CHAPTER II

LITERATURE REVIEW

2.1 Carbofuran

Carbofuran (2,3-dihydro-2,2- dimethyl-7-ly-methylcarbamate) is a broad spectrum carbamate insecticide (Figure 2.1) used to control insect pests such as corn rootworm, wireworm, brow plant hopper (*Nilaparavatalugens stal*), white grubs, weevils, stem borers, aphid, etc. on contact or after ingestion (Kale et al., 2001). The widely use of carbofuran is on rice, sugarcane, crops, tobacco, maize, potatoes, and vegetable fields (Extonet, 1996). It has two formulations: (i) wettable powder containing 75% of the active ingredient and (ii) granule containing 3% of the active ingredient (DC Chemical Co., Ltd, 2003). Trade name includes Furadan, Curator, Yaltox, Kenofuran, ENT27164 (EPA, 2003).
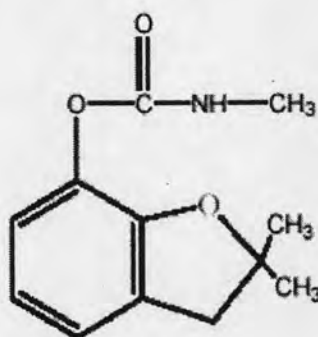

Figure 2.1 Structure of carbofuran

[DC Chemical CO., Ltd, 2003]

In the United State of America carbofuran is used on corn 48%, alfalfa bay 19%, and sorghum 8% (DeVries and Evans, 1999). Two states releasing the majority of carbofuran into the environment are Louisiana and Iowa (DeVries and Evans, 1999). In 2003, Thailand imported carbofuran in liquid and solid forms up to 826.6 mt and 45.5 mt, respectively, (Chulalongkorn University, 2004). In rice fields, Furadan granules 3% (3G) were applied into young plants at the rate of 33.3 kg/ha (Aiumsupasit, 2005). Carbofuran was detected in rice field soils at 0.2 mg/kg soil in rice field, North-Eastern, Thailand (Aiumsupasit, 2005).

2.2 Properties and characteristics of carbofuran

Carbofuran is an odorless and colorless crystalline solid (Extonet, 1996). Carbofuran is soluble in water (320 mg/l at 25 °C). It is also soluble in acetone, acetonitrile, benzene, etc. and has a low vapor pressure (8.3×10^{-6} mmHg at 25°C) resulting in a high potential for environmental contamination (Howard, 1991). The physicochemical properties of carbofuran were shown in Table 2.1

Table 2.1 Physicochemical properties of carbofuran (IDPID, 1993)

Physicochemical properties	Values
Common name	Carbofuran
Chemical name	2,3-dihydro-2,2-dimethylbenzofuran-7-yl- methylcarbamate
Trade name	Furadan
Empirical formula	$C_{12}H_{15}NO_3$
Molecular formula	$C_8H_6O(CH_3)_2(OOCNHCH_3)$
Molecular weight	221.25
Physical form	Crystalline solid
Melting point	150-153 ^{o}C
Vapor pressure	8.7×10^{-4} mm Hg at 25 ^{o}C
Henry's Law constant	3.9×10^{-9} atm m^3/mol
Octanol/water Partition Coefficient (K_{ow})	17 for 1 mg/L at 20 ^{o}C 26 for 10 mg/L at 20 ^{o}C
Adsorption Coefficient (K_{oc})	22

Table 2.1 Physicochemical properties of carbofuran (IDPID, 1993) (Cont.)

Physicochemical properties	Values

Solubility at 25 ^{o}C
   -in water
   -in acetone
   - in xylene
	
	 0.07%
	 15%
	 1.0 %

Specific gravity	1.18
Stability-Hydrolysis (half-life at 25 ^{o}C)	> 20,000 h at pH 3.1 > 7,000 h at pH 6.2 13.3-16.4 h at pH 9.1 2.2 h at pH 9.9
Half-lives	
-Hydrolysis	27.7 days (pH 7, 25 ^{o}C) 2.73 days (pH 8, 25 ^{o}C) 0.54 days (pH 9, 25 ^{o}C)
-Aqueous photolysis half-life	7.95×10^{3} days (pH 7, 28 ^{o}C)
-Soil photolysis half-life	138 days (27 ^{o}C, pH 5.7, sandy-loam, 2.1% organic carbon, 21% moisture)
-Aerobic degradation half-life	22 days (25 ^{o}C, pH 5.7, sandy-loam, 2.1% organic carbon, 21% moisture)
-Anaerobic degradation half-life	30 days (25 ^{o}C, pH 5.7, sandy-loam, 2.1% organic carbon, 21% moisture)
-Field dissipation half-life	13 days (pH 7.3, sandy-loam, 38% organic carbon)

2.3 Environmental fate of carbofuran

The widespread use of carbofuran in liquid and solid forms to kill insects in agricultural areas provided many possible sources of carbofuran released to the environment. The carbofuran and pesticide contaminations in the environment resulted from direct use of pesticide in agricultural areas, runoff of from agricultural

areas, accident between transportation, as well as rinsate from cleaning pesticides containers and application equipments (Ferrell, 2003).

Carbofuran's low vapor pressure and low Henry's Law constant of 8.7×10^{-4} mm Hg at 25 °C, 3.9×10^{-9} atm m^3/mol, respectively, indicates that it has a low tendency to volatilize from water or moisture soils (Duel et al., 1979). Shibamoto et al., (1993) reported a concentration of carbofuran in air ranging from 0.03 to 0.66 μg/m^3 observed after a 44 hour sampling period following an application of 44% carbofuran solution. Carbofuran is not persistent in the environment at basic condition. The half-lives of carbofuran in water at 25 °C were 8.2 weeks, 1.0 weeks, and 13 years at pH of 6.0, 7.0, and 8.0, respectively (Spectrum laboratory Inc, 2003). Moreover, its high water solubility (320 mg/l at 25 °C) and low soil sorption coefficient ($K_{OC} = 22$, Table 1) resulted in a mobility in soil and in surface runoff. Accordingly carbofuran has the potential to contaminate groundwater, streams, and lakes. Fate of carbofuran in the environment is shown in Figure 2.2. It was detected (1 to 5 μg/L) in water table aquifers beneath sandy soils in New York and Wisconsin (Howard, 1991).

2.4 Health effect of carbofuran

Routes of carbofuran exposure to human are inhalation, ingestion, and dermal adsorption (Baron, 1991). Carbofuran is toxic to mammals. It has acute oral LD$_{50}$ in rats 8-14 mg/kg, and dogs 19 mg/kg. Acute dermal LD$_{50}$ in rats and rabbits are 500 and 2,550 mg/kg, respectively. The US EPA reported that the maximum contaminant level (MCL) for carbofuran is 40 μg/l (EPA, 2003). EPA (2003) reported that carbofuran has highly toxic to mice through cholinesterase inhibition (LD$_{50}$ = 2 mg/kg) and affects nervous system indicating that carbofuran is highly toxic to birds, fish and invertebrates (EPA, 2003). Kidd and James (1991) reported that carbofuran is toxic to rainbow trout with an LD$_{50}$ of 0.38 mg/l and 0.24 mg/l in bluegill sunfish. The red-shouldered hawks were reported to be found poisoned after eating preys from carbofuran-treated fields (Smith, 1992).
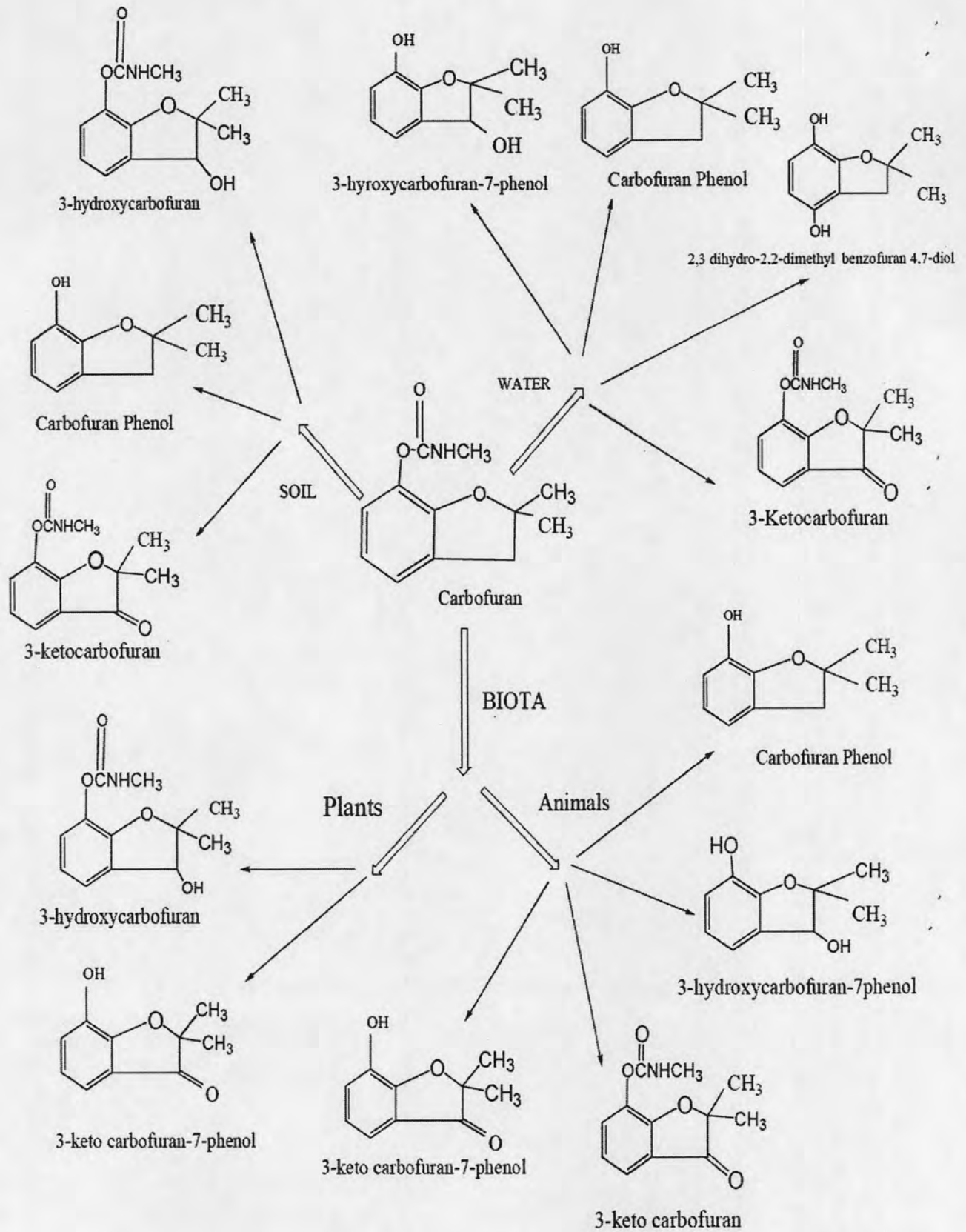
Figure 2.2 Environmental fate of carbofuran (Evert, 2002)

2.5 Degradation of carbofuran

2.5.1 Physical degradation of carbofuran

Physical degradation of carbofuran depends on temperature and moisture content of the soil (Qu et al., 1982). Volatilization influences a dissipation of carbofuran in water or moisture soils (Deuel et al., 1979). Photolysis of carbofuran is an important physical degradation of carbofuran. A study conducted by Benitez et al. (2002) found that the combination of ozone plus UV radiation enhanced the degradation rate of carbofuran. Huston et al. (1999) reported that carbofuran can be degraded by photo-fenton reaction in which 99.9% of carbofuran was lost in 30 minutes. Photometabolites include 2,3 dihydro-2,2-dimethyl benzofuran-4,7-diol and 3-ketocarbofuran (Raha and Das, 1990).

2.5.2 Chemical degradation of carbofuran

Hydrolysis of carbofuran is a major degradation pathway of carbofuran in water and sediment (Yu et al., 1974; Seiber et al., 1978). Degradation products include carbofuran phenol (Seiber et al., 1978) and 3-hydroxy-7-phenolcarbofuran (Chiron et al., 1996). Johnson and Lavy, (1995) reported that the dissipation of carbofuran in paddy water was rapid with a half-life of 3 days compared to 10 days in paddy soil. Hydrolysis of carbofuran is much more rapid in natural paddy water than deionized (DI) water at the half-lives 240 hours and 846 hours, respectively (Seiber et al., 1978). Seiber et al. (1978) found that the hydrolysis of carbofuran was more than 700 times faster at pH 10 than pH 7 with the half-lives of 1.2 hours and 846 hours, respectively.

2.5.3 Biological degradation of carbofuran

Bioremediation is an attractive approach for removal environmental contaminants. Bioremediation can be defined as any process that uses microorganisms or their enzymes to treat or remove the environmental contaminant until its concentration below detectable limits or less than the maximum concentration level (Vidali, 2001). Microorganisms are the key to a successful bioremediation. In general, bioremediation uses indigenous microorganisms from the contaminated area as the degrader. Advantages of bioremediation technique are perceived by public because it is a natural process and the residues from treatment are usually harmless products such as carbon dioxide, water, and cell biomass. Moreover, the contaminants can be

completely degraded. Bioremediation can be applicable over large areas and cost-effective.

Microorganisms capable of degrading the pesticide of interest can be isolated from the site with an application history as well as the pesticide contaminated site. For example, Plangklang (2004) isolated *Agrobacterium radiobactor* PCL3 from rhizosphere soil and found that it could enhance carbofuran degradation up to 4 times in comparison to non-inoculated bulk soil. *Pseudomonas cepacia* isolated from carbofuran contaminated rice field soils could degrade carbofuran to carbofuran phenol (Venkateswarlu et al., 1980). Ambrosoli et al. (1996) reported that *Pseudomonas fluorescens* isolated from carbofuran contaminated soil could degrade carbofuran to carbofuran phenol.

Evidents of biologically degradation of carbofuran were widely reported. A study on roles of fungi and bacteria in the mineralization of the pesticides atrazine, alachlor, malathion, and carbofuan in soil by Levanon (1993) indicated that the mineralization of carbofuran in soil was mainly due to bacterial activity. Venkateswaru et al. (1977) reported that microorganisms isolated from carbofuran contaminated soil involved in the degradation of carbofuran because of these isolated bacteria could degrade carbofuran up to 53% and 75% within 20 and 40 days, respectively, compared to 18% removal within 80 days in un-inoculated soil. Effects of inoculum size, microbial distribution, and soil nutrient amendments on the degradation of carbofuran in soil by bacteria strain C28 were studied by Duquenne et al. (1996). Results indicated that an increase in the inoculum size and the equal distribution of C28 applied to soil increased the effectiveness of carbofuran degradation.

Repeated application of carbofuran in soil enhanced the degradation of carbofuran. A study by Read (1983) showed that repeat applications of carbofuran to acid mineral soil reduced the half-life of carbofuran by two times. Venkateswaru et al. (1978) reported that the degradation of carbofuran in soil after application with carbofuran increased the degradation of carbofuran in paddy water and soil samples from both carbofuran treated and untreated rice field soil. Interestingly, applications of carbofuran led to almost 4-folds increased in the bacterial population from 25.6×10^{10}/g soil in control rhizosphere soil to 108×10^{10}/g soil in carbofuran treated rhizosphere soil. The metabolic degradation pathway of carbofuran were shown in the Figure 2.3.
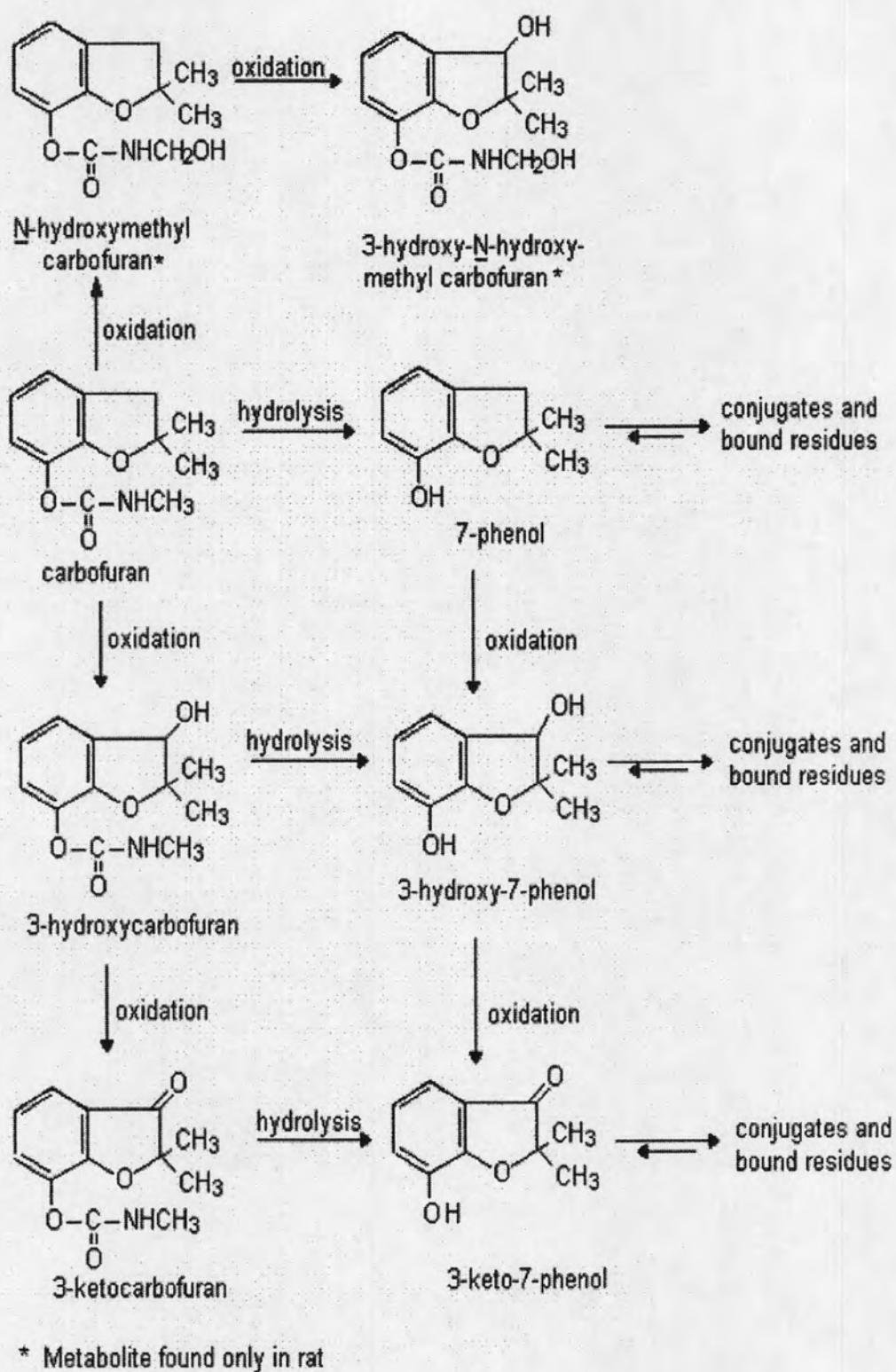
Figure 2.3 Metabolic degradation pathway of carbofuran (ICPS INCHEM)

2.6 Factors affecting carbofuran degradation

There are several factors affect the biodegradation rates of contaminants including pH, temperature, water content, oxygen concentration, microbial nutrients, the availability of contaminants to the microbial population, and water content of soil (Moorman, 2001; Vidali, 2001).

2.6.1 Physical factors

2.6.1.1 Microbial nutrients

Nutrients are substance in the environment that needed for microbial growth and microbial activity. Microorganisms need nutrients for their growth and enhancement of their activities as well as contaminants degradation activity. Two types of nutrient typically classified in microbiological term are macro-nutrient and micro-nutrient. Macronutrients are required in large amounts, while micronutrients (trace elements) are only required in small amounts for microorganisms. Major macronutrients are carbon and nitrogen. Most microorganisms need carbon and nitrogen sources to make new cell materials. The compositions of a microbial cell were shown in Table 2.2.

Table 2.2 Composition of a microbial cell (Vidali, 2001)

Element	Percentage	Element	Percentage
Carbon	50	Potassium	1
Nitrogen	14	Sodium	1
Oxygen	20	Calcium	0.5
Hydrogen	8	Magnesium	0.5
Phosphate	3	Chloride	0.5
Sulfur	1	Iron	0.2

Various types of materials could be used as C-source for microorganism such as glucose (Hollender et al., 2002), sucrose (Karpouzas et al., 1999) and agricultural waste such as rice straw (Venkateswaru et al., 1979), corn cobs (Barahona et al., 2004), compost (Moorman et al., 2001), cassava pulp (Moorman et al., 2001), and cattle manure (Moorman et al., 2001). Compost, NH_4NO_3, NH_4Cl, $NaNO_3$ were widely used as N-source to stimulate microbial growth and degradation activity (Chu

and Cohen 1998). A study on effect of inoculant density, formation, dispersion and soil nutrient amendment on the removal of carbofuran residues from contaminated soil by Doquenne et al. (1996) indicated that a glucose concentration of 1 g/kg soil reduced the lag phase of strain C28 at a low inoculums sizes while a high glucose concentration of 10 g/kg soil reduced the carbofuran degradation rate at all inoculums sizes. Venkateswaru and Sethunathan (1979) added rice straw into carbofuran contaminated soil to improve carbofuran degradation. Results indicated that at the end of 40 days, only 21% of carbofuran remained in amended soils compared to 33.76% remained in un-amended soil. Ferguson et al. (2003) found that an increase in nitrogen concentration resulted in an increase in hydrocarbon mineralization in which the highest hydrocarbon mineralization in diesel contaminated Antarctic soils was obtained at the range of nitrogen equal to 1,000-1,600 mg N /kg soil.

2.6.1.2 Acidity and alkalinity (pH)

Acidity or alkalinity of a matrix is expressed by its pH. Natural environments mostly have pH ranging between 5 to 9. Thus, there are various types of microorganisms in the environment depending on their suitable pH. Most bacteria require neutral pH for their growth. Carbofuran was found to be degraded faster in alkaline soil than in acidic soil. Hydrolysis was found to be a major pathway of carbofuran degradation in alkaline soil. A slow degradation in acidic and neutral soils was dominated by microbial and chemical mechanisms. Getzin (1973) reported a 10-folds difference in DT_{50} (time required for 50% breakdown of carbofuran) in soils at pH 4.3 and 7.8. Bailey et al. (1996) reported that the aqueous hydrolysis rate of carbofuran increased significantly with increasing pH. Carbofuran was removed 80-95% after spiking into the pH 3 soil for 1-6 hours at 25°C comparing to 65% removal of carbofuran from pH 10 soil. Seiber et al. (1978) found that the hydrolysis of carbofuran at pH 10 was 700 times faster than at pH 7. They also studied on hydrolysis of carbofuran from rice paddy water treated with granular formation of carobofuran. It was found that carbofuran was more rapidly degraded in alkaline condition than neutral condition in which the shortest half-lives of 13.9 hours was obtained at pH 8.7 compared with 240 hours at pH 7 in paddy water.

2.6.1.3 Temperature

Temperature affects the growth of microorganisms and metabolic activity. As the temperature decreases the biodegradation rate of contaminants also decreases and stops when the temperature reaches 0 °C (Sims et al., 1993). The suitable temperature for the microorganisms to degrade the contaminants depends on the individual species, in which the optimum temperature for physchrophilic organisms is 15 °C or lower, mesophilic organisms is in between 20 °C and 45 °C, and thermophilic microorganisms is above 45 °C (Pope and Matthews, 1993). The rate of biodegradation of carbofuran in soil is also powerfully affected by temperature. Yen et al. (1997) reported that half-lives of carbofuran in silty clay loam were 105 and 35 days at 15 and 35°C, respectively. Qu et al. (1982) reported that the physical degradation of carbofuran depends on temperature of the soil. The high temperature in tropical zone increased the rate of carbofuran degradation compared to in temperate zone by stimulating enzyme activity of microorganisms responsible for degradation of carbofuran in soil (Kaufman and Edward, 1983). Sahoo et al. (1993) suggested that the degradation of carbosulfan was more rapid in soil at 35°C than 25°C under flooded condition.

2.6.1.4 Water content

The water content of soil is important for the microbial growth and microbial activity, essentially for transportation of nutrients and the contaminants to the microbes and also carries out the toxic products from the cell (Paul and Clark, 1989). Bacteria normally need water activity (A_w) value of higher than 0.98. The effect of soil moisture content on sorption and biodegradation of carbofuran in soil was studied by Shelton and Perkin (1991). They found that the function of soil moisture content was not only activate the microbial population but also increased the biodegradation of carbofuran due to desorption of carbofuran from soil that easily to be degraded by carbofuran degrader compared to bounded carbofuran. A study done by Venkateswaru et al. (1977) found that carbofuran degradation in soil under flooded condition was more rapid than under non-flooded conditions indicated by an increase of degradation time from 40 days to 130 days.

2.6.1.5 Oxygen concentration

Normally microorganisms are separated by oxygen consumption patterns into 4 groups i.e., aerobic, anaerobic, facultative aerobic, and anaerobic microorganisms. The huge majority of microorganisms responsible for bioremediation of contaminants are aerobic microorganisms. These microorganisms require free oxygen for growing and degrading the contaminants (Sims et al., 1993). A study done by Venkateswaru et al. (1978) on degradation of carbofuran in rice field soils indicated that carbofuran was more rapidly degraded in aerobic soils comparing to anaerobic soil. They also reported that under anaerobic condition, its metabolites i.e., carbofuran phenol and 3-hydroxycarbofuran, accumulated in large quantities even at the end of 40 days indicating their resistance to degradation under anaerobic conditions. However, its accumulated metabolites were rapidly transformed to carbon dioxide when the anaerobic condition was turned to aerobic conditions.

2.6.1.6 Bioavailability

The bioavailability refers to an easiness of contaminants to be uptaken and metabolized by microorganisms. It involves the fate and transport of contaminants in surface soil and subsurface soil. A higher sorption of contaminants in soil decreased bioavailability of the contaminants (Alexander, 1994). Several factors affect the sorption of contaminants including pH, temperature, the type and quantity of clay minerals, type of solutes in the surrounding solution, the amount of organic matter in the soil (Alexander, 1994). A study done by Mora et al. (1996) on persistence and degradation of carbofuran in Spanish soil suspension indicated that the organic matter (OM) affected the caroburan degradation. As a level of OM in soil increased the sorption of carbofuran into the soil samples increased, resulting in a decrease of carbofuran bioavailability as well as degradation rate of carbofuran in the soil (Mora et al., 1996). Miles et al., (1981) revealed that carbofuran dissipation rate in sandy loam (organic matter 3.3%, pH7.3) was more rapid in comparison to in muck (organic matter 36%, pH 7.3) indicated by 77% carbofuran remained in sterile muck and 50% remained in sterile sandy loam after 8 weeks. This result also suggested that high OM in soil samples decreased the bioavailability of carbofuran.

2.7 Bioremediation of carbofuran

Bioremediation can be defined as any process that uses microorganisms or their enzymes to remove the contaminants until its concentration is below detectable limits or less than the maximum concentration level (Vidali, 2001). Microorganisms are the key to a successful bioremediation. The bioremediation technique includes natural attenuation, bioaugmentation, phytoremediation, bioventing, landfarming, composting and biostimulation (Skipper, 1999). The major bioremediation techniques are explained as follows:

2.7.1 Bioaugmentation

Bioaugmentation is the addition of microorganisms that can bio-transform or biodegrade a particular contaminant (Gentry, 2004) into the contaminated matrices in order to increase the degradation rate. This approach corresponds to an increase in the metabolic capabilities of the microorganisms present in the soil and in the genetic diversity of that site (Dejonghe et al., 2001). This technique has been applied in many areas including forestry and agricultural areas (Jasper, 1994) and wastewater (Rittman and Whiteman, 1994). For example, a biostimulation and bioaugmentation enhanced polycyclic aromatic hydrocarbons (PAH) degradation in contaminated soil resulting in 100% reduction of all PAHs tested (Atagana, 2006). Plangklang (2004) found that an inoculation of the isolate *Agrobacterium radiobacter* PCL3 into bulk soil could enhance carbofuran degradation up to 4 times comparing to in non-inoculated soil. However, inoculation of the isolate PCL3 did not improve carbofuran degradation in fuzzy flatsedge rhizosphere soil in which the half-lives of carbofuran in inoculated soils were not markedly different from in non-inoculated rhizosphere soil. This might be resulted from the number of carbofuran degrader in rhizosphere soil is higher than bulk soil. Ruberto et al. (2003) reported that bioaugmentation with the *Acinetobacter sp.* B-2-2 strain increased the hydrocarbon bioremediation efficiency in which 75% of the hydrocarbon was removed. They reported that autochthonous bacterial flora from Antarctic soils is able to degrade an important fraction of the gas-oil and suggested that bioaugmentation represents a valuable alternative tool to improve bioremediation. Struthers et al. (1998) reported that the addition of *Agrobacterium radiabacter* J14a at 10^5 cells/g into soil with a low indigenous population of atrazine degraders treated with 50 and 200 mg of atrazine/g soil resulted in two to five times higher atrazine mineralization than in the non-inoculated soil.

2.7.2 Phytoremediation

Phytoremediation is the use of plants to remediate the contaminants in contaminated soil, sludge, sediment, groundwater, surface water, and wastewater. This technique utilizes a variety of plant biological processes and the physical characteristics of plants to aid *in situ* remediation. Phytoremediation has also been called green remediation, botano-remediation, agroremediation, and vegetative remediation (Vidali, 2001). The advantages of phytoremediation are that soil removal is unnecessary, the cost and degree of disruption to site activities may be less than with other remedial technologies, and environmental friendly to the public. Disadvantages of phytoremediation include the necessity for long-term maintenance of the vegetation which may take several years to remediate, restricted to sites with shallow contamination within rooting zone, and possible effect on the food chain due to a consumption of contaminated plant tissue by animals or humans. Phytoremediation had been successfully used to clean up DDT (Gao et al., 2000) and simazine (Wilson et al., 2000). Dushenkov et al. (1997) used sunflowers to remove radionuclides in a small pond near the Chernobyl reactor in Ukraine. Results showed that sunflowers grown for 4-8 weeks in a pond could remove ^{137}Cs and ^{90}Sr from the pond in range of 98.5% to 99.5%. Blaylock et al. (1997) used Indian mustard to demonstrate the capacity of plants to accumulate high concentrations of lead (Pb) when grown in a Pb-contaminated soil. Accumulation of Pb in shoots of *Brassica juncea* could be enhanced through the application of synthetic chelates in the soil and facilitated high biomass accumulation as well as Pb uptake. Atrazine was uptaken, hydrolyzed, and dealkylated to less toxic metabolites by poplar trees (Burken and Schnoor, 1997). The transformation of atrazine occurred in roots, stems and leaves. These findings suggested that hybrid poplar trees had the potential for phytoremediation of sites contaminated with atrazine. *Thlaspi caerulescens* could tolerate Zn and Cd concentrations up to 18,455 mg/kg Zn and 1,020 mg/kg Cd dry shoots (Brown et al., 1994). Ma et al. (2001) found that fern (*Pteris vittata*) could tolerate arsenic concentration of as high as 1,500 µg/g in soil. Wilson et al. (2000) used common cattail (*Typha latifolia*) to uptake metalaxyl and simazine from contaminated water. After 7 days, metalaxyl and simazine activity in solution was reduced by 34 and 65%, respectively, and these activities were detected predominantly in the leaves.

2.7.3 Biostimulation

Biostimulation is an addition of nutrients, air or oxygen into the contaminated systems in order to stimulate the intrinsic microbial population to degrade the contaminants of concern (Vidali, 2001). Advantages of this method are that it is simple to maintain, applicable over large areas, cost-effective, and leads to the complete destruction of the contaminant (Vidali, 2001). Previous research indicated that biostimulation by adding nutrients from agricultural residues is an effective tool to remediate various types of contaminant. For example, a removal of petroleum hydrocarbon in contaminated soil was increased 1.18 times compared to non-stimulated soil at day 15 (Perez et al., 2004). Diesel removal was increased to 67% compared with non-stimulated diesel-contaminated soils when corn and crop residues were added into diesel contaminated soil at C/N ratio of 100:10 (Barahona et al., 2004). Phenanthrene biodegradation was accelerated when zinc was added into the contaminated soil at a concentration of 140 mg/kg (Wong et al., 2005). Selenate in agricultural drainage water was removed when rice straw was added (Zhang and Frankenberger, 2002). Additions of farm manure, straw and nitrogen fertilizer stimulated microbial activity and accelerated atrazine degradation (Hance, 1973). The combination of biostimulation and bioaugmentation increase the biodegradation of cis-DCE and trans-DCE at the range of 14% and 18% degradation rate, respectively, (Olaniran et al., 2005). The additional of rice straw accelerated the hydrolysis of carbofuran to carbofuran-phenol in anaerobic flooded soil. Atagana (2003) reported that additional nutrients in the form of indigenous microbial biosupplement and sewage sludge were effective in creosote removal by increasing the total heterotrophic and creosote degrading microorganisms and increasing the reductions rate of creosote to 88.7 and 86.1%, respectively.

2.7.4 Biostimulation in aged soil

Aged soil is the soils that accumulate or contact with the contaminants more than two months (Moorman et al., 2001). These process enhance the sorption of the contaminants to the soil matrix. The result from the aged process made the contaminants become increasingly resistant with the time to extraction and difficult to remove (Moorman et al., 2001). Previous research indicated that biostimulation by adding nutrients from agricultural residues is an effective tool to remediate the

contaminants from aged soil. For example, the additional of carbon, nitrogen, and phosphorus at the ratio of 100:1.25:1 into an aged soil contaminated with 5.4% of total petroleum hydrocarbon (THPs) resulted in the highest biodegradation efficiency (7.4%) and also the highest TPH removal (14.4%) comparing to natural attenuation of the aged soil (11.5%) (Trindade et al., 2005). Moorman et al. (2001) reported that an addition of amendments such as compost, corn stalks, corn fermentation by product, peat, manure, and sawdust at the rates of 0.5% and 5% into the aged soil (aged for 63 days) enhanced atrazine, metholachlor and trifluralin degradation in the range of 30%, 33%, 44% degradation rate, respectively.

2.8 Kinetic characterizations

Kinetic characterization is a study on the microbial response to the environmental variables such as substrate concentration (Montiel et al., 2006), temperature and dissolved oxygen concentration (Shuler and Kargi, 1992). This study provides the helpful information on the substrate degradation rate and microbial growth rate for the development of using biostimulation of pesticide degradation by agricultural residues and inorganic nutrient as a tool in bioremediation of pesticides in agricultural soil.

The study of kinetic characterization in batch and continuous culture of 2,4 dichlorophenoxyacetic acid (2,4-D) degradation bacteria isolated form soil sample contaminated with 2,4-D indicated that at an early phase of the culture with 2,4-D concentration of 200 mg/L or higher, the instantaneous cell growth yield was lower than the global cell growth yield because 2,4-D was over consumed resulting in an improportional of substrate to the cell mass produced. In continuous culture, 2,4-D removal efficiency was higher than 97% and global cell growth yields were lesser than those obtained in batch (Montiel et al., 2006). The study of Monteiro et al. (2000) using pure culture, *Pseudomonas putida* and mixed cultures for phenol degradation at the varying phenol concentration showed that higher phenol concentration yielded higher biomass concentration. The maximum specific growth rate (μ_m = 0.436 1/h) of *P.putida* is higher than that observed in mixed cultures (μ_m = 0.131-0.418 1/h) led to higher efficiencies of phenol degradation when using *P. putida* to degrade phenol. The variation of salinity at the range of 0, 2, and 3.5% affected the kinetic characterization of toluene-oxidizing cultures, THTO4. At the initial toluene concentration of 30 mg/L, the maximum specific rate of TCE degradation declined from 2.28 to 1.45 days^{-1} when the salinity increased from 0 to

3.5% (Lee et al., 2006). Chen et al. (2006) reported that a C/N ratio of 12:1 (OPEOn (octylphenol polyethoxylates):$(NH_4)_2SO_4$) and C/N ratio of 22:1 (OPEOn:KNO_3) were optimal for specific growth rate of *Pseudomonas nitroreducens* TX1 and OPEOn degradation rate, respectively. Moreover, the kinetic analysis showed that the growth of *P. nitroreducens* TX1 was inhibited when the OPEOn concentration was higher than 18,000 mg/L. This helpful information was used to develop the technique for using of *P. nitroreducens* TX1 as a tool in bioremediation of OPEOn contaminated site.