

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

2.1 Introduction

Gas stations are one of the major pollutant contributors to natural water resources. Activities at gas station are not only fueling but also car washing, cafeteria, lubricant changing, floor cleaning, etc. A high volume of polluted water is produced in the car wash process (Panpanit *et al.*, 2001). Wastewater from car washing operations contains suspended solid, emulsifier and oily wastewater. Oily wastewater not only present in free oil form, but also in oil-in-water emulsion formed by admixture of automotive oil such as lubricant oil with emulsifier and wash water (Delchad, 1992). Generally, conventional treatment systems of gas stations such as the API (American Petroleum Institution) gravitational oil separator and Corrugated Plate Interceptor (CPI) are considered effective and economical, but they can remove only free oil and sludge (Tin and Cheng, 1995). Therefore, oil-in-water emulsion should be treated by other system.

2.2 Sources of car wash wastewater

Typical car washing operation consists of four consecutive steps as shown in Figure 2.1. Each stage in the process is simply described as follows (Panpanit, 2001):

Stage (1) Dust cleaning: high-pressure water is injected on the car body and engine in order to wash away the dirty particles. The wastewater in this stage contains a high concentration of sludge, clay and free oil. In addition, the mechanical force from the high pressure of the water breaks oil into oil-in-water emulsion.

Stage (2) Foaming: The emulsifier solution is sprayed on the car body. The wastewater in this stage consists mainly of the high concentration of emulsifier and later it is combined with oily wastewater from stage 1. Hence, the stabilization of oil-in-water emulsion takes place.

Stage (3) Presoak: This stage is to remove the emulsifier from the car body by spraying with fresh water. The wastewater generated in this stage mostly contains low emulsifier concentration because it is diluted by fresh water.

Stage (4) Spot free rinse and dryer: The objective of this stage is to polish car color. High quality water is sprayed on the car body, which is later dried by hot air. The fresh water contains high impurity such as hardness and organic matter are avoided in this process because it can creates an unpolished appearance problems such as spot on the vehicle's surface color.

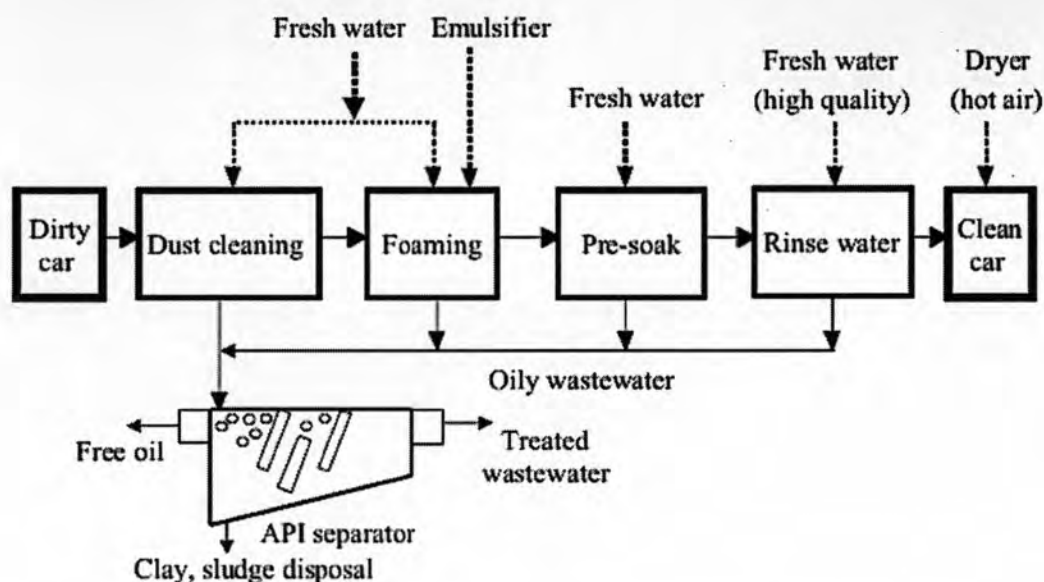


Figure 2.1 Process flow diagram of car wash process (Panpanit, 2001).

2.3 Characteristics of oil-in-water emulsion

Type of oil found in wastewater from gas stations can include fats, lubricants, cutting fluids, heavy hydrocarbons such as tars, grease, crude oil, diesel oils; and light hydrocarbons such as kerosene and gasoline. Whenever two immiscible liquids, such as oil and water, contact each other, one liquid tends to disperse, but not dissolve, in the other. This dispersion of liquid, typically in an aqueous medium, is an emulsion. The two main categories of emulsions are oil-in-water (OW) and water-in-oil (WO). However, an emulsion in wastewater from gas station is an oil-in-water type (Panpanit *et al.*, 2001). Majority of naturally occurring oil-in-water emulsions is negatively charged (Alther, 1998). Therefore, the oil-in-water emulsions preferentially adsorb on colloids from their surrounding solution such as mineral clay and protein. Emulsion oil can be divided into two types as follows:

2.3.1 Mechanically emulsified oil

Mechanical emulsions are formed by mixing of oil and water through pumping, valves (especially globe valves), other restrictions in flow, vertical piping, and other means, and present as droplets 5 to 20 microns in size (Arizona Department of Environmental Quality, 1996). The factors which govern the mean droplet diameter of the emulsion are the quantity of dissipated energy necessary to create turbulence and the oil-water interfacial tension (Supawimol, 2002). Turbulence generation should be avoided where possible; so as to avoid the formation of mechanically emulsified oil.

2.3.2 Chemically emulsified oil

Chemical emulsions are usually intentionally formed using detergents, alkaline fluids, or other reagents, and having a droplet size less than 5 microns (Arizona Department of Environmental Quality, 1996). Chemically emulsified oil wastewater can be generated from foaming and presoak process in car washing operation. Car wash shampoo contains emulsifier and it acts as bridging agent, lowering the interfacial tension of oil, resulting in very small oil droplet size (Alther, 1998). The additional emulsifier affects the size of oil-in-water emulsion. The increase of volume of emulsifier slightly causes the smaller oil droplet and greater stability (Vijay *et al.*, 1995). This very small emulsion droplet size causes a greater stability. Therefore, an emulsion is not settled by gravity force.

2.4 Pollutants in car wash wastewater

Wastewater from gas station is generated from various activities. Generally, high volume of wastewater are generate from car washing operation. Mixed lubricating oils and emulsifier are always found in car wash wastewater (Panpanit *et al.*, 2001). Thus, car wash wastewater is considered as hazardous industrial wastewater because it contains petroleum hydrocarbons, plasticizers, and washing agents. The example of car wash wastewater characteristics and their concentrations of organic pollutants are in table 2.1 and 2.2, respectively.

Table 2.1 Wastewater characteristic of car washing and other facilities in gas stations

Parameter	Car washing (Thailand) ^a	Commercial parking lot (USA) ^b	Radiator repair shop (USA) ^b	Gas station (Thailand) ^c
	Mean	Mean (SD)	Pretreated	Mean (SD)
pH	5.67 (1.13)	6.4 (0.4)	-	7.78 (0.18)
BOD (mg/L)	229.50 (79.54)	-	192	87.10 (8.22)
COD (mg/L)	497.75 (174.55)	171.7 (205.0)	-	193.2 (84.57)
DOC (mg/L)	-	40.1 (57.1)	-	-
TOC (mg/L)	-	-	-	-
Hydrocarbons (mg/L)	-	-	-	-
Oil & Grease (mg/L)	37.0 (16.82)	7.4 (10.3)	17	22.13 (7.23)
TSS (mg/L)	79.0 (19.97)	55.1 (71.6)	160	84.23 (59.06)
VSS (mg/L)	-	38.6 (60.5)	-	-
Total P (mg/L)	6.62 (0.98)	-	-	-
NO ₃ ⁻ (as NO ₃ ⁻ N) (mg/L)	-	1.0 (0.2)	-	-
Aluminium	-	2235	-	-
Cadmium	-	-	-	-
Chromium	-	-	-	-
Copper	-	103	192	-
Lead	-	45	287	-
Nickel	-	75	-	-
Zinc	-	2601	232	-

'-': no data,

a: adapted from Chawadech (1998),

b: adapted from Ratpakdi (2005),

c: adapted from Supawimol (2002)

Table 2.2 Concentrations of organic pollutants in car wash effluents from automatic vehicle washing facilities in Göteborg, Sweden (Paxéus, 1996).

Parameters	Concentration of pollutants (mg/L)					
	Light vehicles			Heavy vehicles		
	Mean	Median	Range	Mean	Median	Range
Total oil	291	242	10-1750	550	460	65-1200
COD	1253	1180	120-4200	4600	4500	1700-7500
Aliphatic hydrocarbons						
C8-C16	29	22	1-139	103.86	76.72	41-220
C17-C-30	0.6	0.4	<0.001	1.84	1.87	0.9-3.0
Aromatic hydrocarbons						
Benzene	0.01	0.01	<0.01-0.2	0.02	0.02	0.02-0.03
Toluene	0.08	0.05	<0.01-0.6	0.10	0.06	0.03-0.2
Naphthalene	0.17	0.13	<0.001-0.7	1.1	0.75	0.3-3
Biphenyl	0.015	0.005	<0.001-0.1	0.12	0.11	0.04-0.2
Dibenzofuran	0.001	0.002	<0.001-0.03	0.011	0.011	0.009-0.012
Phenathrene	0.005	<LOD	<0.001-0.03	0.021	<LOD	0.005-0.03
Pyrene	0.003	<LOD	<0.001-0.01	0.009	<LOD	0.01-0.02
Fluoranthene	0.003	<LOD	<0.001-0.01	0.004	<LOD	0.002-0.006
Plasticizers						
Diethyl -phthalate	0.005	0.01	2E-3-0.06	0.01	0.01	0.01-0.02
Dihexyl – phthalene	0.05	0.03	<0.001-0.15	0.03	0.21	<0.001-0.7
DEHP	0.52	0.38	0.03-4.1	1.50	1.30	0.04-3
Washing agents						
p-nonylphenol	0.60	0.26	0.01-4	0.43	0.41	0.1-0.8
2-Botoxyethanol	25	15	<0.001-270	15	17	<0.001-27

2.4.1 Lubricating oils

Lubricating oils are manufactured in various formulations for different applications. Typical lubricating oil is composed of 95% base stock and 5% additives (Stachowiak and Batchelor, 2005). Base stock is the term used to describe plain mineral oil. The physical properties of oil depend on its base oil. In most case it is chemically inert. There are three sources of base oil: biological, mineral and synthetic. However, mineral oils and synthetic oils are the most commonly used lubricants throughout industry. Some of the main characteristics of lubricants are summarized in Table 2.3. They are petroleum based and are used in applications where temperature requirements are moderate. Main toxic problem of lubricating oils is that they can contain as much as 4 to 8% polycyclic aromatic hydrocarbon before use (Wright, 1993).

2.4.1.1 Mineral oil

Typical applications of mineral oils are to gears, bearings, engines, turbines, etc. The major part of mineral oils consists of hydrocarbons with approximately 30 carbon atoms in each molecule. The structure of each molecule is composed of several aliphatic (straight) chains and cyclic carbon chains bonded together (Wright, 1993). The mineral oils are also impure. The impure nature of mineral oils results in a range of useful and harmful properties, e.g., trace compounds provide anti-oxidants properties and boundary lubrication properties but they also cause deposits which can impede lubrication (Stachowiak and Batchelor, 2005).

There are three basic chemical forms of mineral oil: paraffinic, naphthenic, and aromatic (Stachowiak and Batchelor, 2005). As shown in Figure 2.2 paraffinic implies straight chain hydrocarbons, naphthenic means cyclic carbon molecules with no unsaturated bonds and aromatic oils contain benzene type compounds. Oils are distinguished based on the relative proportions of paraffinic, naphthenic, and aromatic components present.

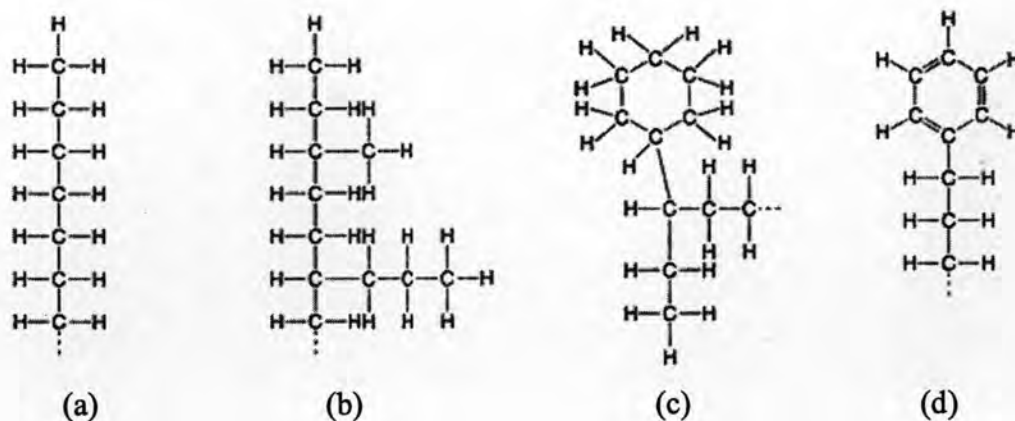


Figure 2.2 Types of mineral oils: a) straight paraffin, b) branched paraffin, c) naphthene, and d) aromatic (Stachowiak and Batchelor, 2005).

2.4.1.2 Synthetic oil

Synthetic lubricants were originally developed early this century by countries lacking a reliable supply of mineral oil. The use of synthetic oils increased gradually, especially in more specialized application for which mineral oils were inadequate (US army corps of engineer, 1999). Despite many positive features such as availability and relatively low cost, mineral oils also have several serious defects, such as oxidation and viscosity loss at high temperatures, combustion or explosion in the presence of strong oxidizing agents and solidification at low temperature (Stachowiak and Batchelor, 2005). This led to the development of synthetic lubricants that can withstand high temperatures without decomposing.

There are three basic types of synthetics lubricant currently in use (Stachowiak and Batchelor, 2005):

- Synthetic hydrocarbon which provide a lubricant that is similar in price to mineral oil but has superior performance,
- Silicon analogues of hydrocarbon which are resistant to extremes of temperature and vacuum but do not provide good adsorption or extreme pressure lubrication and are expensive,
- Organohalogens which can offer effective lubrication by adsorption and extreme pressure lubrication mechanisms and resist extreme of temperature or chemical attack, but are also expensive.

2.4.2 Emulsifier

Emulsifier is the major constituent in car wash shampoo. Normally, the emulsifier used in industries for cleaning purposes can be divided into two groups, e.g., anionic emulsifier and nonionic emulsifier. More than 90% of the wastewater in industrial streams contains nonionic emulsifiers (Alther, 1998). The emulsifier has contaminated surface water with an objectionable foaming property. Moreover, depending on their chemical structure, some emulsifiers are resistant to chemical attack and cause a higher COD in wastewater (Panpanit, 2001). In the case of non-ionic emulsifier such as polyethylene glycol, its chemical oxidation rate is slow and requires high dose of strong chemical such as ozone and long contact periods to achieve a significant removal (Langlais *et al.*, 1991).

Emulsifier or surfactant can cause harmful effects to bacteria in the environment. Hrenovic and Ivankovic (2007) investigated the toxicity of anionic and cationic surfactant to a pure culture of *Acinetobacter junii* that is a phosphate (P)-accumulating bacterium. They reported a high acute toxicity of sodium dodecyl sulfate (SDS) and hexadecyltrimethylammonium bromide (HDTMA) against *A. junii*. The estimated EC₅₀ values of the HDTMA for the inhibition of CFUs in the pure culture of *A. junii* was $3.27 \pm 1.12 \times 10^{-7}$ mol/L and for the inhibition of the P-uptake rates $2.47 \pm 0.51 \times 10^{-6}$ mol/L. For SDS, estimated EC₅₀ values for the inhibition of CFUs in the pure culture of *A. junii* was $5.00 \pm 2.95 \times 10^{-6}$ mol/L and for the inhibition of the P-uptake rates $3.33 \pm 0.96 \times 10^{-4}$ mol/L. The negative effects of these toxicants could greatly decrease populations of P-accumulating bacteria, as well as eukaryotic organisms, inhabiting activated sludge systems, which in turn could result in the decrease of the system efficiency. For nonionic surfactant, Anastácio *et al.*, reported that the estimated EC₅₀ values of the Genapol OX-80 (nonionic surfactant) was 0.5 mg/l.

2.5 Oil-in-water emulsion treatment systems

Most oily wastewater is a combination of free, non-emulsified oil, stable emulsified oil and insoluble solids, including grit, metal fines, carbon, paint, pigments and corrosion products. There are several methods for treatment oily wastewater. Typical oily wastewater treatment devices are API (American Petroleum Institute) gravitational oil separation and Corrugated Plate Interceptor (CPI).

API gravitational oil separation is the most widely used method for the separation of oil dispersion from a contaminated water stream. It is usually employed as a primary stage of separation where no more than a coarse separation is required. Oil and water mixture will separate into two discrete layers of oil and water if allowed to stand undisturbed for an extended period of time. The rate at which an oil droplet will rise to the liquid surface is governed by the density difference and can be described by Stokes Law (Tin and Cheng, 1995).

Corrugated plate separators consist of a fiberglass or steel separation tank, with inlet diffuser, oil and water baffles, and adjustable oil skimmer with a v-shaped hopper for settling out solids (Alther, 1998). A typical design consists of 50 plates, arranged in parallel on 3/4-in, centers. The plates are supported by a series of gutters, which isolate the collected contaminants from the wastewater stream. These plates are encased in a polyvinyl chloride or fiberglass pack, and placed in a separation tank at a 45-degree angle. As wastewater flows through the pack, oil droplets coalesce on the plate and rise to the surface. Sediments settle into the sludge hopper.

The API gravity separator removes oil globules of 150 microns or greater where CPI separators can remove oil droplets down to 30 microns (Plebon *et al.*, 2005). However, these two methods are inefficient in the removal of emulsified oil and dissolved oil from water (Tin and Cheng, 1995). Therefore, oil-in-water emulsion should be treated by the following systems:

2.5.1 Air flotation

Oil droplets and light solids can be removed from water by introducing small bubbles of air or gas into the water. The air bubbles act as scavengers, attaching themselves to oil droplets and solids, and floating them up to the liquid's surface. The two systems based on air flotation technique are dissolved air flotation (DAF) and induced air flotation (IAF).

There are three basic flow sheets for DAF process: (a) total pressurization of influent wastewater, (b) partial pressurization, and (c) recycle pressurization, where a stream consisting of 20-50% of clarified effluent flow is being recycled, pressurized and mixed with the raw influent (Zouboulis and Avranas, 2000). In this system, fine solid particles and oil droplets attach to small air bubbles, which are derived from

compressed air that is injected into the bottom of the flotation tank. As the air bubbles rise to the surface of the flotation tank, solids and oils can be skimmed off the surface

IAF uses a pump to induce air into the system, either on the suction side or with a venturi. The entrained air in water produces bubbles up to 1,000 μm dia (Alther, 1998). With IAF, which yields larger air bubbles than those provided by DAF, the percent removal is lower (Meysami and Kasaeian, 2005). However, IAF can remove larger amounts of oil-solids flocs, which are brought to the surface as froth.

In 2000, Zouboulis and Avranas reported that the destabilization of oil-in-water emulsion is successfully performed by the application of a combination of coagulation and dissolved-air flotation, which shows a synergistic enhancement for the effective separation of emulsified oil. The addition of cationic coagulant ferric chloride is necessary to allow effective agglomeration of individual droplets. The increase of droplet size and the hydrophobic nature of agglomerates, induced by the addition of anionic collector sodium oleate, are found to increase the effectiveness of flotation. This research showed that dissolved-air flotation system needs a pre-treatment such as addition of coagulant to increase the oil-in-water emulsion treatment efficiency.

2.5.2 Sequence Batch Biological Reactor (SBR) Process

The sequencing batch reactor is a large, open-top concrete tank or basin that is equipped with aerators and mixers. In the reactor, the waste cycles between aerated conditions, when the aeration and mixing equipment is running, and anoxic conditions, when the waste is not aerated. Cycling between an aerated and anoxic environment creates conditions favorable for microbes to concentrate phosphorus from the waste stream into microbial cell mass (Classen and Liehr, 2008). Zilverentant (1997) found that the use of powdered activated carbon (PAC) in combination with the SBR process gave no positive results. After an adaptation period, the following average characteristics were achieved COD removal in range from 85% to 90%, oil removal less than 100 mg/L by SBR (Zilverentant, 1997). However, biological processes are relatively slow reaction and oil always inhibits the biological system.

2.5.3 Filtration

Membrane filtration is playing a more prominent role in the treatment of oily wastes because it provides undeniable advantages: no chemical additives are needed to destabilize the emulsion, high COD removal efficiencies are achieved, and treatment facilities are quite compact and fully automated (Chang *et al.*, 2001). Furthermore, membrane permeates can be reused as make-up water for emulsification and discharged directly into a receiving water body (Panpanit *et al.*, 2001). Typical processes of the treatment of oily wastewater by previous investigators included ultrafiltration (UF), UF combined with ion exchange, microfiltration (MF), rotary disc membranes, and ceramic membrane (Chang *et al.*, 2001). There is little information available on the field application of the membrane system because most studies were performed with laboratory-scale experiments. Panpanit *et al.* (2001) was investigated the potential of Ultrafiltration (UF) and Nanofiltration (NF) membrane for treating oil-in-water emulsion from car wash wastewater. They found that nanofiltration achieved the TOC removal significantly higher than the ultrafiltration membrane. The average TOC removal was about 60-75% for three UF membrane types: PS-100, C-100, C-30, and 99% for NF membrane of DK type. Moreover, backwashing or flushing and subsequently disposal of suspended solids represent a major operational problem (Panpanit *et al.*, 2001).

2.5.4 Adsorption

Absorption is an interfacial phenomenon. Oil is selectively absorbed onto a solid surface and coalesces to form a layer only few molecules thick (Supawimol, 2002). The absorption process is dependent on a combination of factors including such forces as; adhesive, cohesive, electrical, surface tension, etc (Tin and Cheng, 1995). Contact between the oil and solids is usually achieved by passing an emulsion through a bed of the absorption solids in granular form. The adsorbent has been developed widely such as silica base material, organic polymer, polygorskite clay, pine and fire bark, etc. (Mansouri, 1997). The use of bentonite powder, pre-mixed with aluminium salts, polymers are alternatives for de-emulsification (Alters, 1998). The advantages of adsorption treatment system are good for polishing oil and emulsifier (less than 1 ppm) and low capital cost; whereas, the disadvantages are can

not use for high oil and emulsifier concentration, difficult in term of operation, regeneration of spent adsorbent, and sludge from spent adsorbent (Panpanit *et al.*, 2001). The review of oil sorbents and their applications are in section 2.6.

2.6 Oil sorbents and their applications

Media or sorbent are the most important parts of some wastewater treatment system. These materials have been developed by several manufactures to remove specific contaminants such as metal, oil and grease, or petroleum hydrocarbons.

2.6.1 Commercial sorbents

2.6.1.1 Bentonite

Bentonite is a type of clay consisting dominantly of smectite minerals. The important properties of bentonite include its ability to exchange cations, its swelling and hydration capacity, it could act as a binder, its permeability, viscosity and thixotropy (Sandhya and Tonni, 2003). Their sorption capabilities come from their high surface area and ion exchange capacities.

Bentonite, whose main clay constituent is montmorillonite, has an ion exchange capacity of 70-90 milliequivalent per 100 grams. The quaternary amine has a hydrophilic nitrogen end that is positively charged. This nitrogen ion can exchange into the clay, replacing sodium, potassium, calcium and other metals. When bentonite is placed into water, the amine chain stands perpendicular to the clay surface (montmorillonite clays have the shape of a rectangular platelet). The result is a hydrophobic, oil-loving end of the quaternary amine with a halide attached to it, extending into the water in an undissolved state (Althers, 1998). However, the use of bentonite requires the addition of conventional coagulant (alum and polyacrylamide) to enhance the removal of oil (Qiu *et al.*, 1995).

2.6.1.2 Activated carbon

Activated carbon is a form of carbon that is produced by a carefully controlled oxidation process to develop a porous carbon structure with a surface area greater than 500 m²/g (Clark and Lykins, 1995). Water treatment with activated carbon is

accomplished by percolating the water to be treated through fixed-adsorption beds of activated carbon (Sahairaksa, 1999). For a substance to adsorb onto activated carbon, it has to diffuse into the carbon's pores. Nonionic surfactants adsorb well onto carbon, especially in alkaline environments (Althers, 1998).

Activated carbon requires complexing agents to improve its removal performance for inorganic matters (Sandhya and Tonni, 2003). Normally, activated carbon is used for separation technology, i.e., color and organic compounds removal in waste and water treatments (Ahmad *et al.*, 2005). Despite of its prolific use, activated carbon is still assumed as an expensive material and not much work has been done in order to adsorb residual oil.

2.6.2 Non-commercial sorbents

2.6.2.1 Straw

The chemistry of the cereal straw is complicated and not fully understood. Straw consists mainly of three groups of organic compounds cellulose, hemicelluloses, and lignin (Staniforth, 1979). Straw also contain silica and fly ash. The chemical compositions of straw acted to adsorb pollutants with the pore of interval structure of cellulose, hemicelluloses, silica, and fly ash (Shairaksa, 1999).

2.6.2.2 Chitosan

Chitosan is a natural, non-toxic, biodegradable, high molecular weight biopolymer that derived from chitin by deacetylation of chitin using concentrated alkali at high temperature. Chitin is first prepared from shells of Crustacea at low-cost by removing other components, such as calcium, and proteins, by treatment with acids and alkalines. The chemical structures of chitin and chitosan are in Figure 2.3. Chitosan has many potential applications in food, cosmetics, and pharmaceutical industries because of its unique nutritional and physiochemical properties (Rinaudo, 2006). Chitosan has high proportions of amino function that provide novel binding properties for many pollutants in wastewater (Feng *et al.*, 2008). Chemically, it is a heterogeneous polysaccharide that consists primarily of acetylglucosamine (uncharged) and glucosamine ($pK_a \approx 6.3 - 7$) subunits (Dergunov *et al.*, 2004). At relatively low pH ($pH < 6.5$), chitosan is positively charged and tends to be soluble in

dilute aqueous solutions, but at higher pH, it tends to lose its charge and may precipitate from solution due to deprotonation of the amino groups (Dergunov *et al.*, 2004).

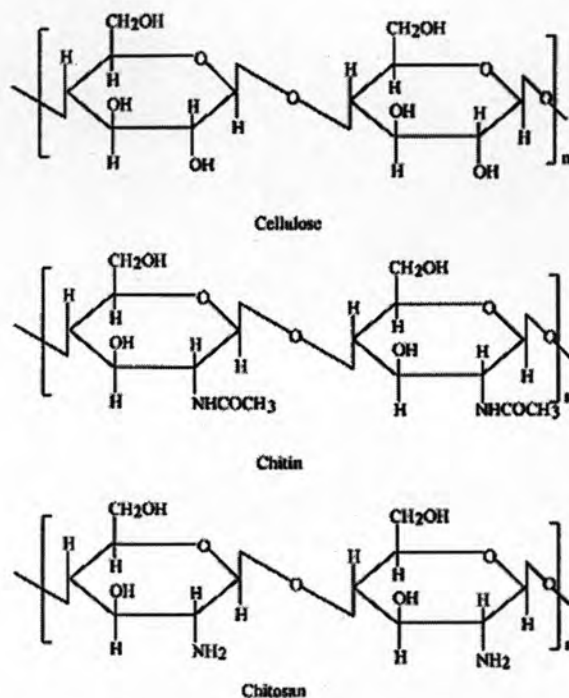


Figure 2.3 Structure of chitin, chitosan and cellulose (Majeti, 2000).

Chitosan is available in a variety of useful forms and its unique chemical biological properties make it a very attractive biomaterial. Key properties of chitosan are its ability to act as a cationic flocculent, humectant, viscosifier, and selective chelator of metal ions (Ahmad *et al.*, 2005). Chitosan has ability to be made into films, fibers, beads, powders and solutions, which lead to many commercial applications.

Chitosan has been recommended as a suitable coagulant resource material because of its excellent properties such as adsorption property and flocculating ability (Kumar, 2000). It has been used for various application such as; coagulation of colloidal particle, as a coagulant for suspended solids in food processing plants, peatland water treatment, rubber factory effluent treatment, as a chelator of heavy metals and flocculation of food emulsion waste and river silt (Ahmad *et al.*, 2006). Cationic properties and biological properties of chitosan are summarized in table 2.4.

Table 2.4 Cationic and biological properties of chitosan (Sandford, 1988)

Cationic properties	Biological properties
1. High charge density	1. non-toxic
2. Linear Polyelectrolyte	2. biodegradable
3. Excellent flocculent	3. natural polymer
4. Adheres to negatively charged surfaces	4. wound healing accelerator
5. Substantive to hair, skin	5. reduce blood cholesterol level
6. Chelates metal ions	6. immune system stimulant

In addition, chitin and chitosan are excellent natural adsorbents with high selectivities due to the following reasons (Mohan, 2007; Roussy *et al.*, 2005):

(1) Large numbers of hydroxyl and amino groups give chitosan high hydrophilicity.

(2) Primary amino groups provide cationic charge and high reactivity.

(3) The polymer chains of chitosan provide suitable configurations for efficient complexation with metal ions.

Ahmad *et al.* (2005) found that chitosan has the highest ability to adsorb residual oil in palm oil mill effluent compared to bentonite and activated carbon. The application of adsorbent in treatment system resulted in higher than 90% reduction of residual-oil content in the supernatant and suspended solid. In 2006, Ahmad *et al.* investigated the potential and effectiveness of chitosan compare to aluminum sulphate (alum) and poly-aluminum chloride (PAC) as a coagulant to coagulate suspended solid and residue palm oil. They found that chitosan was comparatively more efficient to alum and PAC which showed more than 95% removal of suspended solid and residue oil removal. These researches showed that chitosan has similar potential as conventional coagulants to treat oily wastewater from palm oil mill effluent. Moreover, it is a non-toxic and environmental friendly material.

2.7 Lubricating oil biodegradation

A large number of microbes have the potential to growing on hydrocarbons as sole carbon source, so biodegradation can be used to treat hydrocarbon containing wastewater. The right microbes are bacteria or fungi, which have the physiological

and metabolic capabilities to degrade the pollutants (Boopathy, 2000). A complex mixture of hydrocarbons is the main fraction in lubricating oil (Vazquez-Duhalt, 1989). There are many types of hydrocarbons varied from short-chain alkane to heavy hydrocarbons such as polycyclic aromatic hydrocarbons (PAHs) and long-chain alkane (Van Beilen and Funhoff, 2007). Thus, the fundamental lubricating oil degradation metabolism can be described by biodegradation pathways of each type of hydrocarbons. Most researches have focused on the biodegradation pathways of alkanes and PAHs.

2.7.1 Alkane degradation pathway

Many alkane degraders are able to grow on a wide range of alkanes (Van Beilen *et al.*, 2003). The initial step of alkane biodegradation is the action of alkane monooxygenase (Figure 2.4). The predominant mechanism of alkane degradation involves terminal oxidation to the corresponding primary alcohol, which is further oxidized by alcohol and aldehyde dehydrogenases. The resulting fatty acids enter the β -oxidation cycle. Alternatively, ω -hydroxylation by a fatty acid monooxygenase or alkane hydroxylase may take place, leading to dicarboxylic acids. Subterminal oxidation gives rise to secondary alcohols, which are oxidized to ketones. A Baeyer-Villiger monooxygenase converts ketones to esters, which are subsequently cleaved by an esterase to an alcohol and a fatty acid. Subterminal oxidation has also been detected for longer alkanes, e.g. in *Bacillus*, *Pseudomonas* (Britton, 1984), and more recently in *Rhodococcus* sp. Q15 (Whyte *et al.*, 1998). In some cases, both ends of the alkane substrate are oxidized, which has been exploited for the production of dicarboxylic acids by yeasts as well as bacteria (Van Beilen *et al.*, 2003).

Branched alkanes are less readily degraded in comparison to *n*-alkanes. Methyl branching increases the resistance to microbial attack since only few alkane degraders can overcome the blockage of beta-oxidation (Venosa *et al.*, 2003). Highly branched isoprenoid alkanes, such as pristane and phytane, which were earlier thought to be resistant to biodegradation, have also been shown to be readily biodegradable. Cycloalkanes, however, are particularly resistant to biodegradation. Complex alicyclic compounds such as hopanes and steranes are among the most persistent compounds of petroleum spills in the environment (Atlas, 1981).

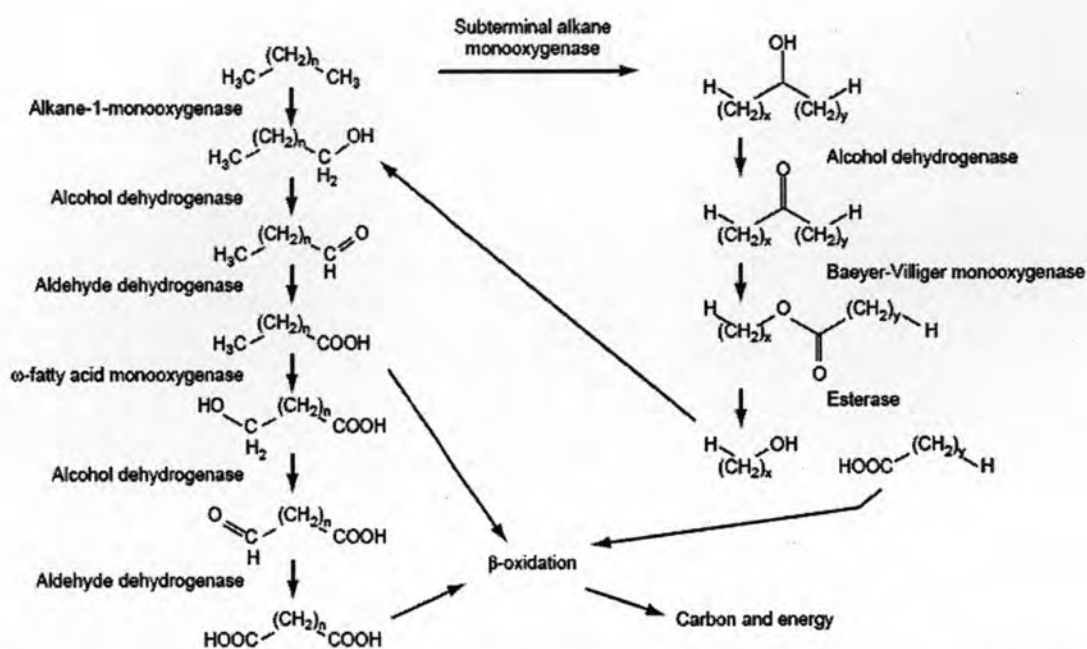


Figure 2.4 Aerobic biodegradation pathways of alkanes (Van Beilen *et al.*, 2003)

2.7.2 Aromatic degradation pathway

For the biodegradation pathways of aromatic compound, bacteria initially oxidize aromatic hydrocarbons to *cis*-dihydrodiols (Figure 2.5). The oxidation of aromatic compounds involves dioxygenase enzyme which incorporate two oxygen atoms from atmosphere into the aromatic nucleus. *cis*-Dihydrodiols are re-aromatized through a *cis*-dihydrodiol dehydrogenase to form dihydroxylated derivative. Then, *cis*-dihydrodiols are further oxidized to form catechols which are substrates for other dioxygenases that bring about enzymatic cleavage of the aromatic ring. Catechol can be oxidized via two pathways which are *ortho* pathway and *meta* pathway. Finally, ring cleavage results in the production of succinic, fumaric, pyruvic and acetic acids and aldehydes. All of these products are utilized by the micro-organism for the synthesis of cellular constituents and energy (Wilson and Jones, 1993).

Although the aromatics are generally more resistant to biodegradation, some low-molecular-weight aromatics such as naphthalene may actually be oxidized before many saturate (Venosa, 2003). Monoaromatic hydrocarbons are toxic to some microorganisms due to their solvent action on cell membranes, but in low concentrations they are easily biodegradable under aerobic condition. PAHs with 2-4

rings are less toxic and biodegradable at rates that decrease with the level of complexity. PAHs with five or more rings can only be degraded through co-metabolism, in which microorganisms fortuitously transform non-growth substrates while metabolizing simpler hydrocarbons or other primary substrates in the oil (Venosa, 2003).

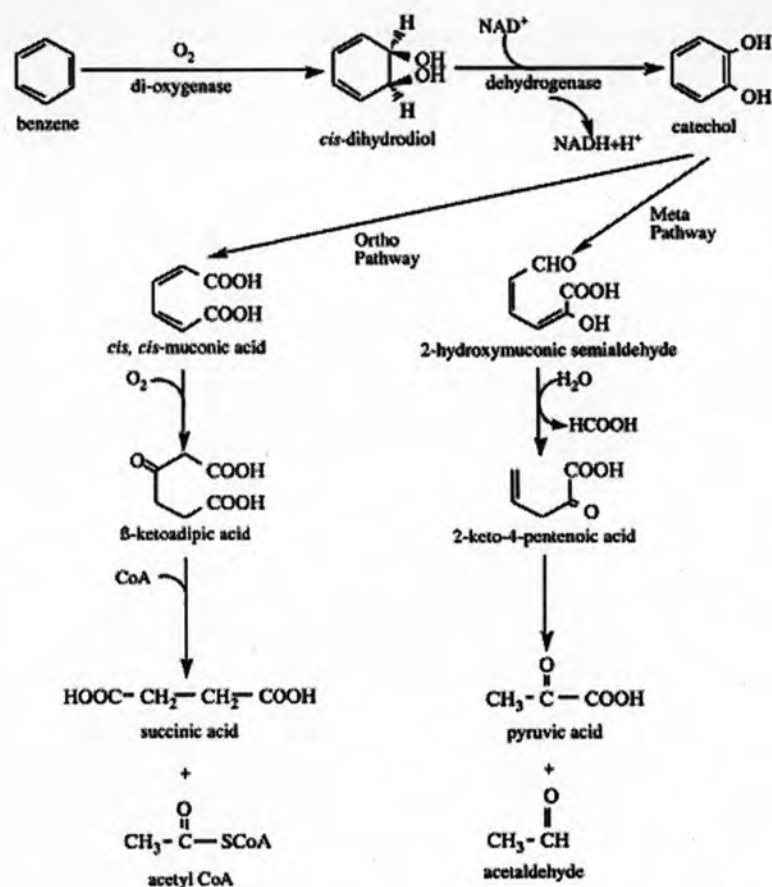


Figure 2.5 Aerobic biodegradation pathways of PAHs (Juhasz and Naidu, 2000)

2.8 Biological treatment of oily wastewater

Generally, conventional activated sludge (CAS) process is used to decompose organic substances in municipal wastewater because of its low cost and reasonable efficiency (Ahmad *et al.*, 2006). However, the characteristics of petroleum oil contaminated wastewater are different from municipal wastewater. For example, oil field wastewater is low in N and P compounds, thus CAS process cannot operate efficiently over the long term, as bulking and foaming tend to occur (Tellez *et al.*, 2002). The high salt concentrations of oil field wastewater also affects the metabolism

of the microorganisms in activated sludge, causing plasmolysis and attenuating endogenous respiration (Zhao *et al.*, 2006). Moreover, oily wastewater originating from road and rail car cleaning installation is known to be potentially toxic and inhibitory for biological treatment (Zilverentant (1997). Therefore, the development of new biological process is required for treatment of wastewater from gas station.

The use of oil sorbent materials, for example chitin, chitosan, bentonite, and activated carbon, can enhance oil contaminated water biodegradation. Setti *et al.* (1998) studied the degradation of heavy oil by a *Pseudomonas* sp. in an aqueous system containing natural and synthetic sorbents. They found that all three natural sorbents tested i.e. keratin, chitosan, and chitin was capable of adsorbing large amounts of heavy oil as well as enormously increasing the n-alkane biodegradation rate. In the presence of natural sorbents, n-alkane biodegradation was complete after 7 days while in their absence the same result took 40 days.

Another method for enhancing the biodegradation of pollutants includes immobilization method which offers a promising potential for the improvement of efficiency of bioprocess. One of the most widely used techniques for cell immobilization is cell entrapment, in which the microbial cells are enclosed in a polymeric matrix which is porous enough to allow the diffusion of substrate to cells and of product away from the cells (Jianlong and Yi, 1998).

The immobilization of microorganisms to the bedding material can be divided into two main immobilization process (Cohen, 2000):

- (1) The self-attachment of microorganisms to the filter bedding material, which is defined as 'attached growth'

The main microbial structure which is involved in attachment is the glycocalyx that consists of extracellular polysaccharides. It has several forces are involved in microbial attachment to a surface. Usually, none of the forces could be considered as the dominant force. The strength of the attachment generally depends on environmental conditions, microbial species, material surface properties and fluid properties.

Advantages of attached growth systems are easy to provide, low diffusion restriction, great variety of microorganisms present within attached biofilms, and population changes within attached biofilms when waste sources changes with time.

The systems which treat wastewater with attached biofilm can be divided, generally, into four main groups according to the main mode of operation, are the following:

- Trickling filter; the wastewater is uniformly dosed over the bedding material and trickles downward through it.
- Rotating biological contactors; circular discs made of a lightweight material are half submerged in the wastewater and rotate at low speed.
- Submerged biofilters; the bedding material is completely submerged in the treatment basin. Usually, air diffusers located at the bottom of the bedding material aerate the basin.
- Fluidized bed reactors; the biofilm is grown on particle of medium such as sand. The wastewater is pumped through the reactor at a high rate, thereby suspending the medium particles.

(2) The artificial immobilization of microorganisms to a support material.

There are many methods for artificial immobilization of microorganisms to a support material as described below;

- Microencapsulation; the microencapsulation method consists of wrapping droplets containing microorganism with a thin membrane. The microorganisms can freely move within their own capsule, consuming substrates that penetrate through the membrane cover.
- Membrane separation; the main principle of this method is to separate the microorganisms from the bulk fluid by the use of sheets of membrane. The membrane will allow the substrates to penetrate to the microorganism's zone, while preventing the microorganism from mixing with the fluid to be treated.
- Covalent bonding and covalent crosslinking; the outer surfaces of microbial cells contain large quantities of a variety of reactive groups. The covalent bonding method includes the creation of covalent bonds between those reactive groups and different ligands on the bedding material. The covalent crosslinking method is support free and involves the joining of microorganisms to each other to form a large, three-dimensional complex structure. This structure is used as a bedding material.

- Entrapment within polymers; this method consists of trapping microorganisms within a three-dimensional polymer matrix. The pores in the matrix are smaller than the microbial cells, keeping them trapped within the material, but the pores still allow the penetration of substrates through the polymer matrix towards the trapped microorganisms.

Kermanshahi pour *et al.* (2005) studied the biodegradation of petroleum hydrocarbons in an immobilized cell air lift bioreactor. An “immobilized cell air lift bioreactor” was used for the aerobic bioremediation of simulated diesel fuel contaminated ground water and tested with *p*-xylene and naphthalene in batch and continuous regimes. The innovative design of the experiments consists of two stages. At the first stage “immobilized soil bioreactor” (ISBR) was used to develop an efficient microbial consortium from the indigenous microorganisms, which exist in diesel fuel contaminated soil. The concept of ISBR relies on the entrapment of the soil particles into the pores of a semi-permeable membrane, which divides the bioreactor into two aerated and non-aerated portions. The second stage involves inoculating the “immobilized cell air lift bioreactor” with the cultivated microbial consortia from the first stage. Immobilized cell air lift bioreactor has the same configuration as ISBR except that in this bioreactor instead of soil, microorganisms were immobilized on the fibers of the membrane. Results of the present study show a high biodegradation rate of *p*-xylene and naphthalene in the “immobilized cell air lift bioreactor”.

Gentili *et al.* (2006) demonstrated that the removal of crude oil from seawater microcosm that contained hydrocarbon-degrading bacterial strain (QBTo) immobilized in chitin and chitosan had greater efficiency than the microcosm with free QBTo cells. The researchers mentioned that the principle reason for this result was due to the protective effect of carrier material and the biofilm structure that the cells have developed on it. The QBTo strain has been characterized by 16s RNA gene sequences and proposed as *Rhodococcus corynebacterioides*. The bioremediation experience also showed that the native population in the positive control microcosm (seawater without QBTo) could not produce any significant reduction in the hydrocarbon concentrations.