Chapter II

LITERATURE REVIEW

2.1 POLYCHLORINATED BIPHENYLS

2.1.1 What are Polychlorinated Biphenyls?

The term polychlorinated biphenyls (PCBs) refers to a class of synthetic chlorinated organic compounds with biphenyl as the basic structural unit. PCBs were commonly produced as complex mixtures for a variety of industrial uses. Their chemical and physical stability and their electrical insulating properties led to the widespread commercial utility of PCBs. In the United States, complex PCB mixtures were manufactured under the trade name Aroclor (Yadav et al., 1995). The major producer, Monsanto Company, marketed Aroclors in North America from 1929 until their manufacture was banned in 1977 (Erickson, 1986; Nies and Vogel, 1990). Because of their widespread use, improper disposal, chemical stability, potential toxicity, and possible health effects, PCBs remain a ubiquitous environmental concern (Berkaw et al., 1996; Davis and Bradlow, 1995; Safe, 1994).

2.1.2 PCBs: Chemical Formula, Structure, Composition, and Nomenclature

PCBs are a family of compounds consisting of a biphenyl nucleus carrying from 1 to 10 chlorine atoms. Theoretically, there are 209 possible PCB congeners (chlorobiphenyls) that differ in the number and position of the chlorine on the biphenyl structure (Bedard et al., 1987b; Mousa et al., 1996; Quensen III et al., 1990; Waid, 1986a). The empirical formula for PCBs is thus $C_{12}H_{10-n}Cl_n$, where n=1 to 10; i.e., monochlorobiphenyl through decachlorobiphenyl (Erickson, 1986; Exner, 1982; WHO, 1993). The structural formula of the unsubstituted biphenyl molecule with the numbering system of the carbon atoms in each ring, is given in Figure 2.1.

The Aroclors are complex mixtures, because each of the 10 positions on the biphenyl molecule may be substituted with either chlorine or hydrogen. The various Aroclors produced differed in the percentage of chlorine by weight. The degree of chlorination of any Aroclor may vary between 19 and 71% (Crine, 1988).

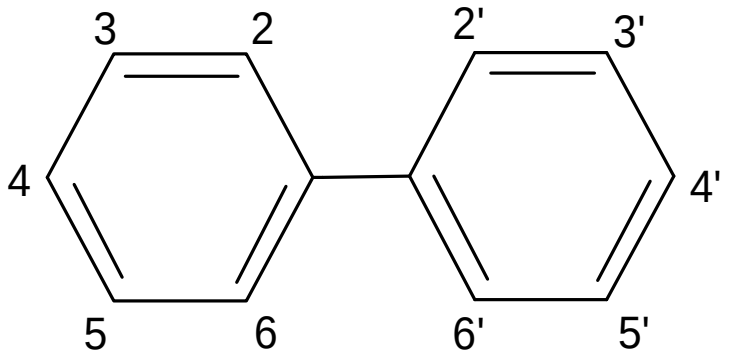

Figure 2.1. Numbering in the biphenyl ring system.

Individual manufactures had their own system of identification for their products. In the Aroclor series, a 4-digit code is used. Most are given a numerical designation beginning with 12 (in the first two positions) for 12 carbon atoms and ending with two digits expressing the percentage by weight of chlorine in the mixture (Quensen III et al., 1990; WHO, 1993). Thus, Aroclor 1242 is 42% chlorine by weight and averages three chlorine per molecule. Three other Aroclors, 1248, 1254, and 1260 contain 48, 54 and 60% chlorine by weight, with an average of four, five and six chlorine per biphenyl molecule, respectively (Quensen III et al., 1990; Yadav et al., 1995).

The entire set of 209 chlorobiphenyls forms a set of congeners. When PCBs are subdivided by the degree of chlorination, the term isomer group or homolog is used. For example, the 12 different PCB compounds with two chlorine atoms are called dichlorobiphenyl isomer group. PCBs of a given isomer group with different chlorine substitution positions are called isomers. Thus, 2,3-dichlorobiphenyl and 2,4-dichlorobiphenyl are two of the 12 dichlorobiphenyl isomers. PCB nomenclature is summarized in Table 2.1. Table 2.2, summarizes the compositions and number of isomers of the ten PCB isomer groups.

2.1.3 Physical and Chemical properties of PCBs

The unique physical and chemical properties of polychlorinated biphenyls have made them desirable components in a wide range of industrial applications. Some of these same properties make PCBs environmentally hazardous. PCBs are among the most stable organic compounds known. Individual pure PCB congeners are colorless, often crystalline compounds, but commercial PCBs are mixtures of these congeners with a clear, light yellow or dark color (WHO, 1993). They do not crystallize at low temperatures, but turn into solid resins. Because of the chlorine atoms in the molecule, their density is quite high. Other properties of PCBs include low vapor pressure at ambient temperatures; thermal stability; resistance to acids, bases and other chemical agents; compatibility with organic materials; and resistance to oxidation and reduction (Safe and Hutzinger, 1987).

PCBs are practically insoluble in water, whereas they dissolve easily in hydrocarbons, fats, and other organic compounds and they are readily absorbed by fatty tissues (WHO, 1993). This

Table 2.1. PCB nomenclature categories.

Category	Number of individual compounds
Congener	209
Isomer Group/ Homolog	10
Isomers/Group	1-46

Table 2.2. Composition of PCB isomer groups.

PCB isomer groups	Empirical formula	Percent chlorine	Number of isomers
Monochlorobiphenyl	$C_{12}H_9Cl$	19	3
Dichlorobiphenyl	$C_{12}H_8Cl_2$	32	12
Trichlorobiphenyl	$C_{12}H_7Cl_3$	41	24
Tetrachlorobiphenyl	$C_{12}H_6Cl_4$	49	42
Pentachlorobiphenyl	$C_{12}H_5Cl_5$	54	46
Hexachlorobiphenyl	$C_{12}H_4Cl_6$	59	42
Heptachlorobiphenyl	$C_{12}H_3Cl_7$	63	24
Octachlorobiphenyl	$C_{12}H_2Cl_8$	66	12
Nonachlorobiphenyl	$C_{12}HCl_9$	69	3
Decachlorobiphenyl	$C_{12}Cl_{10}$	71	1

Total: 209

hydrophobic property of PCBs makes them resistant to breakdown. One of the most important properties of PCBs is their excellent dielectric (electrically insulating) properties (Hutzinger et al., 1983). Properties like low electrical conductivity and high heat capacity have made PCBs ideal for use as cooling liquids in electrical equipment.

2.1.4 Production and Uses of PCBs

The polychlorinated biphenyls constitute a large class of compounds produced by the partial (or complete) chlorination of the biphenyl molecule. PCBs were first synthesized in 1864 (WHO, 1993), but the commercial production of PCBs began in 1929. Commercial PCBs have been produced in several countries including the United States, Great Britain, France, Germany, Italy, Japan, Czechoslovakia and Russia, and have been marketed under several trade names including Aroclor, Kanechlor, Phenoclor, Clophen, Fenclor, Pyranol, Pyroclor, Sovol, and Hyvol (WHO, 1993). All the PCBs that were produced in North America came from a single manufacturer, Monsanto Industrial Chemicals Corporation in the United States. The manufacture of PCBs was banned in the United States in 1977, due to potential health hazard. From 1929 to 1980, the cumulative world production of PCBs was approximately 2.4 billion pounds (Davila et al., 1993).

Commercial production of PCBs began in the United States in 1929 in response to the electrical industry's need for a safer cooling and insulating fluid for industrial transformers and capacitors. PCBs were also used in hydraulic fluids; heat transfer fluids; as flame retardant in lubricating oils; as plasticizer in sealant, adhesives, paints, textiles, synthetic resins, rubbers, waxes, and asphalt; as surface coatings for carbonless copy papers (Davila et al., 1993); and as solvent extenders and organic diluents (Yadav et al., 1995). PCBs are also reported to increase the insecticidal properties of DDT, lindane, organophosphorus compounds, and carbaryl (Hutzinger et al., 1983).

2.1.5 PCBs in the Environment

The widespread use of PCBs coupled with improper disposal practices has led to significant environmental contamination by commercial PCB formulations. Because their hazardous nature has only recently been understood, PCBs have been routinely disposed of over the years, without any precautions being taken. As a result, large volumes of PCBs have been introduced into the environment. Some estimates suggest that up to one third of the total United States production of PCBs (approximately 1.4×10^9 lbs) has entered the environment (Safe and Hutzinger, 1987).

Two important routes of entry into the environment have been losses during the process of manufacture and leakage from electrical equipment and other products containing PCBs. PCBs have entered the environment through open burning or incomplete incineration; by vaporization from paints, coatings and plastics; by direct leakage into sewers and streams; by dumping in non-secure landfill sites and municipal disposal facilities; and by other disposal techniques, such as ocean dumping, which did not destroy material. Despite regulation, some PCBs have been illegally dumped through ignorance or negligence. Accidental spills and leaks, while of local significance, have been relatively minor sources of PCB contamination of the global environment.

In 1966, PCBs were first identified as environmental contaminants during the analysis of environmental extracts for DDT and related metabolites (Crine, 1988). It is now apparent that PCBs are among the widespread pollutants in the global ecosystem and have been identified in the air, water, soils and sediments, fish, wildlife, plants, domestic animals, and human adipose tissue, blood and milk (Crine, 1988; WHO, 1993). PCB residues have also been reported in regions of no industrial activity, such as in snow deposits in Antarctic (Safe and Hutzinger, 1987). PCBs are highly stable in the environment and are readily transported from localized or regional sites of contamination throughout the global ecosystem. In the atmosphere, PCBs exist primarily in the vapor phase. The tendency to adsorb on particulates increases with the degree of chlorination. In water, PCBs are adsorbed on sediments and other organic matter. Estuarine and marine sediments are the ultimate global sinks for worldwide accumulation of PCBs sorbed to particulate material (Berkaw et al., 1996). The major routes of entry of PCBs into the soil environment are the disposal sites and atmospheric transport. At present, the major source of PCB exposure in the general environment appears to be the redistribution of PCBs, previously

introduced into the environment. PCBs are truly pervasive in the environment and will remain so for a long period of time.

Ironically, one of the properties of PCBs which most contributed to their widespread industrial use, their chemical stability, is also one of the properties which causes the greatest amount of environmental concern. This unusual persistence coupled with its tendency to accumulate in living organisms, means that PCBs are stored and concentrated in the environment. This bioconcentration raises concern because of the wide dispersal of PCBs in the global environment and the potential adverse affects they can have on various organisms, including humans.

2.1.6 Toxicology of PCBs

Due to their worldwide distribution, persistence in the environment, and their possible health effects, PCBs have attracted great concern over the past years. Their ability to bioaccumulate in fatty tissues and their lipophilic behavior, pose a serious threat to mammalian systems (Brunner et al., 1985). PCBs can enter the body through skin contact, by the inhalation of vapors or by ingestion of food containing PCB residues. PCBs are not particularly toxic in acute or short-term chronic testing. High dosages are necessary to induce a lethal response in most trophic levels. Chronic exposure to sublethal concentrations combined with high lipid solubility and resistance to metabolism can result in PCB accumulation and a toxic response (Exner, 1982).

PCBs are known to elicit a spectrum of toxic responses in humans, laboratory animals and wildlife. Some of the biologic and toxic effects of PCBs are as follows (Crine, 1988; Mousa et al., 1996; Safe and Hutzinger, 1987):

1. acute lethality at relative high dose levels (>1000 mg/kg) in most animal species;
2. a wasting syndrome: a progressive body weight loss;
3. thymic and splenic atrophy and immunosuppressive effects;
4. skin disorders: acneform eruptions or chloracne, alopecia, edema, and hyperkeratosis;
5. hyperplacia of the epithelial lining of the extrahepatic bile duct and gall bladder;
6. hepatomegaly and liver damage: necrosis and hemorrhage;
7. hepatotoxic effects including porphyria;

8. teratogenesis: cleft palate and kidney malformations;
9. reproductive, developmental and neurotoxicity;
10. role in moduling carcinogenesis and carcinogenicity;
11. the induction of diverse enzyme systems

The occurrence and severity of the toxic symptoms noted above are species, strain, sex and age dependent and not all the responses are observed in any single animal species.

PCBs can be toxic to higher plants, marine biota, and birds. PCBs may interfere with the overall growth of some plant species, by inhibiting cell division, and also disturb photosynthesis in some plants. PCBs can induce mixed function oxidase (MFO) enzymes and can inhibit ATPases in marine biota. PCBs are known to effect hormones in fish, and in birds, they can cause teratogenic and behavioral effects (Waid, 1986b). PCBs can also be toxic to some microorganisms, including the fresh-water, marine and soil microorganisms (WHO, 1993). PCBs may effect respiration and photosynthesis in some algae, whereas, they can reduce the micelial growth and increase the relative RNA content of the mycelium in some fungi (WHO, 1993).

Because PCBs are water insoluble and resistant to chemical decomposition and biodegradation, these compounds persist in the environment. PCBs pose a serious health hazard due to the potential for long-term toxic effects. Therefore, remediation of the PCB contaminated sites is required to reduce the potential for further dissemination.

2.2 REMEDIATION

While PCBs are not readily degraded, under certain conditions, they may be destroyed by chemical, thermal, and by specific biological processes.

2.2.1 Destruction

2.2.1.1 Thermal Destruction

Thermal destruction of PCBs include incineration systems, such as liquid injection, rotary kiln, fluidized bed, multiple chamber, catalytic combustion, and pyrolysis (Ackerman et al., 1983). These incinerators are designed to combust waste materials. In additions to incinerators, conventional, high efficiency boilers can also be used to destroy PCBs if proper combustion conditions are maintained. Gas-fired, oil-fired, and pulverized coal-fired boilers are frequently used for PCB destruction (Ackerman et al., 1983).

2.2.1.2 Chemical Destruction

PCBs can be chemically degraded by Fenton's reaction. Fenton's reaction is an advanced oxidation process for destruction of toxic organic compounds in water. In Fenton's reaction, H_2O_2 is combined with Fe^{3+} in the presence or absence of light to generate hydroxyl radicals ($HO^{\bullet}$):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + HO^{\bullet}$$

The $HO^{\bullet}$ initially adds to the aromatic ring to give a substituted hydroxycyclohexadienyl radical which may further react by acid catalyzed elimination. Chen and Pignatello (1997) proposed the catalytic role of quinone intermediates in Fenton's oxidation of aromatic compounds. According to these researchers, quinone intermediates shuttle electrons from $HO^{\cdot}$ radical adduct of the starting aromatic compound to Fe^{3+}, thus facilitating the degradation of the starting aromatic compound. The presence of co-oxidants like oxygen and quinones, plays a crucial role in steering oxidation into the desired pathways.

2.2.2 Photolysis

Physicochemical process like photolysis is a possible route of environmental breakdown for PCBs. Photolysis of PCBs occurs at wavelengths greater than 290 nm (Ackerman et al., 1983). In order for photolysis to occur, the PCB molecule must first absorb light energy above 290 nm or receive energy from another molecule through an energy transfer process. The initial step of the photolytic reaction usually involves fission of the parent molecule to form free radicals. These intermediates are unstable and react further with the solvent. Other organic molecules,

inorganic species, radicals, etc., may be formed. The product may be a complex mixture in which isomerization, substitution, oxidation or reduction process have occurred.

2.2.3 Biodegradation

Microbial biodegradation of PCBs is a potential means of remediating a contaminated soil or sediment. The biodegradability of a PCB molecule depends upon the degree of chlorination and the position of the chlorine atoms on the biphenyl molecule. Chlorine substitution positions on the biphenyl ring appear to be important in determining the biodegradation rate. PCBs containing chlorine atoms in the *para* positions are preferentially biodegraded (WHO, 1993).

PCBs can be biodegraded via two distinct microbially mediated mechanisms: aerobic biodegradation, involving the oxidative destruction of PCB molecules through a series of degradation intermediates, and anaerobic reductive dechlorination, involving the removal of chlorine atoms from PCBs in the absence of oxygen (Abramowicz, 1990).

2.2.3.1 Aerobic Biodegradation

The aerobic biodegradation of PCBs is generally limited to congeners with five or fewer chlorines and two adjacent unsubstituted carbon atoms (Bedard et al., 1987; Boyle et al., 1992). The more chlorinated congeners are generally recalcitrant to aerobic degradation (Yadav et al., 1995). Aerobic PCB biodegradation by a variety of naturally occurring bacteria has been extensively studied in the laboratory (Bedard et al., 1987) and in natural environments (Flanagan and May, 1993).

The principal route of PCB biodegradation in aerobic bacteria appears to involve 2,3-dioxygenase attack at an unsubstituted 2, 3 (or 5, 6) position of the biphenyl ring (Abramowicz, 1990; Abramowicz and Olson, 1995; Bedard et al., 1987; Boyle et al., 1992, and Furukawa, 1982). As a result of this attack, chlorinated 2,3-dihydro-2,3-dihydroxybiphenyl is formed. This product is further degraded to a chlorinated 2,3-dihydroxybiphenyl, followed by enzymatic cleavage of the hydroxylated ring to ultimately form the corresponding chlorobenzoic acid

(CBA) and a five-carbon fragment (Flanagan and May, 1993). The chlorobenzoic acid can then be readily degraded by indigenous bacteria, producing carbon dioxide, water, chloride and biomass (Abramowicz and Olson, 1995).

There are additional correlations between PCB structure and biodegradability, under aerobic conditions including: (i) biodegradability decreases as chlorine substitution increases. (ii) congeners with two *ortho* chlorines are extremely resistant to biodegradation. (iii) PCBs containing an unsubstituted ring are generally degraded more rapidly then isomers that are chlorinated on both rings. (iv) tetra- and pentachlorobiphenyls containing a 2,3-chlorobiphenyl ring are more susceptible to biodegradation than other tetra- and pentachlorobiphenyls. (v) ring fission generally occurs on the unchlorinated or less chlorinated ring of a congener (Bedard et al., 1987; and Furukawa et al., 1978).

2.2.3.2 Anaerobic Dehalogenation

PCBs undergo reductive dechlorination under anaerobic conditions, leading to the formation of less-chlorinated congeners. Lower chlorinated congeners and biphenyl are resistant to further degradation in anoxic environments. Anaerobic reductive dechlorination is an important step toward the bioremediation of PCBs since it reduces the chlorine content of PCB mixtures thereby reducing their bioaccumulation potential and, in some respects, toxicity (Rhee et al., 1993). Dechlorination of PCBs in anaerobic environments is generally considered to be slow, with major change occurring over period of months or years.

Anaerobic reductive dechlorination of PCBs involves replacement of a chlorine atom with a hydrogen atom (Mousa et al, 1996; Nies and Vogel, 1990). The mechanism of reductive dehalogenation can be represented by the reaction:

$$R - X + 2\,[H] \rightarrow R - H + HX$$

in which R represents an organic molecule, X a halogen, and [H] an electron plus proton.

The reduction potential of PCBs increases with increasing chlorine number (Nies and Vogel, 1990; Rusling and Miaw, 1989). However, the positions of the chlorine substituents also

influence ease of reductive dechlorination. Generally, chlorines in the *meta* and *para* position are most readily removed (Abramowicz, 1990; Abramowicz and Olson, 1995; Boyle et al., 1992; Mousa et al., 1996; Nies and Vogel, 1990). Chlorines substituted in *ortho* position are generally recalcitrant to anaerobic dechlorination (Yadav et al., 1995). However, reductive *ortho* dechlorination of PCBs has also been shown (Berkaw et al., 1996). A general relationship between reduction potential, chlorine substitution number, chlorine substitution pattern, and dechlorination rate seems to exist in case of reductive dechlorination of PCBs (Nies and Vogel 1990).

Anaerobic dechlorination could potentially be used as the first step in a remediation process. The decrease in highly chlorinated congeners resulting from anaerobic reductive dechlorination would decrease the Cl content of a given congener resulting in a PCB congener much more susceptible to degradation by aerobes possessing oxygenases. All lightly chlorinated congeners that are products of reductive dechlorination are biodegraded by aerobic PCB-degrading microorganisms (Bedard et al., 1986). Thus, aerobic degradation could be used as the second step in a bioremediation process (Nies and Vogel, 1990). A sequential anaerobic-aerobic system has an additional advantage, and the potential to mineralize PCBs. Figure 2.2 shows a two-step combined anaerobic/aerobic process to biodegrade PCBs.

2.2.4 Methanogens

2.2.4.1 Methanogenic Microorganisms

Methanogenic fermentations play an important role in the anaerobic dehalogenation process. Reductive dehalogenation of a wide spectrum of halogenated organic compounds, such as polychlorinated biphenyls (PCBs) (Quensen III et al., 1990; Williams, 1994; Ye et al., 1995), tetrachloroethylene (Perchloroethylene, PCE) (de Bruin et al.1992; Fathepure and Boyd, 1988; Vogel and Mc Carty, 1885), trichloroethylene (TCE) (Jablonski and Ferry, 1992), and Chloroform (Egli et al., 1987; Mikesell and Boyd, 1990) have been observed.

Biological methanogenesis occurs in a diversity of anaerobic habitats such as the rumen, the lower intestinal tract of animals, sewage digesters (Berry et al., 1987; Ferry, 1992), landfills,

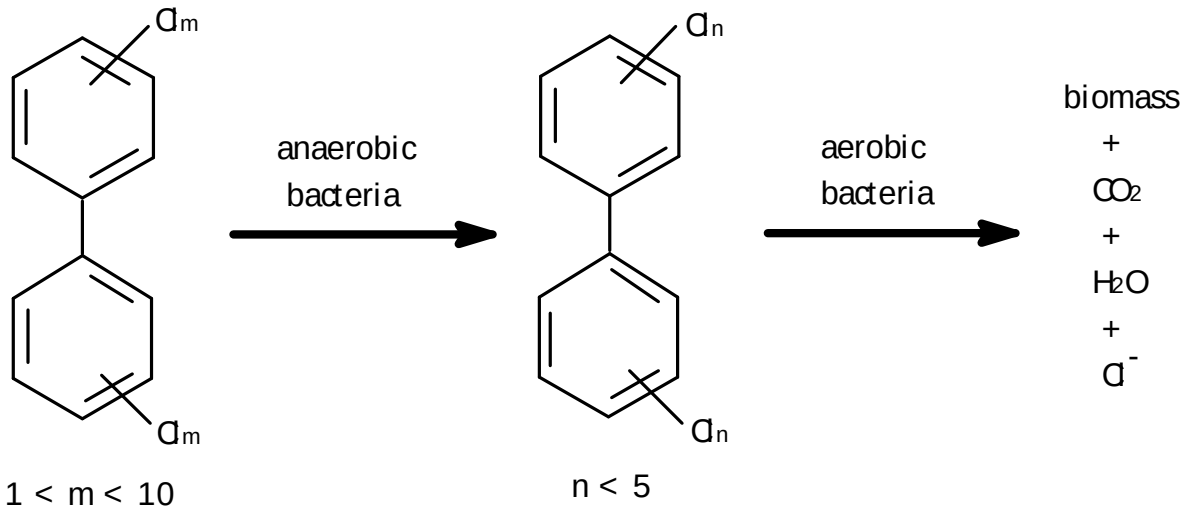

Figure 2.2. Two-step reaction mediated by microorganisms that can result in the elimination of PCBs.

freshwater sediments of lakes and rivers (Ferry, 1992; Holland et al.,1987; Zhender and Svensson, 1986), rice peddies, hydrothermal vents, and coastal marine sediments (Ferry, 1992). These microbes are also active in subsurface (groundwater) (Frederickson et al., 1989; Frederickson et al., 1991; Gibson and Suflita, 1986; Kuhn and Suflita, 1989; Kuhn et al., 1990). Active methanogenic microbial assemblages are usually found in anoxic environments, which lack both sulfate and nitrate.

Biological methanogenesis plays a major role in the carbon cycle on Earth. Under anaerobic conditions, a syntrophic association is required to degrade complex organic compounds. The consortium consists of at least three interacting metabolic groups of anaerobic microorganisms: (i) fermenters which degrade complex organic molecules to H_2, CO_2 and organic acids; (ii) acetogenic proton-reducing bacteria which convert the higher volatile fatty acids and organic acids to acetate and either H_2 or formate; (iii) hydrogen and acetate consuming methanogens. So, methanogenesis is the terminal step in carbon flow in many anaerobic habitats.

Some of the biochemical features of methanogens include ferredoxin (Terlesky and Ferry, 1988) and iron-sulfur proteins (Ferry, 1993). Coenzyme M (2-mercaptoethanesulfonic acid; HS-CoM) which is the simplest coenzyme know to date (Taylor and Wolfe, 1974) is exclusively found in methanogenic bacteria (Balch and Wolfe, 1979) and is considered to be the terminal methyl carrier for methane production from H_2-CO_2, methanol and methylamine (Lovley et al., 1984). The reduction of methyl-CoM to methane is catalyzed by the methyl CoM reductase system. Methanofuran is a C_4-substituted furfurylamine found in all methanogenic bacteria (Ferry, 1993; White, 1988). Methanofuran (MFR), tetrahydromethanopterin (H_4MPT), and CoM are the three C_1-unit carriers found in all methanogens (Di Marco et al., 1990; Keltjens et al., 1990). Methanogens are also rich in reduced transition metal cofactors (e.g., F_{430}) (Ye et al., 1995).

Hydrogenotrophic methanogens, e.g., *Methanobacterium thermoautotrophicum* can use the carbon dioxide reduction pathway to reduce CO_2 to CH_4 with H_2 or formate as the electron donor (Vogels et al., 1988). The CO_2 reduction pathway can be divided into 4 steps: (i) reduction of CO_2 to the formyl level; (ii) reduction of the formyl group to the formaldehyde level; (iii) reduction of the methylene group to the methyl level; (iv) conversion of the methyl group to

methane. Energy is derived from CO_2 reduction pathway via electron transport linked phosphorylation (ETLP) (Thauer et al., 1977).

$$4\ H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

$$\Delta G^{\circ\prime} = -\ 130.4\ \text{kJ/mol}\ CH_4$$

Acetogenic microbes reduce $2CO_2$ to acetyl-CoA as a terminal electron-accepting process producing acetate as the major end product (Ferry, 1995). Carbon monoxide dehydrogenase (CODH) catalyzes the synthesis of acetyl-CoA from CO_2. The reductive synthesis of acetyl-CoA by acetogenic microbes is estimated to contribute as much as 10% of the total production of acetate in natural environments.

Acetate is the end product of fermentation of several organic compounds including carbohydrates (hexoses and pentoses), organic acids (benzoic acid, lactic acid, fumaric acid, citric acid, ferulic acid, syringic acid, etc.) and amino acids (alanine, histidine, cysteine, etc. (Brock et al., 1994). These organic compounds serve as both primary electron donors and ultimate electron acceptors for many facultative or obligate anaerobes.

The acetate produced in anaerobic food chain is consumed by acetotrophic (aceticlastic) methanogens. These group of microbes ferment acetate to CO_2 and CH_4 (Ferry, 1995; Fukuzaki et al., 1990):

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$$

$$\Delta G^{\circ\prime} = -36\ \text{kJ/mol}$$

Some species of methanogenic anaerobes can utilize CO as a sole energy source (Daniels et al., 1977; Uffen, 1981), converting it to CO_2 and H_2:

$$4\ CO + 4\ H_2O \rightarrow 4\ CO_2 + 4\ H_2$$

$$\Delta G^{\circ\prime} = -20\ \text{kJ/mol}$$

CODH, as its name indicates, oxidizes CO to CO_2 (Ragsdale, 1991). CO_2 produced here can be used in the CO_2 reduction pathway or by acetogenic microbes for energy production.

Some methanogens, such as the members of the Methanosarcinaeae family can derive their energy for growth from the conversion of methanol to methane and CO_2:

$$4\ CH_3OH \rightarrow 3\ CH_4 + CO_2 + 2H_2O$$

$$\Delta G^{\circ\prime} = -106\ \text{kJ/mol}\ CH_4$$

CoM is the terminal methyl carrier in methanogenesis from methanol (Ferry, 1993).

2.2.4.2 Methanogenesis from Acetate

Acetate is the major intermediate in the bioconversion of organic matter to CH_4 and CO_2 and about 70% of the total methane produced in anaerobic degradation originates from the methyl group of acetate (Gujer and Zehnder, 1983). Thus, the production of CH_4 from acetate is an important step in anaerobic degradation process. Generally, acetate that is formed via fermentation of sugars and amino acids or via oxidation of volatile fatty acids is converted to CH_4 and CO_2 by aceticlastic (acetotrophic) methanogens. Only two genera, *Methanosarcina* and *Methanothrix* and a few species are known to ferment acetate to CO_2 and CH_4 (Ferry 1993;Ferry, 1995; Fukuzaki et al., 1990; Jones et al., 1987. Methanogenesis from acetate can proceed well from pH 6 to pH 8 (Fukuzaki et al., 1990).

The initial step in the formation of CH_4 and CO_2 from acetate is the activation of acetate to acetyl-CoA. The acetate kinase enzyme first activates the acetate to acetyl-phosphate followed by derivatization to acetyl-CoA by the phosphotransacetylase (Ferry, 1993).

The central enzyme in the pathway of methanogenesis from acetate is a CO dehydrogenase (CODH) complex, which catalyzes the cleavage of acetyl-CoA. The Ni/Fe-S component of the CODH complex catalyzes the cleavage of the C-C and C-S bonds of acetyl-CoA (Raybuck et al., 1991). The Ni/Fe-S component oxidizes the carbonyl group to CO_2 and reduces a ferredoxin (Ferry, 1993). The methyl group is transferred to the Co/Fe-S protein in the complex (Ferry, 1995; Lovely et al., 1984; Terlesky et al, 1986). This process involves tetrahydrosarcinapterin (H_4SPT) as a methyl-transfer cofactor (Ferry, 1995).

The final step is the formation of CH_3-S-CoM, and subsequent reductive demethylation to methane by action of CH_3-S-CoM methylreductase (Nelson and Ferry, 1984). The two electrons required for the reduction are derived from the sulfur atoms of CH_3-S-CoM (Ferry, 1993) and 7-mercaptoheptanoylthreonine phosphate (CoB) (Ferry, 1995; Peer et al., 1994). The heterodisulfide CoM-S-S-CoB, formed as a consequence of the reaction, is reduced to the active sulfhydryl forms of the cofactors with electrons originating from oxidation of the carbonyl groups of acetyl-CoA (Hedderich, 1989; Schworer and Thauer, 1991). No exogenous electron acceptors are required. ATP is generated by electron transport phosphorylation (Ferry, 1995). A relatively small amount of energy ($\Delta G^{\circ\prime}$ = –36 kJ/mol) is available from the conversion of acetate to CH_4 and CO_2 and an equivalent of one ATP is already expended in the activation of acetate. Thus, these organisms have developed a very efficient mechanism for energy conservation.

2.2.4.3 Methanogens and Anaerobic Dehalogenation

Fathepure and Boyd (1988) while studying the dechlorination of PCE by a *Methanosarcina* sp., proposed a hypothetical scheme for the transfer of electrons to PCE during methanogenesis, resulting in the formation of TCE. According to the scheme, the methyl group was transferred to vitamin B_{12}, followed by the formation of CH3-S-CoM and subsequent reductive demethylation to CH_4. Krone et al. (1989) reported that vitamin B_{12} can catalyze the reductive dechlorination of carbon tetrachloride and other chlorinated methanes. Gantzer and Wackett (1991) reported reductive dechlorination of TCE by vitamin B_{12}. Smith and Woods (1994) studied reductive dechlorination of pentachlorophenol (PCP) and 2,3,5-trichlorophenol (2,3,5-TCP) by vitamin B_{12} in the presence of Ti(III) citrate. The ability of vitamin B_{12} to catalyze reductive dechlorinations has led to the assumption that vitamin B_{12} can serve as an abiotic model of biological reductive dechlorination (Smith and Woods, 1994).

The CODH complex in methanogenic bacteria consist of modified cobamides, which consist of vitamin B_{12} corrinoid ring structure, also known as Factor III (Ferry, 1993). Krone et al.(1991) reported that reductive dehalogenation of one-carbon compounds are catalyzed by corrinoids which are present in high levels in the CO dehydrogenase complex of aceticlastic methanogens.

The CODH complex in methanogens consists of a two-subunit CO-oxidizing nickel/iron-sulfur (Ni/Fe-S) component, and a two-subunit corrinoid/iron-sulfur (Co/Fe-S) component containing the corrinoid, factor III. Jablonski and Ferry (1992), showed that purified CODH from the aceticlastic methanogen *Methanosarcina thermophila*, reductively dechlorinated TCE. According to these researchers, factor III was the site for dechlorination of TCE in CODH complex, in the presence of Ti(III) citrate. Even though, no direct evidence has been shown so far on the role of CODH in the reductive dehalogenation of PCBs, it is possible that CODH plays a key role in the anaerobic dehalogenation of polychlorinated biphenyls in environments where methanogenic fermentation dominates.

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