# CHAPTER II

LITERATURE REVIEWS

2.1 PHYSICAL AND CHEMICAL PROPERTIES OF CADMIUM

Cd is a metal chemically similar to zinc and mercury because they all have similar arrangments of electron in the outermost shells. (O'neill, 1993). Cd is in group II, period V of the periodic table. Its atomic number is 48 and atomic weight is 112.41. Cd was first discovered in association with Zn ores, and was concentrated in flue dusts from the extraction of Zn (McCutcheon and Musgrave, 1954; Stoeppler, 1992). It is a relatively rare metal, being 67th in the order of elemental abundance (Alloway, 1995).

Cd is almost divalent, Cd(II), in all of its stable compounds but evidence exists for Cd(I) in Cd_2^{2+} species. Cd and its compounds have general density of 8.64 g/cm^3, lower melting (321°C) and boiling points (767°C) than zinc compounds. In metallic from, it is silver-white, lustrous, ductile and have a low coefficient of friction. They are concentrated in sulfide ores of zinc, mercury, lead and copper. The principal Cd mineral is hexagonal, CdS, greennokite. Cd does not react with water at room temperature, but dissolves in inorganic acid and some organic acid; nitric acid is the best solvent. Unlike zinc, Cd is not soluble in alkaline, however, it is soluble in ammonium nitrate solution, forming a Cd-ammonium complex. Salts of Cd with strong acids are readily soluble in water. Less soluble compounds are the sulfide, the carbonate,

the fluoride and the hydroxide (McCutcheon and Musgrave, 1954; Burn, Townshend and Carter, 1981; Fergusson, 1990).

Aqueous solution of Cd is colorless and weak electrolyte. Cd below 0.1 M is in the form of $CdOH^+$ ion,e.g.,

$$Cd^{2+}(aq) + H_2O \iff CdOH^+(aq) + H^+$$
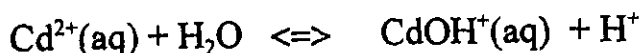
For more concentrated Cd solution, the principle species is $CdOH^{+3}$;

$$2Cd^{+2}(aq) + H_2O \iff Cd_2OH^{+3}(aq) + H^+$$

In the presence of complexing anions (e.g., halide), species such as $Cd(OH)Cl$ or $CdNO_3^+$ may be obtained (Cotton and Wilkinson, 1980).

2.2 APPLICATIONS OF CADMIUM

World production of Cd currently was estimated at 15,000 metric tons per year, about 50% greater than the present rate of mercury production. The major producing countries are the United States, Japan, Canada, Belgium and France (Laws, 1981). In Thailand, Cd are produced from by product of Zn smelting works at Phadang Industry, Co. in the province of Tak since 1990. The amounts of Cd production is estimated to be 1 ton per month and it is export to Japan. However, modified and unmodified Cd used in the country are imported from Germany, USA, England, Australia and Switzerland (Pollution control, 1997). In Thailand Cd was used in many industrial, as described below.

2.2.1 Electroplating or Anticorrosive Coating

About 45% of the total amount of Cd are used widely in automobile construction, e.g., bearings, races, nuts, bolts, and diskbreakes because Cd is much easier to deposit, smoothly and has greater resistant to evenly atmospheric and galvanic corrosion than zinc

coating. Further, Cd is resistant to alkalines (McCutcheon and Musgrave, 1954; Fergusson, 1990).

2.2.2 Pigments

About 21% of the total amount of Cd are widely used in plastics, paints, printing inks and ceramics. Cd and its products are stable to heat and ultra violet radiation and not darken in a H_2S atmosphere, as lead pigment do. The color of range pigments contained CdS, range from yellow to red. The pigments become darker as the proportion of Cd to Zn increases and CdSe produces darker shades.
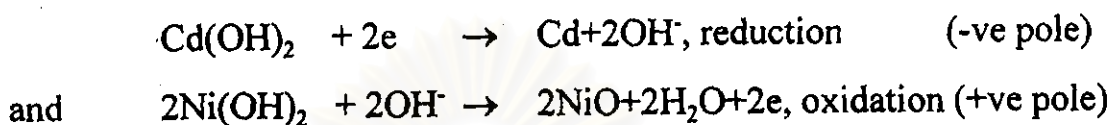
2.2.3 Plastics Stabilizers

It is estimated that 15% of the total amount of Cd are organocadmium compound and also used as catalysts in organic hydrogenation and polymerization reactions. Mixtures of Cd and barium combined with organic acid anion are used as heat stabilizers in plastic to retard degradation due to elevated temperatures.
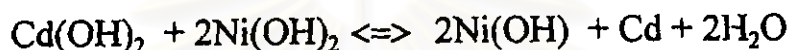
2.2.4 Alloy

It is estimated that 7.5% of the total amount of Cd are used in bearing (99% Cd, 1% Ni) for increase speed in cars, aircraft, and marine engines that operate at high temperatures. Fusible alloys with low melting points contain Cd and are valuable in five-detector units protection devices, such as spinkdlers (e.g. 44.7%Bi, 22.6%Pb, 8.3%Sn, 5.3%Cd). A level of 0.02-1.0% Cd in Cu hardens the metal without significantly lowering its electrical conductivity.

2.2.5 Nickel-Cadmium Batteries

It is estimated that 3.0% of the total amount of Cd were used in batteries with a potential of 1.1-1.3 volt. The two poles consist of Ni $(OH)_2$ and $Cd(OH)_2$ in the uncharged state, and the electrolyte is KOH. The charging reactions are

$$Cd(OH)_2 + 2e \rightarrow Cd + 2OH^-, \text{ reduction} \qquad \text{(-ve pole)}$$

and $$2Ni(OH)_2 + 2OH^- \rightarrow 2NiO + 2H_2O + 2e, \text{ oxidation (+ve pole)}$$

The combined reaction is

$$Cd(OH)_2 + 2Ni(OH)_2 <=> 2Ni(OH) + Cd + 2H_2O$$

During the use of this battery, the reverse reaction occurs.
(Fergusson, 1990)

2.2.6 Miscellaneous

About 8.4 % of the total amount of Cd are found in other products, i.e,

(i) Cd selenide is used in photoconductors, photoelectric cells and phosphorus on screens, such as TV screens.

(ii) Cd salts have been used as fungicides and pesticides both in agriculture (e.g. on broad beans, tomatoes, and wheat) and in non agriculture applications (e.g. on goft courses).

(iii) The metal is also used as the principle material in the control rods of nuclear reactions because Cd is effective in absorbing neutrons.

(iv) Cd is used in the Weston standard cell, which is the working standard the United States in maintaining the value of the volt. The absolute standard cell is composed of a 12.5% (by weight) Cd-87.5 mercury amalgam.

2.3 PROBLEMS AND TOXICITY OF CADMIUM

In 1955, two Japanese physicians reported the occurrence of a mysterious disease in the Jintsu River, Minamata area of Northern Japan. The disease was characterized as lumbago-type pains that progressed to become severe bone damage with multiple fractures of softened bones. Death was attributed to kidney failure. These health problems from Cd were suddenly brought into focus when high Cd intake was identified as the probable cause of the terrible Itai Itai disease, loosely in Japanese "ouch-ouch", because of the severe pain experienced by the more serious patients. (Laws, 1981; Sittig, 1976; O'neill, 1993)

The problems in Japan arose from lead-zinc mining and smelting operation upstream from where the victim lived. Cd was contaminated in the mine waste and sediment. Because of its lower boiling point, Cd is more concentrated, relative to Zn. Rice grown in paddy fields flooded by the river contained up to 3.4 mg Cd per kg rice. (O'neill, 1993)

The mining and smelting are important sources of soil contamination of Cd. The other sources are the atmospheric pollution from metallurgical industries; the disposal of wastes containing Cd, such as, the incineration of plastic containers and batteries. In many agricultural soils have led to significant increases of Cd contents. (Alloway, 1995)

Biological role of Cd is unclear but may be required since metallothionein contains Cd (Cotton and Wilkinson, 1980). Human are

exposed to Cd orally mainly from the diet, and by inhalation from tobacco and air pollution. The total daily human Cd intake has been estimated to be 15-80 μg per person (Meijer, Beems, Janssen, Vaessen and Speijers, 1996) If high Cd intake occurs in human, it is able to cause toxicity. Both acute and chronic toxicity depend on the level and time of contact. Generally, Cd accumulates predominantly in the kidneys with a biological half-life between 10 to 30 years (Stoepple, 1992).

2.3.1 Effect on Kidney

The renal damage, which is first seen by the appearance of low molecular weight proteins (MW 12000-13000) in the urine, is caused by the impaired re-adsorption function of the proximal tubules. A later, and may be an independent effect is the appearance of high molecular weight proteins, such as albumin and transferrin from a glomerular dysfunction of the kidneys. Later effects of further renal damage are aminoaciduria, phosphaturic, glucouria and calcium in urine. (Fergusson, 1990)

2.3.2 Effect on Gastrointestinal Tract

Effects are excess salivation, nausea, vomiting, abdominal pains, diarrhea, vertigo and for larger dose, loss of consciousness. A lethal dose of Cd is over 350-500 mg (Fergusson, 1990).

2.3.3 Effect on Respiratory System

The initial symptoms from Cd exposure are dry or sore throat, followed by cough, headache and dizziness. This is followed by fever and shortness of breath. Apparently, there is acute inflammation of

the lungs which produces edema; the fluid progressively reduces the lung area until the blood cannot absorb oxygen and death ensure (McCutcheon and Musgrave, 1954).

2.3.4 Effect on Circular System

High blood pressure or hypertension is more conclusive in animals than human. Some people with hypertension have been found with elevated Cd in their kidneys and arterial wall. Slight anemia has been associated with Cd toxicity. This may occur because of competition between Cd and iron in the body, giving the appearance of iron deficiency (Fergusson, 1990).

2.4 PHYSICO-CHEMICAL AND BIOLOGICAL TREATMENT

Heavy metals are one of many different hazardous wastes including Cd. They come from many industrial plants, e.g., electroplating, pigment, battery and alloy manufacture (Sittig, 1976). In Thailand, the waste from smelting work is estimated to be 922,893 metric tons per year in 1996 and it may be increased to 1,620,190 metric tons per year in 2001.Hence, chemical and physical treatment processes are used to reduce this problem. There are many methods used for the removal of metal from aqueous wastes.

2.4.1 Physico-Chemical Treatment

2.4.1.1. Precipitation

The law of mass action is the principle of precipitation process for metal removal. It affects the equilibrium that exist between

crystals of a metal salt compound (solid state) and its ions in solution by the following expression:

$$M(OH)_n \iff M^{n+} + n(OH)$$

$$Ksp \iff [M^{n+}][OH^-]^n$$
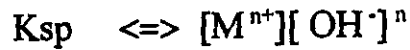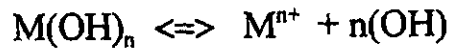
Where n=2,3,4 and Ksp is the solubility product. (Cheremisinoff, 1995)

In general, chemical precipitation depends upon several variables such as the maintenance of an alkaline pH through the precipitation, addition of a sufficient excess of treatment ions to drive the precipitation reaction to completion and addition of an adequate supply of sacrificial ions (such as iron or aluminium) to ensure precipitation.

Cd can be removed by precipitation as hydroxide, sulfide and carbonate at pH between 7.5-11.

Hydroxide precipitation with lime is commonly used for precipitating metals. The process converts soluble metal ions into less soluble hydroxide compounds (Kuhre,1995; Wentz,1989; Batstone,Smith and Wilson,1989 Maruyama, Hannah and Cohen, 1975). Disadvantages are voluminous sludge production, which tends to increase the cost of sludge disposal, limitation of metal removal due to solubility constraints attributed to matrix effects and interference associated with complexing agent (e.g., cyanide, ethylenediaminetetra-acetic acid EDTA, citrate) when stabilizing metal hydroxide sludge (Cheremisinoff, 1995).

Sulfide precipitation may be an effective alternative to hydroxide precipitation due to the low solubility of metal sulfides yielding high metal removal efficiencies. However, disadvantages are the produced hydrogen sulfide. The sulfide ion is toxic and must be rigidly controlled to minimize to avoid post treatment.

Carbonate precipitation process improves settle ability, reduces sludge volumes, and is easier for the filtration. Other advantages include the buffering capability, superior handing characteristics and its availability. (Cheremisinoff, 1995)

Hence the neutralization of an acidic waste can cause precipitation of heavy metals and allow them to be removed as a sludge residue by clarification, sedimentation, or filtration. But in the presence of some complexing agents (e.g. cyanide), it is impossible to precipitate out the cadmium ion. Therefore, pretreatment to remove the agents would be required. (Sittig, 1976)

2.4.1.2. Oxidation-Reduction

Oxidation is a chemical reaction with an increase in valence from a loss of electrons. Reduction is a reaction with a decrease in valence from a gain of electrons. Chemical reactions that involve both oxidation and reduction are known as REDOX reaction.

Kastone process, hydrogen peroxide oxidation precipitation system, was developed for the removal of cadmium, which simultaneously oxidizes cyanides and forms the oxide of cadmium

(rather than the hydroxide). Cadmium oxide is claimed to be much easier to remove from solution than cadmium hydroxide. This process is reported as suitable for small plating operation. A solid removal is readily accomplished with simple filtration apparatus (Sittig, 1976). Other metals, chromium is reduced chemically from highly toxic (Cr^{6+}) to non toxic form (Cr^{3+}) by interaction with reducing reagent, including sulphur dioxide (SO_2), sulphite salts (SO_3^{2-}), bisulphite salts ($H_2SO_3^{3-}$) and ferrous salts (Fe^{2+}). Soluble chromium (Cr^{3+}) is usually removed through alkaline precipitation (Batstone, Smith and Wilson, 1989).

2.4.1.3. Ion Exchange

Ion exchange method is the chemical treatment which involves the use of resins for the removal of heavy metal from solution. In principle, ions on the resin surface are exchanged by ions with similar charge from the solution in which the resin is immersed. The process depends upon the electrochemical potential of the ion to be recovered versus that of the exchange ion, and also upon the concentration of the ion in solution (Kuhre,1995). Ion exchange materials should have ion active site throughout their entire structure, high capacity, selectivity for ionic species, capability of regeneration, chemical and physical stability, and low solubility. Synthetic organic cationic resins have a high ion exchange capacity and have reactive groups, such as the sulfonic, phenolic and carboxylic groups (Wentz,1989). Trace quantities of cadmium by a pilot plant cation exchanger were reduced 99% and 99.9% removal by cation plus anion exchange (Linstedt, Houck and O' Connor, 1971). But this method is unsuitable for recovery of cadmium cyanide solution (Sittig, 1976).

2.4.1.4 Sedimentation

Sedimentation is the removal of suspended solids from liquids by gravitational setting. The velocity of the liquid must be reduced to the point that the retention time in the sedimentation vessel is sufficient for solid to settle by gravity. Chemicals are added to cause flocculation or coagulation, which produces faster-setting particles. The setting rate is affected mainly by the size, shape, and density of the solid particles as well as by the density of liquid phase. Then this method is not suitable for wastes consisting of emulsified oils (Wentz, 1989; Kuhre, 1995).

2.4.1.5 Flotation

Flotation is the opposite of sedimentation. Suspended solids are cause to float to the surface of a tank, where they can be removed. This is commonly used to remove metal hydroxides. It involves the release of gas bubbles or foam, which attach or actual formation at the solid-liquid interface. They may also be come entrapped under larger particles or become an integral part of flocs. The particles or flocs then rise to the surface for removal by skimming (Wentz, 1989; Kuhre, 1995).

2.4.1.6 Filtration

Filtration is used after precipitation and sedimentation to remove residual suspended solids that do not settle out. Principle, the liquid is passed through a porous medium and the solids deposited add to the thickness of the porous medium. Filter medium can include silica sand, anthracite coal and garnet supported on beds of gravel, screen and various membranes. Sand filters have been commonly use as a final

polishing step in treatment of wastewater to produce a high quality effluent. Filtration should not be used with sticky or gelatinous sludge due to the linklihood of filter media plugging (Wentz, 1989; Kuhre, 1995).

2.4.1.7 Adsorption

Use of an adsorbent to remove a targeted substance from a solution is called adsorption, which is the physical adhesion of molecules or particles to the surface of a solid adsorbent without chemical reaction. This contrasts with absorption, which involves the penetration of the molecules or particles into the solid absorbent. Sorption is a commonly used term that refers to both adsorption and absorption.

Activated carbon is widely used to adsorb for removal of organic and inorganic substances from aqueous waste. It is used in either granular or powdered form, depending upon the application and the process economics (Wentz, 1989). Marzal, Seco and Gabaldon (1996) had studied the adsorption characteristic of cadmium and zinc onto a granular activated carbon. Darco 12-20 mesh granular activated carbon was shown to be effective for the removal of cadmium and zinc from aqueous solutions. The removal of Cd and Zn increased by raising pH and temperature, and decreased by raising the molar metal/carbon ration.

This treatment is not specific to metal ion. The organic and organic substance wastes are adsorption onto a carbon matrix. Most adsorption is a physical process caused by van der Waals molecular forces, hence the complex is not stable.

2.4.2 Biological Treatments

Microbial treatment of inorganic contaminants usually involves the immobilization or removal of metals and metal salts. Microorganisms can alter the chemical characteristics of metals and particularly their abundance in the environment by mechanisms such as precipitation, accumulation inside the cells, alteration in the valence state of the metal through oxidation or reduction reactions and transformation by methylation and demethylation. Many microbial metal treatment processes are system evolved by microorganisms to provide resistance against toxicity. (Ensley, 1994). Hence, for application of microbial treatment that should the study of each resistance mechanisms, following: metal transformation, efflux system and immobilization. Cell wall, sulfide, siderophore, binding protein and exopolysaccharide can be used in immobilization (Reichardt, 1996).

2.5 MECHANISM OF METAL RESISTANT IN MICROORGANISMS

2.5.1 Metal Transformation

Living microorganisms can carry out chemical transformation of heavy metals such as oxidation, reduction, methylation and demethylation. For well known example, the mercuric ion, Hg^{2+} (high toxic) is converted Hg^0 (less toxic). This detoxification process is plasmid-encoded and is regulated by an operon consisting of several genes. The most important gene is the *mer A* gene, which is responsible for the production of mercuric reductase, the enzyme that catalyzed the transformation of Hg^{2+} to Hg^0 (Bitton, 1994; Brierley, Brierley and Davidson, 1989; Gadd, 1990a). Organomercurial compounds are

enzymatically detoxified by organomercurail lyase, which cleaves the Hg-C bond of, e.g. methyl-, ethyl- and phenyl mercury. to form Hg and methane, ethane and benzene, respectively. Hg can then be volatilized by mercuric reductase (Gadd, 1990a and 1990b). The other metal transformations and conditions are shown in **Table 2.1**.

2.5.2 Efflux System

Metabolism-dependent intracellular transport may be a slower process than binding. A relationship between metal transport into microbial cell and toxicity is often observed, with resistant strains taking up less metal ion than sensitive strains. They are decreased transport, impermeability or the occurrence of metal efflux systems that are resistance mechanisms in many organisms.

Gram positive bacteria, such as *Staphylococcus aureus* and *Bacillus subtilis* have mechanism of Cd resistance by efflux system, Cd can be pumped out again rapidly by highly specific for Cd^{2+} as well as Mn^{2+}. This system may use energy from the membrane potential or more directly from ATP. (Burke and Pfister, 1986; Perry and Silver, 1982). In *S. aureus*, this system is called *cadA* determinant from plasmid pI258. Cd accumulation occurs via the chromosomally-determined manganese transport system in sensitive cells and less net Cd accumulation occurs in resistance cells. Rather than a direct block on Cd^{2+} uptake, Tynecka, Gos and Zajac (1981) demonstrated that an energy-dependent efflux system functioned in resistance cells but was missing in sensitive cells. Fundamental mechanism was a $Cd^{2+}/ 2H^+$ exchange (**Figure 2.1** and **Figure 2.2**) (Rayner and Sadler, 1989; Ji and Silver, 1995; Silver and Phung, 1996; Silver and Walderhaug, 1992; Silver and Misra, 1988).

Table 2.1 Aerobic and anaerobic transformations of heavy metals

(Adriaens and Hickey, 1994)

Reaction Type	Metals	Products	Environmental conditions
Methylations	Hg	Mono-,dimethyl Hg	Anaerobic, presence of SBR*, complicated by the presence of sulfide
	Se	Dimethyl Selenide	Organic C-amendment, soil fungi
	As,Pb,Sn	Mono-,di-,trimethylated	Nutrient depleted environments
Demethylations	Hg	Divalent Hg	Cd,Cu/prior exposure to organo-Hg compounds required
	Se	CH_4, CO_2	Methanogenic conditions
Oxidations	Hg	Divalent Hg,organic Ligands	Oxygen
	Se,As,Pb, CN and metallo-CN	Selenic acid (H_2SeO_4) Amminia,bicarbonate, $Ni(CN)_2$	Oxygen Methanogenic conditions
Reductions	Hg	Volatile Hg^0	Inhibition by Cd and Cu prior exposure to organo-Hg cpds required
	Se	Insoluble Se^0,hydrogen Selenide	Presence of SRB
	As	Arsine(AsH_3), insoluble Arsenite	Aerobic/anaerobic conditions
	Cr	Cr(III) hydroxides, free Cr^{3+}	Aerobic/anaerobic conditions

*SBR; Sulfate reducing bacteria.

Gram   negative   bacteria,   *Alcaligenes   eutropuus*,
Pseudomonas putida and *E. coli* also exhibits reduced uptake that
function in response to the cell membrane potential but does not involve
ATP (Higham, Sadler and Scawen, 1985; Trevors, Stratton and Gadd,
1986: Nies and Silver, 1989). This system is called *Czc* (for Cd^{2+}, Zn^{2+}
and Co^{2+} resistance) and *Cnr* (for Co and Ni resistance). They consist of
three proteins (**Figure 2.1** and **Figure 2.3**), *CzcA* is the basic inner
membrane transport protein. There is no evidence to distinguish whether
Czc function as a dimer complex or as a complex consisting of one
molecule each of *CzcA*, *CzcB* and *CzcC* polypeptides. *CzcC* is thought to
be an outer membrane protein, although there remains some uncertainly
about its location, and *CzcB* appears to be a 'membrane fusion protein'
that bridges the inner and outer cell membranes of gram negative bacteria
(Silver and Phung, 1996; Silver, 1994).

The mechanism of arsenate resistance in *S. aureus* and *E.coli*
is the reduced accumulation. Arsenate, function as a phosphate analogue,
is accumulated by phosphate transport systems that similar Cd can into
bacterial cell via a Mn^{2+} system. The toxic cation may be accumulated by
means of existing transport system for essential nutrients. Therefore,
efflux mechanisms of Cd^{2+} and AsO_4^{3-} must be highly specific in order
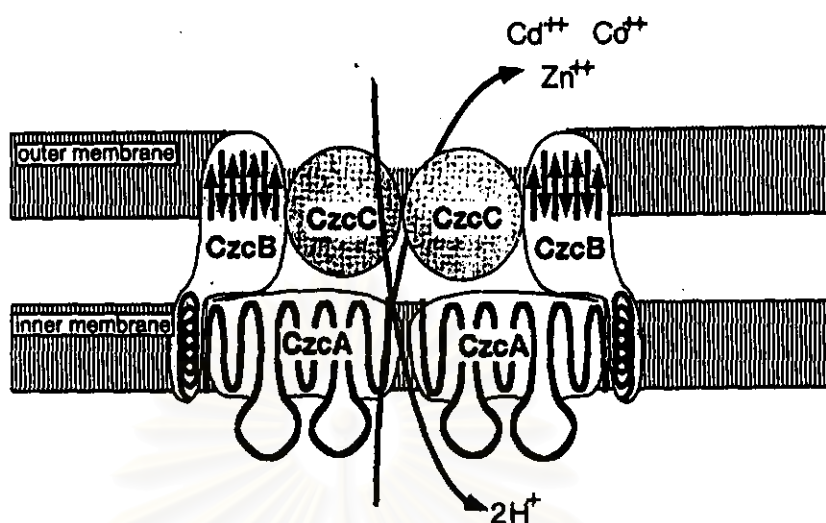to prevent loss of the essential nutrient (Gadd, 1990a).
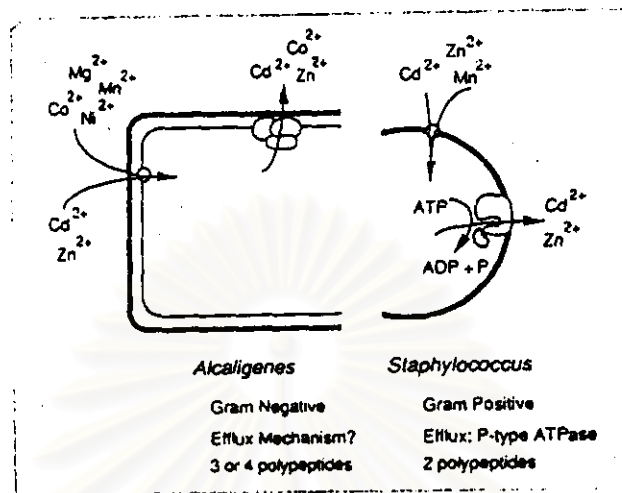
Figure 2.1 Pathways for Cd, Mn and Zn uptake and efflux in the

   A. eutrophus Czc system and *S. aureus CadA* system.

   (from Silver and Walderhaug, 1992)
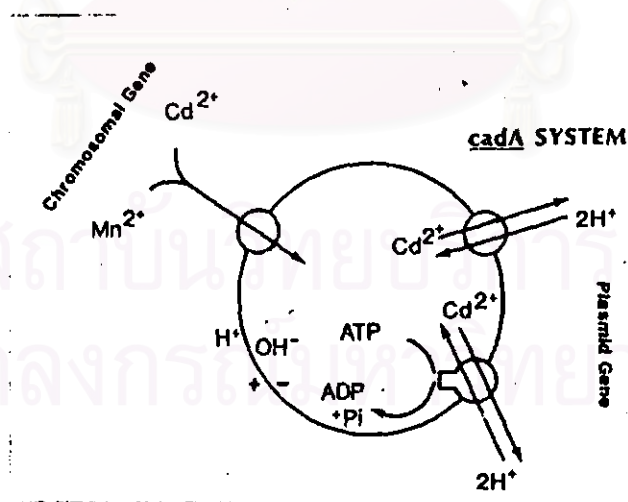
Figure 2.2 Cadmium uptake by the manganese transport system and

   efflux by the *cadA* Cd efflux ATPase.

   (from Silver and Misra, 1988; Rayner and Sadler, 1989).

Figure 2.3 Czc model for Cd, Zn and Co efflux system functioning as
proton/cation antiporter consisting of inner membrane
(CzcA), outer membrane (CzcC) and 'membran fusion'
(CzcB) protein functioning as a dimer.
(from Silver, 1996; Silver and Phung, 1996).

2.5.3 Accumulation

2.5.3.1 By Cell Walls

Microbial walls are anionic owing to the presence of
carboxyl, hydroxyl, phosphoryl and other negatively charged sites.
Hence, cationic metals rapidly bind to these sites by energy-independent
reaction. (Brierley, Brierley and Davidson, 1989)

Gram-positive bacteria contain reasonably large amounts of
peptidoglycan and an anionic polymer, such as a teichoic or a teichuronic
acid. **Figure 2.4** shows a segment of the cell wall structure of *Bacillus
subtilis* 168. Anionic sites include carboxylate (from peptidoglycan) and

phosphate (from teichoic acid). Positively charged sites are exclusively ammonium, from D-alanine (teichoic acid), amino sugar (glycan) and diaminopimelic acid (peptide portion of peptidoglycan). These are the charges that mediate the interaction between cell walls and metal ions (Doyle, 1989; Flemming, Schmitt and Marshall, 1996).
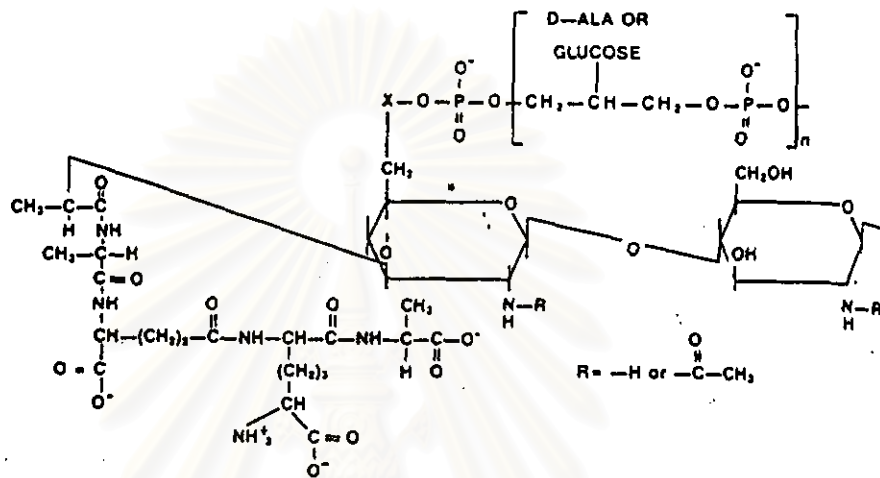
Figure 2.4 Segment of the cell wall of *Bacillus subtilis* 168. The structure
reveals both positively and negatively charged sites. The teichoic
acid portion may contain a D-alanine residue in place of the
α-D-glucose as shown.The "X" represents the linkage between
a uramic acid residue and a teichoic acid polymer (Doyle, 1989).

The major molecular constituents of the Gram-negative outer membrane are phospholipids, lipopolysaccharide (LPS), and proteins (**Figure 2.5**). The amounts of these various components can vary substantially, depending on the growth conditions and type of organisms. However, the outer membrane usually contains between 20 and 25% phospholipid, 30% LPS, and 45-50% protein by weight. The most abundant reactive electronegative sites in the outer membrane capable of binding metal cations are the phosphoryl groups of LPS and phospholipids (Ferris, 1989: Sidhu and Olsen, 1997).
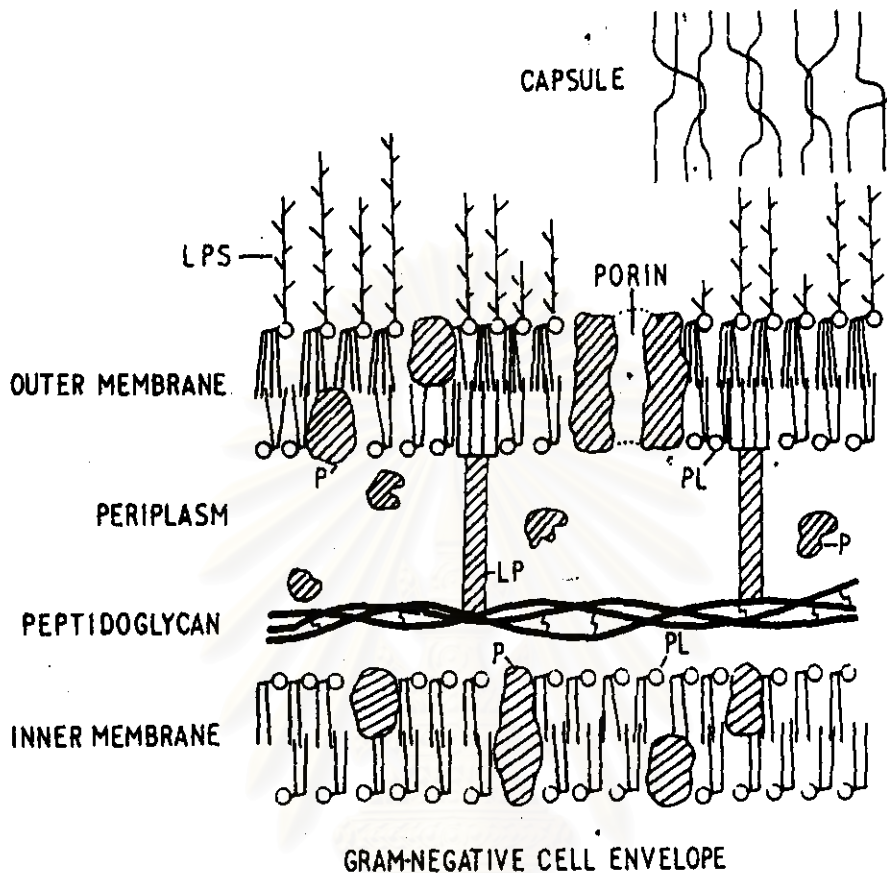
Figure 2.5 The cell envelope of a Gram-negative bacterium. LP, lipoprotein;LPS, lipopolysaccharide; P, protein:PL, phospholipid (Sleytr and others, 1988).

2.5.3.2 Sulfide

Extracellular precipitation of metals occurs when microorganisms produce metabolic product such as ammonia, organic bases or hydrogen sulfide that are excreted and result in precipitate metals as hydroxides or sulfides. The sulfate-reducing bacteria such as *Desulfovibrio* spp, *Desulfotomaculum* spp. and *Desulfomonas pigra*, are

the productions of hydrogen sulfide for extracellular precipitation (Hutchins, Davidson, Brierley and Brierley, 1986). This mechanism is oxidize organic matter and reduce sulfate to sulfide in anaerobic environments, such as bogs, anoxic soils, and sediment. Show following:

$$H_2SO_4 + 8(H) \rightarrow H_2S + 4H_2O$$
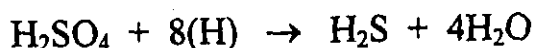
The H_2S readily reacts with soluble heavy metals to form insoluble metal sulfide. The metal is removed by this mechanism such as covellite (CuS) and sphalerite (ZnS; Gadd, 1990a; Gadd, 1990b).

Metal resistance strains of *Klebsiella aerogenes* and *Klebsiella pneumoniae* are able to detoxify Cd , Pb, or Hg, as insoluble sulfide particles deposited on outer cell surface (Aiking, Govers and Riet,1985; Justin and others,1997). Similarly, a new strain of *Pseudomonas aeruginosa* was sulfide production for removal Cd by precipitation on the cell walls that used energy dispersive microanalysis (Wang and others, 1997).

2.5.3.3 Siderophore

Many microorganisms synthesize chelating agents that have a high binding efficiency for metals such as siderophores are produced by microorganisms to facilitate uptake of iron into the cell. Iron specific siderophores, which are catechol or hydroxamate derivatives, can be employed for removal of specific metals from solution. The catechol derivative can be modified by substituting electrophilic ion on the benzene ring. These substitution, such as Cl, Br, NO, and NO_2, draw electrons from the benzene ring that are changing the binding capacity of the entire molecule. This modification of siderophores is using for

selective capture of base metals, such as Cd, Cr, Cu, Pb, Hg, Ni, and Zn and radioactive (Brierley, Brierley and Davidson, 1989; Gadd, 1990; Nieland, 1989). Some characteristic of siderophore are shown in Table 2.2.

2.5.3.4 Binding Protein

Metallothionein (MT) production is one of the most mechanisms that living organism has respond to toxic effects of heavy metals. MT production is induced after uptake of the metals into the cells that have been found in bacteria, cyanobacteria, algae, fungi and yeast. MTs are broadly defined as a class of low molecular weight cysteine-rich proteins, which bind heavy metals (Butt and Ecker, 1987). For instant, three cysteine-rich proteins of molecular weight 4000 to 7000, containing 4 to 7 gram atoms of Cd, Zn, and Cu per mole was isolated from *Pseudomonas putida* growing in 3 mM Cd (Higham and Scawen, 1984). But Yoshida, Morinaga and Murooka (1993) reported isolation of a novel type of heavy metal binding protein, high molecular weight of the protein was about 150 kDa and it bound 6.5, 5.2, and 1.5 gram atoms of Cd, Cu, and Zn per mole of protein. The protein contained abnormal levels of histidine residues and low levels of cysteine residues. MTs and other metal-binding proteins were interested since they can bind valuable metal, e.g. Au and Ag, as well as those of low value, e.g. Cu, Cd and Zn (Gadd, 1990b; Wood and Wang, 1983; Brierley, Brierley and Davidson, 1989; Shaikh and Tewari, 1990).

Table 2.2 Some well-characterized siderophores from bacteria and

fungal species (Neilands, 1989).

Microorganism	Siderophore
Bacteria	
Escherichia coli, Salmonella typhimurium	Enterobactin, aerobactin
Agrobacterium tumefaciens	Agrobactin
Rhizobium meliloti DM4	Rhozobactin
Pseudomonas sp.	Pyochelin, Pseudobactins, Pyoverdines
Anabaena sp., *Bacillus megaterium*	Schizokinen
Arthrobacter sp.	Arthrobactin
Azotobacter sp.	Azotobactin
Vibrio sp.	Vibriobactin, anguibactin
Actinomyces sp.	Ferrioxamines
Mycobacteria	Mycobactins
Fungi	
Penicillia, Aspergilli, Neurospora, Ustilago	Ferrichromes, coprogen
Fusaria	Fusarinines
Rhodotorula sp.	Rhodotorulic acid

2.5.3.5 Exopolysaccharides

Microorganisms exopolysaccharide have anionic properties, carboxyl group that play an important role in the adsorption of metal ion from solution. This mechanism of metals accumulation is most evident in activated sludge systems for biological wastewater treatment (Brown and Lester, 1979; 1982a and 1982b).The detail is described in the next topic.
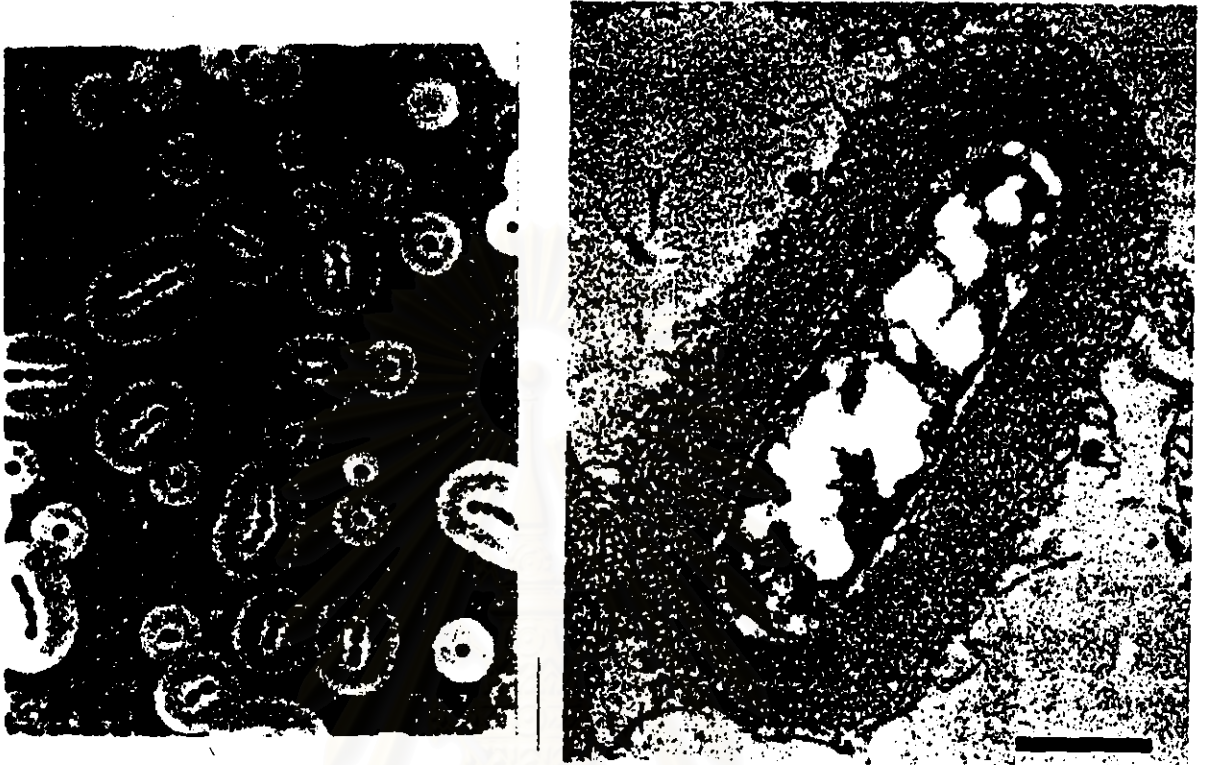
2.6 Exopolysaccharides

2.6.1 Introduction

The exopolysaccharides (EPS) has been widely used to describe polysaccharides found external to the structural outer surface of the microbial cell that concluded three structure, capsules, slime-layers and sheaths, but they can occur singly or in combination (Beveridge, 1989). The presence of EPS associated with microbial cells grown on solid surfaces in the mucoid colony morphology. In the liquid medium, EPS-production cultures may become very viscous or may solidify as a gel. Visualization of EPS under the light microscope is possible through negative staining (**Figure 2.6a**). Techniques such as scanning electron microscopy (SEM) frequently provide strong evidence for the presence of EPS, but are not capable of providing confirmatory chemical evidence. Equally, the modern techniques of transmission electron microscopy (TEM) can reveal considerable information about the surface structures (**Figure 2.6b**), including the polysaccharides involved, but are unable to distinguish between different chemotypes (Sutherland, 1990).

2.6.2 Composition and Structure

Exopolysaccharides are primarily composed of carbohydrates, but in addition to the various sugars, there may be organic and inorganic substituents. A distinction between eukaryotes and prokaryotes can be seen in the presence of pentoses. Eukaryotic polysaccharides may contain pentoses such as D-ribose or D-xylose, but they are of less common occurrence in extracellular polymer derived from prokaryotes (Sutherland, 1990).

Figure 2.6 The characteristic of EPS detecting by negative stain

(left; Taylor and Juni, 1960) and TEM techniques

(right; Beveridge, 1989).

Polysaccharide may be classified into two types, namely, homopolysaccharides, which contain only one type of sugar moiety, and heteropolysaccharides which contain two or more sugar moieties. Heteropolysaccharides are usually produced by microorganisms from any utilizable carbon source and complex enzyme systems are usually involved, where as homopolysaccharide synthesis involves a single or simple enzyme system (Margaritis and Pace, 1985). For example is shown in **Table 2.3**.

Table 2.3 Conclusion of exopolysaccharide production form many organisms (Sutherland, 1990; Sutherland, 1985).

Exopolysaccharide	Organisms
1. Homopolysaccharides	
1.1 β–D-glucan	
1.1.1 cellulose	*Acetobacter xylinum*, eukaryotic plant, slime mould, algae
1.1.2 curdlan	*Alcaligenes faecalis, Agrobacterium* sp., *Rhizobium* sp.
1.1.3 scleroglucan	*Sclerotium rolfsii,S. glucanicum, Schizophyllum commune*
1.2 α–D-glucan	
1.2.1 dextran	*Leuconostoc mesenteroides*
1.2.2 pullulan	*Aureobasidium pullulans, Trmella mesenterica, Cyttaria Harioti*
1.3 Sialic acid	*Neisseria meningitidis*
2. Heteropolysaccharides	
2.1 bacterial alginates	*Azotobacter vinelandii, Pseudomonas* sp.
2.2 emulsan and related polysaccharide	*Acinetobacter calcoaceticus*
2.3 hyaluronic acid and heparin	*Escherichia coli* serotype K5, *P.aeruginosa* and group A and group C streptococci
2.4 rhizobrium heteroglycans	*Rhizobium trifolii, R. meliloti, R. leguminosarum*
2.5 succino glycan	*Rhizobium* species.
2.6 xanthan	*Xanthomonas campestris*
2.7 XM6	*Enterobacter* strain (XM6)
2.8 zooglan	*Zoogloea ramigera*

    Another important characterization of polysaccharide is basd on their charge properties and they may be classified as naturally anionic, neutral or cationic. Some anionic such as xanthan gum, phosphomannan

and alginate. Neutral EPS examples include scleroglucan, pullulan, dextran and curdlan. Some polysaccharides have anionic properties and they contain acidic groups, such as carboxyl, phosphate or sulfate (Margaritis and Pace, 1985).

The structures of polysaccharide are presented in some type, for example *Enterobacter* strain (XM6) is produce a polysaccharide with gelation properties. This polymer proved to resemble very closely polysaccharide from *Klebsiella aerogenes* type 54. Each polysaccharide was composed of the same tetrasaccharide repeat unit (**Figure 2.7**). However, the *Klebsiella* EPS carry *O*-acetyl groups either on every L-fucose residue or on alternate ones, whereas the XM6 EPS is devoid of acyl substituents. The most available alginates are isolated from marine algae. The main structure composed of D-mannuronic (M) acid and L-guluronic acid (G) that also found in bacterial alginates but the proportion of M and G and amount of acetyl ester are differing. The structure of bacterial and algal was presented in **Figure 2.8.**

$$......\rightarrow 4)\text{-D-GlcA-}(1^{\alpha}\rightarrow 3)\text{-L-Fuc-}(1^{\alpha}\rightarrow 3)\text{-D-Glc}(1\text{-}......$$

$$\uparrow 4$$

$$\beta\text{-D-Glc} \_\_\_$$
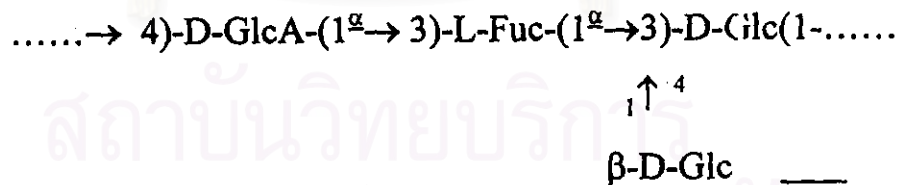
Figure 2.7 The structure of the polymer from *Enterobacter aerogenes* strain XM6. (from Sutherland, 1990)

```
algal              — M — M — M — G — G — G — M — G — M — G —


A. vinelandii      — M — M — M — G — G — G — M — G — M — G
                     \   \   \                       \
                     OAc OAc OAc                     OAc


P. aeruginosa      — M — M — M — M — M — M — M — M — M
                     \       \   \   \       \
                     OAc     OAc OAc OAc     OAc


P. putida,         — M — M — M — M — G — M — G — M — G —
P. fluorescens               \
                             OAc


P. phaseolicola    — M — M — G — M — M — M — M — M — M
                                 \
                                 OAc
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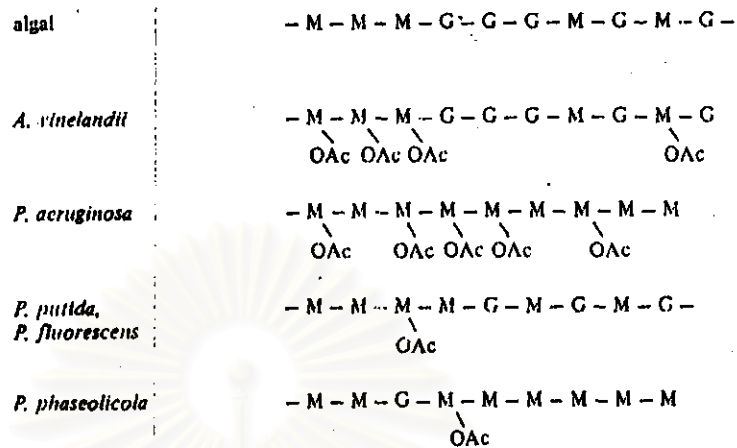
Figure 2.8 Bacterial and algal alginate structures, M, mannuronic acid;
G, guluronic acid; OAc, acetyl ester. Ester may be on C_2, C_3
or both (Sutherland, 1990).

2.6.3 Genetics of Exopolysaccharide Synthesis

Some common aspects in the genetic control and regulation of exopolysaccharide synthesis are the concept of a 'cassette' of biosynthesis genes unique for each polysaccharide.

The EPS gene are clustered at a single chromosomal locus in *E. coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Haemophilus influenza*, etc. This gene allows the coordinate regulation of a large number of genes that may be involved in the biosynthesis and export of EPS (Robert, 1996).

The capsule gene cluster of *E. coli* is the most studied that it can produce over 80 chemically distinct capsule polysaccharides (K antigent). The K antigens were divided into group I and II on the basic of

their biological and chemical properties. Recently, a different of two group at the serA locus on the *E.coli* chromosome were identified. Group I K antigens have higher molecular weights than those of group II and expression at all growth temperatures. They contain hexuronic acid as acidic components may contain amino sugars. Group I K antigens have been subdivided into groups Ia and Ib base on the presence of amino sugars. Group Ia K antigens do not contain amino sugars and they resemble the capsular polysaccharides of *Klebsiella* species (Roberts, 1996). Group II K antigens have a higher charge density than those of group I. They are coexpressed with many O antigens and are not expressed at growth temperature below 20 °C.

In other microorganisms, *P. aeruginosa* are the one of alginate synthesis. It have four of the gene (A-D) in control the enzymes yielding the activated precursor Guanosine diphosphomannuronic acid (GDP-mannuronic acid).

Xanthomonas campestris has demonstrated a cluster of 12 genes involved in Xanthan production in a 16 kilobase region of DNA. The function of the gene products has been identified by *Bam*HI restriction enzyme. All seven transferase activities needed for the production of a fully acetated repeat unit were identified.

The polysaccharide-producing strains of *Heamophilus influenzae* type b have not been observed in other bacteria producing exopolysaccharides. A region of the chromosome involved in expression of capsule contains a directly repeated duplication of a DNA segment of approximately 17 kb.Loci in each copy are involved in exopolysaccharide

production. The other *H. influenzae* serotypes lack the duplication (Sutherland. 1990 and 1985).

2.6.4 Metal Adsorption

The components of polysaccharide are involving to metal binding reaction. Metal tend to form bonds with electron-donating groups in order to fill their outer electron shall. The carboxyl residue is the most effective electron donor group associated with acidic capsule. In addition, Oxygen atoms and hydroxyl residues on the sugar subunits are also weak electron donors in the acidic and neutral polysaccharides.

The capsules of most bacteria are containing 5 to 25 percentage uronic acids. They contain free carboxyl groups, which confer a net negative charge to the polysaccharide.

The metal interactions with charged polysaccharides based on the shape of the adsorption isotherms. They involved salt bridges with carboxyl groups on acidic polymer and those involved week electrostatic bonds with hydroxyl groups on neutral polymers (Geesey and Jang, 1989).

The complexes between metal ion and the capsule of *Zoogloea ramigera* 115 was proposed to involve primarily hydroxyl groups of the glucose subunits even the polysaccharide contain free carboxyl groups. This type of metal binding is believed to be the most important mechanism of metal removal in activated sludge (Brown and Lester, 1979).

The carboxylated polysaccharides is prefer binding to cations with large ionic radii. Exceptions, carboxylated polyanions exhibit a higher selectivity for transition metals than the alkaline earth metals.

One of the most microbial polysaccharide is the alginate that can binding vary metals. Affinity of alginic acid for the transition metals decrease in the order Cu > Ni> Co> Zn > Mn. The specific site binding is seen in circular dichroism when addition of Ca^{2+} to alginate solution or to polyguluronic acid (**Figure 2.9**).
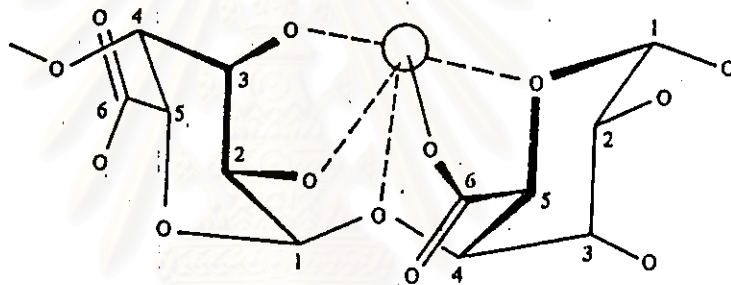
Figure 2.9 Cation binding site of the dimer of L-guluronic acid in polyguluronic acid portions of alginate. (Sutherland, 1990)