve as a carbon and energy sources because the energy yielding and biosynthetic processes cannot function. The initial phases of the biodegradation involve modification of the novel substrate to yield a product that is itself an intermediate or, following further metabolism. This need to convert

the synthetic molecule to intermediates is characteristic of both aerobes and anaerobes as they derive carbon and energy from the substrate.

However, that an organic compound need not be a substrate for growth in order for it to be metabolized by microorganisms. Two categories of transformations, first, the biodegradation provides carbon and energy to support growth and the process therefore is growth-linked. Second, the biodegradation is not linked to multiplication; the reasons will be considered in the following. During the growth-linked mineralization about by bacteria, the cells use some of the energy and carbon of their organic substrate to make new cells, and this increasing large population causes an increasing rapid mineralization. Example, bacteria capable of metabolizing 4-nitrophenol increase in sewage samples as the chemical disappears from the water (Wiggins et.al., 1987). The mineralization reflects the population change. Some species are efficient in capturing the E in the organic substrate and converting the carbon to cells. For example, 93-98% of benzoate and phenol added to samples of sewage at levels below 300 μg/L was converted to CO_2 (Subba-Rao et.al., 1982).

For heterotrophic microorganisms in most natural ecosystems, the limiting element is generally carbon, and usually sufficient N, P, S and other nutrient elements are present to satisfy the microbial demand. The biodegradation usually will still lead to the mineralization of the organic compounds as carbon and energy sources.

2.5.1 ENERGETIC AND BACTERIAL GROWTH

Microorganisms oxidize inorganic and organic materials in order to obtain energy for growth and maintenance. Heterotrophic organisms use a portion of organic material metabolized for energy, which in turn is used to convert another portion of the organic matter into

cells. Autotrophic organisms on the other hand oxidize inorganic materials for energy and in turn use the energy released to reduce carbon dioxide to form cellular organic. Electrons are needed to reduce the carbon dioxide and are obtained by oxidizing another portion of the inorganic electron donor. Thus, whether heterotrophic or autotrophic growth is considered, a portion of the electron donor is used for energy and a portion is used for synthesis. These two uses for the electron donor are shown schematically in **Figure 2.5.**

2.5.2 EFFECT OF PHENOL ON OTHER COMPOUNDS IN BACTERIAL ISOLATES

Two circumstances are common in which the presence of inhibitors may affect the length of the period prior to the start of rapid biodegradation. First, the chemical of interest may be present at high levels that few of the biodegrading microorganisms present may be able to grow or metabolize and biodegradation will not be detectable until the rare species, is able to increase to reach a biomass sufficient to cause appreciable chemical loss. Such high concentration is known in waste discharges from chemical manufacturing. Second, many sites containing toxic chemicals have a mixture of compounds, may be inhibitory to the organisms destroying the test substance. When that toxicants disappears by biodegradation, nonenzymatic destruction, sorption or volatilization, the period in which no destruction of the test substance is evident, replaced by a period in which the destruction become marked. The inhibition in many hazardous waste sites may be complete so that readily utilizable substrate are not metabolized, but degradation will occur as the pollutant plume in the groundwater moves away from the source and the inhibitors become diluted (Murakami and Alexander, 1989).

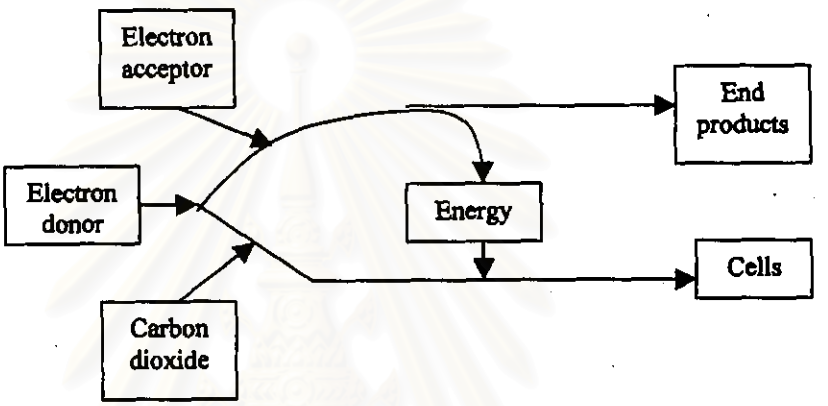

Figure 2.5 Schematic diagram of biological oxidation of an electron donor for energy and transfer of the energy for cell synthesis. Either an organic electron donor or carbon dioxide may provide the cellular need for carbon.

Source : Sawyer et. al. (1982)

The toxicant may act in several ways. (i) In the first and second circumstances just indicated, it may only act to slow the growth rate of the degrading species and then lengthen the period during which no loss of the chemical being measured is evident. (ii) The toxicants may be eliminated so that the degraders are able to grow and the acclimation then represents the sum of the time for lowing the level of the antimicrobial agent to a noninhibitory concentration plus the subsequent time for increase of the degrading species to a density sufficient for significant chemical loss. For example, in wastewater containing both 4-nitrophenol and 2,4-dinitrophenol, the acclimation for the mineralization of the first compound resulted from the toxicity of the second to the 4-nitrophenol-degrading species, but when 2,4-dinitrophenol was biodegraded, the 4-nitrophenol utilizers increased and the acclimation was ended (Wiggins and Alexander, 1988b). For example, the acclimation for 4-nitrophenol mineralization in the presence of high phenol concentrations is result of the antimicrobial effect of the second chemical, but this period is markedly shortened, as phenol is mineralized (Murakami and Alexander, 1989). (iii) The toxicant may suppress the faster-growing species that usually predominance in a mixture of species capable of metabolizing the contaminants, but a resistant and slower-growing species will then have a selective advantage that it did not previously have and the longer acclimation is simply a reflection of the longer time needed for the appearance of the large biomass of the slow-growing organisms. (iv) The toxins may not be present initially, but they may be generated during biodegradation. This possibility is suggested by the observation that phenol mineralization may be suppressed by products generated microbiologically from 4-nitrophenol that is present together with phenol (Murakami and Alexander, 1989).

2.5.3 DIAUXIE

Pure cultures of bacteria growing in the media containing relatively high concentration of two carbon sources often do not show the single exponential phase that is characteristic of the same organisms growing in media with a single carbon source. They have two exponential growth phases, which are separated by an interval with little or no growth. The first exponential period, only one of the substrate is metabolized by the organism to support growth, and the second corresponds to growth on and degradation of the second organic compound. This growth and utilization of two substrates in sequence is known as diauxie. The carbon source that permits faster growth usually is metabolized first. Diauxie is characterized by repression of synthetic of the enzymes concerned with initial steps in the metabolism of the second carbon source as the bacteria uses the first.

The utilization of one organic compound in preference to a second has been advanced as an explanation for acclimation; the chemical of interest is the second substrate and its loss does not begin until the supply of the first is depleted. For example, it has been suggested that bacteria in seawater only attack 4-chlorophenol after certain naturally occurring organic constituents of the water are consumed by bacteria. Enrichment cultures that are probably dominated by a single bacterial type and thus behave much like a pure culture, also show sequential destruction of organic substrates and diauxie growth. Diauxie utilization of substrates and the acclimation before use of the second substrate.

The occurrence of diauxie in biodegradation or its role in acclimation in natural ecosystems or in waste-treatment systems is scarce. Diauxie does not appear to account for the acclimation prior to mineralization of low concentration of 4-nitrophenol in sewage, but it may be important in other environments, especially those in which the

one or both of the compounds needed for diauxie biodegradation are present at high concentrations (Wiggins et.al., 1987). However, in a heterogeneous microbial community, two available compounds will be used by different species, rather than one of the molecules persisting while the bacteria active on the first are growing and using their preferred nutrient.

2.5.4 THE GROWTH OF *Pseudomonas* sp. ON A MIXTURE OF GLUCOSE AND PHENOL

These bacteria were isolated from oil-polluted soil and are able to utilize with almost equal efficiency both glucose and phenol (Panikov, 1995). Batch culture of ***Pseudomonas*** sp. was grown on a glucose-phenol mixture with the usual concentration of mineral components. Glucose was assimilated first, then growth ceased and during 2-3 days bacterial numbers gradually declined. After lysis of about one-third of the cells there was the second growth phase on phenol (**Figure 2.6**). Repeated addition of the glucose-phenol mixture resulted in a very interesting effect of change in substrate preference: phenol was taken up first followed by glucose.

Thus, diauxie on the glucose-phenol mixture was characterized by the following features: (i) a long midway lag phase preceding the consumption of the second substrate, during which a significant fraction of cells grown on the first substrate perished from starvation or toxicity exerted by the second one. (ii) Substrate preference is not absolute and depends on the prehistory of the culture inoculum (i. e., the substrate that had been used to obtain it). It is clear that the transition from one substrate to another is accomplished via the modification of the metabolic machinery of all cells of the populations rather than by an autoselection of mutants. Otherwise, it would not be

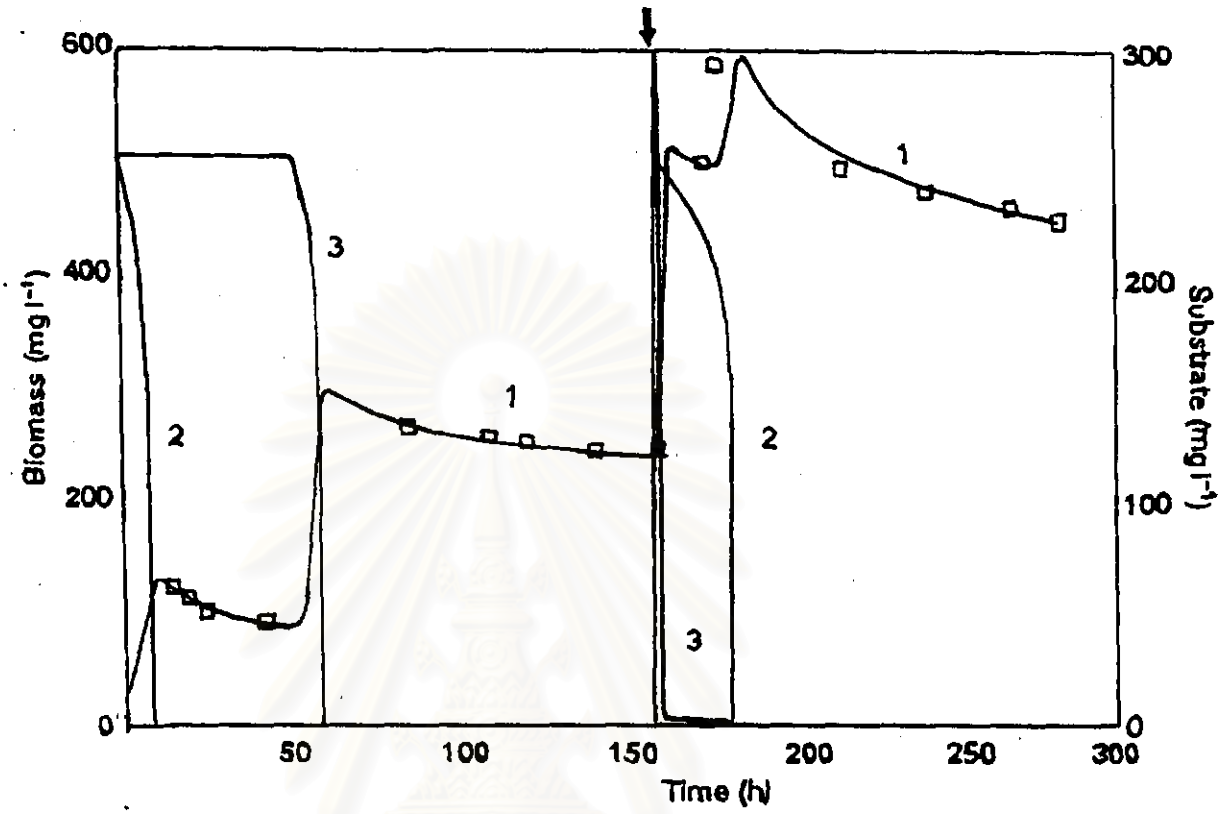

Figure 2.6 Batch growth of ***Pseudomonas*** sp. on a mixture of glucose and phenol: cell biomass (curve 1), residual glucose (curve 2), and residual phenol (curve 3). The medium containing 250 mg/L glucose and 250 mg/L phenol was inoculated by cells initially grown on glucose (note that glucose was the preferential substrate). At the time indicated by the arrow the glucose-phenol mixture was added for the second time (note that in this case phenol was preferential substrate).

Source : Panikov (1995)

observed such a steep increase in biomass during the second growth phase.

Thus, the two considered pairs of substrates (glucose-starch and glucose-phenol) revealed two types of diauxie. Quantitative interpretetion of the first type is straightforward and is allowed by relatively simple kinetic models. The second type of diauxie is much more complicated and requires structured models for its explanation. They should take into account the profound modifications in the physiological state of cells, which occur during the intermediate lag phase.

2.5.5 BACTERIAL COCULCURES IN MULTIPLE SUBSTRATES

More attention has been given to explanations of how second compounds inhibit biodegradation. (i) Undoubtedly, the suppression in many highly polluted sites results from toxicity of the second compound-a toxicity that slows or prevents growth or that diminishes activity of the microorganisms. If both compounds individually are at levels just below those that are toxic, a combination of the two could then exceed the tolerance of the active microorganisms (Smith et.al., 1991). (ii) One substrate could be converted to products that are detrimental to the population acting on the second, as with the products of 4-nitrophenol metabolism by a pseudomonad, which inhibits phenol oxidation by different bacterium (Murakami and Alexander, 1989). (iii) Studies of the biodegradation of two substrates by two bacterial species, each of which can metabolize only one of the molecules, show that competition between the organisms for limiting concentrations of phosphorus may be reflected in a reduction in the rate of biodegradation of one or both of the compounds as compared to media with only one substrate. The two bacteria are competing for an inadequate supply of a limiting factor, and

this competition is manifested in an effect on the transformation. (iv) Similarly, competition for O_2 or another electron acceptor, if present in amounts insufficient for the microbial demand, may be the reason why the organisms degrading one substance apparently have an effect on the utilization of a second. (v) The number of bacterial cells will be greater if two rather than a single carbon source is present, and this larger population would result in more intense grazing in environments in which protozoa are active; a likely outcome would be a lower rate or extent (or both) of biodegradation of a compound when a second substrate is being degraded by a different bacterial species. This reason for an inhibition is suggested by an investigation of degradation of a mixture of two substrates by two bacteria. (vi) A single species should be responsible for the biodegradation of both organic molecules, the inhibition may result from a repression of further synthesis of enzymes needed for the catabolism of one substrate by an intermediate formed in the catabolism of the second (catabolite repression), the inhibition by an intermediate of the activity of already existing enzymes, or by an interference by one substrate in the uptake by the cell of the second substrate.

The effects of one compound on the biodegradation of a second are frequent and occur in many environments. However, given the number of compounds, the undefined populations causing their destruction and the multitude of chemical mixtures, generalizations on whether there will or will not be an effect, whether the effect will be stimulatory or inhibitory and the reasons for the enhancement or suppression are premature.

2.5.6 SYNERGISM

Many biodegradations require the cooperation of more than single species. These interactions may be necessary for the initial step in

the conversion, a later phase of the transformation, or the mineralization of the compound. These various interactions represent several types of synergism, in which two or more species carry out a transformation that one alone cannot perform or in which the process carried out by the multispecies mixture is more rapid than the sums of the rates of reactions effected by each of the separate species. Thus, some reactions take place in mixtures of species but not in pure culture or take place more readily in multispecies associations.

A number of mechanisms for synergistic relationships have been described, but undoubtedly other mechanisms have yet to be discovered. (i) One or more species provide B vitamins, amino acids, or other growth factors to one or more of the other organisms. (ii) One species grows on the test compound and carries out an incomplete degradation to yield one or several organic products and the second species mineralizes the products that otherwise would accumulate. The second species commonly grows on the intermediate in the sequence. (iii) The initial species cometabolizes the test compound to yield a product that it can no longer metabolize and the second species destroys that product. This mechanism differs from the second in the type of activity of the initial population, that is, whether it uses the parent compound as a carbon source for growth or only cometabolizes it. (iv) The first species converts the substrate to a toxic metabolite that then slows the transformation, but the reaction proceeds rapidly if the second member of the association destroys the inhibitor. This detoxification may sometimes be a consequence of the use of the inhibitor as a carbon source for growth, so that it is somewhat analogous to mechanism (ii). On the other hand, it may involve interspecies hydrogen transfer, in which H_2 or reducing equivalents generated by one population is used by another.