



CHAPTER II

LITERATURE REVIEW

2.1 Palm oil industry in Thailand

Oil palm plantations and the palm oil industry in Thailand are comparatively young. In 1979, oil palm plantations covered 155,000 rai (2.5 rai = 1 acre) and increased to 950,000 rai by 1993 with the production yield of 1,530,000 tons of fresh fruit bunches (FFB), valued at about 2,600 million Baht. More than 98% of the plantations are in Southern Thailand, particularly in the provinces of Krabi, Surat Thani, Chumporn, Trang, Satun, and Songkhla. The palm oil industry has developed very fast in recent years. In 1995 there were 49 palm oil mills in operation with an overall production capacity of 405,000 tons of crude oil/year. Both the plantations and the mills have a high potential for further optimization in terms of agricultural and production technology. Because of the concentration of palm oil plantations and mills in Southern Thailand, this industry is dominant in terms of work provision and the generation of income to local people.

2.2 Oily wastewater treatment technologies

The treatment of oily wastewater has the following two main objectives:

1. To adjust the existing insufficient quality of oily wastewater to a load level (i.e. oil and grease) suitable to the individual fertilizing conditions; here partial treatment would be sufficient. This treatment will not significantly reduce the content of dissolved mineral substances.

2. To meet the requirements for effluent discharge into surface waters; in this case full treatment would be necessary.

And after the treatment, the effluent has to be corresponded to the standard as shows in Table 2.1.

Table 2.1 Effluent standard for palm oil mill industry (Bureau of Industrial Environment Technology, Department of Industrial Works, Ministry of Industry, 1997)

Parameter	Unit	standard
BOD	mg/L	< 100
COD	mg/L	< 1,000
Suspended solids	mg/L	< 150
Oil & Grease	mg/L	< 25
Total Kjeldahl Nitrogen (TKN)	mg/L	< 50
pH		5 to 9
Temperature	°C	< 40

2.2.1 Physio-chemical treatment

The main technique for separating fat and oil from wastewater is the grease trapping method (gravity separation of floatable fat/oil). A fat trap is a rectangular or circular vessel through which the wastewater passes under laminar flow conditions at a rate that allows the fat/oil particles to rise to the surface by the time they approach the outlet end of the trap. Alternatively, oil/water separators (tilted plate separators–TPS), flotation systems, and physical–chemical treatments have been employed to reduce wastewater lipid content. The TPS was developed in the petrochemical industry and takes advantage of the fact that its surface area, rather than the depth, is the most important factor in the oil separation. Physical–chemical treatments reduce the organic load by protein and fat precipitation or flotation with different chemical compounds. Aluminum sulfate, ferric chloride, or more commonly, lime, may be used to break the fat emulsion and coagulate fat particles, which can then be readily separated by flotation or sedimentation. This involves the addition of low levels of polyelectrolyte (0.5–5.0 mg/L) to the wastewater. The widespread utility of physical–chemical treatments however is questionable because the cost of reagents is high, the removal efficiency of the dissolved and/or emulsified oil

and grease is low, and highly problematic sludge is produced when flocculating agents such as polyelectrolytes and salts are used (Commarota and Freire, 2006).

2.2.2 Biochemical treatment

Chemical hydrolysis with NaOH increases the ratio of soluble COD to total COD and reduces the volatile solid content during anaerobic digestion. The lipid on waste activated sludge can be reduced by 28% with chemical (HCl, NaOH, and Ca(OH)₂) hydrolysis pretreatment, but there is no effect of biological (acid fermentation) hydrolysis pretreatments on the sludge because the lipid portion of waste activated sludge is difficult for the organic fraction to hydrolyze. However, the alkali added to neutralize acidic wastewaters and to maintain an ideal alkalinity level, enhanced the breakdown of fat and enhancing fat biodegradation (Commarota and Freire, 2006).

Masse *et al.* (2001) studied the effect of alkaline and enzymatic pretreatments on the solubilization and size reduction of fat particles in slaughterhouse wastewater. They concluded that the high additions of NaOH required (more than 12 g/l) result in increasing pH, and alkaline pretreatment is a poor choice for the hydrolysis pretreatment of fat particles. Moreover, they also studied with lipase LG-1000 and their conclusions were that bacterial lipase LG-1000 was also efficient in reducing the average fat particle size, but high doses (>1000 mg/L) and lipase PL-250 was the best pretreatment for hydrolyzing fat particles in slaughterhouse wastewater.

2.2.3 Biological treatment

The biological treatment of fats and oils under thermophilic conditions is advantageous because of the favorable changes in most physical properties of hydrophobic compounds at high temperatures. In the liquid state, these substances become more accessible to microorganisms and their lipolytic enzymes. Both diffusion coefficients and the solubility of fatty acids in aqueous media increase significantly with rising temperatures, allowing a better mass transfer.

Becker *et al.* (1999) studied the biodegradation of olive oil and the treatment of the lipid-rich wool scouring wastewater under aerobic thermophilic

(65°C) conditions using the newly isolated strain *Bacillus thermoleovorans* IHI-91. They concluded that lipid degradation rates obtained under thermophilic conditions were markedly greater than that obtained with mesophilic processes.

2.2.4 Treatment using microorganisms, surfactants and/or commercial enzymes

Nowadays, there are many developments in using microorganisms and/or enzymes for the biological treatment of effluents with high fat and oil concentrations; for example, a lipase from *Candida rugosa* (Lipase-MY) for fat removal in equipment of effluent treatment plants and bioorganic catalytic formulations that rapidly break down organic contaminants including fats, oils, and greases due to the difficulty of successfully treating these substances with conventional products and equipments. Applications of hydrolytic enzymes, especially, lipases in wastewater treatment were summarized by Mendes *et al.* (2006). Lipid degradation in grease-traps that involves the addition of lipase-producing bacteria and which are immobilized on non-biodegradable support material and immersed in the surface of grease-traps, achieve hydrolysis rates greater than 90%. They mentioned the use of a mixture composed of emulsifiers, microorganisms, and enzymes (protease, amylase, lipase, cellulose, and pectinase) for lipid removal from wastewaters produced by the cleaning of industrial machines.

Another alternative method to deal with high fat content in wastewater is the use of surfactants to facilitate the biodegradation by dissolving the fat and oils. Biosurfactants can easily be incorporated directly into the biological process. Nakhla *et al.* (2003) demonstrated the effectiveness of a biosurfactant derived from cactus (BOD-Balance TM) in improving the anaerobic digestion of high strength oil and grease rendering wastewater within a mesophilic temperature range. They found an improvement in the overall anaerobic biodegradability with total and soluble COD reduction of 62% and 74%, respectively.

Enzymes and pure cultures have also been used to increase hydrolysis during or prior to biological treatment processes. Cail *et al.* (1986) evaluated an enzymatic mixture containing protease, amylase, cellulase, and lipase, and *Bacillus subtilis* spores on wool scouring wastewater with high lipid content; this mixture in-

creased the COD reduction from 59% to 78%, increased grease removal from 47% to over 70%, and improved solids reduction from 34% to over 70%. Lipases have also been used for the degradation of wastewater contaminants from olive oil processing. De Felice *et al.* (2004) cultivated the yeast *Yarrowia lipolytica* ATCC 20255 on wastewater from an olive oil mill under batch culture conditions. They found that the yeast was capable of reducing the COD value (100–200 g/L) by 80% in 24 h.

2.3 Problems in the biological treatment of effluents with high fat content

2.3.1 Operational problems

In aerobic bioreactors, oils and greases block the gas transfer required for the biological degradation. Specifically they reduce the oxygen transfer rates to the biological microbial consortium by the formation of a lipid coat around the floc. Oils and greases cause problems in the pumping and aeration systems that arise due to the development of filamentous microorganisms (*Sphaerotilus natans*, *Thiothrix*, *Beggiatoa*, *Nocardia* and *Microthrix* *genuses*). These microorganisms are involved in the formation of scum and stable foams on the surface of the aeration tank. Moreover, high oils and greases contents also generate agglomerates or pellets inside the secondary sludge flocs, which hinders sedimentation, generating unpleasant odors, and reducing the efficiency of the treatment station and oils, and greases may solidify at lower temperatures and thus cause operational damage associated with clogging. This, too, can cause unpleasant odors (Cammarota and Freire, 2006).

2.3.2 Effect of long chain fatty acids on the biological treatment

In addition to the necessity of longer residence times for fat particles to be degraded, and the design of a proper bioreactor to avoid undesirable fat accumulation, long chain fatty acids (LCFA) produced from the hydrolysis of oils and greases can exert toxic effects and inhibit anaerobic bacteria. Fats in wastewaters produce glycerol and LCFA (saturated fatty acids with 12–14 carbon atoms and unsaturated fatty acids with 18 carbon atoms). Glycerol was found to be a non-inhibitory compound and LCFA have been reported to inhibit the activity of various

microorganisms, so glycerol and LCFA content affects the amount of hydrogen produced by acetogenic bacteria (Cammarota and Freire, 2006).

Vidal *et al.* (2000) evaluated the influence of absolute and relative concentrations of carbohydrates, fats, and proteins on the anaerobic biodegradability of dairy wastewaters. They found that the anaerobic biodegradation rate of wastewaters rich in fat was slower than that of wastewaters with a low fat content, because of the slower rate of the fat hydrolysis step. However, this reduced hydrolysis rate prevented volatile fatty acid (VFA) accumulation and as a result the overall process was favored.

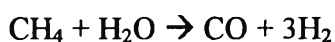
2.4 Hydrogen production

2.4.1 Hydrogen production from chemical method

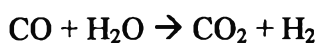
2.4.2.1 *Steam reforming of natural gas*

Steam reforming of natural gas is currently the least expensive method for producing hydrogen and is used for about half of the world's production of hydrogen. Steam, at a temperature of 700–1,100°C, is mixed with methane gas in a reactor with a catalyst at 3–25 bar pressure.

The formula for this process is:



It is usually followed by the shift reaction:

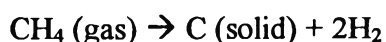


The percentage of hydrogen to water is 50%.

2.4.2.2 *Thermal cracking of natural gas*

H₂ can be produced via the thermal decomposition of CH₄. However, this method requires a high reaction temperature, about 2000°C.

The reaction of this process is:



2.4.2.3 *Coal gasification*

Gasification of coal is the oldest method for the production of hydrogen. Generally, coal is heated to 900°C with a catalyst and without air. There are also more complex ways of gasifying coal. These techniques are similar in that

by using steam and oxygen at temperatures over 1400°C, carbon is changed into H₂, CO, and CO₂. In addition, these create some sulfur and nitrogen that must be treated. It is almost twice as expensive to produce hydrogen from coal as from natural gas.

2.4.2.4 Electrolysis of water

Electrolysis uses an electric current to split water into hydrogen at the cathode (+) and oxygen at the anode (-). If renewable energy is used to produce electricity, which is used to split the water into hydrogen and oxygen, the hydrogen will be an even cleaner form of energy. The efficiency of the electrolyzer is an important factor because the consumption of energy makes up 80-90% of the production costs at an electrolysis plant.

Disadvantages of this method are that it needs to have a high temperature heat source at above 2500 K for a reasonable degree of dissociation possibility, recombination of H₂ and O₂, or end up with an explosion. H₂ from electrolysis is extremely pure, but expensive at the same time; being ten times more costly than from steam reforming of natural gas.

2.4.2.5 Photoelectrochemical systems of water

Photoelectrochemical systems use semi-conducting materials (like photovoltaics) to split water using sunlight. This is the cleanest way to produce hydrogen, by using sunlight to directly split water into hydrogen and oxygen. Multi-junction cell technology developed by the photovoltaic industry is being used for photoelectrochemical (PEC) light-harvesting systems that generate sufficient voltage to split water and are stable in a water/electrolyte environment.

2.4.2.6 Direct thermal decomposition or thermolysis of water

Thermal water splitting uses a high temperature (approximately 1000°C) to split water. Concentrated solar energy can also be used to generate temperatures of several hundreds to over 2000 degrees, at which thermochemical reaction cycles can be used to produce hydrogen. At such high-temperature, high-flux solar driven thermochemical processes offer a novel approach for the environmentally benign production of hydrogen. Very high reaction rates at these elevated temperatures give rise to very fast reaction rates that enhance the production rates significantly and more than compensate for the intermittent nature of the solar resource.

2.4.3 Hydrogen production from biomass

The problem of limited amounts of fossil fuel, increasing demand and the crisis in the Middle East, is pushing the price of fossil fuel to increase rapidly. Moreover, combustion of fossil fuel emits greenhouse and toxic gases (such as CO₂, SO_x, NO_x, and other pollutants), causing global warming and acid rain. Biomass is one of the most renewable resources to gain energy. It is formed by fixing carbon dioxide in the atmosphere during the process of plant photosynthesis. But one of the major drawbacks is the low efficiency in utilization, such as cooking and heating through biomass burning with 10% and 30%, respectively. The efficient way is to convert biomass to gaseous and aqueous fuels, electricity, and especially hydrogen. The major problems in biohydrogen production from waste are the low rates and yields. Large reactor volumes are required due to the low production rate. Low yields and rates may be overcome by selecting and using more effective organisms or mixed cultures, developing more efficient processing schemes, optimizing the environmental condition, improving the light utilization efficiency, and developing a more efficient photo-bioreactor (Kapdan and Kargi, 2006).

Hydrogen production from biomass can be divided into thermochemical and biological processes. Pyrolysis and gasification are the two thermochemical processes. Direct biophotolysis, indirect biophotolysis, biological water-gas shift reaction, photo-fermentation, and dark-fermentation are the five biological processes (Ni *et al.*, 2006).

2.4.3.1 Biomass pyrolysis

Biomass pyrolysis is the heating of biomass at high temperature (650–800 K) and low pressure (0.1–0.5 MPa) in the absence of air (O₂) to convert into gaseous, liquid and solid products.

Gaseous products include H₂, CH₄, CO, CO₂, and others, depending on the nature of the biomass.

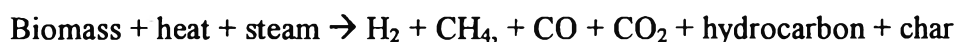
Liquid products are tar and oil.

Solid products are mainly composed of char, carbon, and other inert materials.

Although most pyrolysis processes are designed for biofuel production, hydrogen can be produced with high temperature, high heating rate, and sufficient volatile phase residence time.

2.4.3.2 Biomass gasification

Gasification of biomass under partial oxidation and high temperature (above 1000 K) with moisture content less than 35% gives gas and charcoal. Finally charcoal is reduced to H₂, CH₄, CO, and CO₂ as follow:



The problem of biomass gasification is the production of tar and ash; tar cause formation of tar aerosols and polymerization to more complex structures but ash causes deposition, sintering, slagging, fouling, and agglomeration. If biomass has moisture content over than 35%, the gasification can be done under the supercritical water condition with heating water to a temperature above its critical temperature (647 K) and compressing it over its critical pressure (22 MPa). Then the biomass is rapidly decomposed to small molecules and gases with no tar and ash formation.

2.4.3.3 Direct biophotolysis

Direct biophotolysis is a biological process to produce hydrogen using a microalgae photosynthetic system to convert solar energy into chemical energy in the form of hydrogen with an overall conversion of 10%. This conversion process can be expressed as:



2.4.3.4 Indirect biophotolysis

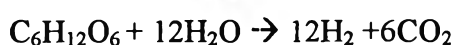
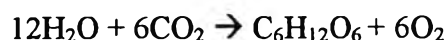
Hydrogen production from indirect biophotolysis involves four steps:

1. Biomass production by photosynthesis.
2. Biomass concentration.

3. Aerobic dark fermentation yields 4 moles hydrogen per mole of glucose and 2 moles of acetate.

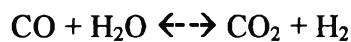
4. Conversion of 2 moles of acetate into hydrogen.

In typical indirect biophotolysis with *Cyanobacteria*, hydrogen production can be expressed as in the following reactions:



2.4.3.5 *Biological water-gas shift reaction*

Biological water-gas shift reaction is the same reaction as the thermochemical water-gas shift reaction but it is done by bacteria. Some photolytic bacteria, such as *Rhodospirillum rubrum* can survive in the dark by using CO as carbon source to generate adenosine triphosphate (ATP) by coupling the oxidation of CO to the readdition of H^+ to H_2 as follows:



In equilibrium, the main products are CO_2 and H_2 , and this reaction is thermodynamically favorable for hydrogen production with ΔG° of -20.1 kJ/mol.

2.4.3.6 *Photo-fermentation*

The production of hydrogen through the action of photosynthetic bacteria using solar energy and biomass or organic acid is called the photo-fermentation process. Some waste, such as industrial and agricultural wastewater, is used in this process, but the main drawbacks of this process are the high energy demands of hydrogenase enzyme, low solar energy conversion efficiency, and the large area required for the elaborate anaerobic photobioreactor.

2.4.3.7 Dark-fermentation or anaerobic digestion

Dark fermentation is the fermentation of anaerobic bacteria on carbohydrate-rich substrates with no requirement of solar energy to produce hydrogen. The products of dark fermentation are mostly H₂ and CO₂, with some CH₄ or H₂S depending on the reaction process and substrate used. With glucose as the substrate, H₂ is produced as follows:



With high hydrogen production by dark-fermentation, low pH values, high hydraulic retention time (HRT), and low partial pressure of H₂ are required. Because of no requirement of solar energy, hydrogen production by dark-fermentation does not use much area and is not affected by the weather conditions.

For the degradation pathways as shows in Figure 2.1, in hydrolysis step, fermentative bacteria hydrolyze protein to polypeptide and amino acids, while lipids are hydrolysed via β -oxidation to long-chain fatty acids (LCFAs) and glycerol and polycarbohydrates to sugars and alcohols. After that, fermentative bacteria convert the intermediates to volatile fatty acids (VFAs), H₂, and CO₂. Ammonia and sulphide are the by-products of amino acid fermentation. Hydrogen-producing acetogenic bacteria metabolise LCFAs, VFAs with three or more carbons, and neutral compounds larger than methanol to acetate, H₂, and CO₂. Methanogens ultimately convert acetate, H₂ and, CO₂ to CH₄ and CO₂ (Salminen and Lintala 2002). For anaerobic digestion, the optimum condition to produce H₂ was reported at 37°C (Fang *et al.*, 2006 and Chitababu *et al.*, 2006) and pH of 5.5 (Fan *et al.*, 2006).

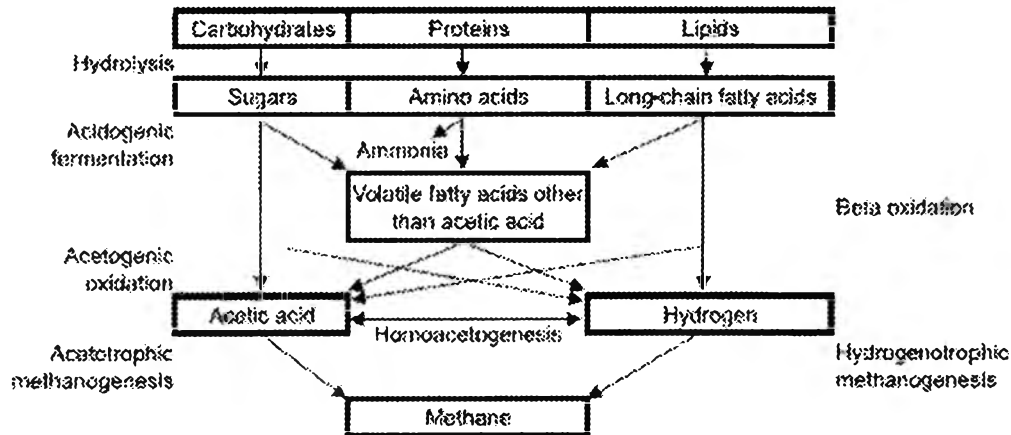


Figure 2.1 Degradation pathways in anaerobic degradation (Salminen and Rintala 2002).

2.5 Hydrogen utilization

Today, environmental pollution is a great concern to the world, mainly due to rapid industrialization and urbanization. So, increasing focus is being placed on clean energy alternatives for satisfying growing energy demand. Hydrogen is considered as a clean energy because it has no emission and moreover, it has much higher in energy content per weight than the others fuel as shown in Table 2.2. Hydrogen has various other uses (Das and Veziroglu, 2001), which can be broadly divided into the following categories:

1. As a reactant in hydrogenation processes: hydrogen is used to produce lower molecular weight compounds, saturate compounds, crack hydrocarbons, or to remove sulfur and nitrogen compounds.
2. As an O₂ scavenger: hydrogen is used to chemically remove trace amounts of O₂ to prevent oxidation and corrosion.
3. As a fuel in rocket engines.
4. As a coolant in electrical generators to take advantage of its unique physical properties.

Table 2.2 Energy content per weight with different fuel (Ni *et al.*, (2006))

Fuel	Energy content (MJ/kg)
Hydrogen	120
Liquified natural gas	54.4
Propane	49.6
Aviation gasoline	46.8
Automotive gasoline	46.4
Automotive diesel	45.6
Ethanol	29.6

2.6 Surfactants and their effect on biodegradation.

2.6.1 Background of surfactant

A surfactant, or surface active agent, is a substance that, when present at low concentration in a system, has the property of adsorbing onto the surfaces or interfaces of the system and reduces the surface or interfacial free energies of those surfaces or interfaces. Molecular structure of a surfactant consists of lyophobic or hydrophilic groups; the group that has very little attraction for the non-polar solvent and lyophilic or hydrophobic group; the group that has strong attraction for the non-polar solvent. This structure is known as an amphipathic structure. The hydrophobic groups usually consist of long chain hydrocarbons and less often halogenated or oxygenated hydrocarbons or siloxane chains. The hydrophilic group is an ionic or polar group. Depending on the nature of the hydrophilic group (Rosen, 2004), surfactants are classified as

1. Anionic surfactant: the surface active portion of the molecule bears a negative charge, such as RCOO^-Na^+ (soap) and $\text{RC}_6\text{H}_4\text{SO}_3^-\text{Na}^+$ (alkylbenzene sulfonate).

2. Cationic surfactant: the surface active portion of the molecule bears a positive charge, for example, $\text{RNH}_3^+\text{Cl}^-$ (salt of a long chain amine) and $\text{RN}(\text{CH}_3)_3^+\text{Cl}^-$ (quaternary ammonium chloride).

3. Zwitterionic surfactant: Both negative and positive charges; are presented in the surface active portion; for example $\text{RN}^+\text{H}_2\text{CH}_2\text{COO}^-$ (long chain amino acid) and $\text{RN}^+(\text{CH}_3)_3\text{CH}_2\text{CH}_2\text{SO}_3^-$ (sulfobetaine).

4. Nonionic surfactant: the surface active portion bears no apparent ionic charge; such as $\text{RCOOCH}_2\text{CHOHCH}_2\text{OH}$ (monoglyceride of long chain fatty acid), $\text{RC}_6\text{H}_4(\text{OC}_2\text{H}_4)_x\text{OH}$ (polyoxyethylenated alkylphenol), and $\text{R}(\text{OC}_2\text{H}_4)_x\text{OH}$ (polyoxyethylenated alcohol).

As shows in Figure 2.2, when adding a surfactant to water, a surfactant molecule may dissolve as a monomer, adsorb to surface or interface with its hydrophobic end pointing away from the water, or aggregate with other surfactant molecules into clusters called micelles with the minimum concentration of a surfactant of critical micelle concentration (CMC). Another of the most important properties of a surfactant that is directly related to the micelle formation is solubilization. Solubilization can be defined as a spontaneous dissolving of a substance (solid, liquid, or gas) by reversible interaction with the micelle of a surfactant in the solvent to form a thermodynamically stable isotropic solution with reduced thermodynamic activity of the solubilized materials. And the solubilization into an aqueous media is a major application, such as the formulation of products containing water insoluble ingredients, removal of oily soil, and enhancing oil recovery. Lin *et al.* (2007) studied the two- and three-phase biodiesel emulsion. They found that the nonionic surfactants, Tween 80 and Span 80, were effectively used to increase the affinity and reduce interfacial tension between water and oil phase. Lif and Holmberg (2006) also studied water in diesel emulsion using Tween 80 and Span 80 as an emulsifier. They indicated that both Tween 80 and Span 80 are not effective emulsifiers when used alone, but the mixture of them becomes a very good emulsifier.

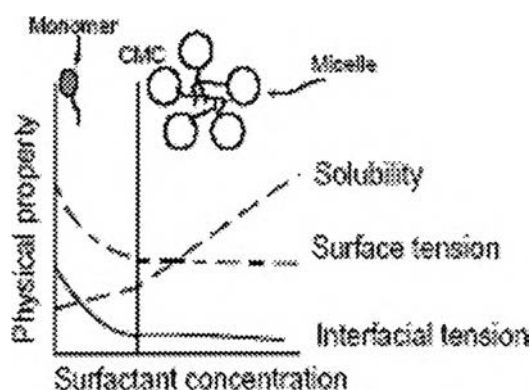


Figure 2.2 Diagram of the variation of surface tension, interfacial and solubility with surfactant concentration.

2.6.2 Surfactant-enhanced biodegradation of hydrocarbon compounds

Surfactants are amphipathic molecules which have hydrophilic and hydrophobic parts in their structures. Due to their outstanding properties, such as reduction in surface or interfacial tension, micellization, and solubilization properties, surfactants can be used for solubilizing hydrocarbon or organic compounds in a system to increase degradation in surfactant micelles. The second mechanism involves the metabolization of contaminants from soil (Rosen, 2004).

Lee *et al.* (2005) studied *in situ* flushing to remediate soil and groundwater at a diesel contaminated area. They found that about 88% of the initial total petroleum hydrocarbon (TPH) was removed from the pilot site by 2% sorbitan monoleate (nonionic surfactant) solution flushing.

Attavavuthichai *et al.* (2005) studied biodegradation of oil sludge in sequencing batch reactor (SBR) at ambient temperature by using polyethylene sorbitan monoleate or Tween 80 to solubilize the oil sludge. They concluded that the optimum surfactant concentration and oil loading rate (OLR) are 0.1% w/v and 4 kg/m³d, respectively. Moreover, they found that 90% of oil removal was reached.

Comchumpoo *et al.* (2006) studied the effect of surfactant concentration, oil loading rate, and number of operation cycle per day on the biodegradation of oil sludge in SBR at ambient temperature. They found that the optimum surfactant concentration, oil loading rate, and number of operation cycle per day are 0.2% w/v,

1 kg/m³d, and 1 cycle operation per day. And at these conditions, 80% of oil removal could be reached.

2.6.3 Surfactant biodegradation

The biodegradability of a surfactant increases with increased linearity of the hydrophobic group and is reduced, for isomeric materials, by branching in that group; particularly by terminal quaternary carbon branching. A single methyl branch in the hydrophobic group does not change the biodegradation rate, but additional ones do. In isomeric alkylbenzene and alkylphenol derivatives, degradation decreases as the phenyl group is moved from a position near the terminal end of a linear alkyl group to a more central position. And in polyoxyethylene (POE) nonionics, biodegradation was retarded by an increase in a number of oxyethylene groups. The inclusion of oxypropylene and oxybutylene groups in the molecule tends to retard biodegradation. Secondary ethoxylates degrade more slowly than primary ethoxylates, even though both have linear hydrophobic groups (Rosen, 2004).

Jahan *et al.* (1999) studied the influence of nonionic surfactants on the biodegradation of phenanthrene and they indicated that Tween 40 has the highest oxygen uptake and cell mass production. But Brij 35 showed that its oxygen uptake was comparable in the control case (without surfactant), but has higher cell productivity because of the biodegradation of Brij 35. And Triton X-114 was similar to the control system (had lower cell productivity than Brij 35 because of the lower biodegradation of Triton X-114). They concluded that Tween 40 is the best to resist biodegradation and Triton X-114 is better than Brij 35. Yeh *et al.* (1998) also presented that Tween surfactants were found to be the least inhibitory and were also partially degradable.

2.7 Bioreactors for biohydrogen production

Biohydrogen, or biological hydrogen, is a byproduct in the acidogenic phase from the anaerobic fermentation process with organic or organic wastewater as the substrate, such as glucose, sucrose, paper mill waste, municipal solid waste, starch effluent, food processing waste, domestic waste, rice winery, daily waste, and palm

oil mill effluent (Vijayaraghavan *et al.* 2006). Matcarf and Eddy (2003) categorized anaerobic bioreactor into 3 groups, anaerobic suspended growth reactor, attached growth anaerobic reactor, and upflow anaerobic sludge blanket reactor. The anaerobic suspended growth reactor including a batch reactor (Ting *et al.*, 2007, Atif *et al.* 2005, Zhu *et al.*, 2006), a continuous stir tank reactor, or CSTR (Wang *et al.*, 2006, Zhang *et al.*, 2006, Lin *et al.* 2006), an anaerobic contact filter reactor (Vijayaraghavan *et al.*, 2006), and anaerobic sequencing batch reactor, or ASBR (Arooj *et al.*, 2007). The attached growth anaerobic reactor, such as an immobilized granular sludge bed reactor (Lee *et al.*, 2004), an anaerobic fluidized bed reactor or AFBR (Zhang *et al.*, 2007). And lastly, upflow anaerobic sludge blanket reactor, or UASB (Han *et al.*, 2005). Due to the different types of substrate and bioreactor, the amount of hydrogen produced is different.

Because an anaerobic wastewater treatment is rapidly growing in the application for hydrogen production, upflow anaerobic sludge blanket (UASB) reactors are studied in many ways because continuous, high rate of production (Perez *et al.*, 2006), low energy requirement, and simple to operate (Matcarf and Eddy, 2003).

Han *et al.* (2005) studied the production of VFAs and alcohols during the hydrogen fermentation of food wastewater in an UASB at pH 7.5 and 37°C. They indicated that the maximum COD removal is 96% at loading 12.9 g COD l⁻¹d⁻¹ with the main VFAs and alcohols being formate, acetate, propionate, butyrate, ethanol, propanol, and butanol.

Kotsopoulos *et al.* (2006) studied biohydrogen production from glucose in UASB reactor using mixed cultured at hyper-thermophilic temperature (70°C). They concluded that the maximum hydrogen yield was 2.45 mol H₂/mol glucose and no methane formation.

In 2003, Chang *et al.* studied biohydrogen production from sewage sludge in UASB reactor at 20 gCOD/l. They found that each gram of biomass produced 53.5 mmol H₂/day with hydrogen gas content of 42.4% v/v.