CHAPTER II

THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Silver nanoparticles

Nanotechnology relates to a wide range of technologies that incorporate materials feature with a range of dimensions between approximately 1 and 100 nm. presents scale of materials related to nanotechnology. Nanoparticles, particles in nano scale, are a part of nanotechnologies claimed to substances, such as silver, iron, gold, carbon nanotubes, and nano-scale organic compounds (Kaiser et al., 2008; Savage and Diallo, 2005).

2.1.1 Properties of silver nanoparticle

Silver nanoparticles (AgNPs) were found in ashes, soil particles or biomolecules. The sizes of the nanoparticles are varied from 1 to 100 nm causing the difference in physico-chemical properties compared to their bulk material. Table 2.1 is shown typical properties of silver nanoparticles.

2.1.2 Application of silver nanoparticles

Silver nanoparticles are widely used in many applications because of their properties. Normally, AgNPs are applied for antimicrobial purpose. Estrin et al. (2008) examined the property of AgNPs as antimicrobial and antifungal agents on *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Penicillium phoeniceum*. The result demonstrated that AgNPs produced from a novel electrochemical method had strong antibacterial and antifungal properties. In food industries, AgNPs were applied as an antimicrobial agent by coating on the food container (Del Nobile et al., 2004; Jain and Pradeep, 2005; An et al., 2008; Tankhiwale and Bajpai, 2009; Fernandez et al., 2009; Fernandez et al., 2010). It was found that number of spoilage-related microorganisms in melon kept in cellulose-AgNPs-hybrid materials decreased compared to the control. Another application of

AgNPs utilization as antimicrobial agent were textiles industry (Lee and Jeong, 2005; Dubas et al., 2006; Benn and Westerhoff, 2008; Yoksan and Chirachanchai; 2010). Lee and Jeong (2005) found that AgNPs at sizes of 2-3 nm were skin-innoxious which can be used as antibacterial agents on fabrics.

Table 2.1 Properties of silver nanoparticles

Properties	Expressions
Shape	Triangular, spherical, rod-shape[a]
Size	9-25 nm[b]
Types	Polymeric silver, colloidal silver, spun silver, nanosilver powder, ionic silver[c]
Specific surface area	9-11 m^2/g[d]
Color	Red, brown , and green[e]

[a] Sun et al. (2003), [b] Choi and Hu (2008), [c] Luoma (2008), [d] Navarro (2008), [e]Van Hyning (1998)

Other utilizations of AgNPs were for medical application. Silver nanoparticles were exploited for medical apparatus (Furno et al, 2004; Biju et al., 2008; Kassaee, 2008). For example, Biju et al. (2008) examined that AgNPs can be applied to in vitro and in vivo imaging of living cell, and in vivo imaging of cancers, tumor vasculature, and lymph nodes. In addition, AgNPs were synthesized and demonstrated to use as a potentiometric redox marker in a glucose biosensor (Ngeontae et al., 2009).

2.1.3 Fate and transport of silver nanoparticles to environment

Silver nanoparticles are one of the most well-liked nanoparticles utilized in the world. Although there has not been published document of AgNPs contamination levels in the environment, the trend of AgNPs utilization significantly increases. Silver nanoparticles could spread throughout the environment.

Fauss (2008) classified AgNPs into the five types which are polymeric silver, colloidal silver, spun silver, nanosilver powder, and ionic silver. First, polymeric silver is AgNPs from handrails, medical devices, food storage containers, dressing for wounds, and female-hygeine products. It forms a complex long chain molecule as gelatin. Second, colloidal silver is AgNPs at the size of 0.6-25 nm. Third, spun silver is AgNPs from fabric, impregnating sheets, clothing, and sportswear. Forth, nanosilver powder is AgNPs from the first wash from sock and shoes. Fifth, ionic silver is AgNPs from washing machines and dishwashers. The highest amount of AgNPs that was released into water is from spun silver as mention in Benn and Westerhoff (2008).

Luoma (2008) calculated the release of AgNPs from manufacturers' information and came up with fate and transport scenarios for three new AgNPs applications including silver sock, silver wash machines, and swimming pool or spa equipments. All applications were applied AgNPs as antibacterial agent. Silver nanoparticles can react and fate into many characteristics to the water as an individual particles, aggregation, dissolution, and dissolved organic matter. Silver nanoparticles could be discharged up to 150 tons per year. Silver nanoparticles could be degraded in acidic condition and room temperature (JR Nanotech, 2002). The abundant of chloride or dissolved organic matter could activate dissolution rate of AgNPs. On the other hand, natural water containing a large amount of dissolved organic matter can reduce the reactivity of AgNPs.

Besides estimated fate and transport in natural water, Mueller and Nowack (2009) predicted that AgNPs released into soil and water as the wet deposition. There are many processes to dispose AgNPs such as, waste incineration plants, landfills, and sewage treatment plants. They analyzed the percentage of releasing in AgNPs from products. The result showed that the highest percentages of AgNPs were released to dissolution form from textiles, cosmetics, spray agents, metal products, plastics, and paint. Blaser et al. (2008) reported that AgNPs could leach from landfill to soil. In addition, the digested sludge from sewage treatment plants is sold as an agricultural fertilizer, this leads to contaminate of AgNPs into soil (FOE, 2007).

For fate of AgNPs in living organisms, AgNPs could transfer from soil to heterotrophic (ammonifying/nitrogen fixing) and chemolithotrophic bacteria (FOE, 2007). Moreover, AgNPs in the suspended particulates form in water transported to sediments. Then, animals consume AgNPs sediment as food (JR Nanotech, 2002). Silver nanoparticles could pass though membranes of the digestive tract though all over animal body.

2.1.4 Effect of silver nanoparticles in the environment

After AgNPs discharged to soil and water, it can damage to organism resulted from an antimicrobial function of AgNPs. The toxicity of AgNPs on human, fish, algae, crustaceans, some plants, fungi, and bacteria was reported (Eisler, 1996; Yeo and Kang, 2008).

2.1.4.1 Human

Silver nanoparticles can cause argyria as reported in White et al. (2003). This study reported the case of a 58-year-old man who drank colloid silver protein solution for at least 1 year. He believed the prevention of silver from various diseases. Consequently, he has a deep blue/grey discoloration of the skin on face, neck, bald scalp, hands, and forearms. The ingestion of AgNPs has also related to neurological problems, kidney damage, stomach upset, headaches, fatigue, and skin irritation (White et al., 2003; Hori et al., 2002)

2.1.4.2 Animal

There are some studies demonstrated the effect of AgNPs to the mammalian cells. For example, Hussain et al. (2005) evaluated the acute toxic effects of metal nanopartciels using the *in vitro* rat liver derived cell line. Silver nanoparticles meaningfully decreased the function of mitochondria. Lactate dehydrogenase leakage significantly extended in the treated cells by AgNPs. Likewise, Braydich-Stolle and colleagues (2005) examined the cytotoxicity on male germline *in vitro* of mouse. Silver nanoparticles were the most toxic to a mouse spermatogonial stem cell line. Moreover, AgNPs could induce the neurotransmitter dopamine depletion which was essential for the normal functioning of the central nervous system (Hussain et al., 2006).

2.1.4.3 Microorganisms

It is well known that AgNPs is a toxic substance for microorganisms (Sondi and Salopek-Sondi, 2004; Pal et al., 2007; Kim, 2007; Estrin, 2008; Fabrega et al., 2009). Sondi and Salopek-Sondi investigated the antimicrobial activity of AgNPs on *Escherichia coli* as a model of Gram-negative bacteria. The result showed that the cell wall of *E. coli* was damaged by the formation of pits. Similarly, Pal and colleagues (2007) revealed the images of treated *E. coli* with AgNPs by energy-filtering transmission electron microscopy. There was a change in the cell membrane on the treated cell, resulting in cell death. Besides, Kim and co-worker (2007) researched the antimicrobial activity of AgNPs against yeast, *E. coli*, and *S. aureus*. Silver nanoparticles at the low concentration obviously inhibited the growth of yeast and *E. coli* while the growth-inhibitory impact on *S. aureus* was light. Moreover, they studied generation of free radicals from AgNPs which attack to membrane lipids and this cause a breakdown of membrane function.

According to Choi and Hu (2008), the relationship between the inhibition and reactive oxygen species (ROS) on nitrifying bacteria was determined. The reactive oxygen species including singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radical, may be free radical species as mentioned in Kim et al. (2007). On the other hand, the toxicity of AgNPs also reacts to HIV-I (Elechiguerra, 2005) by preferential binding to the gp 120 glycoprotein knobs. This interaction inhibits the virus from binding to host cells.

2.1.4.4 Wastewater treatment plant

As mentioned in fate and transport topic, there are many industries that directly release AgNPs to water. The wastewater is contaminated with AgNPs effect on microorganisms in wastewater treatment plants because of the strong antimicrobial properties of AgNPs. The toxicity damages useful bacteria. Choi and Hu (2008) investigated the size-dependent inhibition by AgNPs on nitrifying bacteria. The result showed that the less AgNPs size than 5 nm and the concentration of AgNPs at 1 mg/L could be more toxic to bacteria than their bulk materials. Moreover, the relationships of ROS were different for the various form of silver.

2.2 Nitrification

Nitrification, a two-step nitrogen conversion process, is one of the most important part of nitrogen cycle, since it provide nitrate (NO_3^-) in soil water from ammonia in soil particles for plants and wastewater treatment plants. The nitrification process relates to two groups of lithoautotrophic bacteria which are ammonia oxidizers and nitrite oxidizers (known as nitrifier). The process generates the energy by the oxidation of ammonia to nitrite (ammonia oxidizers) or nitrite to nitrate (nitrite oxidizers) as shown on Equation (2.1) and (2.2).

$$NH_4^+ + 1.5O_2 \quad \rightarrow \quad 2H^+ + 2H_2O + NO_2^- + \text{energy} \qquad (2.1)$$

$$NO_2^- + 0.5O_2 \quad \rightarrow \quad NO_3^- + \text{energy} \qquad (2.2)$$

Nitrifying bacteria are useful for agriculture because it reduces the acidification on unbuffered soils, which are phytotoxic by transforming nitrate in soil particles to mobile phase. In addition, nitrifying bacteria remove nitrogen from wastewater leading to eutrophication and reducing the toxic effect of ammonia on aquatic organisms.

2.2.1 Microbial Nitrification

As mentioned earlier, nitrification processes relate with two groups of bacteria, ammonia oxidizer (AOB) and nitrite oxidizer (NOB). The most frequently determined genus with the step from Equation (2.1) is *Nitrosomonas*. Furthermore, there are the other genera associate with this step which is *Nitrosococcus* and *Nitrosospira* (Watson et al., 1981). *Nitrobacter* is the most frequently determined genus with the step from Equation (2.1), including with *Nitrococcus* and *Nitrospira* (Watson et al., 1981).

The lithoautotrophic ammonia oxidizing bacteria has the ability to use ammonia as the major source of energy and carbon dioxide as the main source of carbon. The characteristics of the AOB genera are shown in Table 2.2 (Koops and Pommerening-Röser, 2006).

Table 2.2 Characteristics of the genera of AOB

Characteristic	*Nitrosomonas*	*Nitrosospira*	*Nitrosococcus*
Cell shape	Spherical to rod shaped	Tightly coiled spirals	Spherical to ellipsoidal
Intracytoplasmic membranes	Peripherally located flattened vesicles	Occasional tubular invaginations	Centrally located stack of membranes
Flagella	Polar flagella	Peritrichous flagella	Tuft of flagella

The major groups of AOB, located within the *Betaproteobacteria*, enclose two clusters (Figure 2.1) by the use of 16S rDNA. The phylogenetic analyses of AOB were demonstrated into two classes which are *Betaproteobacteria* and *Gammaproteobacteria* (Woeses et al., 1984; Woeses et al., 1985). The genera *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio*, and *Nitrosolobus* belong to the class *Betaproteobacteria*, while the genus *Nitrosococcus* is branched with the class *Gammaproteobacteria*.

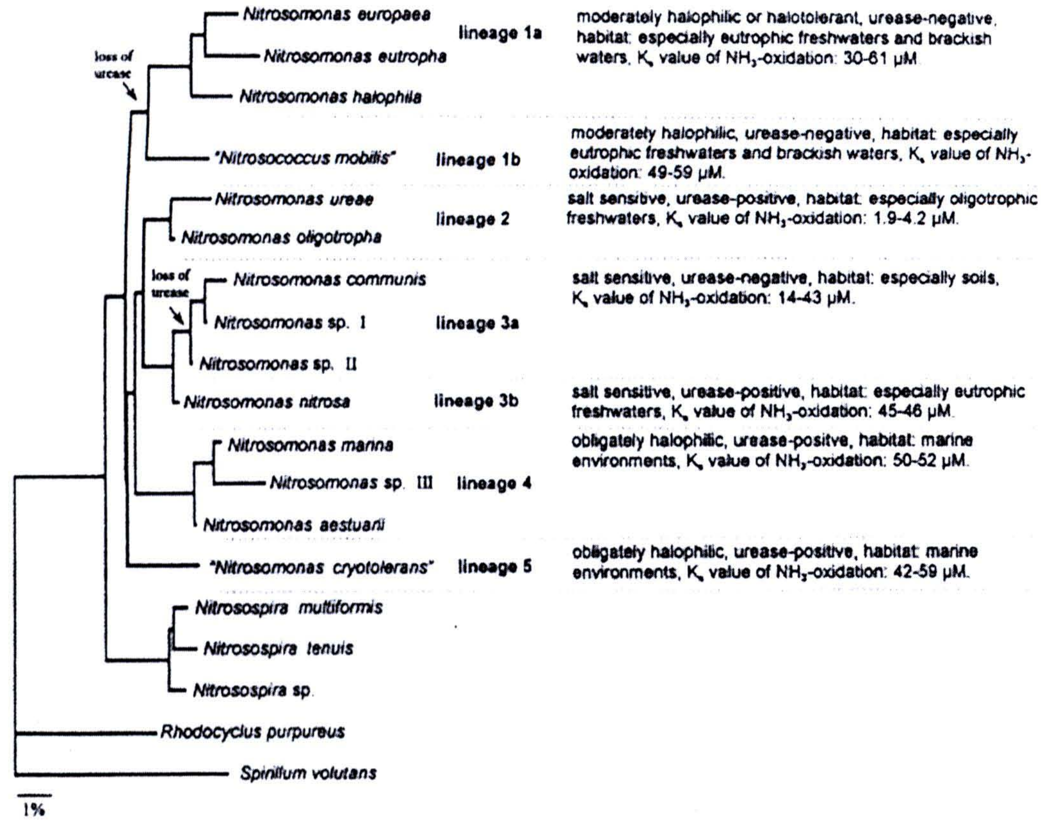

Figure 2.1 16S rDNA-based phylogenetic tree nearly complete sequences, in the class *Betaproteobacteria* and the most important ecophysiological characteristics of the five genera (Koops and Pommerening-Röser, 2005).

The lithoautotrophic nitrite oxidizing bacteria has the ability to use nitrite as the main source of energy and carbon dioxide as the sole source of carbon. The characteristics of NOB are rods, cocci, and spirilla (Spieck and Bock, 2005b). There are four genera classifications which are *Nitrobacter*, *Nitrococcus*, *Nitrospina*, and *Nitrospira* (Table 2.3). Unlike AOB, NOB is more scattered phylogenetically as demonstrated in Figure 2.2. The dominate genus of NOB in most natural environments is *Nitrobacter*. However, NOB is more likely to live in the extreme environments such as concrete and natural stones, desert soils, and sulfidic ore mines. Besides, they can survive long periods of starvation and dryness without endospores.

Table 2.3 Differentiation of the four genera of NOB (Spieck and Bock, 2005)

Characteristics	*Nitrobacter*	*Nitrococcus*	*Nitrospina*	*Nitrospira*
Phylogenetic position	*Alphaproteo-bacteria*	*Gammaproteo-bacteria*	*Deltaproteo-bacteria*	Phylum *Nitrospirae*
Morphology	Pleomorphic short rods	Coccoid cells	Straight rods	Curved rods to spirals
Intracytoplasmic membranes	Polar cap	Tubular	Lacking	Lacking
Size (µm)	0.5-0.9x1.0-2.0	1.5-1.8	0.3-0.5x1.7-6.6	0.2-0.4x0.9-2.2
Reproduction	Budding or binary fission	Binary fission	Binary fission	Binary fission

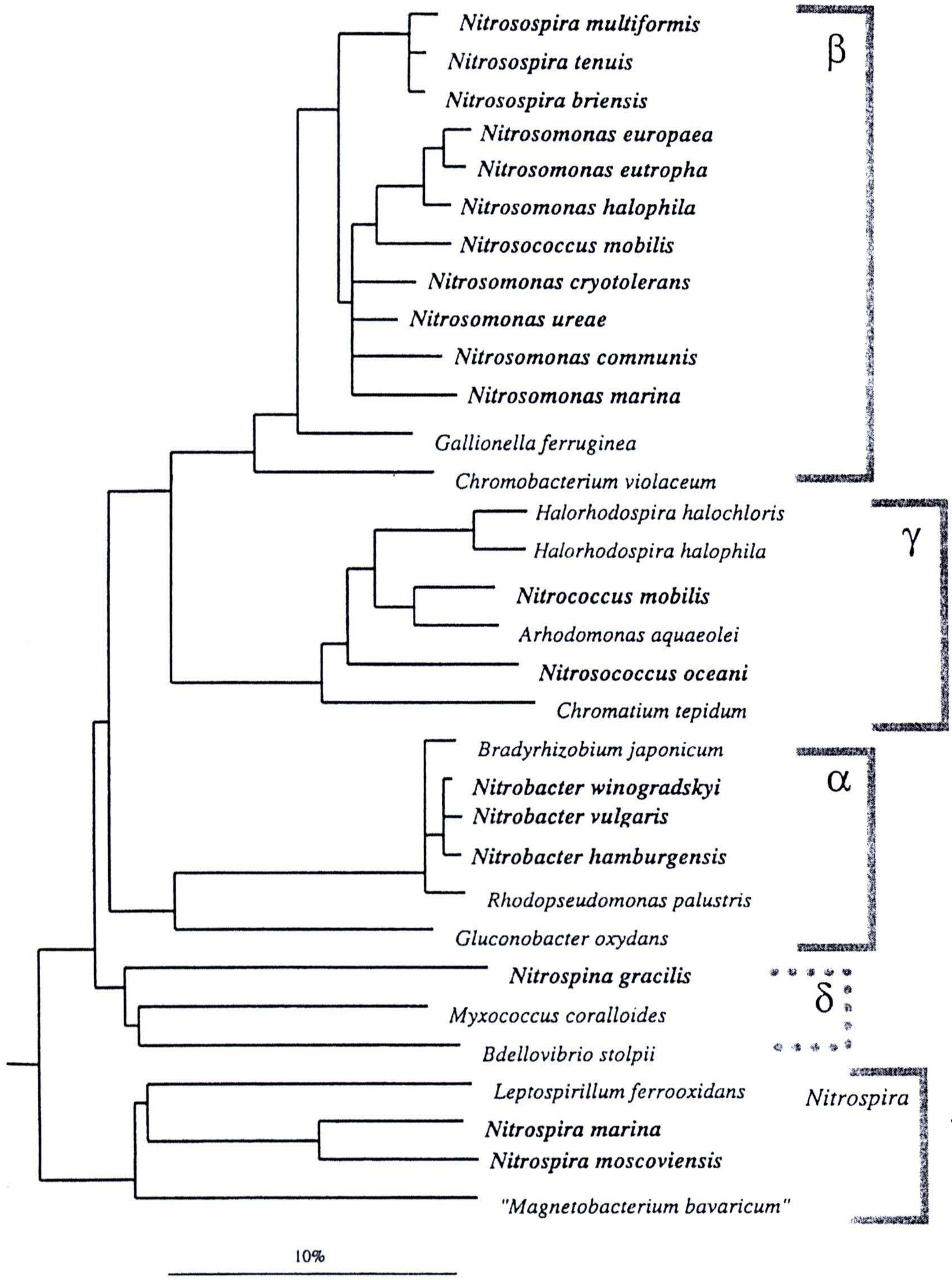

Figure 2.2 16S rRNA-based tree reflecting the phylogenetic relationship of NOB (Spieck and Bock, 2005a).

2.2.2 Influential factors for nitrification

Nitrification process is the first important pathway to remove the reduced nitrogen in term of ammonia and organic nitrogen by nitrification and denitrification processes in wastewater. Since, nitrifying bacteria have a slower growth rate (Prosser, 1989) and lower competitor of oxygen than aerobic heterotrophs, it is difficult to manipulate nitrification in municipal and industrial wastewater treatment plants. Several influential factors affecting nitrification are as follows.

2.2.2.1 Ammonia

From Equation (2.3), ammonia is the substrate in the nitrification process, nitrifiers use ammonia as the sole source of energy. Kemp and Dodds (2002) determined the influence of ammonium on nitrification rates with prairie stream substrata. The conclusion of the study was nitrification response to increasing NH_4^+ concentrations but depending on the substrata type. Furthermore, the 98% of ammonia conversion to nitrite can be produced in the case of saturated oxygen concentration (Ruiz et al., 2003).

2.2.2.2 Biochemical Oxygen Demand

Biochemical oxygen demand (BOD_5) represents the level of organic pollutant in wastewater samples by measuring the oxidized organic matter from bacteria. Wastewater treatment plants are plenty of microorganisms like autotrophs and heterotrophs. The increasing of BOD leads to the competition in both types of microorganism which affect on nitrification process. Therefore, the high amount of BOD_5 could reduce the performance of nitrification process. Parallels to Coskuner and Jassim (2008) indicated that the nitrification performance was decreased by the higher BOD concentrations results from the competition for DO.

2.2.2.3 Sludge Retention Time (SRT)

For biological treatment process in wastewater treatment plants, SRT is one of the most important for operational factor. Longer SRT leaded to accumulation of biomass (sludge), which interrupted with the transfer of oxygen in bioreactor (Huang et al., 2001). Likewise, Hallin et al (2005) also studied the effects of different

SRTs on the nitrification activity. Prolonged SRT reduce the nitrification activity causing by the physiological changes in the AOB community rather than a change in community composition. On the other hand, too short SRT caused reduction of nitrification activity by decreasing of the sludge concentration (Huang et al., 2001).

2.2.2.4 Salinity

High saline concentrations were occurred in the high nitrogen concentrated waste streams. Due to the fact that they contain plenty of ions such as chloride from fish canning industry and wet lime-gypsum desulphurization process, sulphate from tannery wastes (Campos et al., 2002). Organic matter, nitrogen, and phosphorus removal are affected from this situation (Panswad and Anan, 1999; Intrasungkha et al., 1999). Panswad and Anan (1999) investigated that the more increasing of chloride concentration was added, the lower of ammonia and nitrate uptake rate were made. Likewise, Campos et al. (2002) discovered the inhibition of nitrification activity became completed at the higher of salt concentration than 525 mM.

2.2.2.5 Temperature

Temperature is one of the factors that influence for nitrification as Antoniou and colleagues (1990) determined the maximum growth rate of nitrifying bacteria on temperature. They found the maximum growth rate is in the range of 15-25 °c. Besides, Mulder et al. (2001) constructed the SHARON process operating without sludge retention. The optimal operating temperature of 30-40 °c supported N-removal over nitrite. The growth rates of nitrifying bacteria increase with extend the temperature up to 30 °c (Coskuner and Jassim, 2008).

2.2.2.6 pH and alkalinity

The optimum pH for the growth of nitrifying bacteria is in the range of 7.5-8.0 (Prosser, 1989; EPA, 2002). EPA (2002) informed the reduction of alkalinity leads to the change of buffering capacity as bicarbonate which is consumed in the conversion of ammonia to nitrite. Moreover, an increasing in pH to higher than 9 can reduce the nitrification activity.

2.2.2.7 Dissolved oxygen concentration

Since oxygen is the important substrate to nitrification process as can be seen from Equation (2.3). The optimal oxygen concentration in the operation of nitrification process should not less than 2.0 mg/L (Coskuner and Jassim, 2008). Prosser (1989) stated that the low concentrations of oxygen decrease rates of nitrification result from the nitrifiers become a poor competitors at the low concentration of oxygen compare to heterotrophic nitrifiers. The K_s value of hetrerotrophs is lower for oxygen leading them to be a competitive advantage in this situation.

2.2.2.8 Light

There are some studies which investigated in the effect of light through the nitrification process because of the light penetration in the different light sources and intensities (Yoshioka and Saijo, 1984; Diab and Shilo; 1988; Guerrero and Jones, 1996). Guerrero and Jones (1996) demonstrated the effect of light on nitrifying bacteria depends on both types of nitrifiers and the conditions of their environment. Yoshioka and Saijo (1984) concluded that light can be also lethal and bacteriostatic to nitrifying bacteria.

2.2.3 Nitrification process monitoring

Due to the various influential factors of nitrification, many of the monitoring ways were produced to support the researchers. Nitrifying bacteria are sensitive microorganism to the stress condition so the process for monitoring should produce the less effect to the nitrification process.

2.2.3.1 Decrease in dissolved oxygen

This method is applied for determination the nitrification activity which impact from toxic substance, called respirometric method (Surmacz-Gorska et al., 1996; Hu et al., 2002; Moussa et al., 2003; Ciudad et al., 2006; Cecen et al., 2010). The method is convenient, accurate, precise, and less-time consuming. The saturated oxygen concentration which is required for nitrification is available; therefore, it is easy to control the concentration of oxygen.

2.2.3.2 Decrease in ammonia concentration

As ammonia is the main source of energy to nitrifying bacteria, the method to monitor the ammonia concentration is applied. Panswad and Anan (1999) investigated in the specific oxygen, ammonia, and nitrate uptake rates of a biological nutrient removal process treating uplifted salinity wastewater. Moreover, You et al. (2009) examined the effect of heavy metal in nitrification by measuring the specific ammonia uptake rate.

2.2.3.3 Real-time Polymerase Chain Reaction (Real-time PCR)

Nowadays, PCR is widely used for quantification of nitrifying bacteria. by amplification of ammonia monooxygenase (amoA) and *Nitrospira* spp. 16S rRNA genes (Dionisi et al., 2002; Harms et al., 2003). Harms et al. (2003) developed the real-time PCR for the quantification of total bacteria (NOB and AOB) in mixed liquor suspended solids (MLSS) from wastewater treatment plants.

2.3 Cell entrapment

Immobilized cell is widely utilized in the field of sciences and technologies to develop the production. Mostly, it is used in bioreactor, and production of the useful compounds such as amino acids, organic acids, antibiotics, steroids, and enzymes (Cassidy et al., 1996). According to Tampions (1987) introduced the advantages of immobilized cell which are long-term stability of biocatalyst, low leakage of cells, high resistance to abrasion, resistance to microbial degradation, low diffusional limitation, high surface area, cheap support materials, and non-toxic materials. There are many different forms to produce the entrapped cells which can be shown in Table 2.4. There still are two more techniques to form the immobilized cell which are cross-linking of cells and encapsulation in polymer-gel (Cassidy et al., 1996). The application of immobilized cell is used in agriculture, bio-control, pesticide application, and pollutant biodegradation in contaminated soil or groundwater (Connick and William, 1982; Bettmen and Rehm, 1984; Bashan, 1986; Axtell et al, 1987; Hu et al., 1994).

2.3.1 Principle of cell entrapment

Cell entrapment is one of the immobilized cell techniques which contain two main steps (Dulieu, 1999; Siripattankul, 2010). First, the mixture of cells and matrix are well blended by magnetic stirrer to become a homogenous phase. Second, the droplets of cell mixture is formed the gelation by peristaltic pump. The gelation is produced in the formation of cross-linking between a matrix and a cation in gel formation solution such as calcium alginate beads. The spherical shape has been applied for the formation of the beads because there is much surface area than the other shape.

2.3.2 Types of cell entrapment materials

There are two types of matrix materials using for cell entrapment which are natural and artificial types. Natural matrices are polysaccharides made from algae or seaweed, such as calcium alginate, carrageenan, agarose, and gelatin. On the other hand, the artificial types are polymer, such as polyvinyl alcohol, cellulose triacetate, and polyacrylamide.

Table 2.4 Classification of the immobilized cell techniques (Tampion, 1987)

Technique		Advantages	Disadvantages
Adsorption			
	Neutral supports	Cheap	Cell leakage
		Mild	Sensitive to pH changes
		Reusable	
		Simple	
	Charged supports	Mild	
		Reusable	
		Simple	
Flocculation		Simple	Cell leakage
		Mild	Diffusional limitations
Entrapment			
	Natural polymers	Mild	Diffusional limitations
		Simple	
	Synthetic polymers	May be simle	Toxicity
			Expensive
			Diffusional limitations
Covalent coupling		Permanent	Toxicity
			Expensive
Containment		Mild	Diffusional limitations
		Simple	Expensive
		Reusable	

2.3.2.1 Calcium alginate

Calcium alginate is a cross-linking of alginate with divalent cation as Ca^{2+}. Alginate is a non-toxic natural polysaccharide from brown algae, such as *Macrocystis pyrifera*, *Laminaria digitata*, *L. hyperborea*, and *Eklonia cava* and some bacteria, principally *Azotobacter vinelandii* (Fett et al., 1986, Fett et al., 1995). Alginate is provided as a sodium salt of alginate. While the sodium alginate matrix contact with the Ca^{+} solution, a gelation is formed suddenly in its outer layer through the entire alginate bead (Siripattankul, 2010). The powder of sodium alginate is dissolved in DI water at 2% (w/v) by slowly add into stirred DI for overnight. The concentrated microbial cells were centrifuged at 7,00 rpm for 10 min. The mixture of sodium alginate solution and wet cells is dropped into calcium chloride solution of 3.5% (w/v) using peristaltic pump. The droplets are left in the solution for 2.5-3.0 hr. for the hardening of the beads.

2.3.2.2 Carrageenan

Carrageenan is produced from red algae, mainly *Chondrus crispus*, *Eucheuna cottonii*, *Gigartina stellata* and *G. radula* (Guisely, 1989). The gealation of carragenans depend on temperature and the further strengthening of the polymer network with K^+, or Al^{+3} ions. The 2-5% (w/v) of carragenan in physiological saline is warmed to 70-80 °c and maintain at 42 °c, including the cell suspension. The warm cells at 40-50 °c are added to the carragenan solution. The droplets are expelled into cold KCl solution to make a gelation.

2.3.2.3 Polyvinyl alcohol

Polyvinyl alcohol is a non-toxic synthetic polymer. The characteristics of raw PVA are white and free-flowing granule. There are several gelation techniques for producing PVA gels for immobilized cell, for instance boric acid-PVA (BPVA), freezing and thawing of PVA (FPVA), and phosphorylated-PVA (PPVA) methods. The BPVA technique is a one-step droplet gelation method (Hashimoto and Furukawa, 1987) and a cross-linking of boron. The FPVA technique is a physical cross-linking during temperature-induced condition (Lozinsky and Plieva, 1998). PPVA method is less time consuming and cell damage than BPVA. The PPVA technique is a two-step droplet gelation method that is spherical bead formation and hardening. The spherical bead formation is the first step by cross-linking of the PVA-boron as in BPVA. After that, the spherical beads are transfer to a sodium phosphate solution for bead hardening process. A sodium phosphate increases the surface gel strength through PVA phosphorylation.

2.3.2.4 Cellulose triacetate

The other natural polymer used for matrix of entrapped cell is cellulose which containing of a chain of glucose

molecules. The modification of cellulose with esterification and etherification are applied for the entrapped cell. The entrapped cells of CTA are prepared by the plated gelation (Khan et al., 1994; Yang et al., 1997; Jittawattanarat et al., 2007; Siripattankul, 2010). The 10% (w/v) powder of CTA is dissolved in methylene chloride. Concentrated suspension cells are mixed with CTA solution. After that the mixture is plated into toluene for hardening. The hardened CTA is cut to small cube and washed with water.

2.3.3 Applications of entrapped cells

As a result of the entrapped cells properties, they are broadly used in many industries for increased metabolic activity and metabolite production, protection from toxic substances, and increased plasmid stability (Dwyer et al., 1986; Keweloh et al., 1989; Keweloh et al., 1990; Gadkari, 1990).

2.3.3.1 Biomedical

The cell entrapment was used in the biomedical area for the production of pharmaceuticals and reagents. Koshcheyenko et al. (1983) studied on the transformation of steroid by living entrapped cells in polyacrylamide gel. Likewise, O'Shea (1984) concluded that the entrapped cells have great potential in the treatment of diabetes and liver diseases.

2.3.3.2 Food and beverage

In food industries, pack-bead systems are being applied to give flavors for addition to cheese (Cavin et al., 1985). The entrapped cell also is utilized for cream fermentations of milk in cheese manufacturing (Linko, 1984). In addition, the entrapped cell is used in the alcoholic beverage industry for wine treatment and beer brewing (Onaka, 1985).

2.3.3.3 Wastewater treatment

Biodegradation properties of the entrapped cells are used in wastewater treatment by adding mixed culture the matrix (Chen, 1998; Siripattankul, 2010). Siripattanakul et al. (2008) investigated the effect of cell to matrix ratio in PVA on atrazine degradation. Moreover, Chen (1998) examined the carbon and nitrogen removal in wastewater using phosphorylated PVA-entrapped microorganisms. Due to the prevention of eutrophication effect on surface water, the removal of excess nitrogen has concerned. The entrapped cell is occupied for degradation of ammonia in the wastewater treatment plants (Tramper, 1985; Chen, 1994; Hill and Khan, 2008)