Chapter II

Literature Reviews

2.1 Tilapia

2.1.1 Characteristic of Tilapia

Tilapia *(Tilapia Nilotica)* is a native animal of African continent. Tilapia has fairly conventional, laterally compressed, deep body shape. The body is covered with relatively large, cycloid scales, which are not easily dislodged (Ross, 2000). The dosal and anal fins have hard spines and soft anterior in an advanced configuration. The numbers of scales, vertebrae, gill rakers and fin raya and spinners are widely used for species distinction and identification. Tilapia bodies are generally characterized by vertical bars, with relatively subdued colours and with little contrast over the body colours. This provides the fish with a modest ability to change their colours, in response to stress by controlling skin chromatophores.

2.1.2 Tilapia Cultivation

Tilapia cultivation can be categorized into 3 groups based on the initial density of the crops, the quantity of feed, and operation (Abdel-Fattahm and El-Sayed, 2006).

1. Extensive Culture. Extensive culture is done purposely for domestic consumption. Tilapia acquires natural foods available within pond (i.e., earthen ponds) so that it is unnecessary to provide additional diets. The initial tilapia density is approximately 0.5 – 2 tilapia m^{-2}.
2. Semi-intensive Culture. Semi-intensive culture is done purposely for domestic consumption as well as commercialization. Tilapia acquires natural foods available within ponds, and may need supplemental diets occasionally. The initial tilapia density for semi-intensive culture is estimated from 2 – 4 tilapia m^{-2}.
3. Intensive Culture. Intensive culture requires high quality feeds, pond maintenance, heavy aearation, and disease control. Intensive culture

also needs significant water replacement several times per day to maintain good water quality. For these reasons, intensive culture is carried out purposely for commercialization. The initial tilapia density for intensive culture is estimated from 4 – 10 tilapia m^{-2}.

4. Caged Production. Caged production is often used for tilapia cultivation in Thailand at the moment. Cages, made from synthetic materials, are normally available in square shape, rectangular shape, or spherical shape. Different shapes of cages influences the characteristic of water flow, quantity of incoming water, and solid-deposition. In Thailand, square shaped (1.2 x 1.2 x 2.5 m) and rectangular shaped (4 x 2 x 2.5 m) are popular. Deployment of tilapia weighed from 50 – 60 g was often performed to obtain an initial crop density around 4 – 6 kg m^{-3}.

2.1.3 Conditions Affecting Tilapia Growth

1. Temperature. Tilapia grows well in the temperature range from 20 – 35 °C (Balarin and Haller, 1979). Tilapia stops eating when the temperature is lower than 15 °C and die at the temperature below 8 °C (Abdel-Fattahm and El-Sayed, 2006).
2. Alkalinity and pH. Tilapia should be cultivated in water with alkalinity from 200 – 300 mg L^{-1} $CaCO_3$ (Abdel-Fattahm and El-Sayed, 2006). The suitable pH for tilapia was reported from 6.5 – 8.5 (Ross, 2000).
4. Inorganic Nitrogen Compounds. Ammonium is toxic towards tilapia when its concentration exceeded 0.5 mg N L^{-1}. The threshold for nitrite was reported at 2.1 mg N L^{-1}, but it was recommended to keep nitrite in water below 1.0 mg N L^{-1} (Balarin and Haller, 1979)

2.2 Inorganic Nitrogen Compounds and Toxicities

2.2.1 Ammonia

Ammonia is introduced into aquaculture ponds via feeds, aquatic animal excretion, and biological degradation of unconsumed feeds. Ammonia is available in water in two forms (NH_3 or NH_4^+) depending on pH of water. Free ammonia (NH_3) is more toxic towards aquatic animal in comparison to ionized form (NH_4^+). The proportion of free ammonia increases with increasing pH and increasing temperature. Toxic concentrations of ammonia can damage gills of fish, consequently impairing its respiratory system. Ammonia also causes the neurological and cytological failure in fish (Nootong, 2006). The acceptable level of ionized ammonia (NH_4^+) is 1.0 mg N L^{-1}.

2.2.2 Nitrite

The presence of nitrite is generally trivial as it is the intermediate of nitrification process, which converts ammonium into nitrate. However, nitrite accumulation in water is possible due to incomplete nitrification and denitrification, and its consequence is undesirable. Nitrite can combine with Fe^{2+} in hemoglobin forming a compound called methamoglobin, which has lower oxygen transport capability than hemoglobin. The presence of nitrite at high concentration can cause a lack of oxygen in tilapia. In human, nitrite is a potential carginogic compounds. Infant under the age of 6 month may become seriously ill and die, if untreated, after drinking water containing nitrite (Nootong, 2006). For the purpose of aquaculture, it is desirable to keep nitrite concentration under 1.0 mg N L^{-1}.

2.2.3 Nitrate

Nitrate is a stable compound, which is an end-product of nitrification. Nitrate, although far less toxic to ammonium and nitrite, can become toxic towards tilapia when its concentration exceeds 70 mg N L^{-1} (Van Rijin, 1996). Nitrate is also poisonous to human especially in baby under 4 – 6 months old because it replaces hemoglobin methamoglobin. Discharge of nitrate into natural water resource can cause eutrophication, which is a natural aging of freshwater reservoir such as lakes to become organically rich, thereby leading to domination of weeds and eventually transforming into mash land (Tchobanoglous et al., 2003). Discharge of nitrate into

natural water resource quickly accelerates eutrophication by stimulating the growth of microalgae.

2.3 Biological Processes for Inorganic Nitrogen Treatment

Inorganic nitrogen (i.e., ammonia, nitrite, and nitrate) treatment can be accomplished based on using different biological processes in the nitrogen cycles. Common biolological processes for inorganic nitrogen treatment include nitrogen assimilation, ammonification, nitrification, denitrification, and recently discovered anaerobic ammonium oxidation (Annammox).

2.3.1 Nitrogen Assimilation

Nitrogen assimilation can be defined as the overall process in which nitrogen is acquired into cells to form new cell constitutents (i.e., biomass). Nitrogen assimilation by phytoplanktons is an important process of inorganic nitrogen treatment. Assimilated nitrogen is incorporated into proteins of new biomass during photosynthesis. Hargreves (1998) estimated that microalgae with the composition C:N:P of 106:16:1 was capable of assimilating inorganic nitrogen into cells from 150 – 450 mg N m^{-2} day^{-1} at low temperature and from 750 – 1,500 mg N m^{-2} day^{-1} at high temperature. Microalgae was able to utilize all forms of nitrogen in water but appeared to prefer ammonium and nitrite (DeBoer, 1981). Hargreves (1998) further pointed out that microalgae would first assimilate ammonium until its concentration reached 0.3 mg N L^{-1} before switching to nitrate. Heterotrophic bacteria can also incorporate ammonium and nitrite to synthesis protein during cell growth. Addition of organic carbon compounds can quickly enhance assimilating process given that oxygen is presence in sufficient quantity. Nitrogen assimilation by heterotrophic bacteria and microalgae can be described by equation 2.1 and 2.2, with the symbols $C_5H_7O_2N$ and $C_{106}H_{263}O_{110}N_{16}P$ representing chemical composition of heterotrophic bacteria and microalgae, respectively (Ebeling and Timmons, 2007).

$$NH_4^+ + 1.18C_6H_{12}O_6 + HCO_3^- + 2.06O_2 \rightarrow C_5H_7O_2N + 6.06H_2O + 3.07CO_2 \quad \text{(Bacteria)} \quad (2.1)$$

$$16NH_4^+ + 92CO_2 + HPO_4^{2-} + 92H_2O + 14HCO_3^- \rightarrow C_{106}H_{263}O_{110}N_{16}P + 106O_2 \quad \text{(Microalgae)} \quad (2.2)$$

2.3.2 Ammonification

Ammonification is the release of ammonium from organic matters (e.g., proteins and urea). Ammonification is an important process that is responsible for feed degradation in aquaculture ponds. In degradation, proteins in feeds are broken down by bacteria into constituent amino acids, and the subsequent degradation gives ammonium.

2.3.3 Nitrification

Nitrification is the biological process in which ammonium is oxidized in the presence of oxygen successively into nitrite and nitrate. Microorganisms responsible for nitrification are known as chemoautotrophic nitrifying bacteria, which utilize inorganic carbon (i.e., alkalinity) and ammonium as carbon and energy sources, respectively. The first step of nitrification, which involves the conversion of ammonium to nitrite, is carried out by ammonium oxidizing bacteria (AOB) such as *Nitrosomonas, Nitrosolobus, Nitrospira, Nitrosococus,* and *Nitrovibrio* (Bitton, 1994; Nootong, 2008). Nitrobacter is commonly recognized as a species responsible for the second step of nitrification that is the conversion of nitrite to nitrate. Other nitrite oxidizing bacteria (NOB) include *Nitrospina* and *Nitrococcus*, but they are marine-obligated. Recently, *Nitrospira*-like bacteria was found as common NOB in various wastewater treatment facilities (Nootong, 2006). Equation 2.3 and 2.4 represent the nitrifying reactions by AOB and NOB.

$$NH^+_4 + 1.5\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad \text{(AOB)} \quad (2.3)$$

$$NO^-_2 + 0.5O_2 \rightarrow NO_3^- \quad \text{(NOB)} \quad (2.4)$$

Environmental Factors Affecting Nitrification

1. Dissolved oxygen (DO). Effective nitrification was observable when the DO is greater than 2.0 mg L^{-1} (Nootong, 2008). Pure cultures of *Nitrosomonas* and *Nitrobacteria* exhibited a stoppage of nitrification when DO is lower than 0.5 mg L^{-1}.
2. Temperature. Freshwater nitrifying bacteria were reported to grow at the temperature range from 8–36 °C, with the optimal temperature at 30 °C (Bitton, 1994). Marine nitrifying bacteria were reported to have optimal temperature range from 30–35 °C (Bitton, 1994). Temperature dependency of nitrification can be described Arrhenius equation.
3. pH. The optimal pH for nitrification is reported in the range from 7.5–8.5. The pH value lower than 6.0 inhibited nitrification (Nootong, 2006)
4 BOD/N Ratio. Increasing the proportion of organic matters can increase the population of heterotrophic bacteria, which possess higher oxygen affinity. The rate of nitrification was reported to decrease as high as 20–29% when the organic matters, measure as the chemical oxygen demand (COD) increased from 2–6 kg m^{-3} day^{-1}.
5. Inhibitory Compounds. Nitrifying bacteria were partially or completely inhibited by various compounds including organic matters, heavy metals, cyanide thiourea, cresol, phenol, anilines, mercaptan, and halogenated compounds (Lu et al., 1984; Sato et al., 1988; Bitton, 1994)

Table 2-1 Examples of inhibitory compounds for nitrification (Adapted from Lehr, 2005).

Chemicals	Inhibitory Concentrations (mg L^{-1})
Cobalt	0.08 – 0.5
Cromium	0.25
Copper	0.05 – 0.56
Nickel	0.25
Zinc	0.08 – 0.5
Cadmium	14.3
Sulfide	5.0
Sodium Chloride	35,000
Sodium Cyanide	100
Hydrogen Sulfide	50
Sodium Cyanide	1
Potassium Dichromate	6.0

2.3.4 Heterotrophic Denitrification

Heterotrophic denitrification is the biological process in which nitrate is reduced into nitrogen gas by denitrifying bacteria under oxygen-limited or anaerobic conditions. Under aerobic environment, oxygen is the preferred electron acceptor. In contrast, nitrate is the second preferred choice, and in lights of nitrification in which nitrate is abundant and oxygen is limited, denitrifying bacteria is expected to utilize nitrate as the electron acceptor. Common denitrifying bacteria include *Achromobacter, Bacillus denitricans, Flavobacterium, Micrococcus denitrificans, Dinitrobacillus, Spirillum* and *Pseudomonas stutzeri* (US.EPA., 1975; Anderson and Ibahim, 1978; Knowler, 1982). Denitrification also requires appropriate electron donors, which are normally organic carbon compounds such as methanol, ethanol, and acetate. Among available choices, methanol is the most popular due to its price. If using methanol as electron donor, denitrifying reaction can be written as shown in equation 2.7. Note that denitrification process will increase the pH of solution since one of the products is hydroxyl ion.

$$6NO_3^- + 5CH_3OH \rightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH^- \quad (2.7)$$

Environmental Factors Affecting Denitrification

1. Dissolved Oxygen. Low oxygen concentration must be kept in order to sustain successful heterotrophic denitrification. Many reports suggested different threshold for the DO concentration, ranging from 0.2 – 2.0 mg L^{-1}, but the generally accepted DO concentration should not exceed 0.5 g L^{-1} (Christensen and Harremoes, 1977).

2. Temperature. Similar to nitrification, temperature dependency of heterotrophic denitrification can be described by Arrhenius equation. Denitrification is active under wide temperature range from 0 – 50 °C, with the optimal values reported between 35 and 40 °C (Winker, 1984; and Bitton, 1994).

3. pH. It is agreeable that the optimal pH range for heterotrophic denitrification is between 7 and 8 (Winkler, 1984). The rate of denitrification decreases approximately 30% when the pH is outside that range. Moreover, the pH levels can also determine the end product species of the process, for instance the majority of end product is nitrous oxide when the pH is under 7.3, while nitrogen gas is dominant beyond that level (Christensen and Harames, 1977).

4. Inhibitory compounds. Heterotrophic denitrification is inhibited by many substances such as acetylene, pesticides, and nitrifying inhibitors. Sulfide was shown to inhibit nitric oxide and nitrous oxide reduction process. Metal chelating agents such as potassium cyanide, dithiol and o-phenanthroline can inhibit nitrate reductase in denitrifying bacteria.

2.3.5 Anaerobic Ammonium Oxidation (Anammox)

Anaerobic ammonium oxidation (Anammox) is a biologically process that autotrophically converts ammonium to nitrogen gas with nitrate as a terminal electron acceptor. In this process, nitrogen gas is generated without organic carbon requirement (Khin and Annachhatre, 2004). Anammox has the disadvantage since the bacteria responsible for this process, *Plantomecetales*, have an extremely slow growth

with the doubling time at 11 days (Khin and Annachhatre, 2004). Besides, the type of wastewater that was used during Anammox research normally possessed an extremely high concentration of ammonium (i.e., low C/N ratio), a characteristic that is in contrast to wastewater generated from aquaculture systems (Nootong, 2008). The optimal conditions for anammox are comparable to those reported for autotrophic nitrification.

2.4 Inorganic Nitrogen Treatment for Closed Aquaculture Application

The biological processes described section 2.3 are employed for the design of treatment systems for the closed-water aquacultural application. Based on literature reviews, it was possible to categorize inorganic nitrogen treatment systems into attached-growth systems and suspended-growth systems.

2.4.1 Attached-Growth Systems

In attached-growth system, bacteria are immobilized via adsorption onto the surface of cell supporting materials called biofilters. Examples of biofilters include stone, marble, sand or plastic such as PVC, polyethylene, and polypropylene. Plastic biofilters are increasingly popular due to their high surface area to encounter nitrifying bacterial slow growth as well as their durability. Plastic biofilters are available commercially, for example Biocord™, Bioball, HyperDrain™ and etc. Attached-growth systems can be further divided into nitrifying and denitrifying systems.

2.4.1.1 Nitrifying Systems

Nitrogenous wastewater from aquaculture cultivation is normally circulated through aerated nitrifying biofilters located outside production ponds. Dissolved oxygen concentration above 4.0 mg L^{-1} and suspended solid removal are always maintained to ensure optimal growth condition for nitrifying bacteria and aquatic stocks. The disadvantage of nitrifying systems largely involves expensive operational expense and clogging between biofilter pored spaces. Many design configurations of nitrifying biofilters are available including:

Rotating Biological Contactor

In rotating biological contactors (RBC), immobilized cells (i.e., biofilm) are formed on stationary surfaces of large plastic discs amounted on a horizontal shaft rotating slowly from 2 – 5 rpm (Tchobanoglous et al., 2003). Because of the rotation, different sectors of the discs are alternatively exposed to oxygen and wastewater, thus allowing the nitrifying reaction to proceed. RBCs were suggested to reduce the clogging of suspended solids (Brazil, 2006). The rates of inorganic nitrogen removal by RBCs were reported in the range from 0.19 – 0.79 g TAN m^{-2} day^{-1} (TAN = Total Ammonia Nitrogen) (Brazil, 2006; Crab et al., 2007).

Trickling Filter

Tricking filters consists of a bed of highly permeable medium to which microorganisms are attached and through which wastewater is percolated. The filter media usually consist of stones or light plastic packing materials, which possess the specific surface area from 100 – 1,000 m^2 m^{-3} (Crab et al., 2007). Wastewater from aquaculture ponds is introduced evenly at the top of media bed and trickles down between media pore space so that oxygen and inorganic nitrogen mass transfers can take place. The disadvantage associated with trickling filters is clogging from suspended solids in wastewater and from overgrowth of biofilms. The rates of inorganic nitrogen removal by trickling filters were reported in the range from 0.24 -- 0.64 g TAN m^{-2} day^{-1} (Eding et al., 2006; Crab et al., 2007).

Fluidized Filters

Principle of fludized filters is similar to that of the trickling filters. Nitrification is carried out on the surface of cell supporting materials (e.g., sand and polystyrene beads) with average sizing from 1 – 3 mm. Fluidized sand filters have the specific surface area for immobilization in the range 4,000 – 20,000 m^2 m^{-3} (Shieh and Keenan, 1987). The wastewater is introduced upward at the bottom of bioreactor column at high rate to fluidize cell supporting materials. Fluidzed filters are able to treat large volume of wastewater and less susceptible to solid clogging. However, fluidized filters are energy intensive and need efficient external aeration system to maintain aerobic condition within expanded bed. The rates of inorganic nitrogen removal

by fluidized filters were reported in the range from 0.19 – 0.79 g TAN m^{-2} day^{-1} (Sandu et al., 2002; Summerfelt and Sharrer, 2004; Crab et al., 2007).

Microbead Filters

Design of microbead filters is similar to tricking filters but the size of beads (i.e., cell supporting material) is smaller. Size of bead varies from 1 – 3 mm, thereby giving the specific surface area from 1,360 – 3,780 m^2 m^{-3} (Greiner and Timmons, 1998). Wastewater is distributed evenly over the top of the packing column. Inorganic nitrogen treatment occurs in biofilm layers formed on the surface of beads. At the same time, suspended solids are trapped between void spaces. Microbead filters are capable of treating large volume of wastewater and separation of suspended solid. The rates of inorganic nitrogen removal by microbead filters were reported in the range from 0.3 – 0.6 g TAN m^{-2} day^{-1} (Greiner and Timmons, 1998; Sastry et al., 1999; Crab et al., 2007).

Table 2-2 Various types and operating results of nitrifying biofilter for tilapia cultivation system.

Intial density	Type of biofilter	Rate of biofilter	Reference
2.4 kg m^{-3}	Floating bead filter Type polyethylene and Rotating biological contactor	56.2 mg TAN m^{-2} day^{-1} (Bead filters) 257 mg TAN m^{-2} day^{-1} (RBC)	DelosReyes and Lawson, 1996
0.39 kg m^{-3}	Rotating Drum filter	330 mg TAN m^{-2} day^{-1}	Twarowska et al., 1997
20.0 kg m^{-3}	Submerged biofilter	3.46 mg TAN m^{-2} day^{-1}	Al-Hafedh et al., 2003
10.3 kg m^{-3}	PP plastic chips PE blocks	46.5 mg TAN m^{-2} day^{-1} 44.5 mg TAN m^{-2} day^{-1}	Mohanmmad and Emmanuel, 2000
Not specify	Floating bead filter	54 mg TAN m^{-2} day^{-1} (series filter) 81 mg TAN m^{-2} day^{-1} (Solitary filter)	Hargrave et al., 1998
Not specify	Bubble-washed bead filter	0.45 g m^{-2} day^{-1}	Sastry et al., 1999
Not specify	Trickling filter	0.2 mg TAN m^{-2} day^{-1}	Lekang and Kleppe, 2002
140 kg m^{-3}	Microbead and Trickling filters 53 m^3 rearing tank	130 mg TAN m^{-2} day^{-1} 940 mg TAN m^{-2} day^{-1}	Anthony and Timmons., 1998

2.4.1.2 Integrated Nitrifying and Denitrifying Systems

Despite being relatively harmless to aquatic species, the presence of nitrate at extremely high levels may induce stress on aquacultures as well as creating environmental concerns if proper treatment is not met. Denitrification occurs naturally in sediments. However, natural process cannot handle large volume of aquaculture wastewater containing high nitrate concentrations. Literature reviews indicate limited information about combined nitrifying and denitrifying systems for closed aquacultures. The design features of denitrifying systems are similar to those for nitrification in the way that it requires high surface area biofilters to immobilize denitrifiying bacteria. Additional feature of denitrifying systems is the quick oxygen removal from wastewater to ensure anaerobic condition. It is desirable to keep DO concentration in denitrifying bioreactor below 1.0 mg L^{-1}. The research and development of combined nitrifying and denitrifying systems in Thailand was reported by Triyarat (2003). In this work, the tubular denitrification bioreactor was developed. Effluent containing nitrate (DO > 4 mg L^{-1}) from nitrifying bioreactor was slowly introduced into long cylindrical tube filling with plastic Biocall^{TM} biofilters. Methanol, chosen as an electron donor, is delivered via automated ORP control at the beginning section of the tube as mean to remove oxygen in wastewater. The performance of the system, tested with the closed-water shrimp cultivation, showed that the tubular bioreactor was able to grow shrimp without any water exchange for 7 months. The average ammonium concentration was observed below 0.06 mg N L^{-1} without any significant nitrite accumulation. The maximum nitrate concentration in this works was reported at 39 mg N L^{-1}.

2.4.2 Suspended-Growth Systems

In suspended-growth system, microorganisms (e.g., bacteria and microalgae) are free to move within water. Due to the slow growth of both nitrifying and denitrifying bacteria, the use of suspended growth in treating inorganic nitrogen compounds is limited. Based on literature reviews, it becomes clear that nitrification, denitrification, and direct nitrogen assimilation can occur simultaneously in suspended-growth systems. Examples of existing suspended-growth systems are earthen stabilization ponds and biofloc technology ponds.

2.4.2.1 Earthen Stabilizing Pond

Earthen stabilizing ponds or in short as earthen ponds are the least expensive system to build and maintain. Wastewater from aquaculture ponds is introduced into earthen pond with the dept about 0.5 – 1.0 m and thoroughly mixed to attain homogeneity. Wastewater is kept in earthen from 1 – 2 days or as long as a week to ensure a complete treatment. Figure 2-1 displays the biological relationships between different biological processes in earthen ponds. Oxygen is generated from reaeration at water surface and from photosynthesis of phytoplanktons during the day. Oxygen is consumed during nitrification or aerobic degradation to produce ammonium, CO_2, NO_3^- and PO_4^{3-}. Microalgae utilize these inorganic compounds for growth. Nitrate removal is accomplished via direct assimilation or denitrification in bottom sediments to produce nitrogen gas. These biological processes are cylic so that inorganic nitrogen waste can be treated continuously. The capability of earthen ponds depends strongly on the rate of oxygen production by microalgae as well as the ability of both nitrifying and denitrifying bacteria to utilize nitrogen. Earthen ponds with microalgae are capable of treating inorganic nitrogen waste under wide ranges from 176 – 2,113 mg N m^{-2} day^{-1} (Brune et al., 2003; Burford et al., 2003; Hargreaves, 2006).

2.4.2.2 Bioflocs Technology

Inorganic nitrogen treatment can be carried out in suspension in a process known as biofloc technology. This process is also known in different terms namely active suspension pond (ASP), heterotrophic pond, and green soup. Biofloc technology requires heavy aeration, intensive mixing, and addition of organic carbon compounds (e.g., starch, wheat, and molass) to enhance nitrogen assimilation during heterotrophic bacterial growth (Avnimelech, 2006). As cells continue to grow, they tend to form large irregular aggregates known as bioflocs, which posess voidage approximately 65 – 70% (Avnimelech, 2006; Sales and Shieh, 2006). Examination reveals that bioflocs consists of various strains of bacteria, microalgae, protozoa, and suspended solids, which are loosely-held together by excreted polysaccharide compounds called extracellular polymer (EPS) (Burford et al., 2004). Opended structure of bioflocs facilitates the oxygen transport for nitrifying bacteria so that they

can compete effectively with heterotrophic counterparts. Figure 2-2 illustrates the concepts of biofloc technology.

The main feature of biofloc technology is the ability to recycle proteins, which are the most expensive component in aquaculture feeds. Although feed proteins are already converted into ammonium via ammonification, heterotrophic bacteria were still able to utilize ammonia for growth, forming bioflocs, which in turn can be used by several ecomical aquatic stocks such as shrimp and tilapia as supplemental diets. As a result of biofloc consumption, cost of feed can be reduced either by lowering feed protein content or reducing feed ration. Studies show that by utilizing biofloc technology concept it is possible to reduce feed expense as high as 30 – 50% (Boyd and Boyd, 2002; Panjatan, 2004; Avnimelech, 2006; Crab et al., 2007). Recent study also reveals that bioflocs contain essential proteins, vitamins, and probiotic compounds comparable to commercial shrimp feeds (Tacon et al., 2002; Defoirdt, 2007). The disadvantage of biofloc technology stems from a rapid growth of heterotrophic bacteria that is almost 40 folds greater than nitrifying bacteria. As a result, water in biofloc technology tends to be extremely turbid and requires regular solid removal to prevent formation of anaerobic metabolites (e.g., H_2S) at the certain area of the pond. Moreover, aeration and mixing must be provided continuously. Malfuction of aeration equipments can cause a quick decline of DO concentration to reach critical level (i.e., DO < 1.0 mg L^{-1}) within 2 – 3 hours (Boyd and Clay, 2002).

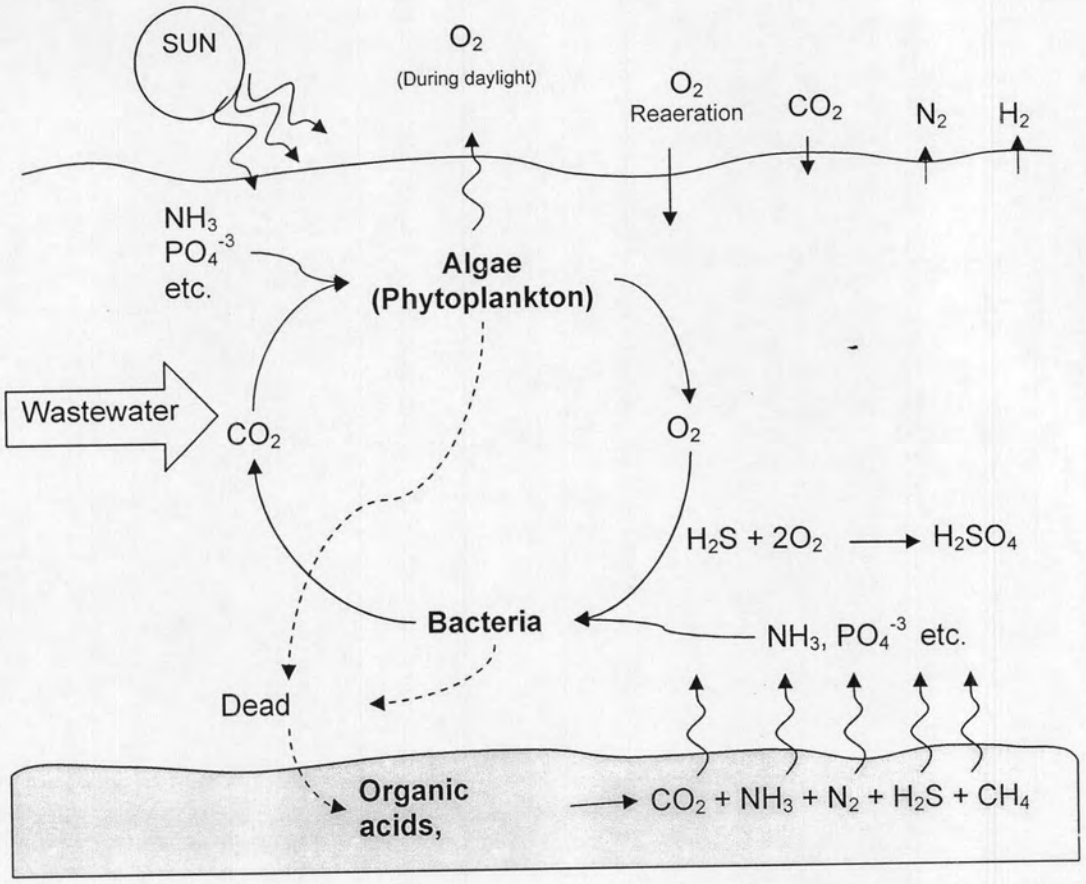

Figure 2-1 Biological relationships between different biological processes existing in earthen stabilizing ponds (Nootong, 2008).

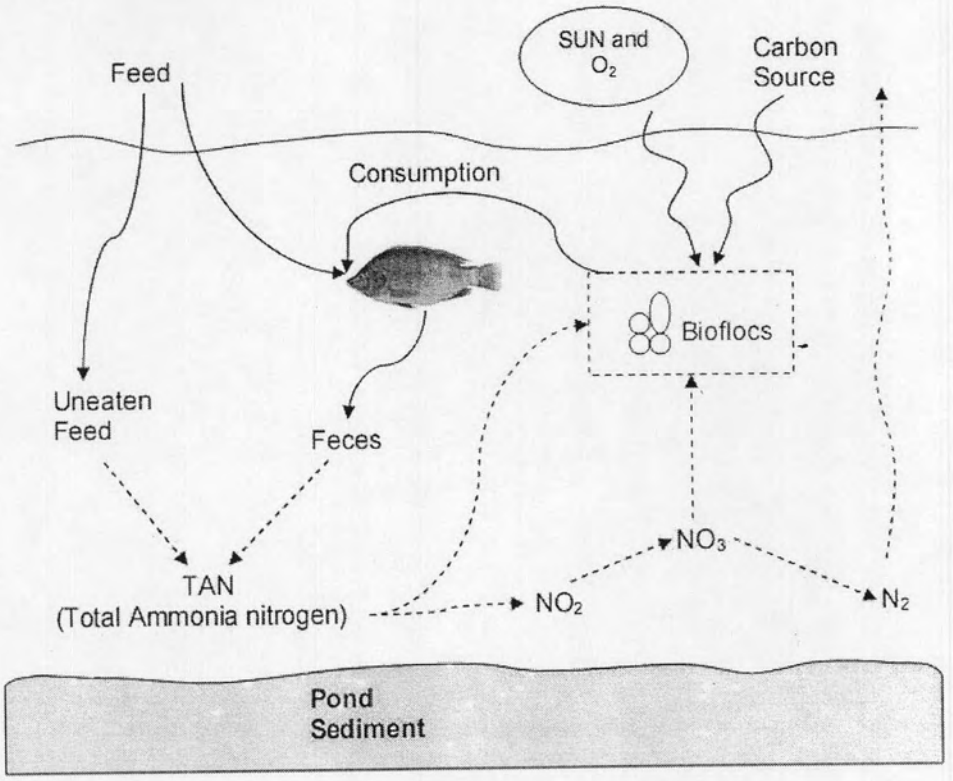

Figure 2-2 Inorganic nitrogen treatment and protein reutilization in biofloc technology pond (Nootong, 2008).