CHAPTER II
LITERATURE REVIEW

2.1 Trichloroethylene (TCE)

2.1.1 Properties of TCE

Trichloroethylene (TCE) is a chlorinated aliphatic hydrocarbon compound with a low molecular weight (131.4 g/mol) (Shang et al., 2001) (Figure 2.1). At room temperature, TCE is a volatile, non-flammable, colorless liquid (Tox Probe, n.d.). TCE is moderately soluble in water (1.1 to 1.4 g/L), and has a low *n*-octanol/water partition coefficient (log K_{ow} 2.29 to 2.42) and a high vapour pressure (8.0 to 9.9 kPa at 20–25°C) (McNeill, 1979; Eisenreich et al., 1981; ATSDR, 1989). Physicochemical properties are shown in Table 2.1.

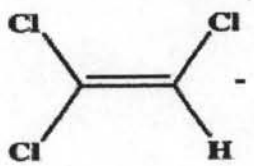

Figure 2.1 Structure of TCE (Shang et al., 2001)

2.1.2 Use of TCE

The major use of TCE is for degreasing and cleaning of fabricated metal parts in the automotive and metals industries (Wu and Schaum, 2000). It is also used in the production of adhesives and copolymers, household and industrial dry-cleaning, textile manufacturing, the cleaning of electronic components, petroleum industry processes involving refining catalysts, paint removers coatings and vinyl resins, and in laboratory reagent/solvent applications. Household or consumer products that may contain TCE include typewriter correction fluids, paint removers/strippers, adhesives, spot removers and rug-cleaning fluids (Frankenberry et al., 1987; Bruckner et al., 1989; ATSDR, 1991).

In Thailand, TCE was widely used for cleaning and degreasing operations in electronic manufacture. The amount of TCE imported to Thailand had been increased from 7363.6 tons in 2002 to 7889.3 tons in 2004 (Thai Customs Department) resulting from a development of industrial sector.

Table 2.1 Physicochemical properties of TCE (Adapted from ATSDR, 1995)

Property	Characteristic
Molecular weight	131.40 g/mol
Molecular formula	C_2HCl_3
Synonyms	Ethinyl trichloride; Acetylene trichloride; ethylene trichloride; 1,1,2-Trichloroethylene; Trichloroethene; Trichloran; Trichloren; 1,2,2-Trichloroethylene; anamenth; benzinol
Color	Clear, colorless
Physical state	Liquid (at room temperature)
Melting point	-87.1°C
Boiling point	86.7°C
Density at 20°C	1.465 g/mL
Odour	Ethereal; chloroform-like; sweet
Odour threshold: Air	100 mg/L
Solubility: Water at 25°C Organic solvents	 1.366 g/L Miscible with many common organic solvents (such as ether, alcohol, chloroform)
Partition coefficients: Log Kow Log Koc	 2.42 2.03-2.66
Vapour pressure at 25°C	74 mm Hg
Henry's low constants at 25°C	0.11 atm-m^3/mol
Flammability limits at 25°C (explosive limits) volume% in air)	8.0-10.5

2.1.3 Fate of TCE in Environment

TCE enters the atmosphere from vapor degreasing operations. Releases to the air could occur at sewage treatment and disposal facilities, water treatment facilities, and landfills (ATSDR, 1995). About half of TCE in the air is photochemically transformed primarily into hydroxyl radicals within days in summer and weeks to months in the winter (CEPA, 1993). When TCE enters surface soils, it will either evaporate or leach into groundwater. TCE is a Dense Non-Aqueous Phase Liquids (DNAPLs) so it does not move with the groundwater flow but instead move downward by gravitation force through an aquifer until reaching an impermeable layer. Thus, TCE can serve as a long-term source for dissolved contaminant plumes at many contamination sites. Consequently, a contamination of TCE occurs in aquifer. TCE is readily mobile in soil. The mobility is primarily affected by the organic carbon content, thus affects sorption of TCE to soil (CEPA, 1993). TCE evaporates from surface water faster than soil. The half-life of TCE in soil and water ranges from several days to several weeks mainly depending on number of microorganisms present. In water, TCE can break down in 2 to 10 days (Department of the Environment and Heritage, 2004).

2.1.4 Health Effect of TCE

Contaction with TCE can irritate and burn the skin and eye with possible eye damage. TCE exposure can cause lightheadedness, dizziness, unconsciousness, visual disturbances, nausea and vomiting in human health (New Jersey Department of Health and Senior Services, 2000). TCE is carcinogenic to human and classified as probably human carcinogenic by International Agency for Research on Cancer (IARC, 1995).

2.1.5 Regulations

In the United States, Environmental Protection Agency (EPA) has set a drinking water standard for TCE to 5 μg/mL. TCE levels in the workplace are regulated by the Occupational Safety and Health Administration (OSHA). The occupational exposure limit for an average concentration of 100 mg/mL TCE in air is an 8 hours workday. In Thailand, TCE level in groundwater must not exceed 5 μg/mL according to Royal Government Gazette, vol. 117 special part 95 D, promulgated on September 15, B.E. 2543 (2000) (PCD, 2004). Soil quality standard has been

established to be not more than 28 mg/kg of TCE in residential and agricultural soil and 61 mg/kg of TCE in industrial soil according to Royal Government Gazette, vol. 121 special part 119 D, promulgated on October 20, B.E. 2547 (2004) (PCD, 2004).

2.2 Biodegradation of Chlorinated Hydrocarbon

2.2.1 Direct Oxidation

Direct oxidation of chlorinated hydrocarbon mainly takes place under aerobic conditions resulting in metabolites such as vinyl chloride (VC), dichloroethene (DCE), dichloromethane (DCM), chloromethane (CM), 1,2-dichloroethane (EDC), and chloroethane (CE) (Figure 2.2). These metabolites can be used as growth substrate for microorganisms existing in the chlorinated hydrocarbon contaminated site (Bradley and Chapelle, 1997). DCE has been shown to be aerobically oxidized and mineralized to CO_2 without adding any co-substrate (Bradley and Chapelle, 1998; Bradley et al., 1998a; Bradley et al., 1998b; Olaniran et al., 2004). However, there is no evident on using TCE as primary substrate for microorganisms under aerobic condition (Olaniran et al., 2004).

2.2.2 Co-metabolism

Co-metabolism describes the metabolism of substrate not required for growth in which no apparent benefit is accrued by metabolizing organism (Horvath, 1992; Perry, 1979). The term co-metabolism indicates that transformation of contaminant is secondary reaction (Figure 2.3). The microbes consume hydrocarbon as their energy source. In this process, microbes produce enzyme that fortuitously degrade target contaminant by consuming the other compounds as their primary substrate (WRHSRC, 2004). Co-metabolism of tetrachloroethylene (PCE) and TCE has been demonstrated under aerobic condition. None of the two compounds has been shown to serve as primary substrate for aerobic microbial degradation (Olaniran et al., 2004). Laboratory studies have shown that aerobic degradation of TCE in soil occurs only in the presence of a co-metabolite such as toluene, propane, or methane (EPA, 1999). Variety of co-metabolites for TCE degradation were discovered. Wilson and Wilson (1985) found that aerobic microbes grown on natural gas promoted the transformation of TCE, potentially into carbon dioxide, chloride ion and water.

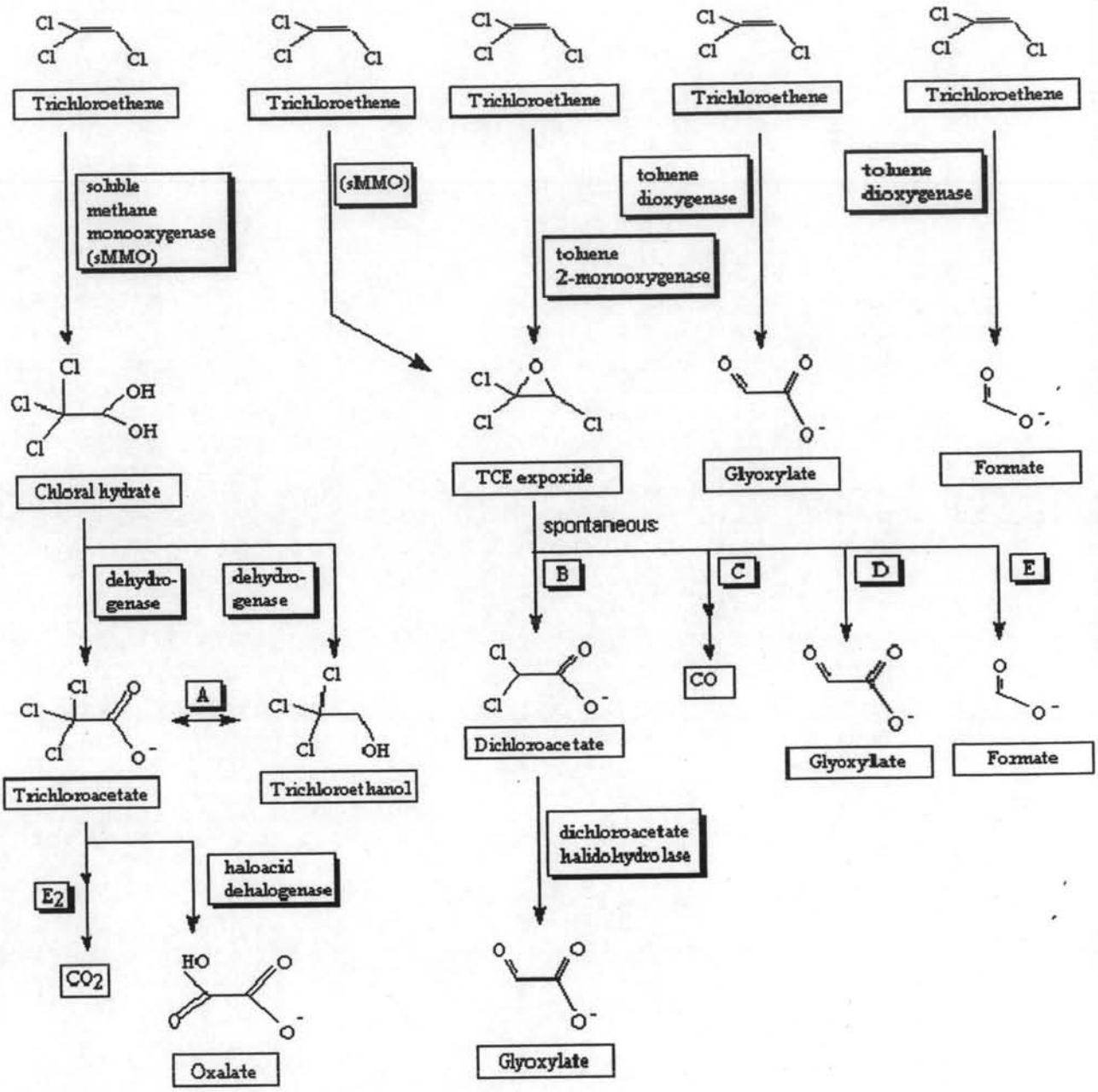

Figure 2.2 Proposed scheme of TCE metabolism (Oh, 2005)

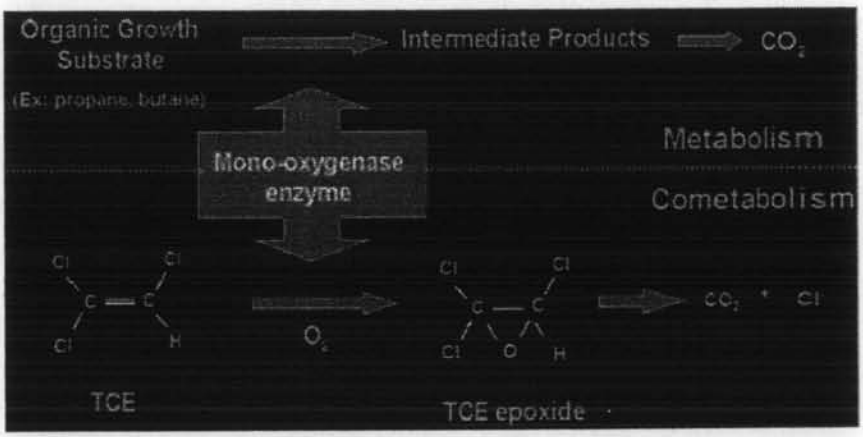

Figure 2.3 Diagram of co-metabolism of TCE (WRHSRC, 2004)

Natural gas was used as a co-metabolite. Hopkins and McCarry (1995) demonstrated that indigenous microbes grown *in situ* on either phenol or toluene could effectively co-metabolize TCE. Hecht et al. (1995) used phenol as the co-metabolic substrate for TCE removal in vapor phase bioscrubber and reported that degrees of TCE transformation ranged from 30% to 80%. Wilcox et al. (1995) found that TCE could be transformed by *Mycobacterium vaccae* JOB5 using propane as a primary substrate. However, the TCE utilization rate and growth rate of *Mycobacterium vaccae* JOB5 were very low. Parvatiyar et al. (1996) treated TCE contaminated air and toluene was used as a co-metabolic substrate. Results indicated that about 30% of TCE was removed.

2.2.3 Reductive Dechlorination (Reductive Dehalogenation)

Reductive dechlorination is the primary mechanism involved in the transformation of chlorinated compounds under anaerobic condition (Mohn and Tiedje, 1992). Chlorinated hydrocarbons are used as electron acceptors (Vogel et al., 1987). The chlorine atoms are removed from the molecule and replaced with hydrogen (Baker and Herson, 1994). Reductive dechlorination of PCE and TCE resulted in metabolites mainly cis-DCE and VC in subsurface and groundwater system (Major et al., 1991; Pill et al., 1991; McCarty and Reinhard, 1993; Weaver et al., 1995; Wilson and Wilson, 1985) (Figure 2.4). Biological reductive dechlorination was reported by Harkness et al. (1999) who reported that non native TCE-dechlorinating culture could dechlorinate 4 mg/L of TCE to ethane after 30 days. This culture was also able to dechlorinate TCE to ethane at higher concentration (170

mg/L). Hydrogen is the only substrate that directly serves as electron donor (Figure 2.4) for reductive dechlorination. Hydrogen (3 mmol/L) amendment enhanced dechlorination in soil and groundwater microcosms (Aulenta et al., 2005). DiStefano et al. (1992) and Holliger and Schumacher (1994) reported that hydrogen was very important electron donor for PCE-dechlorinating bacteria. DiStefano, et al. (1992) suggested that the production of hydrogen should be chosen or method should be developed for direct supply as an electron donor for dechlorination.

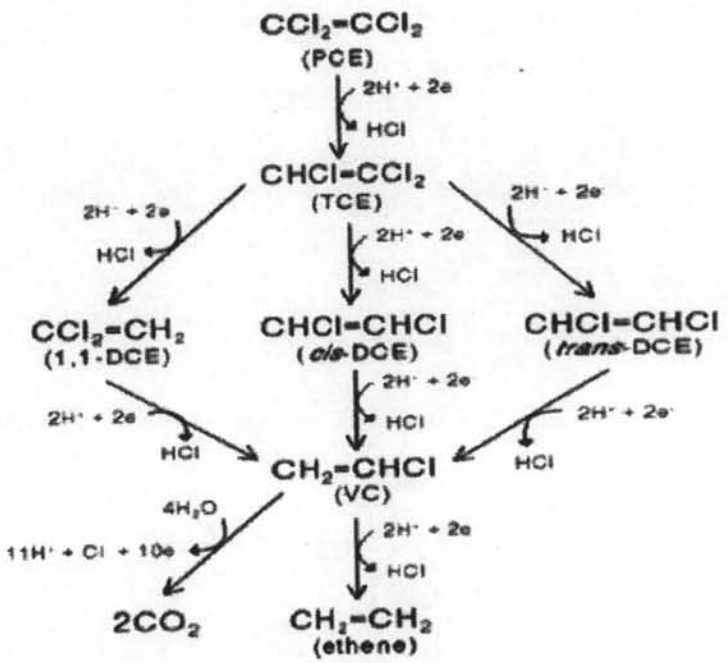

Figure 2.4 Proposed pathway for a reductive dechlorination of PCE (Maymo-Gatelle et al., 1997)

2.3 Bioremediation

Bioremediation is an attractive approach to clean up hazardous chemicals because it is simple to maintain, applicable over large areas, cost-effective and leads to the complete destruction of the contaminant (Frankenberger, 1992). Bioremediation comprises of various techniques, i.e., natural attenuation, biostimulation, bioventing, bioaugmentation, landfarming, composting, and phytoremediation (Skipper, 1999). This study focused on bioaugmentation and biostimulation.

2.3.1 Bioaugmentation

Bioaugmentation is the technique for improvement the capacity of a contaminated matrix to remove pollutant by the introduction of specific strains or consortia of microorganisms (Fantroussi and Agathos, 2005). Methanotrophs (Little, 1988; Oldenhuis 1989; Tsien, 1989), *Mycobacterium aurum* L1 (Hartmans and deBont, 1992), *Nitrosomonas europaea* (Arciero, 1989), *Pseudomonas putida* Fl (Wackett and Gibson, 1988) and *Pseudomonas fluorescens* (Vandenbergh and Kunka, 1988) have been reported to biotransform chlorinated hydrocarbon in aquifer. However, single strain was rarely applied for *in situ* bioremediation because a microbial degradation by pure culture on a large scale can be complicated by natural limitations relating to nutrient amendments, temperature control, and pH adjustment (Cookson, 1995).

An alternative choice to pure culture is the mixed cultures from wastewater sludge. Wastewater sludge is attractive to be used as inocula because sludge contains a high number of microorganisms which may enhance the degradation of toxic substances (Jateau et al., 2003). Moreover, advantage of mixed culture as a key issue in field applications of bioremediation is their ability to survive in a non-sterile environment (Murialdo et al., 2003). Jateau et al. (2003) reported that addition of activated sludge from the wastewater treatment facilities of an oil refinery industry could improve degradation of alkanes and PAHs in contaminated soil. The percentage of alkanes and PAHs degradation in the soil inoculated with activated sludge were 80% and 77%, respectively, which were higher than in soil without inoculation (24% for alkanes and 49% for PAHs).

Over the past two decades, *in situ* bioremediation has gained both acceptance and use as a viable, cost-effective remediation technology. Anaerobic bacteria can reductively dechlorinate TCE (Vogel and McCarty, 1985), but this process is often slow and incomplete (Kleopfer et al., 1985, Parsons and Lage,1985), potentially leading to a buildup of more toxic substances (e.g. vinyl chloride). Aerobic biodegradation of TCE, however, is quicker and leads to the production of harmless byproducts, making it an attractive choice for bioremediation. Groundwater contaminated with 1000-2500 μg/L chlorinated ethenes, TCE, DCE, VC, was treated by *in situ* bioaugmentation with a specialized microorganisms, *Burkholderia cepacia* ENV435. The strain was selected for its limited adhesion to aquifer solids and its ability to degrade chlorinated ethenes in the absence of inducing co-substrate. The

culture was added to achieve an injection concentration of approximately $1x10^{11}$ CFU/mL along with oxygen (20 mg/L) into a semi-confined silty-sand aquifer. The total mass of TCE, DCE, and VC in the treated area was reduced as much as 78% within 2 days after injecting the organisms (Steffan et al., 1999). Duba et al. (1996) has demonstrated that an *in situ* biofilter using resting-state cells effectively remediated groundwater with about 425 μg/L of TCE as the sole contaminant species. *Methylosinus trichosporium* OB3b ($5.4x10^9$ cells/mL) was injected into an aquifer through a single well at a depth of 27 m. The injected groundwater was devoid of TCE and growth substrates but was amended with a phosphate solution (10 mM) to buffer the pH and phenol red (20 μM) to act as a tracer. TCE concentrations in the extracted groundwater decreased from 425 μg/L to less than 10 μg/L during the first 50 h, which is equivalent to a 98% reduction.

2.3.2 Biostimulation

Biostimulation is one of bioremediation techniques that can improve pollutant degradation by optimizing condition such as aeration, addition of nutrient, pH or temperature (Margesin et al., 2000). Biostimulation with organic amendment is an effective tool to remediate various types of contaminant because organic materials can serve as nutrient suppliers and bulking agents to stimulate indigenous microorganisms. Previous research indicated that agriculture residues successfully stimulate degradation of contaminants in soil. Olaniran et al. (2005) reported that when agricultural fertilizer was amended at N:P:K ratio of 3:1:6, the highest degradation of *cis*- dichloroethylene in sandy soil of 33.61% was obtained. Soil microcosm containing 3% corn straw and C:N ratio of 100:10 resulted in approximately 60% diesel removal greater than non-stimulated diesel-contaminated soil (Barahona et al., 2004). Cho et al. (1997) reported that the addition of coconut charcoal in oil contaminated soil could enhance oil degradation from 15% to 33% during 43 weeks incubation. Sandy soil spiked with 1 mg/g soil of each phenanthrene, fluoranthene and pyrene was mixed with each of the following agricultural materials: rice straw, peanut shells and rain tree leaves, at the ratio of 9:1. Results showed that the addition of peanut shells and rain tree leaves could stimulate the degradation of phenanthrene, fluoranthene and pyrene to under detectable concentration within 24 days, 42 days and 42 days, respectively, while rice straw did not enhance bioremediation effect (Charoenchang, 2003).

TCE cannot be served as carbon and energy sources. Thus, biodegradation of TCE requires the primary substrate. A variety of primary substrates such as aromatic substrates (e.g. phenol and toluene) has been reported to stimulate aerobic co-metabolism of TCE under laboratory and field conditions (Semprini et al., 1990, 1991; Hopkins and McCarty, 1995). Toluene oxidizing bacteria appear to be widespread and data from laboratory studies indicate that many of these microorganisms have the potential to degrade TCE (Wackett and Gibson, 1988). McCarty et al. (1998) used a recirculating well system to inject toluene (15 mg/L) thereby stimulating the aerobic co-metabolism of TCE in the contaminated groundwater at Edwards Air Force Base (AFB), CA, US. Results showed that approximately 98% of TCE was removed. Fan and Scow (1993) measured the biodegradation of TCE by indigenous microbial populations in unsaturated soils incubated under aerobic conditions. At a concentration of 1 μg TCE/mL, TCE was not degraded in the absence of toluene while TCE were degraded approximately 60 to 75% in the present of 20 μg of toluene/mL after 70 to 90 h, respectively. Hopkins et al. (1993) injected phenol and oxygen at concentrations of 12.5 mg/L and 35 mg/L, respectively, into a confined aquifer at the Moffett Field test site. Initial concentration of TCE in the range of 62-500 pg/L were removed 88%. When initial concentration of TCE was 1000 pg/L, removal of TCE was lower (77%), but increased to 90% when the phenol concentration was raised to 25 mg/L. These results demonstrated a promise for *in-situ* aerobic co-metabolic biodegradation of TCE with phenol-oxidizing microorganisms.

However, the aromatic hydrocarbon substrates i.e., toluene and phenol have been reported as hazardous substances. Environmental friendly substances were attractive to be used as potential primary substrate on TCE degradation. Plant terpene have been undertaken to overcome this problem. Several of terpene such as limonene, cumene, and carvone have structure similar to aromatic hydrocarbon which can induce enzyme involving in aromatic hydrocarbon biodegradation. *Arthrobacter sp.* Strain B1B were induced with carvone for polychlorinated biphenyl (PCBs) degradation in soil. Results showed that 100 μg of Aroclor 1242/g soil removed 55-59% PCBs after 9 weeks (Singer et al., 2000). Tandlish et al. (2001) studied the effect of carvone and limonene on the biodegradation of DÉCOR 103 by *Pseudomonas stutzeri*. Results showed that 30-70% of the congeners of 10 μg/mL DÉCOR 103 were degraded in the present of 10 mg/mL and 20 mg/mL carvone as potential

inducer and 4 g/L of xylose as a carbon source. Moreover, plant materials containing plant terpene directly stimulated PCBs biodegradation. Hernandez et al. (1997) added orange peels, eucalyptus leaves, pine needles and ivy leaves separately to soil spiked with Aroclor 1242 (100 mg/kg soil) and it was found that this compound disappeared after six months in all the amended soils, but not in unamended soils. In addition, amended soils had much higher levels (10^8/g soil) of biphenyl-utilizing bacteria than the unamended control (10^3/g soil). In addition, 5 isolates were studied further with respect to growth on pure terpenes and metabolism of PCBs. The most effective strains were *Cellulomonas* sp. T109 and *R. rhodochrous* T100 which could metabolize 83% and 80% of Aroclor 1242, respectively, during a six day period of growth on cymene and limonene, respectively. Dabrock et al. (1992) demonstrated that *Psuedomonas sp.*JR1 and *Rhodococcus erythropolis* BD1 were induced with 2 mM of cumene to degrade various concentrations of TCE from 25 mM-200 mM. They found that increasing initial TCE concentrations resulted in increasing initial rates of TCE degradation (3-24 mg/L).

2.4 Immobilization Technique

This technique is used to physically or chemically fix cells, organelles, enzymes, or other proteins onto a solid support, into a solid matrix or retained by a membrane, in order to increase their stability and make possible their repeated or continue use (Iqbal and Saeed, 2005). The cell immobilization tends to have higher level of activity and more flexible to environmental disturbance such as pH, or the toxic of chemical substances than free cell.

Immobilization techniques can be divided into 4 major categories based on the physical mechanisms (Figure 2.5) (Kourkoutas et al., 2004).

[1] Attachment or adsorption on carrier solid

Cell immobilization on a solid carrier is carried out by physical adsorption due to electrostatic forces or by covalent binding between the cell membrane and the carrier.

[2] Entrapment within a porous matrix

Cells are either allowed to penetrate into the porous matrix until their mobility is obstructed by the presence of other cells, or the porous material is formed *in situ* into a culture of cells.

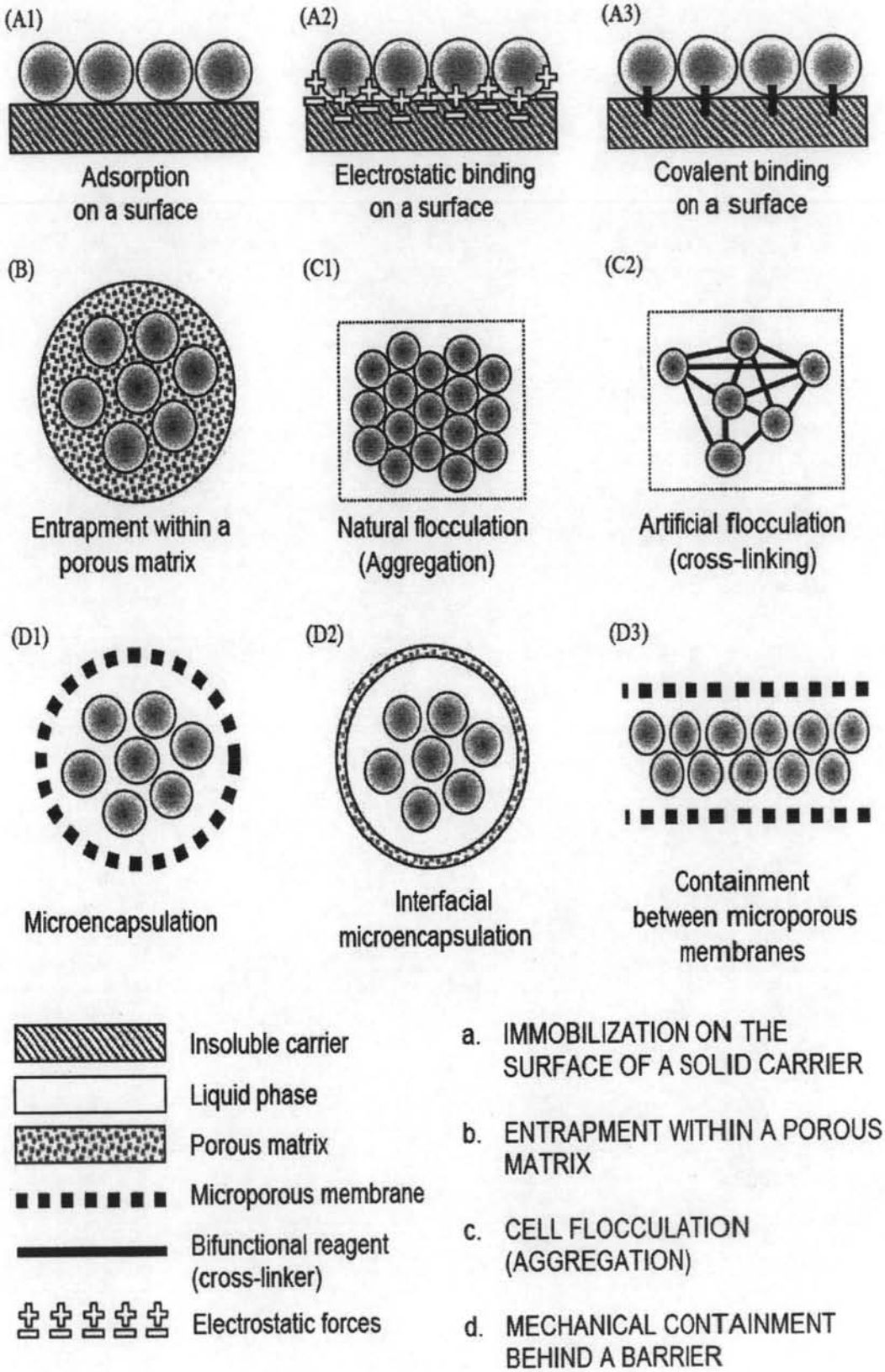

Figure 2.5 Basic methods of cell immobilization (Kourkoutas et al., 2004)

[3] Self-aggregation by flocculation (natural) or with cross-linking agents (artificially induced)

An aggregation of cells to form a larger unit or the property of cells in suspensions to adhere in clumps and sediment rapidly (Jinan and Speers, 1998). The ability to form aggregates is mainly observed in moulds, fungi and plant cells.

[4] Cell containment behind barriers

Containment of cells behind a barrier can be attained either by use of microporous membrane filters or by entrapment of cells in a microcapsule or by cell immobilization onto an interaction surface of two immiscible liquids.

Immobilization of TCE degrading bacteria had been reported. TCE-degrading methanotroph, *Methylocystis sp.* M were immobilized in 2 % calcium alginate. TCE degradation of immobilized and free cells were studied at different TCE concentrations. Degradation rate of the free cells was faster than that of the immobilized cells, but when the concentration increased (to more than 240 μM) the degradation rate was similar in both forms of immobilized and free cells. Immobilized cell showed optimum TCE degradation activity at pH 7.0 and 35°C. (Uchiyama et al., 1995). *Burkhoderia cepacia* PR1 sub 301c (PR1) were immobilized on small porous ceramic pallets (Isolite) and microencapsulated in an alginate gel which introduced into soil. Results showed that the isolite-immobilized cells and microencapsulated cells kept their TCE-degrading activity 4-5 times and 2-3 times, respectively, longer than free cells in non-sterile soil in the presence of TCE (Adam and Reardon, 2001). Shimomura et al. (1997) studied immobilization of *Methylocystis sp.* M with 2 % calcium alginate which further introduced into fluidized-bed bioreactor developing for remedial synthetic groundwater containing TCE. TCE concentration in the reactor decreased from 0.9-1.6 to 0.1-0.2 mg/L when methane was supplied as primary substrate. Results showed that 80-90% of initial concentration of TCE was degraded in the reactor.

Natural polymeric gels such as agar, carrageenan, calcium alginate and synthetic polymer such as polyacrylamide, polyvinyl, polyurethane have been used as support materials for cell immobilization (Katzbauer et al., 1995). However, use of the synthetic polymer is limited by their mechanical strength and the lack of open spaces to accommodate cell growth and cell release into the growth medium (Barbotin and Nava Saucedo, 1998) as well as a disposal problem (Kumar and Das, 2001). Calcium alginate gels were reported to be unstable when it contacts with complex

anions such as phosphate and citrate which are usually used in media (Birnbaum, et al., 1981). Other support materials used to attach methanotrophs were diatomaceous earth, activated carbon (Fennell et al., 1992; Fennell et al., 1993), glass (Phelps et al., 1990; Arvin, 1991) and ceramic packing material (Strandberg et al., 1989), though these materials are costly. Thus, the agricultural residues are attractive to replace these costly support materials because they are waste and priceless. Previous research reported various agricultural residues were effectively used as immobilized matrices such as rice straw, bagasse, coir (Kumar and Das, 2001) for hydrogen production. Sugarcane chip, rice husk, maize coke, palm wood blocks and bamboo ships (Jimoh, 2004) were used as immobilized matrices for ethanol production.

For bioremediation, peat moss and wood chip were reported to immobilize *Thiobacillus denitrificans* for H_2S removal (Ma et al., 2006). Chitin and chitosan flakes obtained from shrimps wastes were used to immobilize *Rhodococcus corynebacterioides* QBTo (hydrocarbon-degrading bacterial strain) to remove crude oil from polluted seawater. The percentage of hydrocarbon removal obtained in the microcosm inoculated with immobilized cells (60%) were higher than microcosm inoculated with free cell (30%). Results indicated that chitin and chitosan flakes improved the survival and the activity of immobilized cells (Gentili et al., 2006). However, as of our best knowledge, the information of agricultural residues used as support material for TCE bioremediation has rarely been reported.