CHAPTER 2

THEORIES

2.1 Benzene

2.1.1 Physical and chemical properties of benzene

Benzene, which has been normally classified as one of volatile organic compounds (VOCs) because of high vapours pressure, small molecule, and evaporation at room temperature, has ring conformation with resonance structure, so this compound could be stable in the environment. The compound contains 6 C and 6 H atoms (C_6H_6) that had been shown in Figure 2.1.

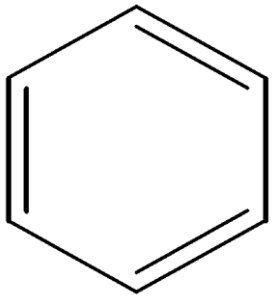

Figure 2.1 Benzene molecules.

Benzene is a hydrophobic compound that could be solubilized easily in oils, hexane, ethanol, and chloroform etc. This compound had been known commonly as explosive vapours and flammable liquid. Physical and chemical properties are presented in Table 2.1 (Sciencelab, 2001). The solution of benzene appears in colourless liquid with sweet odour. The compound could evaporate 100% at room temperature because of high vapours density. Benzene could be solubilized in n-octanol more than in water around 100 times as showing in log K_{ow} that equal to 2.13.

Table 2.1 Physical and chemical properties of benzene (Sciencelab, 2001).

Physical and chemical properties	
Physical state and appearance	Clear liquid
Color	Colorless
Odour	Sweet, solvent-like
Odor threshold	1.5-5 ppm
Vapours density at 0^{o}C	2.8
Boiling point	80^{o}C
Melting point	5.5^{o}C
Solubility	0.1-0.3% in water
Specific gravity	0.88 at 15 ^{o}C
log K_{ow} (octanol/water coefficient)	2.13
Percent volatile	100
Flammability classification	Flammable liquid

In general, benzene could be extracted from petroleum industries (U. S. Environment Agency, 2009). Oil distillation could classify petroleum product such as fuel oil, wax, lubricants, diesel oil, kerosene, gasoline, and petroleum gases. Benzene that contains 6 carbon atoms could be extracted from the group of gasoline, which identify normally to the group of 5-12 carbon atoms (Fig 2.2).

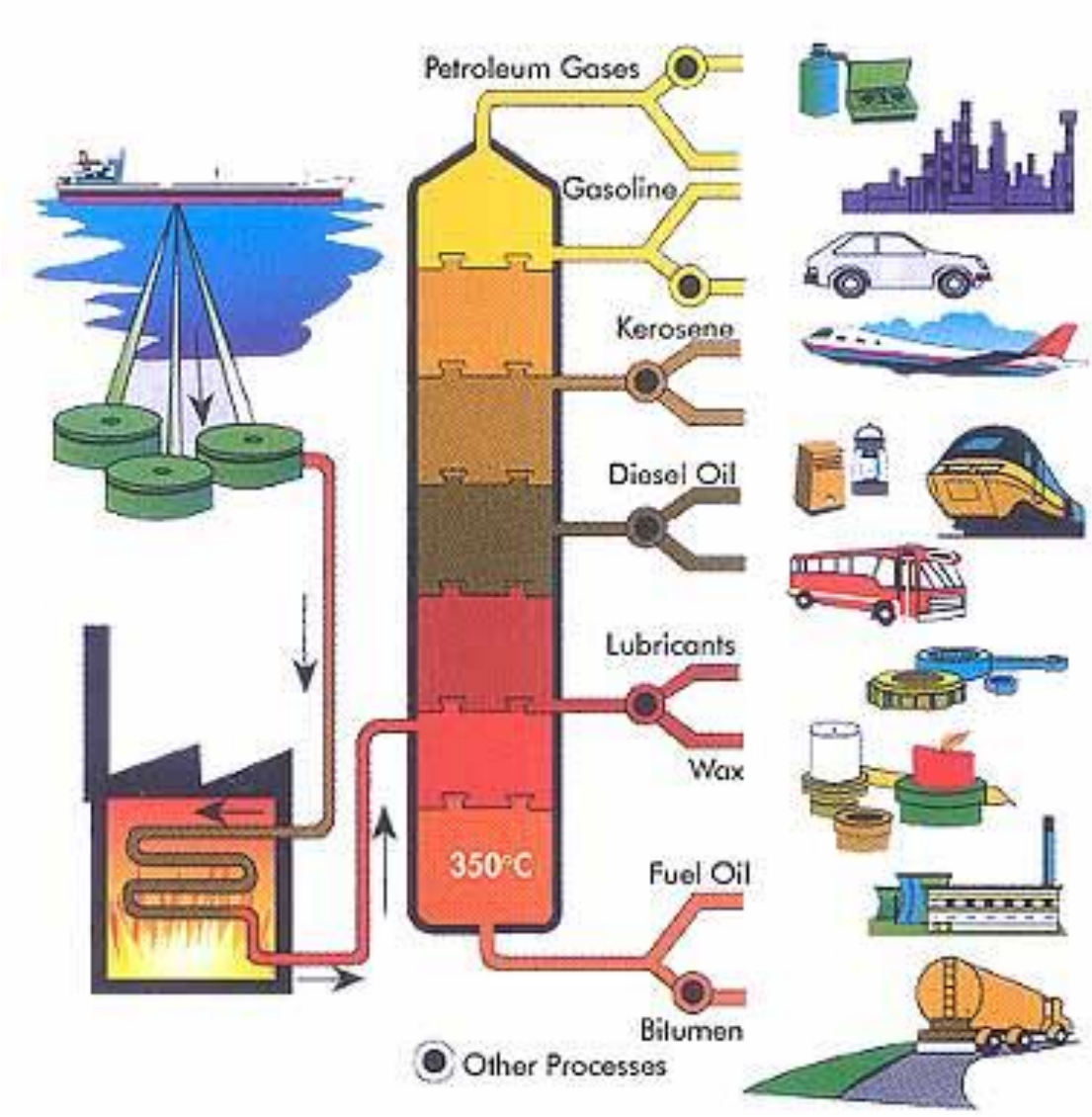

Figure 2.2 Petroleum distillations (Sapref, ND).

Benzene has been widely used as additives, intermediate and/or solvent in several industries for example coating compounds, chemical synthesis, solvent in laboratory, etc. (Atkinson, 2007), so benzene might contaminate in the environment and food chain and create disease and/or symptoms in human.

2.1.2 Source of benzene

There are several source of benzene, for example combustion, petroleum industries, and vehicle. From many survey studies, the results suggested that main source of benzene depend on the structure and composition of the city. For example, previous observation in Thailand showed that vehicle was the most emission source of benzene about 78% of

total benzene emission. The result is shown in Table 2.2 (Pollution control department, 2007).

Table 2.2 Percentage of benzene emission sources in Thailand (Pollution control department, 2007).

Sources	Emission (Ton/Year)	%
Vehicle	1588	77.5
(Gas/uncontrol)	1343	65.6
(Diesel)	181	8.8
(Gas/control)	64	3.1
Industries combustion sources	122	6
Gasoline storage and transportation	323	15.8
Other sources	15	0.7

Although most of benzene had been emitted from vehicle in Thailand, all sources of benzene emission could cause for ambient and indoor air quality problem (Wolverton, 1996). Moreover, sources of benzene emission in Canada, the survey results suggested that more of benzene had been emitted from transportation (Fig 2.3).

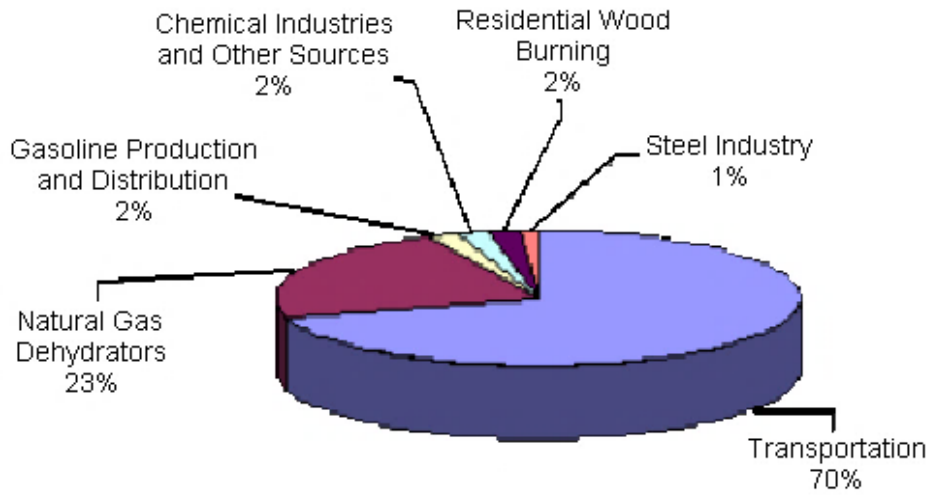

Figure 2.3 Percentage of benzene emission sources in Canada (Canadian Environmental Protection Act, 1999).

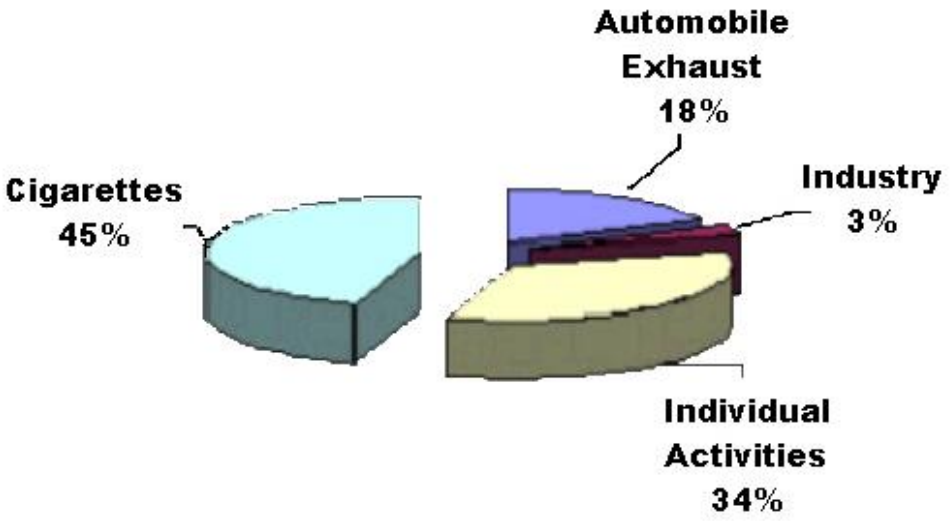

Figure 2.4 Percentage of human benzene exposure sources in Canada (Canadian Environmental Protection Act, 1999).

However, the most important source of benzene exposure in human is not from vehicles, but cigarettes had been reported as the highest percentage of source of benzene uptake by human, which is 45% of total benzene exposure. Automobile exhaust had been reported as a source of benzene exposure in human only 18% (Fig 2.4). Not only direct emission source but also indirect emissions source such as consumer product in home or office could be also found (Table 2.3). Poor ventilation is commonly known as properties of indoor air. Accumulation of benzene in poor ventilation space had been considered to be important problem (U. S. Environment Protection Agency, 2007).

Table 2.3 VOCs emission from each consumer product (U. S. Environment Protection Agency, 2007).

	Emission					
Sources	**Formaldehyde**	**Xylene/toluene**	**Benzene**	**Ammonia**	**Alcohols**	**Acetone**
Adhesive	•	•	•			
Bio effluents		•		•	•	•
Carpeting					•	
Caulking compounds	•	•	•		•	
Ceiling tiles	•	•	•		•	
Cleaning product				•		
Cosmetics					•	•
Draperies	•					
Electro photographic printers		•	•	•		
Fabrics	•					
Facial tissue	•					
Floor covering	•	•	•		•	
Grocery bags	•					
Nail polish remover					•	
Office correction fluid					•	
Paints	•	•	•		•	
Paper towels	•					
Particleboard	•	•	•		•	
Photocopies		•	•	•		
Pre-printed paper forms						•
Stains and carnishes	•	•	•		•	
Upholstery	•					
Wall covering		•	•		•	

2.1.3 Benzene toxicology

The use of benzene in manufacturing and vehicle could create benzene contamination in ambient air, emission air, indoor air, and consumer product. Actually, there are 4 benzene uptake pathways in human such as skin absorption, skin adsorption, gastrointestinal tract, and respiratory system (Fig 2.5). Gastrointestinal tract and respiratory system had been known as the main benzene pathways (Sciencelab, 2001). In benzene uptake by gastrointestinal tract, because benzene molecule is normally stable in environment and shows hydrophobic properties, the molecule could be easily accumulated in non-polar part of living cell. The eating of benzene contaminated food, the molecule could be diffused rapidly in to the human body. For the respiratory system, gaseous benzene could be breathed into human lung, and the molecule could be passively absorbed (Rappaport *et al*., 2012). The emission of benzene from the consumer product appear and accumulate in indoor space, and long term benzene exposure in poor ventilation space, many diseases could be induced that cause people who mostly spend in indoor. The liver, kidneys and central nervous system damage could be generated by benzene (U. S. Environment Agency, 2007). From several studies had confirmed that benzene could be also human carcinogenic molecule, so many organization had classified mostly benzene in dangerous compound group. The International Agency for Research on Cancer (IARC) had classified benzene in group1 that prefer to human carcinogenic. Benzene is grouped in group A by USNTP of USEPA as well that main the agent could be carcinogenic to humans with enough epidemiological evidences. ACGIH and JSOH also classified benzene in group 1A and 1, respectively those are human carcinogenic group (Pollution control department, 2007). For example, one of benzene induced cancer, which is white blood cell cancer in the type of Acute Myelogenous Leukemia (AML), could be induced surely by long-term benzene exposure (Schnatter *et al.*, 2005). Not only chronic diseases but also acute diseases could be induced from high concentration of benzene exposure. Nose discomfort, headache, allergic skin reaction, emesis, dizziness, etc. are the symptoms that happen by short term high benzene concentration exposure (U. S. Environment Agency, 2007). For the suggestion, mouse 50% lethal dose (LD_{50}) and Immediately Dangerous to Life or Health (IDLH) are about 930 mg kg^{-1} and 500 ppm, respectively (Sciencelab, 2001). Not only human toxicology but also environment toxicology was proposed. Benzene is strong aromatic ring conformation, so it is very stable compound. The environmental accumulation of benzene is also happened, and it can affect to animal, fish and some microorganisms (Sciencelab, 2001).

2.1.4 Standard of benzene

Nowadays, several organizations and countries have realized benzene contamination problem in environment, so benzene concentrations have been tried to regulate by many standards. In difference areas such as workspace and ambient, difference values of benzene concentration has been guided however emission standard of benzene from industries are not found. The guideline of benzene in ambient air, workspace, and also water are shown in Tables 2.4 -2.6, respectively.

Table 2.4 International available of ambient benzene (Pollution Control Department, 2007).

Organization		Benzene (µg m^{-3})
WHO Guideline for Air Quality (2000)		5~20
New Zealand		10 (annual)
Canada		-
Japan		3 (annual)
California		-
Rhode Island Air Toxic Guideline	1 hour	200
	24 hour	10
	Annual	0.1
Arizona	1 hour	630
	24 hour	51
	Annual	0.14

Table 2.5 Internationally available of work space benzene concentration (Pollution Control Department, 2007).

Organization	Exposure time	Benzene (ppm)
OSHA (Work space)	8 h/day	1
	15 min	5

Table 2.6 Internationally available of water contamination with benzene (Pollution Control Department, 2007).

Organization	mg L^{-1} (Water)
USEPA	0.005

2.1.5 Thailand situation

Because benzene has been applied to be a additive and/or a solvent in several industries, Thailand manufacturing also use this compound. From demand-supply of benzene data in 2001-2005, the tendency of benzene production had increased from 502,000 tons in 2002 to 742,000 tons in 2005 (Pollution Control Department, 2007). The quantity of imported benzene is ranked as top 10 in CMR (C: Carcinogen, M: Mutagenic, R: Reprotoxic) group in 2002 and 2005. Benzene contamination from several sources can create environmental problem, so pollution control department, Thailand regulate benzene concentration in ambient air when exposure to 24 h accumulation is about 7.8 µg m^{-3} and 1.7 µg m^{-3} for 1 year exposure (Pollution Control Department, 2007). Demand-supply of benzene in 2001-2005 is shown in Figure 2.6.

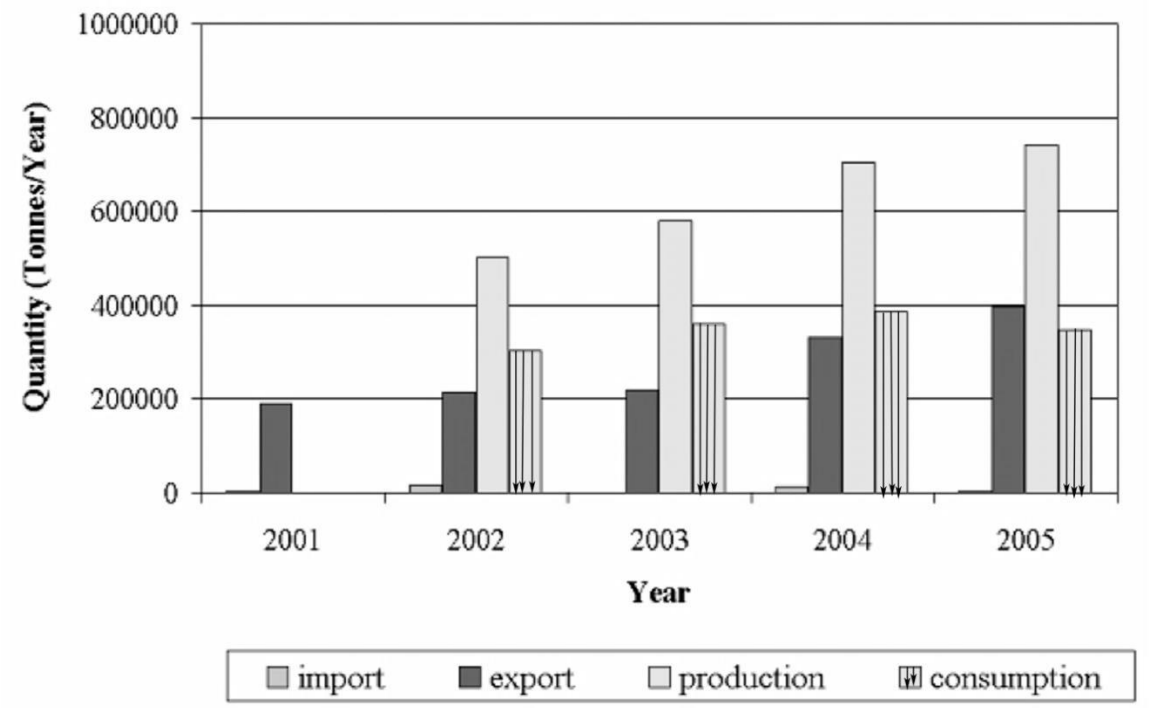

Figure 2.5 Demand-supply of benzene in 2001-2005 (Pollution Control Department, 2007).

The accumulation of benzene had been considered as important problem because although pollution control department of Thailand has benzene standard guide line, higher benzene concentration than standard had been found in many locations around Thailand especially in Bangkok and industrial cities (Fig 2.7).

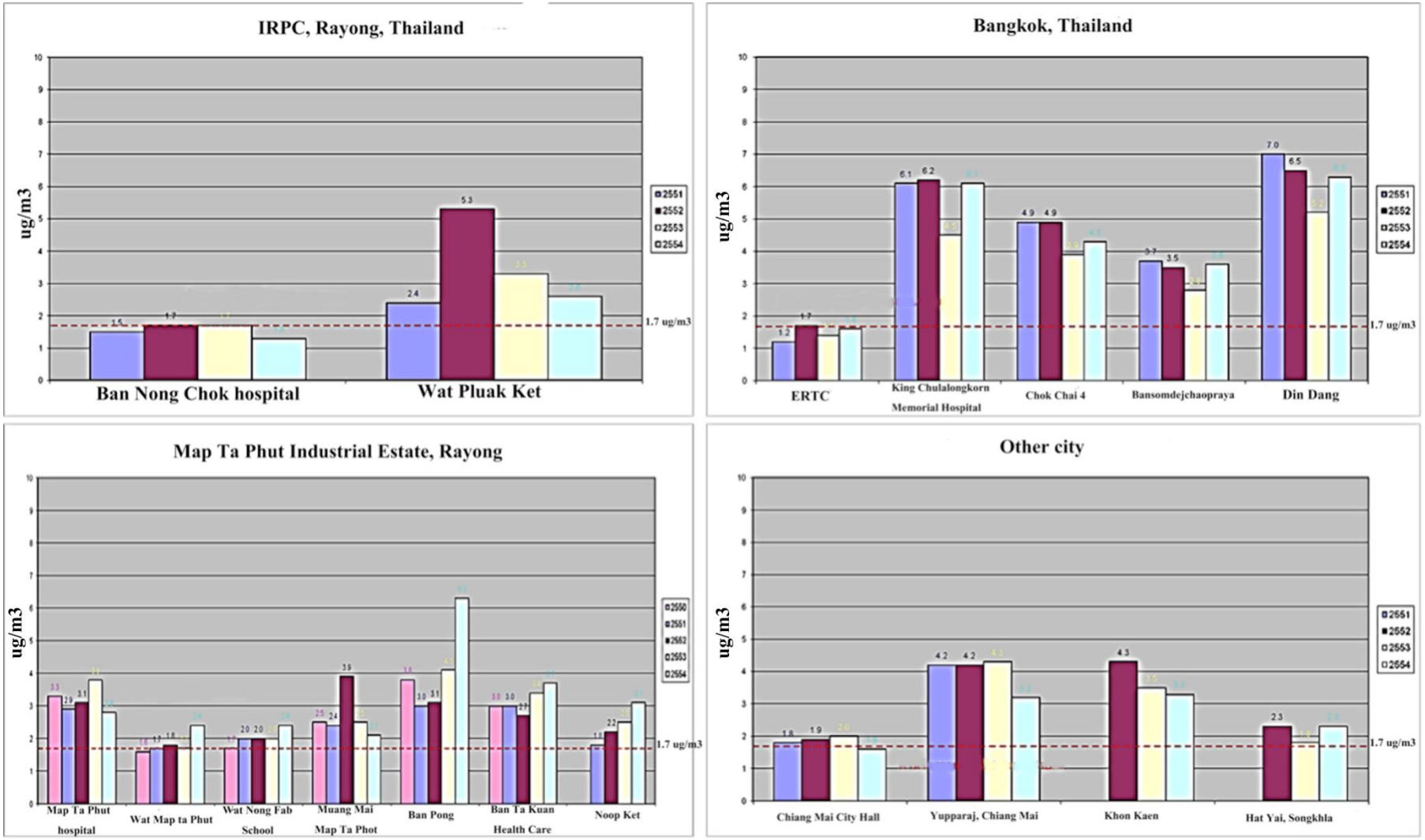

Figure 2.6 Benzene concentration in ambient air around Thailand, 2008-2011 (Pollution Control Department, 2007).

2.2 Adsorption and absorption

Adsorption and absorption process have been generally applied to treat air pollution in several industries. These processes could reduce accumulated energy of pollutant molecule or create bond to pause molecule mobilization. The target molecule could be adhered on the surface of absorbent or adsorbent. For the adsorption, pollutant could be fixed onto a solid matrix typically a surface or a porous material (Michel, 2008). Accumulated energy of pollutant would be decreased when the molecule had been fixed onto the adsorbent. Moreover, the thermodynamic equilibrium is di-variant. In case of absorption, physical or chemical phenomenon or a process in atoms, molecules, or ions enter some bulk phase that might be liquid, solid, and gas. This process, mobilization of pollutant might be slowed down and adhere in to bulk phase of absorbent, which also include the penetration to intra-matrix of absorbent. Also chemical reaction, absorption process might create chemical bond to combined pollutant molecule (McMurry, 2003). The difference between these processes is that in adsorption, pollutant would be adhere only on the surface of matrix of adsorbent, but absorption, the process include also penetration of pollutant to intra-matrix of absorbent (Fig 2.8).

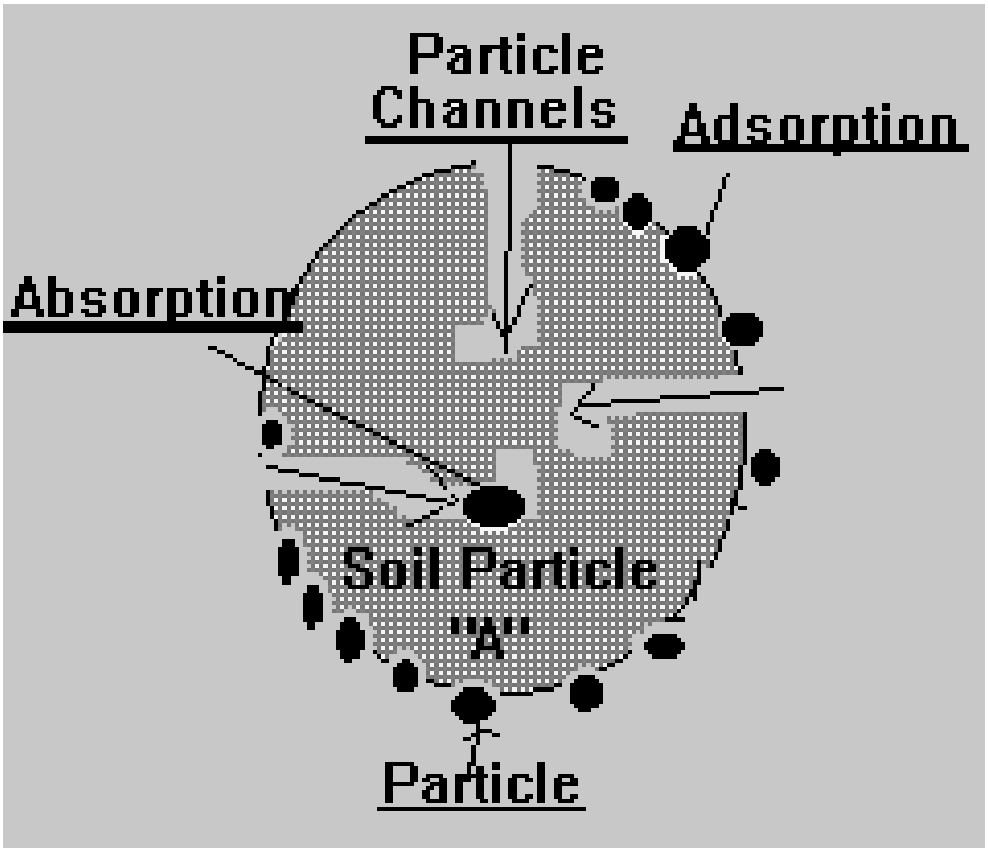

Figure 2.7 Adsorption and absorption process (McMurry, 2003).

Activated carbon has been sometimes called activated charcoal or activated coal. The structure is a form of carbon that has been processed to make extreme porous and create a very large surface area available for adsorption or chemical reactions. Chemical activation and steam activation techniques are normally used by operations of commercial production. The activation of peat and wood based raw materials; Chemical activation is generally used at temperature of 500 – 800 ^{o}C with mixed phosphoric acid (H_3PO_4) or zinc chloride ($ZnCl_2$) into a paste. Chemical activation process could

generate macro-porous activated carbons for the large molecules absorption. In steam activation, Coal and coconut shell are used as raw material. Temperatures of 800 – 1100 ^{o}C in the presence of steam have been normally applied to activate the absorbent. Micro-porous and meso-porous are created in this process. Type of raw material available, desired physical form of the activated carbon, characteristics required for the intended application are the factor for activation technique selection. Activated carbon could be classified following a highly internal pore structure and surface area. The different characteristics depend on the raw material and activation techniques (Caron Link, 2011). The International Union of Pure and Applied Chemistry (IUPAC) define the pore size distribution as:

The macro-pores could be used as the entrance to the activated carbon and large molecule absorption. The meso-pores are important structure for transportation, and finally, the micro-pores could be used to absorb small molecular weight compound. In air pollution treatment, high pollutant removal efficiency and fast absorption was found in micro-pores activated carbon especially benzene, so activated carbon with high micro has been widely used to treat contaminated pollutants in industries. However the adsorption efficiency of activated charcoal depend on concentration of pollution, temperature of vapours stream, relative humidity of vapours stream, flow rates and operating frequency, process operating pressure, pressure drop in system. The activated carbon capacity for chemical fumes, gas, and odors had been classified by the numbers 1 – 4. The description of numbers 1 – 4 was shown in Table 2.7. Some of the contaminated pollutants had been listed and classified following the number of activated carbon capacity in Table 2.8.

Table 2.7 Capacity index numbers and descriptions (Caron Link, 2011).

Index No.	Capacity	Notes
4	High, one pound of carbon can adsorb approx., 20% to 50% of its own weight	Includes most odor causing substances
3	Satisfactory, one pound of carbon can adsorb approx., 10% to 20% of its own weight	Capacity is not high as 4
2	Not highly adsorbed	Might be taken up sufficiently under particular conditions of operation
1	Low	Activated carbon cannot be used satisfactorily to remove chemical gas under ordinary condition

Table 2.8 Chemical absorption index of activated charcoal / activated carbon (Caron Link, 2011).

Chemical	Index number	Chemical	Index number	Chemical	Index number	Chemical	Index number	Chemical	Index number
Acetic acid	4	Carbon disulfide	4	Ethyl chloride	3	Methyl acrylic	3	Nonane	4
Acetic anhydride	4	Carbon dioxide	1	Ethyl ether	3	Methyl alcohol	3	Octalene	4
Acetone	3	Carbon monoxide	1	Ethyl formate	4	Methyl bromide	4	Octane	4
Acetylene	1	Carbon tetrachloride	4	Ethyl mercaptan	1	Methyl butyl ketone	4	Ozone	4
Acrolein	3	Chorine	3	Ethyl silicate	1	Methyl chloride	4	Pentane	3
Acrylic acid	4	Chlorobenzene	4	Ethylene	4	Methyl chloroform	3	Pentanone	4
Acrylonitrile	4	Chlorobutadiene	4	Ethyl chlorhydrin	4	Methyl ether	4	Pentylene	3
Alcoholic beverage	4	Chloroform	4	Ethyl dichloride	3	Methyl ether ketone	3	Pentyne	3
Amines	2	Chloronitropropane	4	Ethyl oxide	4	Methyl formate	4	Perchloroethylene	4
Ammonia	2	Chloropicrin	4	Flurotrichloromethane	3	Methyl iso butyl ketone	4	Phenol	4
Amyl acetate	4	Crotonaldehyde	4	Formaldehyde	2	Methyl mercaptan	4	Propane	2
Amyl alcohol	4	Dichloroethylene	4	Formic acid	3	Methylcyclohexane	4	Propionaldehyde	3
Amyl ether	4	Dichloroethyl ether	4	Heptane	4	Methylcyclohexanol	4	Propionic acid	4
Aniline	4	Dichloropropane	4	Heptylene	4	Methylcyclohexanone	4	Propyl chloride	4
Benzene	4	Dichloromonofluomethane	4	Hexane	3	Methyl oxide	1	Propyl ether	4
Borane	3	Diethylamine	4	Hexylene	3	Methylene chloride	4	Propyl mercaptan	2
Bromine	4	Doethyl ketone	4	Hexyne	3	Methylmethacrylate	4	Propyne	2
Butadiene	3	Dimethylaniline	4	Hydrogen	1	Monochlorobenzene	4	Radiation product	2
Butane	2	dimethylsulfate	4	Hydrogen bromide	2	Monoflurotrichloromrthane	4	Sulfur dioxide	3
Butanone	4	Dioxane	4	Hydrogen chloride	2	Naptha	4	Sulfur trioxide	4
Butyl acetate	4	Dipropyl ketone	3	Hydrogen cyanide	3	Napthalene	4	Tetrachlorethylene	4
Butyl chloride	4	Ethane	4	Hydrogen sulfide	4	Nicotine	4	Toluene	4
Butyl ether	4	Ether	4	Iodine	4	Nitric acid	3	Toluidine	4
Butylene	2	Ethyl acetate	4	Iodioform	4	Nitro benzene	4	Trichloroethylene	4
Butyne	2	Ethyl acrylic	3	Isophorone	3	Nitroethane	4	Trichloroethane	4
Butyraldehyde	3	Ethyl alcohol	4	Isoprene	4	Nitrogen oxide	2	Uric acid	4
Butyric acid	4	Ethylamine	4	Isopropyl alcohol	1	Nitroglycerine	4	Valeric acid	4
Carbolic acid	4	Ethyl benzene	3	Methane	3	Nitromethane	4	Valericaldehyde	4

2.3 Phytoremediation

The use of plants to clean-up the environment, which is called phytoremediation, is one of an effective treatment. In general, phytoremediation had been used for metal cleaning in underground water, surface water, and soil. In addition, VOCs removals by plants had been studied. Pollution degradation, stabilization, or evaporation in soil, sediment, ground water, and also atmosphere could be successes by this method. Low cost, easy to maintain, and no secondary pollutants are the advantages of this method. However; this method requires long time, large lands, and the efficiency relate with spices of the plants. One of important limitation of this method is that plant could not survive under high concentration of toxic compound. Phytoremediation could be classified in many types such as phytoextraction, phytodegradation, phytostabilization, rhizofiltration, phytovolatilization, rhizodegradation. The detail of each method is as follows.

2.3.1 Phytoextraction

Phytoextraction or phytoaccumulation, which is a type of phytoremediation, usually could be used to control metal contaminating land. This method, plants could uptake toxic metal by roots and accumulate the toxic molecules in a part of plant which upon to plant species and metal type. The ion of metal that could solubility easily in water would be transport rapidly to the shoots and leaves of plant. However most of experiment showed that plants accumulated commonly metal in the roots. In this type of phytoremediation, only direct accumulation of metal has been considered.

2.3.2 Phytostabilization

Phytostabilization is a method for inhibition of pollutant distribution in soil and water. In general, plants uptake toxic substances for example lead, cadmium, arsenic, etc. by roots. Toxic substance could be transformed and conjugated to biomolecule, and this process makes normally high stability form of toxic compounds. High stability compounds might accumulate in plant cell organelle or precipitate in the environment, so this method could control distribution of contaminants in the environment. Moreover, phytostabilization could be also processed in both inorganic and organic compounds.

2.3.3 Rhizofiltration

Most of soil and water pollutant could be uptake and reduced rate of pollution distribution by plant roots, which is called rhizofiltration. This process is not only filtrate but also include contaminants absorption. Pollution distribution rate could be reduced by this type of phytoremediation, and some pollution could be uptake and accumulated by plant roots. However filtration rate relates with species of plant and toxic substances.

2.3.4 Phytovolatilization

Some of toxic molecules could be transported from soil and water to atmosphere by plants, which might be called phytovolatilization. The compounds would be uptaken by plant roots and transported to plant shoots and leaves. Conformation of some toxic compounds would be changed to easy evaporated form and emitted to atmosphere through stomata of plant leaves.

2.3.5 Rhizodegradation

Many names could be presented the similar meaning with rhizodegradation such as phytostimulation, rhizosphere biodegradation or enhanced rhizosphere rhizodegradation. This process, the relation of plant and microorganism such as yeast, fungi, and bacteria has been presented. Some essential microorganisms that grow around plant roots could uptake and degrade toxic substances for growing. Some organic compounds could be used as carbon and energy sources for microorganism. Not only pollutants degradation by microorganism but also plant growth promoting could be considered as a benefit of essential microorganism.

2.3.6 Phytodegradation

Phytodegradation, which could be called phytotransformation, is a method for toxic substances degradation by plants. In this process, plants would generate specific catalytic enzymes that suitable for the toxic compound degradation. This type of phytoremediation would happen mostly in organic compounds. Some small molecules, plant could degrade completely to carbon dioxide or essential organic acid in tri-carboxylic acid cycle (TCA cycle). However some complex molecules that contain metal, large molecule or other, plant could only stabilize them after that the stable compound might excrete back to environment or accumulate in plant cells.

2.4 Green liver concept for organic compounds

Principle of chemical compound degradation and/or detoxification in plant had been shown in the model, which could be called "green liver concept". This concept show normally principle of mobilization of compound in plant cells that toxic compounds could be generally separated as heavy metals and organic compounds. In this case, only organic compounds had been presented that normally, organic compounds might be degraded completely to carbon dioxide or changed to less toxic compounds. Green liver concept of organic compounds could be easily classified as 3 processes such as transformation, conjugation, and localization (Kvesitadze and Kvesitadze, 2009). Phase I: transformation, toxic compounds would react with plant enzymes, which molecule could be changed the formation. Some organic compounds could be completely degraded in this process for example benzene, toluene, and xylene, and some compound might be reduced toxicities and accumulated in plant organelle such as trichloroethylene (TCE), trinitrotoluene (TNT), and polyaromatic hydrocarbons (PAHs). There are several reactions that could appear in this process. For example, the non-polar molecules, the polarity would be increased by addition of hydroxyl- group to the molecules by monooxygenase enzyme. Some organic compounds could be activated by addition of functional groups, which is suitable chemical group to conjugate with plant protein or glycosides.

Phase II: conjugation, after transformation process, activated molecule could combine to plant protein or glycosides. The process would decrease toxicities of molecule and stabilize them in a suitable form.

Phase III: localization, when toxic molecules were stabilized by conjugating with plant secretion compounds, the complex molecule would be localized in plant organelles such as cell wall, vacuole, etc. In soluble conjugated compounds, the compounds might be

excreted out of the cells (Singh and Jain, 2003). Green liver concept had been presented in Fig 2.9.

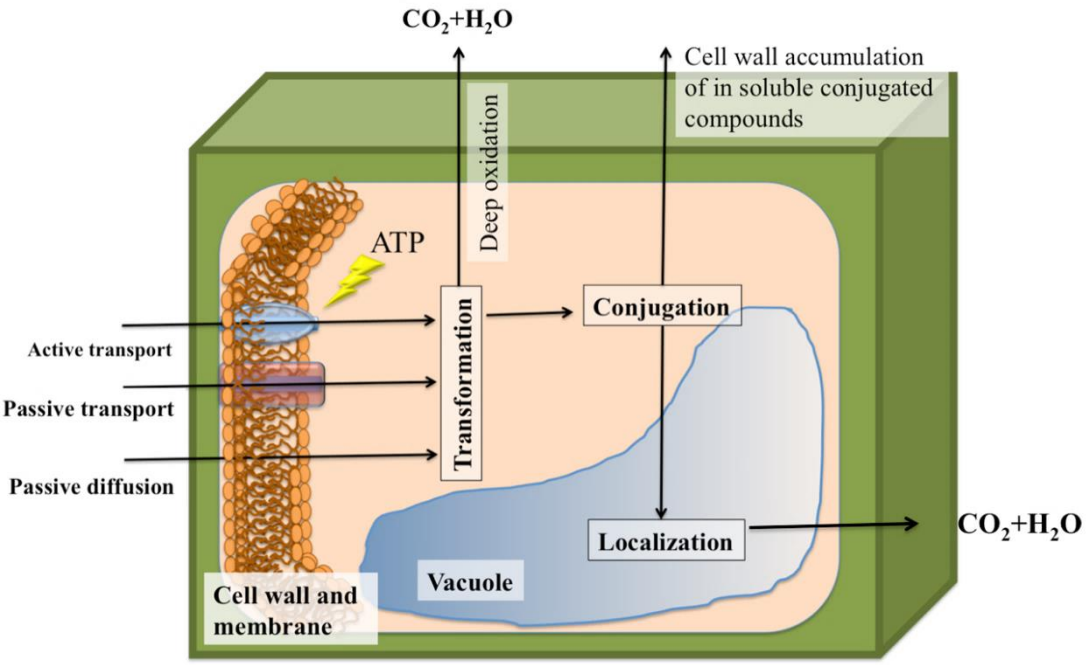

Figure 2.8 Principle of green liver concept.

2.5 Benzene uptake and transformation in plant

2.5.1 Uptake and distribution of benzene by plants

There are three important pathways of plant such as stomata, cuticles, and roots that plants use to uptake benzene, but the root of plants exposed to a little air because it stayed underground, so most of plants do not use roots to be the main pathway for benzene uptake. Stomata and cuticle are considered as main pathways for benzene uptake (Fig 2.10). Ugrekhelidze, *et al.* (1996) had studied on the 1-6^{14}C benzene uptake in 8 hours by 3 species of plants, which the experiment had compared between stomata pathway and cuticle pathway by the use of radioactive techniques. From this experiment, the abaxial side (stomata side) absorbs benzene more intensive than by adaxial side (cuticle side) in every treatment. So most of benzene had been reported that normally uptake by stomata side (Kvesitadze, *et al*., 2009). The result was shown in Table 2.9

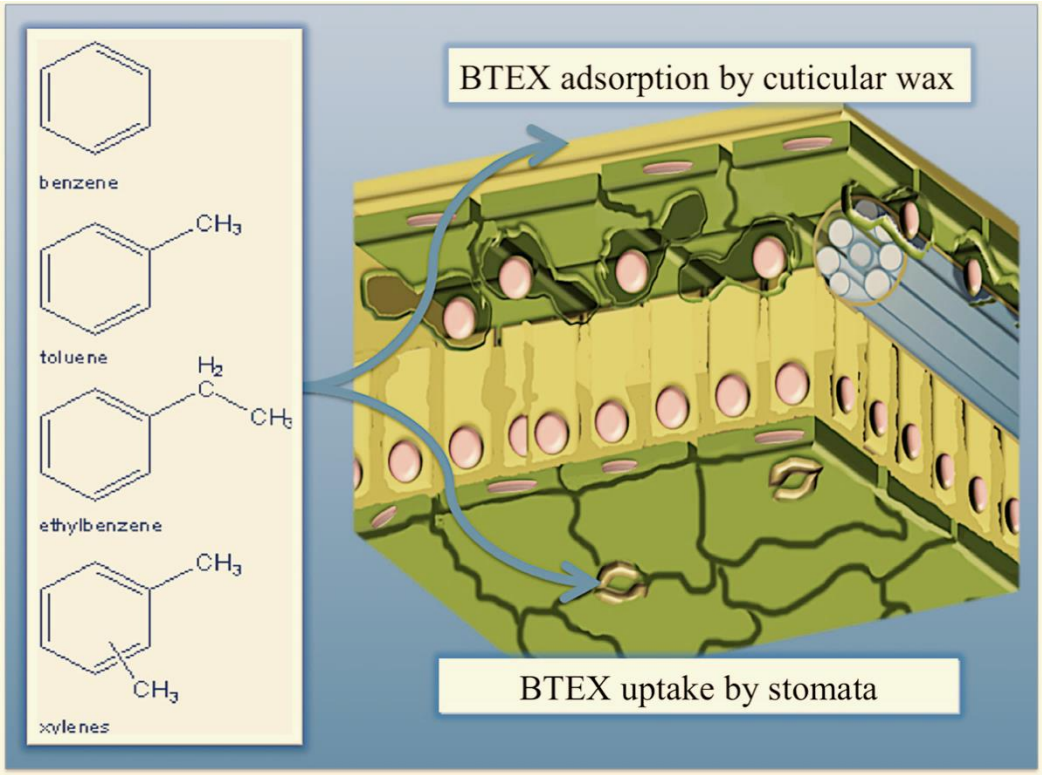

Figure 2.9 Gaseous BTEX uptake pathway in plant (stomata and cuticle).

Table 2.9 Total non-volatile benzene metabolites radioactivity from abaxial and adaxial side of plant after exposure to 0.1 mmol/L of gaseous benzene concentration; 1.76 MBq/mmol of radioactivity at 8 h exposure under light conditions (22-23^{o}C) (Ugrekhelidze, *et al*, 1996).

Species	Age	Absorption side	Stomata number per mm^2	Cuticle thickness (µm)	Total radioactive of non-volatile benzene metabolites (10^{-3} cpm/g fresh weight)
Acer campestre	young	abaxial	860	1.2	66.0
		adaxial		1.1	24.0
	old	abaxial	570	2.1	64.0
		adaxial		2.4	15.5
Viris vinifera	young	abaxial	70	1.0	42.5
		adaxial		1.0	19.0
	old	abaxial	40	1.6	49.0
		adaxial		1.7	17.5

In addition, this experiment also found that young leaves could uptake benzene higher than old leaves in 3 species of plants although young leaves have shown to be lower wax quantity than old leaf. This result, the composition of cuticle might effect on benzene uptake. Distribution of 4 species of plants had been also studied by a radioactive method. Cytosol had been found as the most important fraction in every species that contains the most percentage of total radio activities. Also, chloroplast had been also shown that contain high percentage of total radio activities as well. The result suggested that benzene and benzene metabolites could distribute well and accumulate in these 2 factions of plant cells (Table 2.10).

Table 2.10 Distribution of benzene and benzene metabolite intracellular organelles of plant leaf after exposure to 0.1 mmol L^{-1} of gaseous benzene concentration; 1.76 MBq $mmol^{-1}$ of radioactivity at 7 h exposure under light (22-23^{o}C) (Ugrekhelidze, *et al*, 1996).

Cellular organelles	Percentage of total radioactivity			
	Acer compestre	***Malus domestica***	***Vitis vinifera***	***Spinacia oleracea***
Nuclei and cell walls	5.2	7.3	4.1	6.0
Chloroplasts	33.6	29.8	28.8	30.7
Mitochondria	10.7	11.2	14.6	12.5
Microsomes	4.0	5.3	2.5	3.3
Cytosol	46.5	46.4	5.0	47.5

2.5.2 Benzene degradation by plants

Because benzene is an organic molecule, mechanism of benzene in plants is possible involved organic acids and amino acids from benzene degradation. Radioactive techniques had been used to analyze percent of total radioactivity of 1-6^{14}C benzene metabolites such as organic acids and amino acids in spinach leaves with 72 h 1-6^{14}C benzene exposure. 84% were found as organic acids, and 16% were as amino acids. In organic acids analysis, 37% and 24% were muconic acid and fumalic acid, respectively. In amino acid analysis, tyrosine was found as the main benzene metabolize about 34%, and 26% of phenylalanine had been found as second amino acid. Table 2.11 shows the percent of total radioactivity between organic acid and amino acid in spinach leaves after 72 h exposure.

For benzene transformation enzymes identification, 8-Oxyquinoline was known that is inactivate all the heavy metal enzymes, and sodium diethyldithiocarbamate suppresses the activity of copper-containing enzymes (Sato, 1966). Both inhibitor had been studied the relationship between the total radioactivity of nonvolatile metabolites and the present of inhibitors in spinach chloroplasts with 3 h of benzene exposure. The result was shown in Table 2.12.

Table 2.11 Non-volatile benzene and toluene metabolites, low molecular weight compounds, in plant after exposure to 0.2 mmol L^{-1} of gaseous benzene concentration; 1.76 MBq $mmol^{-1}$ of radioactivity in benzene and 1.5 MBq $mmol^{-1}$ of radioactivity in toluene at 72 h exposure under light conditions (22-26^{o}C) (Ugrekhelidze, *et al*, 1996).

Substrate	Percentage of total radioactivity		Distribution of radioactivity in percentage	
	Organic acid	Amino acid	Organic acid	Amino acid
[1-6 14C]Benzene	84	16	Muconic, 37.2 Fumaric, 24.4 Succinic, 12.5 Malic, 9.6 Oxalic, 9.1 X, 7.2	Tyrosine, 33.8 Phenylalanine, 25.8 Glycine, 16.2 Aspartic acid, 11.3 X1, 7.4 X2, 5.5
[1 14C]Toluene	79	21	Fumaric, 21.7 Malic, 18.9 Oxalic, 9.5 Succinic, 8.3 X1, 33.5 X2, 8.1	Tyrosine, 29.7 Aspartic acid, 26.0 Alpha-alanine, 14.8 Valine, 14.4 X, 15.1

Table 2.12 Transformation of benzene by spinach chloroplasts (Ugrekhelidze, *et al*, 1996).

Chlroplasts with ^{14}C benzene	Total radioactivity of non-volatile benzene metabolites (10^{-3} cpm g^{-1} fresh weight)	
	Dark	Light
Without additives	5.3	9.4
8-Oxyquinoline (10^{-3}M)	>0.1	0.5
α, α'-Dipyridyl (10^{-3}M)	4.7	7.8
Sodium diethyldithiocarbamate (10^{-3}M)	>0.1	0.8

From Table 2.12, the present of 8-Oxyquinoline and sodium diethyldithiocarbamate could inhibit benzene transformation enzymes both under light and dark conditions, but a little radioactive could be reduced by α,α´-dipyridyl that is the iron enzymes inhibitor. This experiment concluded that the benzene transformation enzyme contain copper as main component. Phenoloxidases and ascorbate oxidase that are copper-containing proteins of chloroplasts had been considered to be benzene transformation enzyme. Spinach chloroplasts possess less active ascorbate oxidase than O-diphenoloxidase (Sechneska, *et al*., 1968). So phenoloxidases might be the most important enzymes for benzene transformation. In addition, the interesting result is that NADH and NADPH could enhance benzene mechanism and transformation (Ugrekhelidze, *et al*, 1997). NADH and NADPH show the general reaction that could send $2e^-$ to P450 monooxygenase, and this enzyme contain the function of oxygen molecule activation to combine with hydrophobic molecule. So it is possible that NADH and NADPH can activate benzene metabolism and its transformation. The result was shown in Table 2.13.

Table 2.13 Transformation of benzene by spinach leaf enzyme preparation (Ugrekhelidze, *et al*, 1996).

Enzyme preparation with 14C benzene	Total radioactivity of non-volatile benzene metabolites (10^{-3} cpm g^{-1} fresh weight)	C^{14} phenol formed (10^{-3} cpm g^{-1} fresh weight)	inhibition (-) and activation (+) %
Without additives	650	0	0
8-Oxyquinoline (10^{-3}M)	67	0	-89.7
α, α'-Dipyridyl (10^{-3}M)	538	0	-17.3
Sodium diethyldithiocarbamate (10^{-3}M)	83	0	-87.3
NADH	4660	1.3	+716.9
NADPH	4110	1.2	+632.3

In 2009, Kvesitadze and Kvesitadze (2009) had reported the mechanism of organic molecule in plants. Benzene could be degraded by two general plant enzymes such as phenoloxidases and P450 monooxygenase. P450 monooxygenase can generate hydroxyl group on the benzene molecules. In this pathway NADH and NADPH provide $2e^-$ for cytochrome b_5 reductase activation which b_5 allows $2e^-$ to stimulate P450 monooxygenase. This pathway is shown in Figure 2.11.

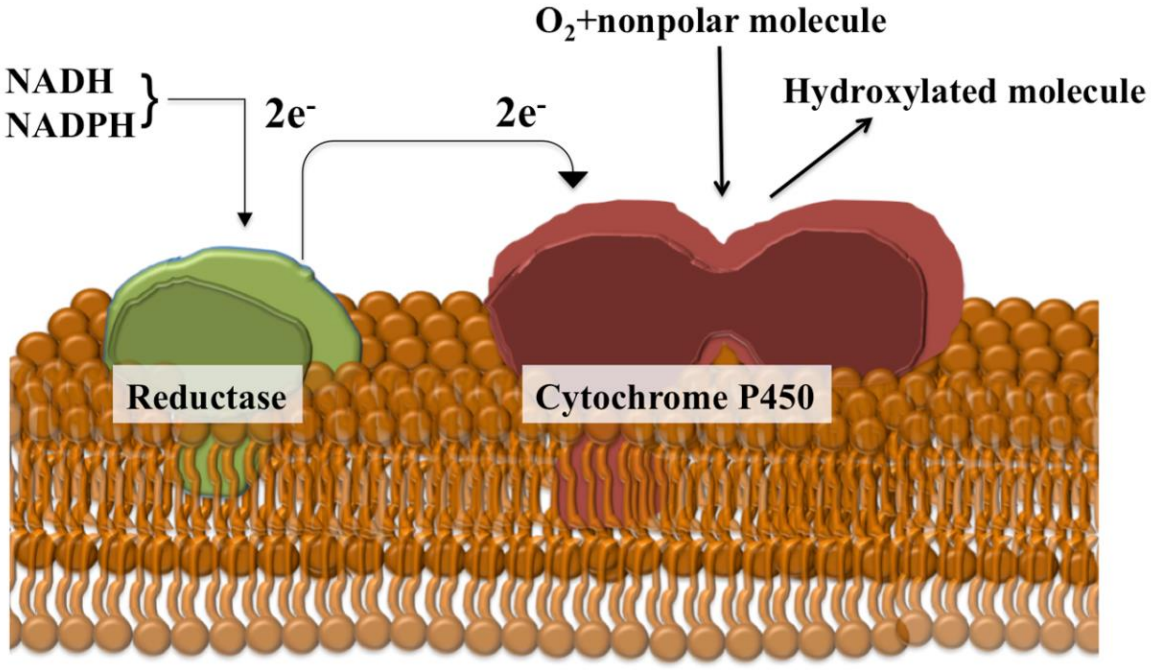

Figure 2.10 P450 monooxygenase function for hydroxyl group addition on xenobiotic molecules.

Benzene could be changed to phenol, and phenol would be added hydroxyl groups by P450 monooxygenase again and changed to catechol. Not only P450 monooxygenase but also phenoloxidases could generate the molecules of catechol. Oxygen active species are also generated by the reaction of o-diphenoloxidase and o-diphenol. This oxygen active species could directly combine with benzene or phenol molecule and create catechol molecule. Figure 2.12, phenoloxidase function for oxygen active species generation and benzene metabolism by oxygen active species.

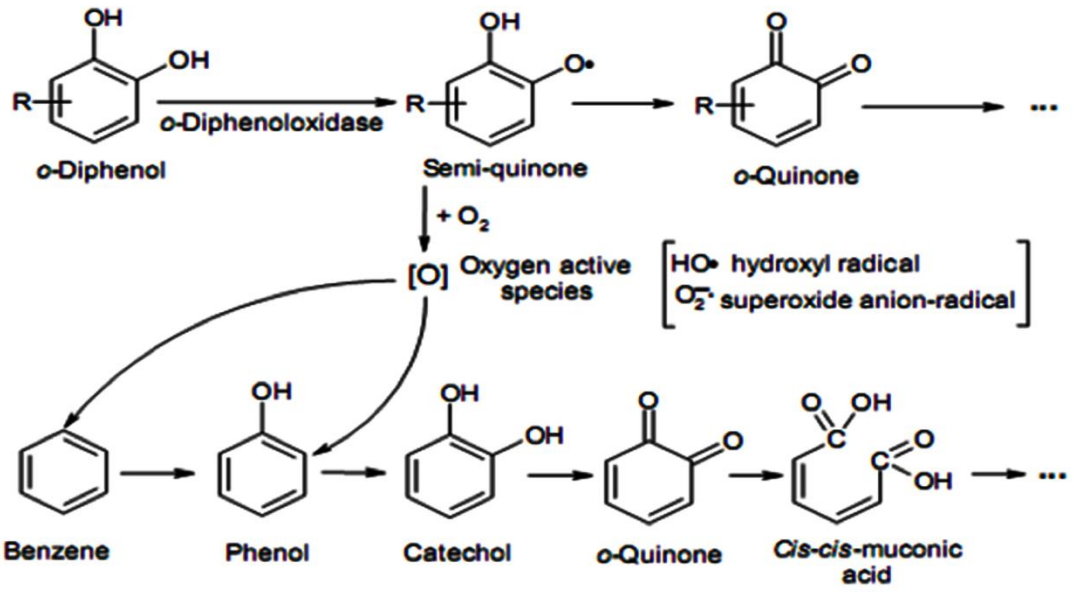

Figure 2.11 Phenoloxidase function for oxygen active species generation and benzene metabolism by oxygen active species.

Benzene metabolism is concluded that after catechol molecule is generated, it could be changed to o-quinone molecule. This o-quinone is normally known as unstable molecule, which could be braked easily the ring structure and changed to muconic acid. This muconic acid, which is general molecule in living cells, is produced from o-quinone ring cleavage. This molecule contains less toxic and can be degraded to CO_2 by TCA cycle (Fig 2.13). The use of benzene as carbon and energy sources had been firstly proposed.

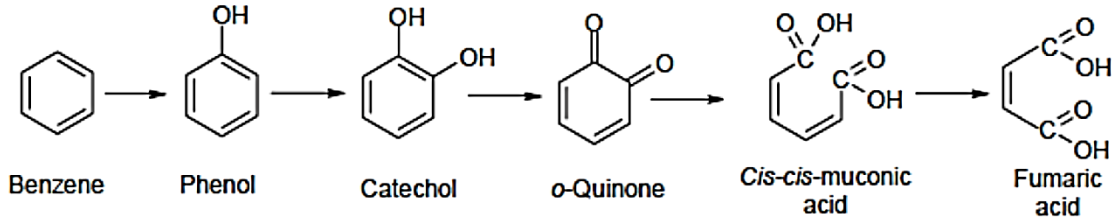

Figure 2.12 Benzene metabolisms in plant cell (Kvesitadze and Kvesitadze, 2009).

2.5.3 Plants application for benzene removal

In 1989, 10 species of plant such as *Gerbera jamesonii, Chrysanthemum morifolium, Hedera helix, Sansevieria laurentii, Dracaena deremensis warneckei, Spathiphyllum, Aglaonema, Dracaena marginata, Chamaedorea seifrizii,* and *Dracaena deremensis* were presented high benzene removal efficiency at initial benzene concentration of 20 ppm (Wolverton, et al., 1989). *Gerbera jamesonii* and *Chrysanthemum morifolium* can uptake highest benzene about 23.5 µg cm^{-2} and 18.2 µg cm^{-2}, respectively. In 2004, Orwell, et al. (2004) screened 7 ornamental plants to remove benzene such as *Dracaena* "Janet Craig",

Epipremnum aureum, Dracaena marginata, Schefflera "Amate", *Spathiphyllum* "Petite", *Spathiphyllum* "Sensation", *Howea forsteriana* at initial concentration of 25-50 ppm. The results showed that *Dracaena marginata* can uptake highest benzene about 23.23 µg cm^{-2}. However the similar species was found only 4 µg/cm^2 of benzene removal in Wolverton study. When *Dracaena* "Janet Craig" in Wolverton study can remove 1.7 µg cm^{-2}, 13 µg cm^{-2} benzene removals was found in Ralph study. The results suggested that growing condition may effect on benzene removal efficiency. In 2007, Liu, *et al*. (2007) had studied 72 species for benzene removal by continuous system at 150 ppb of initial benzene concentrations. High benzene removal efficiency species were shown in Table 2.14.

Table 2.14 Mean benzene removal efficiency in each plant (µg m^{-2} day^{-1}) by dynamic system experiment.

Plant species	**µg m^{-2} day^{-1}**
Crassula portulacea	724.9
Hydrangea macrophylla	293.7
Cymbidium golden	267.4
Ficus microcarpa	255.5
Dendranthema morifolium	204.9
Citrus medica	166.7
Dieffenbachia amoena	115.2
Spathiphyllum supreme	106.9
Nephrolepis exaltata	73.5
Dracaena deremensis	59

Tarran, *et al.* (2007) had showed that at the first cycle benzene exposure, plant could uptake toxic molecules slower than other benzene exposure cycles in every plant in *Zamioculcas* and *Aglaonema*. This tendency suggests that plant required the time for its adaptation. The result of this experiment was shown in Figure 2.14.

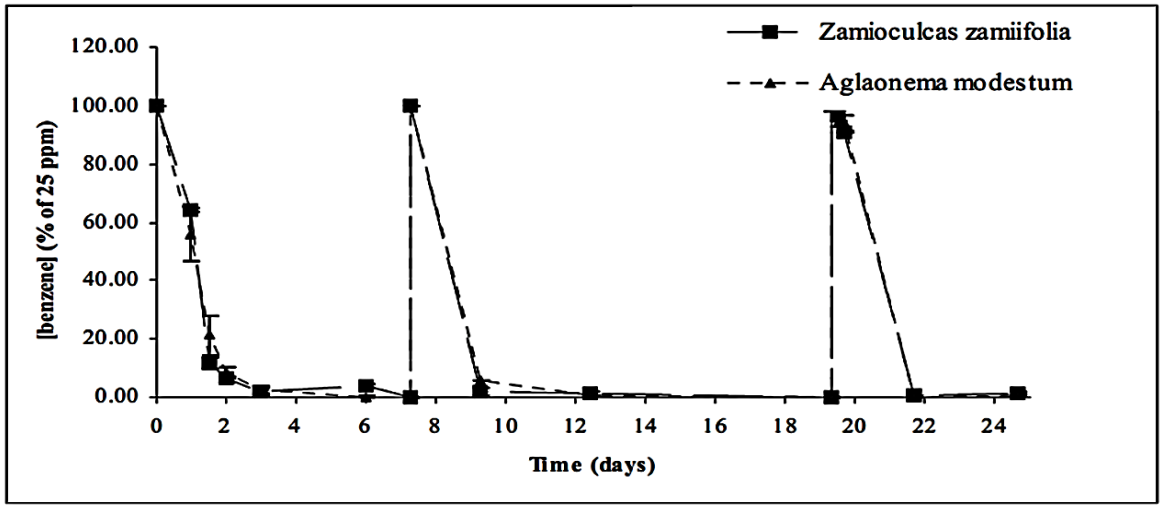

Figure 2.13 Removal of benzene from test-chamber air by potted *Zamioculcas zamiifolia* and *Aglaonema modestum,* challenged with three consecutive doses of 25 ppm benzene.

The similar result had been found in 2001. Wood, *et al*. (2001) studied on the benzene removal efficiency of *Spathyphyllum var Petite* plants when was exposure with 25-30 ppm of benzene in both light and dark. In light condition, the tendency of benzene removal efficiency was similar to Jane Tarran's study. First cycle of benzene exposure, plant required time to adaptation. In addition, slower benzene removal tendency was found in dark condition. The result was shown in Figure 2.15. Light might be important factor for benzene removal in plant. 2 reasons that might be possible to explain this event is that NADH and NADPH, electron donator for benzene degrading enzyme, are required photosynthesis to generate and the open of stomata under light condition could promote gaseous uptake by plant.

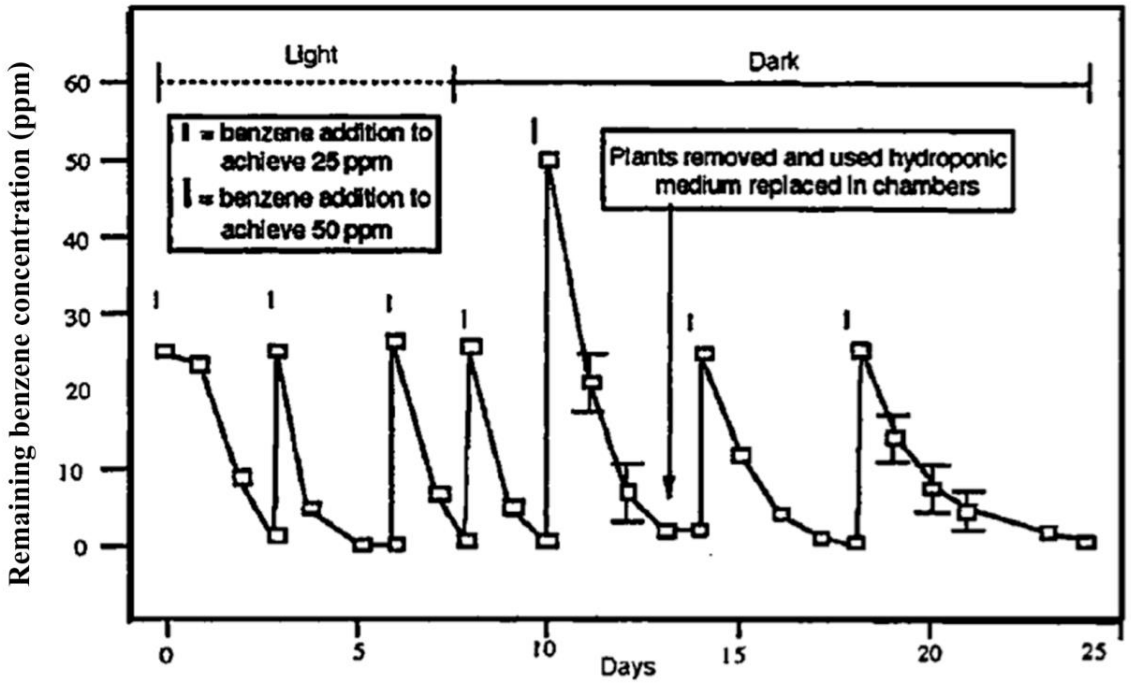

Figure 2.14 Benzene levels in air of test chambers containing Spathyphyllum var Petite plants maintained in hydroponic medium.

2.6 Possibility of benzene adsorption by plant leaf wax

2.6.1 Principle of plant leaf wax

Almost hydrophobic molecule on plant leaf was found as glycerol lipids and fatty acids. Glycerol lipids that have been known as main composition of wax containing one glycerol and 1-3 fatty acids. In monoglycerides, glycerol had been esterified by 1 fatty acid in *sn-1* or-*2* positions. Diglycerides are the main composition on the cell membrane however polar head with phosphate group was often occurred. Finally, triglycerides are the common lipid form in living cells. In fatty acid composition, R-COOH is the common form of organic acid and fatty acids. R is identified to alkyl or alkane groups. In general, fatty acids and organic acids were separated by the alkyl or alkane groups if alkyl or alkane groups contain less than 4 carbon atom contents, which will be classified as only organic acids.

2.6.2 Benzene adsorption and accumulation by plant cuticle

The 1-6^{14}C benzene uptake pathway in 3 species of plants by the use of radioactive technique was studied (Ugrekhelidze, *et al*., 1996). Plant can uptake gaseous benzene though stomata and cuticle on the surface of leaf. In addition, in dark condition, plant can still grow and uptake benzene well that should be the benzene uptake by only waxes when stomata was closed under dark condition (Orwell, *et al*., 2004). The accumulation of benzene in wax layer of plant leaf had been found in many researches (Gorna-Binkul, *et al*., 1996; Slaski, *et al*., 2000; Tsiros, *et al*., 1999; Collins, *et al*., 2000; Poborski, 1988; Reiderer, 1990; Kylin, *et al*., 1994). In 1996, Gorna-Binkul, *et al*. (1996) found the benzene and it derivatives accumulate on orange shell, and parsley. Black berry, apple, and

cucumber were also investigated benzene accumulation. The result found that black berry and apple can well accumulate benzene (Collins, *et al*., 2000). Not only benzene but also poly-aromatic-hydrocarbon (PAHs) was also reported the accumulation in many part of plant that grown in contaminated environment (Slaski, *et al.*, 2000). Benzene, contains 2.13 of log K_{OW}, can diffused rapidly into the plant (Kamath, *et al*., 2004). Ugrekhelidze (1996) observed also the ^{14}C-benzene and non-volatile benzene metabolites both old and young plant leaves. The result suggested that although high quantity of cuticle was found in old leaf, young leaf have more benzene and it metabolites accumulation intensity than old leaf. This result, the composition of wax may be a main effecting factor for VOCs adsorption.

2.7 Biofilter

Biofilter, which is general application of biological treatment to remove contaminate air, could be suitable technology in low concentration and high volume of gas (Joseph, 1999), so many industries have utilized this technology. In biofilter system, air pollutant would transferred through porous of packing bead which contain microorganism in column where liquid is continually recirculated through the packing bed, and the liquid provide moisture, nutrients, pH regulator, and other (Fig 2.16) (Zarook, 2005).

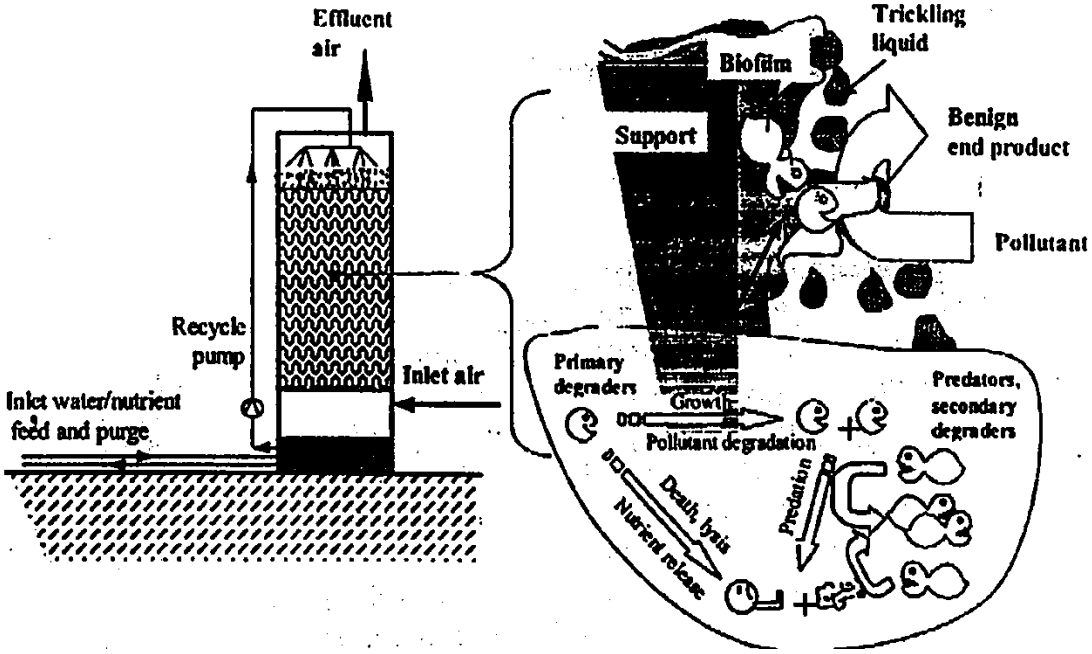

Figure 2.15 Basic set up and principle of biotrickling filter (Zarook, 2005).

In general, air pollution, which might be either organic or inorganic substances, could be used as carbon sources for microorganisms growing in biofilter system. So microorganisms in bio filter, which are important living cells, could catabolize many substances to carbon dioxide, water vapour, and/or organic biomass and decrease a toxicity of pollutants. Several researches reported that both aerobic and anaerobic could be applied to treat VOCs, which had been shown in Table 2.15.

Table 2.15 Pollutants biofiltration by aerobic and anaerobic bacteria (Zarook, 2005).

Substances	Rate ($mg\ L^{-1}\ day^{-1}$)	Microorganism species	Reference
Aerobic bacteria			
2-Hydroxy-benzothiazole	138	*Rhodococcus Rhodochrous*	Wever, *et al*., 1997
Naphthalene	57	*Pseudomonas sp. and Rhodococcus sp.*	Bouchez, *et al*., 1995
Fluoranthene	6.6	*Rhodococcus sp.*	
Pyrene	6.6	*Rhodococcus sp.*	
Benzene	-	*Pseudomonas putida ML2*	Swift, *et al*., 2001
Benzene	-	*Pseudomonas putida F1*	Gibson, *et al*., 1968
Toluene	-	*Pseudomonas putida F1*	Parales, *et al*., 2000
Toluene	57	*Pseudomonas putida*	Heald and Jenkins, 1996
Phenol	188	mixed immobilixed culture	Morsen and rehm, 1987
Benzene, Toluene, and Phenol	-	*Pseudomonas putida F1*	Reardon, *et al*., 2000
Benzene, Toluene, Xylene	-	*Pseudomonas putida F1*	Yu, et al., 2001
o, p, m- Cresol	259	mixed immobilixed culture	Morsen and rehm, 1987
n-Propylbenzene	-	*Pseudomonas putida F1*	Choi, *et al*., 2003
Alkylbenzene	-	*Pseudomonas putida 01G3*	Chablain, *et al*., 2001
Anaerobic bacteria			
Pentachlorophenol	107	methanogenic mixed culture	Juteau, *et al*., 1996
Pentachlorophenol	90	methanogenic mixed culture UASB	Wu, *et al*., 1993
Pentachlorophenol	4.4	methanogenic granular	Kennes, *et al*., 1996
Benzene	0.029	sulfate-reducing mixed culture	Edwards and Gribic-Galic, 1992
Phenol	1000	methanogenic mixed culture	Knoll and Winter, 1987
Phenol	31	syntrophic mixed culture	Knoll and Winter, 1989
Benzoic acid	600	syntrophic culture	Kobayashi, *et al*., 1989
Toluene	0.1-1.5	sulfate-reducing mixed culture	Edwards, *et al*., 1992
Toluene	4.6	methanogenic mixed culture	Edwards and Gribic-Galic, 1994
Xylene	0.1-1.5	sulfate-reducing mixed culture	Edwards, *et al*., 1992
Xylene	5.3	methanogenic mixed culture	Edwards and Gribic-Galic, 1994

Although biofilter could be effective pollutant removal method, there are many factors which should be controlled in the system for example moisture, temperature, pH, pressure drop and several criteria to design biofilter.

2.7.1 Moisture content

Metabolic activates in microorganisms require water to survive (Ottengraf, *et al*., 1986; shimko, et al, 1988; Marsh, 1994). In approximation, moisture in biofilter should be 30-60% by weight (Ottengraf, *et al.*, 1986; Van lith, *et al*, 1990). In addition, surface area,

porosity, and other factors effect to moisture quantity in biofilter (Hodge, *et al*, 1991). Little moisture can be cause bed drying and microorganism decreasing, and too much moisture is not only oxygen transfer inhibiting hydrophobic pollutant expose the biofilm but also increase back pressure (Zarook, 2005).

2.7.2 Temperature

Temperature is also very important factor in biofilter because enzyme activities need a suitable temperature. There are three levels of temperature of aerobic microorganism. First of all, microorganism which grows best below 20 ^{o}C is called psychrophilic-microorganism. Second, mesophilic-microorganism require temperature between 20-40 ^{o}C. Finally, themophilic-microorganism grows best above 45 ^{o}C (Zarook, 2005). In general, the suitable temperature in biotrickling filter is between 15-40 ^{o}C (Leson and Winter, 1991; Bohn, 1992), so inlet gas temperature should be detected for quantity of microorganism controlling in the system.

2.7.3 pH

Although pH range is so specific for species of microorganism. In general, microorganism grow best at pH between 7 and 8 which is found in compost bed. However, pH range may be decreased by carbon dioxide which is made from microorganism metabolism. In pH controlling, soil buffer agents, sodium and magnesium hydroxide bed is used to be pH value regulator in the system (Zarook, 2005).

2.7.4 Nutrient

Nutrient which is not only carbon but also many elements such as nitrogen, phosphorous, mineral, and others is required to grow microorganism in biotrickling filter (Auria, *et al*, 1996). Carbon, which obtains from air pollution, will be degraded to be energy for bacteria. In natural packing bed (soil, peat, and compost), nutrient is contained, but nutrient should be provided for better performance in artificial packing material (Weckhuysen, *et al*, 1994; Morgenroth, *et al*, 1996).

2.7.5 Pressure drop

Microorganism accumulation effect to air flow resistant increasing in the packing bead (Kinney, *et al*, 1996) and void space decreasing in biofilter column. In general, pressure drop happen in natural packing bed such as wood, soil, etc. (Zarook, 2005) However, bed shape changing may be suitable solution in this problem.

2.7.6 Packing media

Packing media high surface area, long term physical stability, low pressure drop, good moisture retention, pH buffering capacity, and nutrients are required for best properties of media (Zarook, 2005). There are two type of packing bed. First, adsorbent and artificial packing beds are utilized in some biofilter. Although these medium can reduce the quantity of pollutant very well, many factor must be controlled because these media is poor to

contain nutrients, to control pH, and to carry moisture. In addition, they may be cause of secondary pollutant. Second, natural medium components such as compost, soil, wood ship, peat, and other are generally used in simple biofilter because they are easy to find and inexpensive (Zarook, 2005). Soil is general media in biofilter because it is not expensive, found easily, and has a large in digenous microbial population. In addition, soil has a high bearing strength more than 30 years (Devinny, *et al*., 1999). Bohn (1992) reports that soil are naturally hydrophilic and contain a lot of nutrients. However, soil has a large pressure drop (Joseph, 1999), so plastic waste and fertilizer in the proportion of 40: 60 by volume is used to decrease this problem.

2.7.7 Biofilter terminology

Since the first works published on biofiltration, a common terminology has been established in biological air treatment system, facilitating communication and comparison among the various processes. This terminology, with the most common units, is defined below

Empty Bed Residence Time (EBRT)

Empty Bed Residence Time (EBRT) is related to the time that the air spends in the biofilter under considering that the reactor is empty. The volume of the reactor instead of a more real air residence time that would include the porosity of the packing had been considered to calculate EBRT. The difference might be substantial for some configuration. In some cases, the real residence time is used. EBRT and true retention time are defined (Eq 2.1)

$$EBRT = V_b / Q \qquad \text{(Eq 2.1)}$$

EBRT define to the empty bed residence time (min), V_b is the bed volume of the reactor (m^3 reactor), Q is the inlet air flow (l/min).

Percentage removal efficiency

Percentage removal efficiency of benzene in the system could be calculated by Eq 2.2

$$\%\ Removal\ efficiency = \frac{(C_i - C_o)\times 100}{C_i} \qquad \text{(Eq 2.2)}$$

Where, C_i and C_O are the benzene concentration in inlet of treatment and outlet of treatment, respectively.

Benzene loading rate

Benzene loading rate could be calculated following Eq 2.3. Q and V_b in the equation were airflow rate and volume of bed, respectively. The relationship of loading rate and elimination capacity should be calculated.

$$Loading\ rate = \frac{QC_i}{V_b} \qquad \text{(Eq 2.3)}$$

Elimination capacity

Elimination capacity was calculated follow Eq 2.4. Q and V_b in the equation were airflow rate and volume of bed, respectively.

$$Elimination\ capacity = \frac{Q(C_i - C_o)}{V_b} \quad \text{(Eq 2.4)}$$

2.8 *Pseudomonas putida*: principle and benzene removal

P. putida is identified as a Genus: *Pseudomonas* and Species: *Pseudomonas putida. Pseudomonas putida* has rod-shaped, nonsporeforming, gram-negative bacteria that utilizes aerobic metabolism. Multiple polar flagella for motility is also found in this species (Fig 2.17). *Pseudomonas putida* is sensitive microorganism to the environment. The movement of bacterial is very helpful in guiding the *Pseudomonas putida* to propel towards the seeds of the plants, which provides nutrients to miccroorganism (Gibson, *et al*., 1968; Harwood, *et al*., 1989; 1990; Costura and Alvarez, 2000). It grows optimally at 25-30 ^{o}C and can be easily isolated. Certain strains of *Pseudomonas putida* are not pathogenic. It is lack of certain genes that include cell wall and cell membranes degradation enzymes. *Pseudomonas putida* is aerobic metabolism that able to degrade organic solvents such as benzene, toluene, etc. For example, some previous report on benzene degradation by *Pseudomonas* spp., *Pseudomonas cepacia* G4 and *Pseudomonas mendocina* KR1 first attack the aromatic ring of toluene with monooxygenase enzymes. Neither *P. cepacia* G4 nor *P. mendocina* KR1 could grow with benzene as sole carbon source (Shields, *et al*. 1989; Whited and Gibson 1991; Gibson and Parales, 2000; Bagnéris, *et al*., 2004).

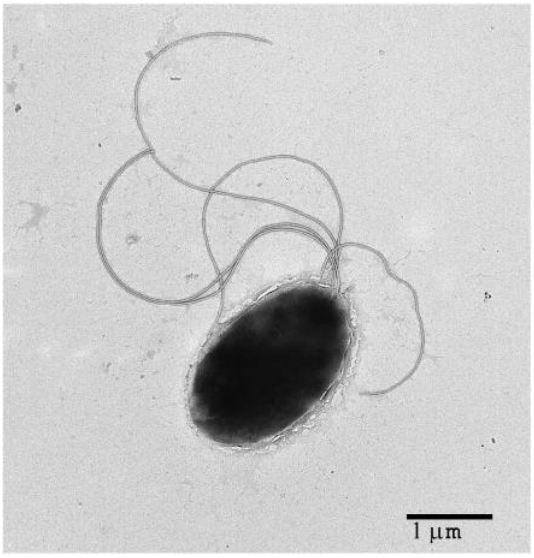

Figure 2.16 *Pseudomonas putida* cell.

For the mechanism of benzene and benzene derivative degradation by *P. putida*, several researches had reported possible transformation pathway, and literature review found that

difference species of *Pseudomonas* have difference pathways to remove benzene and benzene derivatives. Parales (2000) reported on toluene first transformation in difference kind of *Pseudomonas* spp. such as *Pseudomonas putida* F1, *Pseudomonas cepacia* G4, *Pseudomonas mendocina* KR1, *Pseudomonas pickettii* PKO1, and *Pseudomonas putida* PaW15. Difference toluene metabolites had been reported in difference species of *Pseudomonas* sp. (Fig 2.18)

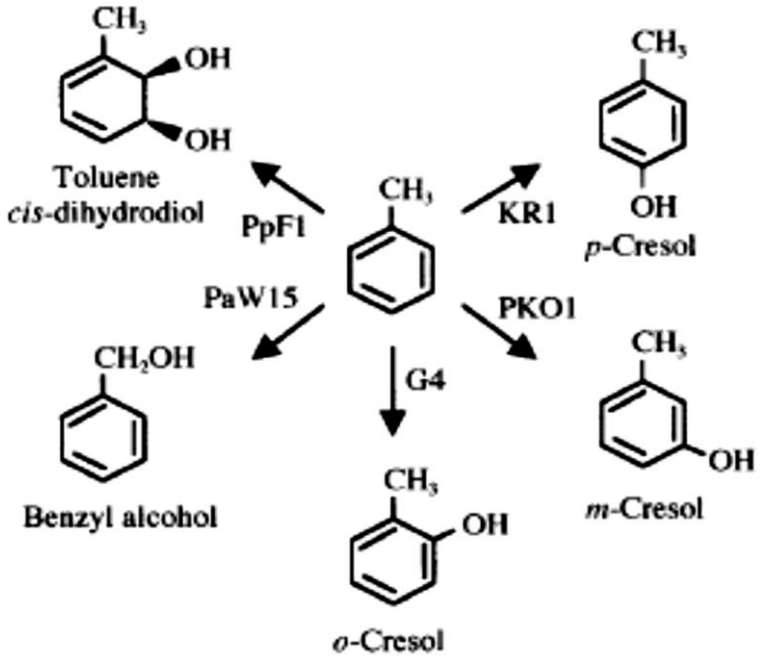

Figure 2.17 Initial transformation of toluene by difference kind of *Pseudomonas* sp. (Parales, 2000).

In 2001, there is a report that confirmed the toluene transformation in difference kind of *Pseudomonas* sp. (Yu *et al*., 2001). Although difference species of *Pseudomonas* sp. could generate difference toluene metabolites. The report in 2001 presented that all toluene metabolites is derivatives of catechol that contain 2 hydroxyl groups (Fig 2.19). These groups of hydroxyl could be changed o-quinone and o-quinone derivatives, which is the main pathway to break benzene ring of the molecule. After benzene ring cleavage, toluene metabolites have been normally known as an organic compound that might be completely degraded to carbon dioxide or used to be intermediate in TCA cycle (Choi *et al*., 2003). Benzene and benzene derivatives ring cleavage was shown in Fig 2.20.

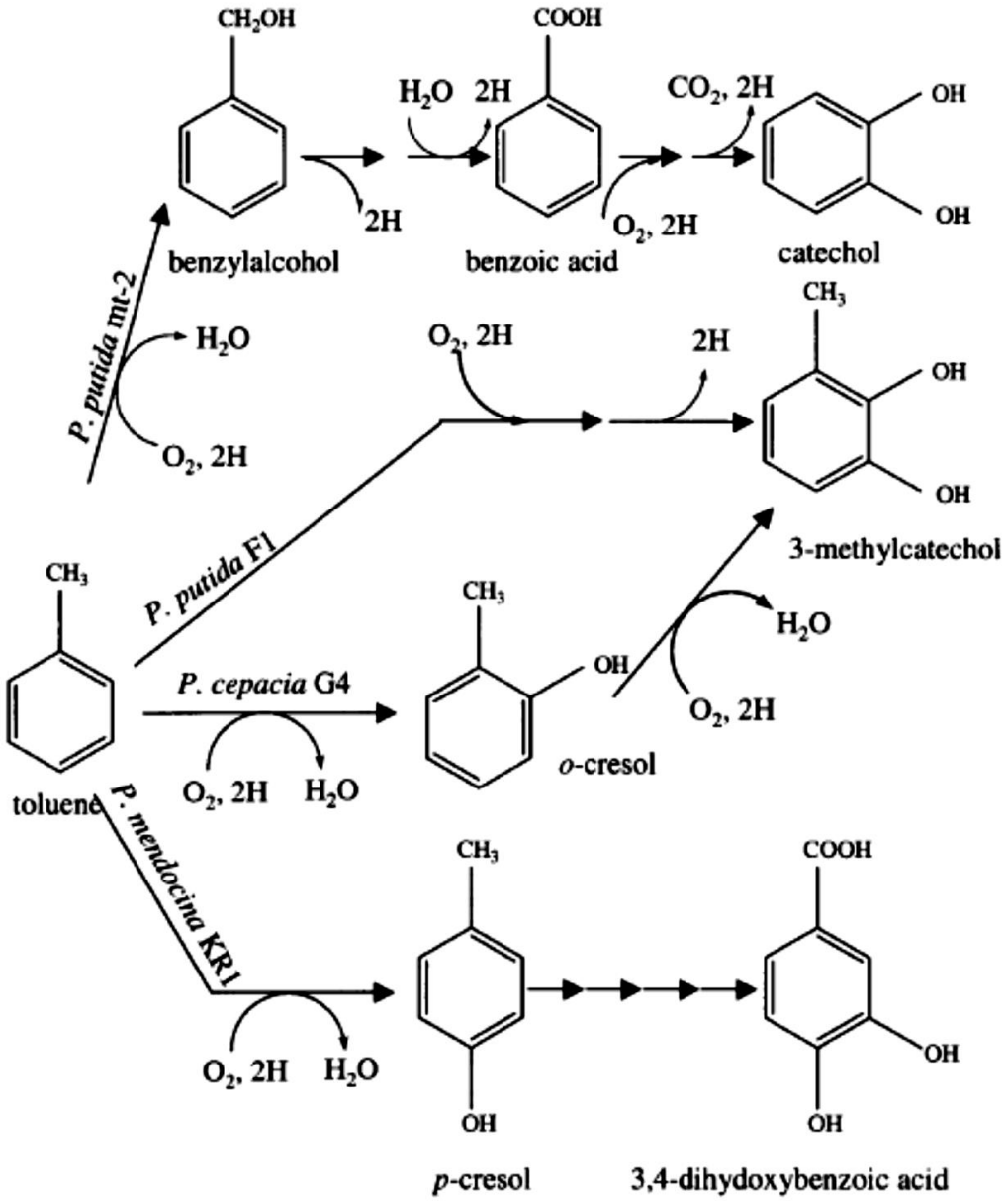

Figure 2.18 Transformation of toluene by difference kind of *Pseudomonas* sp. and generation of derivatives of catechol (Yu *et al*., 2001).

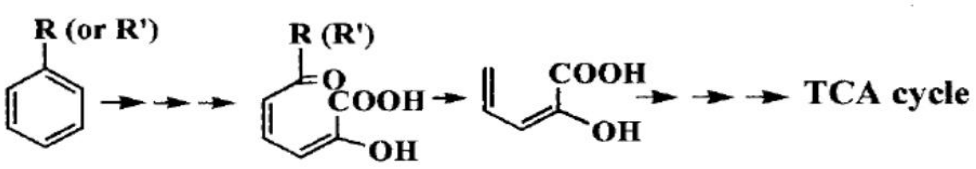

Figure 2.19 Benzene and benzene derivatives ring cleavage and transformation to TCA cycle by *Pseudomonas* sp. (Choi *et al*., 2003).

To conclude benzene and benzene derivatives transformation in *Pseudomonas* sp., *Pseudomonas putida* F1, which had been investigated widely, is used as a model. Benzene and phenol could be added hydroxyl group and changed to catechol. In toluene, 3-methyl catechol could be generated commonly. Unstable molecule could make benzene ring cleavage, and organic acid would be created (Reardon, *et al*., 2000) (Fig 2.21).

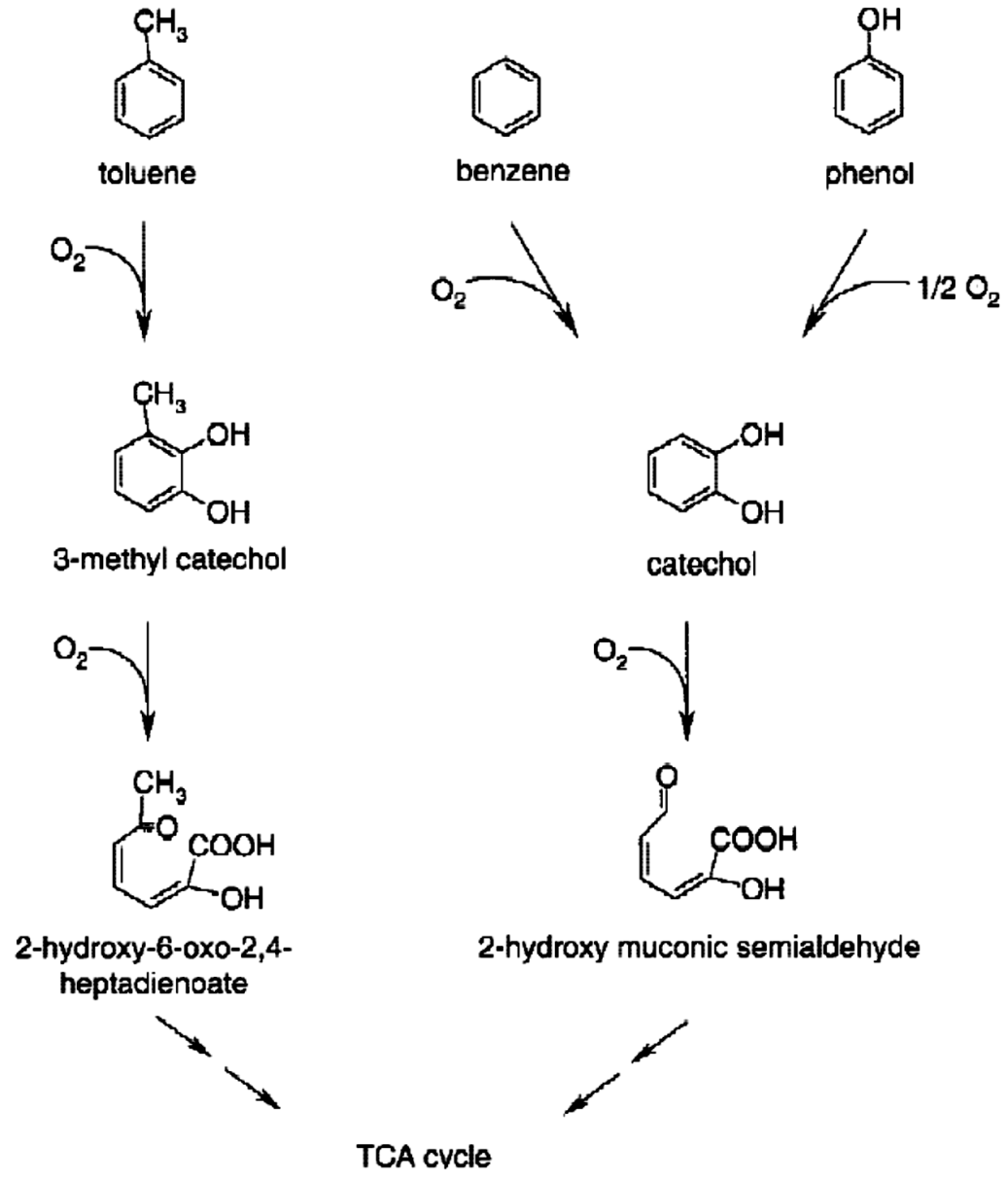

Figure 2.20 Benzene and benzene derivatives transformation in *Pseudomonas putida* F1 (Reardon, *et al*., 2000).