CHAPTER 2
BACKGROUNDS AND LITERATURE REVIEWS

2.1 Backgrounds: Nitrification and Denitrification

Nitrogen compounds found in raw sewage may be biologically oxidized to nitrates, provided that proper aerobic environment is maintained in the biological treatment process. Should the nitrified effluent be subjected to a period of anaerobiosis, the bacteria can utilize nitrate as electron acceptors. Under these conditions, nitrates and nitrites are reduced to nitrogen gas. This process leads to a reduction in the nitrogen content of the wastewater as it escapes from solution in gaseous form. The simplicity of the structures and the fact that no liquid or solid waste byproducts are associated with the process have led considerable attention to the investigation of the behavior of this system. (Canale, 1971)

All of the system designs proposed for nitrogen removal process are based on the sequential steps of

1) Oxidation of nitrogenous material or ***Nitrification***
2) Reduction of nitrates or ***Denitrification***

2.1.1 Nitrification processes

Nitrification is a sequential, two-step oxidation of ammonia to nitrate. The process is mediated by predominately two autotrophic bacterial genera. The oxidation of ammonia to nitrite is mediated by *Nitrosomonas,* and the oxidation of nitrite to nitrate is by *Nitrobactor.*

The stoichiometric reaction for oxidation of ammonium to nitrite by *Nitrosomonas* is:

$$NH_4^+ + 1.5\ O_2 \xrightarrow{\textit{Nitrosomonas}} 2H^+ + H_2O + NO_2 \qquad (2.1)$$

The stoichiometric reaction for oxidation of nitrite to nitrate by *Nitrobactor* is:

$$NO_2^- + 0.5\ O_2 \xrightarrow{\textit{Nitrobactor}} NO_3^- \qquad (2.2)$$

The overall nitrification reaction which is the combination of the two stoichiometry can then be expressed by:

$$NH_4^+ + 2\ O_2 \longrightarrow NO_3^- + H_2O \qquad (2.3)$$

From the overall nitrification reaction, the oxygen requirement for the oxidation of ammonia, is 4.57 g O_2/g NH_4^+, which is consisted of 3.43 and 1.14 g O_2 for the oxidations of ammonium and nitrite, respectively (neglect cell synthesis). Should cell synthesis be considered, the following overall nitrification reaction is obtained.

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \longrightarrow 0.98NO_3^- + 0.021cell + 1.88H_2CO_3 + 1.041H_2O \qquad (2.4)$$

The oxygen requirement for the oxidation of ammonia in this case is 4.33 g O_2 /g NH_4^+. This oxygen requirement is not significantly different from what is obtained in the case that cell synthesis is not considered (Eq. 2.3). For the ease of further calculation, it is presumed that this cell synthesis have very little effect on the overall oxygen requirement for the nitrification and be neglected.

2.1.2 Factors controlling nitrification processes

Literatures showed that nitrification is affected by a number of variables including dissolved oxygen concentration (DO), temperature (T), substrate concentration (S), and pH.

A. pH

pH has a major effect on the rate of nitrification. Figure 2.1 presents the relation between the rate of nitrification and the level of pH in an activated sludge operating at 20 °C where the optimal pH for nitrification process was found to be in the range of 7.8-8.9. (Wild et al., 1971) US. EPA (1975) reported that the rate of nitrification declined as the pH moved to the acid range, and this was found to be true for both unacclimated and acclimated cultures, although acclimation tended to moderate the effect of pH. Nitrifiers could adapt to pH levels as low as 5.5 where a sudden decrease in pH (5.8-7.2) was shown to inhibit nitrification but not having a residue toxic effect. The nitrification process itself can depress the pH to undesirable levels. From stoichiometry in Equation 2.4, alkalinity (HCO_3^-) is destroyed by the oxidation of ammonia, and at the same time carbonate (H_2CO_3) is produced. This tends to lower down the level of pH in the system. Thus in nitrification process, calcium carbonate ($CaCO_3$) is usually required as a buffering agent for the wastewater.

B. Temperature

The rate of nitrification is also strongly affected by temperature. Nitrification can occur in a wide range of temperature, *i.e.* from 4 to 45 °C. Figure 2.2 presents this effect for nitrification where it can be seen that the rate of nitrification increased with the temperature. Although the same figure suggests that nitrification could occur throughout a wide range of temperature, the optimum temperatures for *Nitrosomonas* and *Nitrobacter* were reported to be 35 °C and 35-42 °C, respectively. (Wild et al., 1971; US. EPA, 1975)

In terms of kinetics, the temperature has direct effects on the maximum growth rate of bacteria, μ_m, for both *Nitrosomonas* and *Nitrobacter*. This relation can be expressed in the form of Arrhenius equation as summarized in Table 2.1.

The optimum temperature range for the growth of pure nitrifying cultures is fairly narrow (25 to 30 °C), although the bacteria can actually grow in a much wider range of temperature (3 to 45 °C). (Focht and Verstraete, 1977; Wortman and Wheaton, 1991)

C. Dissolved Oxygen

The concentration of dissolved oxygen (DO) has a significant effect on the growth rate of nitrifying bacteria. Figure 2.3 shows that Nitrification could be achieved even at a very low DO level, *e.g.* 0.5 mg O_2/L, but a significantly higher rate could be obtained at higher DO levels. (Nagel and Haworth, 1969) Literature indicated that, in activated sludge, the nitrification ceased at DO below 0.3 mgO_2/L. (Painter et al., 1977)

D. Ammonia concentration

Nitrification rates were relatively unaffected by NH_4^+-N if the concentration of NH_4^+-N was greater than 2.5 mg/L. As NH_4^+-N concentration dropped below 2.5 mg/L, the rate of nitrification decreased sharply. (US. EPA, 1975) Similarly, van Rijn and Rivera (1990) reported that, at low range of ammonia concentration (< 2 mgNH_4-N/L) a higher rate of ammonia removal could be obtained at higher level of ammonia concentration.

With respect to dissolved oxygen and ammonia concentration, the kinetics of nitrification process is usually reported in the form of Monod equation where both ammonium-nitrogen and dissolved oxygen are treated as essential substrates and the mathematical expression for this kinetics was given by Denac et al., 1983 as followed:

$$\mu = \mu_m \frac{NH_4\text{-}N}{(K_{NH4\text{-}N}+NH_4\text{-}N)} \frac{DO}{(K_O+DO)} \quad (2.5)$$

where

μ = Specific growth rate [d^{-1}]

μ_m = Maximum specific growth rate [d^{-1}]

NH_4-N = Ammonium concentration [mg NH_4-N /L]

DO = Dissolved oxygen [mg O_2/L]

$K_{NH4\text{-}N}$ = Saturated constant of Ammonium-nitrogen [mg NH_4-N /L]

K_O = Saturated constant of Dissolved oxygen [mg O_2/L]

The rate of ammonia or nitrite oxidation depends strongly on substrate concentrations (ammonium and dissolved oxygen). Nitrite is not considered an essential substrate in this kinetic equation because the rate of oxidizing nitrite by *Nitrobacter* is by far greater than the oxidizing rate of ammonia by *Nitrosomonas*. Hence, ammonia oxidation by *Nitrosomonas* becomes the rate limiting step and the concentration of ammonia, not nitrite, becomes the controlling parameter for the overall nitrification process. Table 2.2 summarizes the values of parameters for various parameters in Equation 2.5.

E. Organic carbon

The nitrification process was strongly inhibited when organic carbon was present. The addition of carbon source with a carbon/nitrogen (C/N) ratio of C/N 1.0 or 2.0 reduced the total ammonia nitrogen removal rate by almost 70% compared with a pure nitrification process (C/N=0). (Zhu and Chen, 2001)

F. Toxics

Certain heavy metals, complex anion, and strong organic compounds are toxic to nitrifiers. Examples of toxic organic compounds are thiourea, allyl-thiourea, 8-hydroxyquinoline, salicyladoxine, histidine, amino acids, perchorethylene and mercaptobenzthiazole, whereas inorganic toxic substances are Zn, OCN^-, CLO_4^-, Cu, Hg, Cr, Ni, Ag, etc. (US. EPA, 1975)

It was reported that 10-20 mg/L of heavy metals could be tolerated by the bacteria because at the range of pH in the culture medium (7.5-8.0), most of the heavy metals exhibited low ionic concentrations. However, precipitated metals in the activated sludge at this pH range could cause serious problems if the pH fell and the precipitate dissolved. High concentrations of ammonia or nitrite, between 1400-2500 mg N/L, could also be temporarily toxic to nitrifiers. (US. EPA, 1975; Painter, 1977)

G. Salinity

In 1990, Nihof and Bovendeur studied the characteristics of fixed film nitrification in a fish culture system. The present of salinity in the water caused a reduction in the nitrification rate and it was shown that the nitrification rate in a fresh water and higher salinities (34 ppt.) systems were found to be 0.69 and 0.28 g NH_4-N/m^2 d, respectively.

H. Other essential requirements

Other requirements for growth of nitrifying bacteria include carbon dioxide, carbonate or bicarbonate, and ammonia or nitrite. Phosphate, magnesium, iron, and copper in small quantity are also essential for growth. (Painter, 1977) Table 2.3 summarizes all other essential requirements for proper growth of *Nitrobacter* and *Nitrosomonas*.

2.1.3 Denitrification Processes

Denitrification is the dissimilatory reduction of NO_3^- or NO_2^- to N_2 gas. In other words, NO_3^- and NO_2^- are the electron acceptors used in energy generation metabolism. Denitrification is widespread among heterotrophic and autotrophic bacteria, many of which can shift between oxygen respiration and nitrogen respiration. In environmental biotechnology, denitrification is applied when a complete removal of nitrogen is required. The reduction steps of nitrate can be illustrated as follows:

$$NO_3^- \xrightarrow{\text{Nitrate reductase}} NO_2^- \xrightarrow{\text{Nitrite reductase}} NO \xrightarrow{\text{Nitric oxide reductase}} N_2O \xrightarrow{\text{Nitrous oxide reductase}} N_2 \qquad (2.6)$$

The biological process of denitrification involves the conversion of nitrate nitrogen to a gaseous nitrogen species, primarily nitrogen gas. As opposed to nitrification, a relatively broad range of bacteria can accomplish denitrification, *e.g. Pseudomonas, Micrococcus, Achromobacter* and *Bacillus*. (US. EPA, 1975) Many bacteria can shift between using oxygen and nitrate (or nitrite) rapidly and without difficulty. Denitrification is achieved by contacting nitrified wastewater with biomass in the absence of oxygen. The reduction of nitrate, however, proceeds too slowly to be practical without the addition of biologically degradable organic material to anaerobic step. Several early investigators added raw sewage to the denitrification basin to speed up the reaction, but this has the limitation of adding unoxided nitrogen compounds and additional BOD to the final effluent. Most recent investigators have used methanol to accelerate the biological denitrification.

Stoichiometries involved in the denitrification are:

First step of Denitrification:

$$NO_3^- + 1/3\ CH_3OH \longrightarrow NO_2^- + 1/3\ CO_2 + 2/3\ H_2O \qquad (2.7)$$

Second step of Denitrification:

$$NO_2^- + 1/2\ CH_3OH \longrightarrow 1/2\ N_2 + 1/2\ CO_2 + 1/2\ H_2O + OH^- \qquad (2.8)$$

Overall denitrification reaction:

$$NO_3^- + 5/6\ CH_3OH \longrightarrow 1/2\ N_2 + 5/6\ CO_2 + 7/6\ H_2O + OH^- \qquad (2.9)$$

Based on the above stoichiometry, one mole of nitrate requires at least five-sixth mole of methanol for complete denitrification, or 1.9 mg of methanol is required for each mg of nitrate-nitrogen. If the effluent contains dissolved oxygen, then it must

be removed before denitrification can occur. This biological reaction can be accomplished by the addition of methanol as follows:

$$O_2 + 2/3\ CH_3OH \longrightarrow 2/3CO_2 + 4/3H_2O \qquad (2.10)$$

Each mg of dissolved oxygen (DO) requires 0.67 mg of methanol (CH_3OH) for its removal. Methanol must also be supplied to satisfy the requirements for bacterial growth. The quantity of methanol is about 1.3 time of the amounts given in the stoichiometric equation (Eq. 2.9). From these considerations, the following formula may be used for estimating the total amount of methanol required:

$$C_m = 2.47\ NO_3\text{-}N + 1.53\ NO_2\text{-}N + 0.87 DO_i \qquad (2.11)$$

where

C_m = required methanol concentration [mg/L]
NO_3-N = initial nitrate-nitrogen concentration [mg/L]
NO_2-N = initial nitrite-nitrogen concentration [mg/L]
DO_i = initial dissolved oxygen concentration [mg/L]

The value of C_m calculated above is somewhat conservative in that it does not make any allowance to the residual BOD entering the denitrification step. A methanol to nitrate-nitrogen ratio of 3.0 was suggested as a design guideline. (US. EPA, 1975)

2.1.4 Factors controlling denitrification processes

A. pH

US. EPA (1975) reported that denitrification rates significantly depressed at pH below 6.0 and above 8.0, with the highest rates occurring between 7.0 – 7.5. Figure 2.4 presents the effects of pH on denitrification rate. Similarly, Schroeder (1968) also reported that denitrification was not an unusually pH sensitive process, but sudden wide swings in pH, or operation at values below 6.5 or above 8.2 could be expected to result in decreases in denitrification rate.

B. Temperature

Figure 2.5 indicates that denitrification takes place best at high temperature (in the range of temperature between 10-25 °C). Laboratory and pilot studies on the effect of temperature on the denitrification rate usually resulted in quasi-Arrhenius-type relations: (Delwiche, 1981; Barnard, 1982)

$$q_{D,T} = q_{D,20}\,\theta^{(T-20)} \qquad (2.12)$$

where

$q_{D,T}$ = The denitrification rate at temperature T °C [mgNO_3^-N/gVSS d]

$q_{D,20}$ = The denitrification rate at temperature 20 °C [mgNO_3^-N/gVSS d]

θ = Arrenhius constant

T = Temperature [°C]

However, the denitrification was also reported in soil at temperatures as low temperature as 5-10 °C. (McCarty et al., 1996)

C. Dissolved oxygen

The dissolved oxygen in water is a significant factor in denitrification process. Denitrifiers are facultative aerobes with the ability to use both oxygen and nitrate as electron acceptor in their metabolic processes. Oxygen is a preferred electron acceptor for denitrifying bacteria as they obtain high energy per mole of oxygen consumed. The quantities of energy generated from utilizing oxygen and nitrate as electron acceptors are 686 and 649 kcal/mole, respectively. (US. EPA, 1975) However, at sufficiently low level of dissolved oxygen (0.2-1.5 mgO_2/L), denitrifying bacteria was found to switch from using oxygen to nitrate as electron acceptor. (Painter, 1977)

D. Nitrogen loading

Nitrate-nitrogen is an important substrate for denitrification process and the rate of denitrification usually depends on the nitrate-nitrogen concentration in the

form of Monod kinetics (Eq. 2.13). It was shown that nitrate removal rate decreased with nitrate concentration below 2 mg NO_3-N/L. (Painter, 1977) However, nitrate concentration below 0.5 mgNO_3-N/L was reported to be limiting to the growth of bacteria in aquaculture ponds. (Hargreaves, 1998)

E. Organic carbon

In denitrification process, denitrifiers require organic carbon as substrate for respiration and growth. Methanol, acetate, ethanol, acetone and sugar are used as carbon sources in this process. Stoichiometry in Equation 2.9 illustrates the overall denitrification reaction where methanol is employed as an organic substrate. Methanol is often selected as the carbon source for denitrifying bacteria because it is economically attractive with reasonably good performance in terms of removal rate. (McCarty, 1966) However, ethanol was considered to be a more readily available carbon source than methanol. The growth rate of denitrifiers with ethanol as a carbon source was 2-3 times higher than with methanol. (Christensson et al., 1994; Tam et al., 1994)

There also exists an optimal ratio between carbon and nitrogen sources for optimal growth of denitrifying bacteria. Past experiment suggested that a carbon-to-nitrogen ratio of 1 to 2 in the culture medium was most suitable for nitrate removal. (Balderston and Sieburth, 1976)

In denitrification process, the kinetic expression is usually written with respect to the concentration of nitrate and organic carbon concentrations. Monod equation is commonly employed to explain the kinetics of this reaction where

$$\mu = \mu_{DM} \frac{D}{(K_D+D)} \frac{S}{(K_s+S)} \qquad (2.13)$$

μ = Specific growth rate of denitrifying bacteria [d^{-1}]

μ_{DM} = Maximum specific growth rate [d^{-1}]

D = Nitrate concentration [mg NO_3-N/L]

S = Organic carbon concentration [mg/L]

K_D = Saturated constant of Nitrate [mg NO_3-N/L]

K_S = Saturated constant of Organic carbon [mg /L]

Equation 2.13 illustrates that the denitrification rate increases linearly with substrate concentrations until concentrations reach some specific values that the reaction rate no longer depends on substrates concentrations. The kinetic parameters are depicted in Table 2.2.

F. Oxidation reduction potential

Oxidation-reduction potential (ORP) was suggested for monitoring denitrification in sewage. Several ranges of ORP were used in literatures. Balderson and Sieburth (1976) used ORP [mV] to indicate the principal terminal electron acceptor. Denitrification process was achieved by maintain ORP between 0 mV and +150 mV which was the range where nitrate removal from wastewater could take place without generating toxic byproducts such as H_2S. Sulfide production in aquaculture was extremely toxic to most aquatic animals (less than 1 mg S^{-2}/L) and could occur at a much lower ORP, *e.g.* ORP<0. (Jones, 1964; Balderston and Sieburth, 1976) However, this range of ORP is not definite and various investigators reported different level of ORP for denitrification. For instance, Lee (2000) suggested ORP range between -200 to -400 mV for a proper control of denitrification process.

G. Alkalinity

According to McCarty et al. (1969), denitrification resulted in a theoretical production of 3.57 g. alkalinity (as $CaCO_3$) per gram of nitrate or nitrite reduced when methanol is the substrate. This was based on the following stoichiometries:

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \longrightarrow 0.056C_5H_7O_2N + 0.47N_2 + 1.68H_2O + HCO_3^- \quad (2.14)$$

$$NO_2^- + 0.67CH_3OH + 0.53H_2CO_3 \longrightarrow 0.04\ C_5H_7O_2N + 0.48N_2 + 1.23H_2O + HCO_3^- \quad (2.15)$$

where $C_5H_7O_2N$ is an empirical representation of denitrifying bacterial cell. This is a fortunate occurrence because about twice this amount of alkalinity is destroyed during nitrification. It was noted by McCarty et al. (1969) that Equations 2.14 and 2.15 assumed a constant stoichiometry coefficients for all operating conditions which was often not the actual case. However, these equations were found to provide satisfactory predictions as initial estimates of process performance.

2.2. Literature reviews

2.2.1. Nitrification processes

In seawater culture systems, the nitrification process was carried out in various closed seawater systems. Literature concerned with these systems is delineated as follows.

A. Trickling filters

Trickling filter is a simple unit for nitrification process. The operation of trickling filter is performed simply by spraying wastewater at the top of the filter tank and let it flow down through an immobilized nitrifying bacteria on the packing material by gravity. The heat of reaction, despite only a slight quantity, is enough to force the fresh air to flow up counter-currently with the water flow. Oxygen required for microbial growth from fresh air will then transferred to the biofilm on the surface of the packing. Tricking filter was a preferable method in aquacultural nitrification. Past works indicated that trickling filters had a good performance for nitrification process. The advantage of tricking filter includes low maintenance, cheap installation and great tolerance to differences in hydraulic and organic loads.

Otte and Rosenthal (1979) used a trickling filter as nitrification process in fish (Tilapia and eel) culture system. An average efficiency of the trickling filter in this system for ammonia removal was 31% of ammonium loading. Rogers (1985) applied the trickling filter for nitrification in fishponds and achieved a 50% removal of ammonia loading with a removal rate of 0.3 gNH_4-N/m^2d. van Rijn and Rivera (1990) employed this process for the oxidation and reduction of inorganic nitrogen in aquaculture system. The maximum removal rate of ammonia was 0.34 gNH_4-N/m^2d. In eel farms, the maximum nitrification rate of trickling filter was 0.55 gNH_4-N/m^2d. (Kamstra et al., 1998) Lakeng and Kleppe (2000) studied efficiency of different filter media type between plastic media and crushed dried expand clay (Leca) in tricking filters. The result showed that Leca filter media provided a 100% nitrification rate.

B. Fixed-film biological filters / Submerged filters

Fixed film biofilter or submerged filter column was a conventional nitrification process in recirculating seawater systems. The column is packed with filter media (coarse sand, gravel media, crushed rock media, plastic media and oyster shell) to support nitrifying bacteria. In the operation of this system, the wastewater is passed through an aerated box for oxygen exchange with air, and then pumped through the fixed film biofilter column (see Fig. 2.6 for illustrating example).

Koller and Avtalion (1985) used a fixed-film biofilter column to remove ammonia-nitrogen for Tilapia breeding. In prawns and lobsters culture system, the maximum nitrification rate of fixed film column was 0.43 g NH_4-N/m^2d. (Wickin, 1985) For the culture of Loliginid Squids, the biological filter, filled with crushed oyster shell, was reported to provide good wastewater effluent quality where the ammonium-nitrogen concentration was below 0.1 mg NH_4-N/L (Yang et al., 1989). MacMillan et al. (1994) designed a closed artificial seawater system for Bivalve Shellfish culture. Fix-film biofilter with activated carbon filter media was employed as a treatment unit and the average effluent ammonium-nitrogen concentration was below 0.004 mg NH_4-N/L. For the culture of *Penaeus monodon*, submerged biofilter was also reported to be a successful system for the treatment of wastewater containing ammonia-nitrogen and the treated water could be recirculated to the culturing pond. (Tseng et al., 1998)

C. Rotating biological contactor (RBC) and Biodrum

Rotating biological contactors consist of a series of closely spaced disks (lightweight plastic, 10-12 ft in diameter) which are mounted on a horizontal shaft and rotated while about one-half of their surface is immersed in wastewater. As disks rotate, they carry a film of wastewater into the air where it trickles down the surface of the disks, absorbing oxygen. The speed of rotation is adjustable. The attached growths are similar in concept to tricking filter, with the exception that the microbes are through the wastewater rather than the wastewater being passed over the microbes. Biodrum is a very similar process to the RBC with the

difference at the rotating disks. In Biodrum, the disks are replaced by the perforated drums on which the bacteria grow (Fig. 2.7).

Kaiser and Schmitz (1988) used a rotating disk filter as biofilter in a closed recirculating fish culture system. Wortman and Wheatton (1991) used biodrum for nitrification. The maximum removal performance of biodrum was 400 mgNH_4-N/L d. In the culture of black tiger shrimp and sea bass, biodrum-biofiter was successful in maintaining a low level of ammonia-nitrogen (0.5 mg-N/L) and nitrite-nitrogen (0.6 mg-N/L). (Kittimasak et al., 1997) In a rainbow trout culture system, the rotating disk biofilter was used as nitrification unit and the nitrification rate of 10 mgNH_4-N/L.d could be achieved. (Schuster and Stelz, 1998)

D. Floating immobilized carriers

In floating immobilized system, the carriers with attached biofilm are left floating in the nitrifying column. These carriers might be conveyed through various parts of the reactor in airlift style, or can just be left floating on the top of the column. The attached bacteria on the surface of the carriers are responsible for the nitrification reaction (see illustration in Fig. 2.8).

Greiner and Timmous (1988) used floating polystyrene bead to remove ammonium-nitrogen in recirculating tilapia production facility. The performance of floating micro bead filter could be accepted. Sakairi et al. (1990) designed a nitrification in an airlift reactor (15.7 L). Nitrifying bacteria were immobilized in floating micro-porous cellulose carriers. The maximum ammonium-nitrogen removal rate was 99-100 % and the rate of removal was reported at 1.30 kg-N/m^3-carrier d.

E. Activated Sludge

Early experiment of activated sludge was performed as nitrification in a recirculating aquacultural system in Korea, the performance of the activated sludge reactor containing nitrifiers immobilized in Ba-alginate, Ca-alginate, carrageenan and agar beads was investigated. An airlift bioreactor was used in

this experiment (Fig. 2.9). It was found that using immobilized Ba-alginate and Ca-alginate beads could remove 94 and 87% of the loaded ammonia in 3.4 h, respectively. The amount of ammonia removal rate was 2.8-82 g NH_3-N/m^3d but this rate also varied with hydraulic retention time. (Kim et al., 2000)

Table 2.5 summarizes the conditions and ammonia/nitrite removal efficiencies of various nitrification units mentioned above.

2.2.2 Denitrification processes

Due to anaerobic nature of the denitrifying bacteria, elimination of oxygen is one of the most important factors in the design of reactor for denitrification. Biofilm provides an ideal mechanism for preventing oxygen mass transfer into the reaction zone where denitrifying bacteria reside. Hence, biofilm processes become the fundamentals behind most denitrification applications.

A. Denitrification columns or fixed-film biological filters

This process is used to remove nitrate from culturing seawater system. The denitrification column is prepared by filling the column with various filter media such as limestone, glass bead, crushed brick granules, polypropylene pall ring, crushed oyster shell and plastic ball. Culture water needed to be pretreated before entering the column to remove oxygen by purging nitrogen gas through. The process should be operated with a carbon-to-nitrogen (C/N) ratio of 1 to 2. The retention time of denitrification varies between 1.75 to 3.33 h. (Turk, 1996) Most of the results showed that denitrification column removed nitrate-nitrogen from the closed seawater system at acceptable level. However, the disadvantage of this method is the high cost for deoxygenated seawater.

In aquacultural seawater systems (Balderston and Sieburth, 1976), the denitrification column, filled with limestone for lab scale (1.5 L), was used to remove nitrate-nitrogen in culture water. Methanol was added as a carbon source with a C/N ratio of more than 1. The results showed that the column could remove

95-100% from the total 100 mgNO_3-N/L in 20-22 days with removal rate of 0.007 mg NO_3-N/Lmin. It was reported that hydrogen sulfide production, which was toxic to most culture animal, was produced in the system when the oxidation-reduction potential was below 0 mV.

In 1996, Turk designed a system for reducing nitrate-nitrogen in a culture system. Methanol was used as a carbon source with 2.74 mL CH_3OH/mg NO_3-N. The denitrification column was packed with glass beads for bacterial attachment. This system was designed to control the ORP in the range between -50 and +200 mV which was believed to be the range that nitrate acted an electron acceptor. The amount of methanol added to the system displayed inverse relationship with the ORP. Lee et al. (2000) revealed that one of the problems in the denitrification column was the production of hydrogen sulfide in anoxic condition (ORP below - 400 mV). Hence, it was important to have a good control of ORP where ORP from -325 to -400 mV was found to be optimal for the reduction of nitrate to nitrogen gas.

Menasveta et al. (2001) used a denitrification column as a treatment system in a shrimp culture recirculating system. This system comprised 2 connecting columns, (i) deoxygenating column through which nitrogen gas was purged to remove all dissolved oxygen in the culture water, and (ii) the denitrification column packed with plastic bioballs and crushed oyster shell where most denitrification occurred. Ethanol was added as a carbon source. The results showed that nitrate concentration could be controlled in acceptable range (<50 ppm). Illustration in Figure 2.10 shows the schematic diagram for this system. This system was later modified by Singhabhandhu et al. (2000) to remove the need for nitrogen purge. It was achieved by the use of a long tube, 50 m, packed with plastic balls (PVC) or crushed shell for nitrate removal in artificial shrimp culture seawater. It was shown that this system was capable of reducing nitrate from 145.4 to 2.9 mgNO_3-N/L in 8 days (0.0124 mgNO_3-N/min). However, this system was still subject to the problem of hydrogen sulfide (H_2S) generation.

B. Activated sludge tank

Activated sludge was reported to be able to remove nitrate in a closed recirculating system. Activated sludge was also used as a denitrification unit for Tilapia and European eel farm. This system was left working under anaerobic condition where wastewater at 2 L/min was fed from the bottom of the tank and stirred with a propeller. Glucose solution and methanol was added as carbon sources to the activated sludge tank. The results showed that wastewater with initial nitrate concentration of 200-400 mgNO_3-N/L could be treated with a maximum nitrate removal rate of 98%. (Otte and Rosenthal, 1979)

C. Fluidized bed columns

The fluidized bed was also reported to provide good performance as a nitrate removal process. Commonly, the fluidized bed column was filled with sand and the top of column was equipped with an impeller. This impeller sheared excess biofilm and gas bubbles from the sand particles whereby wash-out of the sand particles was prevented. The average denitrification rate of fluidized bed column was 0.20 mgNO_3-N/Lmin. The advantages were the high rate of nitrate removal and short retention time. The disadvantages of this method were the high-energy requirement for driving the sand in the column and the complicated scale up and design. (van Rijn et al, 1990) Illustration for the fluidized bed column in Figure 2.11 was selected from the work of van Rijn and Rivera (1990) who also reported a success in using fluidized bed in treating nitrate containing wastewater.

D. Floating Immobilized carriers

Floating Immobilized carrier was employed to remove nitrate for seawater treatment. The floating carriers were circulated in a driving liquid reactor (volume 9 L) by liquid flow induced by a driving jet in the reactor. The carriers were cellulose immobilized with denitrifying bacteria. The carbon source such as methanol was required for this system. It was reported that the floating carrier had a high denitrifying rate (20.79 kgNO_3-N/m^3carrier.d). The rate of denitrification depended on the volume of carrier in the reactor. The disadvantages were the cost for

carriers and the reactor. (Sakairi et al., 1996) Figure 2.12 illustrates the immobilized carrier reactor employed as a denitrification unit by Sakairi et al. (1999).

Table 2.6 summarizes the conditions and nitrate removal efficiencies of various denitrification units mentioned above.

Table 2.1 Relationship between specific growth rate and temperature for nitrifying bacteria (Painter and Loveless, 1983)

Source	$\mu_m = \mu_{m15}$ x exp {C(T-15)} [d^{-1}]
Mixed domestic-industrial sewage	μ_m = 0.064x exp {0.031x(T-15)}
Stevenage domestic sewage	μ_m = 0.183x exp {0.0729x(T-15)}
Thames water	μ_m = 0.462x exp {0.096x(T-15)}

Table 2.2 Kinetic parameters for nitrification process (Tchobanoglous,1991; Hargreaves, 1998)

Parameter	Unit	Range
Nitrosomonas		
μ_m	d^{-1}	0.3-2.0
K_s	mgNH_4^+-N/L	0.2-2.0
Nitrobacter		
μ_m	d^{-1}	0.4-3.0
K_s	mgNO_2^--N/L	0.5-5.0
Overall		
μ_m	d^{-1}	0.3 - 3.0
K_s	mgNH_4^+-N/L	0.2 - 5.0
K_o	mg DO/L	0.3 – 0.9

Table 2.3 Essential requirements for proper growth of *Nitrobacter* and *Nitrosomonas*. (Painter, 1977)

Requirement	[mg/L]
Phosphate (P)	5
Magnesium (Mg)	n.a.
Iron (Fe)	n.a.
Copper (Cu)	0.03
Sodium (Na)	0.002-0.005
For *Nitrobacter*	
Zinc	1
Molydenum	0.001
For *Nitrosomonas*	
EDTA	5

n.a. = not available

Table 2.4 Range of Oxidation-Reduction Potential (ORP) for various types of principle electron acceptor

Source	ORP		
	O_2	NO_3^- and NO_2^- ion	SO_4^{-2}
Balderson and Sieburth,1976	above +200 mV	-50 to +200 mV	below -100 mV
Lee et al., 2000	0 to -200 mV	-200 to -400 mV	below -400 mV

Table 2.5 Kinetic parameters for denitrification process (Tchobanoglous et al. , 1991)

Parameter	Unit	Range
μ_{DM}	d^{-1}	0.3 - 0.9
K_D	mg NO_3-N/L	0.06 - 0.2

Table 2.6 Details on the operation of various types of nitrification processes

Reference	Nitrification rate [gN/m^2d]	Type Volume [m^3]	Packing	Flow rate [L/min]	Retention time [min]	DO [mgO_2/L]	Specific surface area [m^2/m^3]	pH	Temperature [^oC]	Salinity [$^0/_{00}$]	NH_4-N [$mgNH_4$-N/L]
Tricking filter					-						
Otte and Rosenthal (1979)	0.75	1.06	Plastic foil filter	83.33	-	4.5- 6.0	480 m^2	7.0	22 - 26	8	15
Rogers (1985)	-	0.04	Slag	0.16	-	5-6	18.3	7.8	25 - 30	20	10
van Rijn et al (1990) and Arbiv et al (1995)	0.15-0.43	2	-	217 and 250	-	6.5-7.5	200 (400 m^2)	7.0	27	-	2
Knosche (1994)	0.4-1.4	-	-	4.02	-	-	200	7.0-7.3	20	-	5
Greiner and Timmons (1998)	0.94-3.92	-	-	-	-	> 5	-	6 – 7	26.4	-	-
Kamstra et al (1998)	0.24-0.55	-	-	-	-	7 – 8	100-150	7 – 8	22-24		-
Nihof and Bovendeur (1990)	0.28-0.69	3.5	Plastic	b	15 - 60	-	200	8.2	24	33-34	5-7
Ninof (1995)	0.7-0.8	0.005	Plastic	48-239	-	7.4-8.2	200	7.0-7.5	25	-	5
Singh et al (1999)	-	-	Plastic	150	-	7-8	1300	-	-		-
Lakang and Kleppe (2000)			Finturf artificial				248				
			glass								
	0.1-0.2	-	Kaldnes rings	0.5	2.98	6.7-10.7	500	6 - 7	14-16		1.5
			Plastic rings				220				
			Leca (clay)				500-1000				
Fixed film biofilter					-						
Davis and Arnold (1998)	0.59	0.72	Polypropylene	280	25.71	10.2	223.1				
Bower and Turner (1982)	-	-	Limestone	-	-	-	-	8.1-8.4	25 ±1	30-31	10
Greiner and Timmons (1998)	0.13-0.57	-	Microbeads	-	-	> 5	-	6 – 7	26.4	-	-

Table 2.6 (cont.)

Reference	Nitrification rate [gN/m^2d]	Type Volume [m^3]	Packing	Flow rate [L/min]	Retention time [min]	DO [mgO_2/L]	Specific surface area [m^2/m^3]	pH	Temperature [^oC]	Salinity [$^0/_{00}$]	NH_4-N [$mgNH_4$-N/L]
Koller and Avtalion (1985)	-	-	Gravel	0.09	3-5	3.4-5.6	3500 m^2	6.9-7.5	26-28	-	3
Tseng et al (1998)	0.23	0.72	Plastic	b	20	3.6	150	7.48-7.96	32	33	3.64
Menasveta et al (1991)	-	-	Plastic bioball	-	-	5-6	-	7-8	27-29	30	-
Menasveta et al (2001)	0.068	6	Plastic bioball	b	-	-	-	7.5	-	-	2
Macmillan et al (1991)	0.083	0.0035	Activated carbon	7	-	-	-	8-8.4	22-24	26-30	-
Millamena (1994)	-	0.1	Sand, gravel & crushed rock	-	1.6	> 4	-	7.8-8.3	28-31	30-32.5	-
Reyes and Lawson (1996)	0.056	0.17	Polyethylene	0.14	-	5.3	178 m^2	7.98	30.4		
Tschui (1994)	0.58-1.35	-	Biocarbon Polystyrene Plastic	3-4 6-7 > 10	$1.97x10^3$ $1.47x10^3$		1450 1050 240	-	10		
Tseng et al (1998)	-	0.72	-	36	20	5.4-6.9	-	7.5-8	29-33	33	-
Sauthier et al (1998)	-	-	Crushed brick granules	-	-	9.2	2200	-	20	-	-
Sastry et al (1999)	0.33-0.45	-	Polyethylene	30-41	-	> 2	-	-	26-30	-	-
Shanableh and Hijazi (1998)	1.5	-	Polypropylene	b	-	5-7	115				8-9
Wickins (1985)	0.43	-	Plastic & gravel	0.083	120	-	-	-	28±2	20-34	-
Yang et al (1989)	0.69	-	Crush oyster shell	81	-	-	-	8.0	30±1	34-36	-
Yang et al (2001)	-	-	Plastic & carbon	70	150	5	80 - 300	6 - 9	0	0	1
Zhu and Chen (2001)	0.5-1.5	0.0025	-	0.11-0.13	-	6	623	6-8	27-28	-	12-30
Rotating biofilter contactor (RBC)											
Reyes and Lawson (1996)	0.257	1.4	-	73.6-78.2	-	5.3	246 (197 m^2)	7.98	30.4	-	-
Rogers (1985)	-	0.04	-	0.08	-	5-6	18.3	7.8	25-30	20	10

Table 2.6 (cont.)

Reference	Nitrification rate [gN/m^2d]	Type Volume [m^3]	Packing	Flow rate [L/min]	Retention time [min]	DO [mgO_2/L]	Specific surface area [m^2/m^3]	pH	Temperature [^oC]	Salinity [$^o/_{oo}$]	NH_4-N [$mgNH_4$-N/L]
Kaiser and Schmitz (1988)	-	-	-	-	-	-	-	6.8-7.0	15±1	-	2
Schuster and Stelz (1998)	-	0.12	-	-	-	-	-	6-7	15	-	-
Biodrum											
Rogers (1985)	-	0.04	Slag	0.08	-	5-6	18.3	7.8	25-30	20	10
Wortman and Wheaton (1991)	0.4-1.6	0.009	Polypropylene	0.62	-	4.6-11	278.83	7.5-8.5	25	7-35	8-9
Menasveta (1991)	-	-	-	-	-	5-6	-	7-7.8	28-30	30	-
Immobilized in porous carrier											
Sakairi et al (1996)	-	0.0157	Cellulose carrier	0.052	-	5± 0.4	-	8	28		
Greiner and Timmons (1998)	-	-	-	-	-	> 5	-	6-7	26.4		
Malone and Beecher (2000)	-	-	Polyethylene	-	-	> 3.0	1150-1475 (2-3mm)	6.5-8.0	20-30	-	-
Seo et al (2001)	2.63 mg/L-h	0.045	Polyvinyl alcohol (4.5 mm)	4.5	60	5.2	-	7.8-8.2	23-27	0-30	10
Moving bed bioreactor											
Tal et al (2003)	0.59-0.75	0.15	Polyethylene	166	-	7	500	8	26	20	3
Fluidized bed filter											
Reyes and Thomas (1995)	-	170	Polyethylene	-	-	5.3	178	7.98	30.4	-	-
Skjolstrup et al (1998)	0.21-0.27	0.053	-	50	-	6-8	1000	7	17.6	-	2.2
Sequence batch reactor											
Zhu and Chen (1999)	1.86	0.047	Plastic	0.016	0.79	6	-	7.5-8.6	26.8-27.6	-	60

Table 2.6 (cont.)

Reference	Nitrification rate [gN/m^2d]	Type Volume [m^3]	Packing	Flow rate [L/min]	Retention time [min]	DO [mgO_2/L]	Specific surface area [m^2/m^3]	pH	Temperature [^oC]	Salinity [$^0/_{00}$]	NH_4-N [$mgNH_4$-N/L]
Sliekers (2002)	0.15	1	-	1.45	690	-	-	7.8	30	-	14
Strotmann and Windecker (1997)	-	0.0035	-	0.048	240	> 3	-	6-7	-	-	100-400
Activated sludge											
Campos et al (1999)	-	0.004	-	-	78	2-6	-	9.8	20	-	150
Campos et al (2002)	-	0.004	-	0.0007-0.002	-	> 2	-	7.8	20	-	500-3300
Kim et al (2000)	-	-	Ba-algenated Ca-algenated Carageenan Agar bead	-	18	-	7.5-7.9	-	25	-	20
Pond											
Gross et al (2000)	0.07	-	-	-	-	-	-	-	-	-	5.9

* b as operate batch type

Table 2.7 Details on the operation of various types of denitrification processes

Reference	Denitrification rate [$mgNO_3$-N/Lmin]	Volume [L]	Packing Type	Flow rate [L/min]	Retention time [min]	DO [mgO_2/L]	ORP [mV]	pH	Temperature [°C]	C:N ratio	Salinity [‰]	N-NO_3 [$mgNO_3$-N/L]
Fixed film column												
Balderston & Sieburth (1976)	0.007	1.5	Limestone (11 mm) Plastic Koch Flexiring (25 mm)	0.0075	200	< 1.2	0 to –200	-	20±1	> 1 Methanol	18	100
Abeysinghe et al (1996)	-	-	Perspex plastic	-	-	-	-	-	-	-	1	20
Turk (1996)	0.02-0.025	60	Glass beads	0.33	105-135	< 1.5	-50 to +200	-	-	1.5	-	80
Sauthier et al (1998)	8 g/m^3h	30	Crush bricks	0.14	200	0.5-1	0 to –200	-	20-25	1-2 (Met)	-	60
Shanableh & Hijazi (1998)	-	-	Polypropylene pell rings	0.10	135		-			1-4		8
Nagadomi et al (1999)	-	-	Polyvinyl alcohol beads	-	-		-			0		
Lee et al (2000)	-	-	Glass bead	-	200	0.8-1.2	-325 to 400	7-8		Methanol		
Boley et al (2000)	-	82.5	Polymer pellet	0.01		-	-	8	27-28	0	-	40
Menasveta (2001)	-	143	Plastic ball & crushed oyster shells	0.04-0.1	-	-	-	7-8	25	0.92 (Met)	28-32	200
Sinhabhandhu (2001)	0.0124	-	Plastic bioballs	0.043	-	-	-	-	29 ± 2	1-2 (Met)	30	100
Activated sludge tank												
Otte and Rosenthal (1979)	0.008	1060	-	2	-	6.0-7.5	-	7	22-26	-	8	1208(max)
Fluidized bed column												
van Rijn et al (1990) and Arbiv et al (1995)	0.2	131.5 (Lab scale)	Sand (0.3-0.9 mm)	5-40	12-13	< 0.2	-	7	27	-	-	50

Table 2.7 (cont.)

Reference	Denitrification rate [$mgNO_3$-N/Lmin]	Volume [L]	Packing Type	Flow rate [L/min]	Retention time [min]	DO [mgO_2/L]	ORP [mV]	pH	Temperature [°C]	C:N ratio	Salinity [‰]	N-NO_3 [$mgNO_3$-N/L]
Floating immoblilized												
Sakairi (1996)	1.44 per carrier	9	Cellulose carriers (3 mm)	0.022	-	1	-	8	30	1.3	7.23	20
Boley (2000)	0.02-2.77 0.03-3.84 g/m^2-d	82.5	Biodegradable polymer pallet (0.39-0.52 m^2)	0.003-0.01	-	-	-	6 -8	20-25	0	-	5-40
Pond												
Gross et al (2000)	0.038 g/m^2-d						-	7-9	21-28	0		

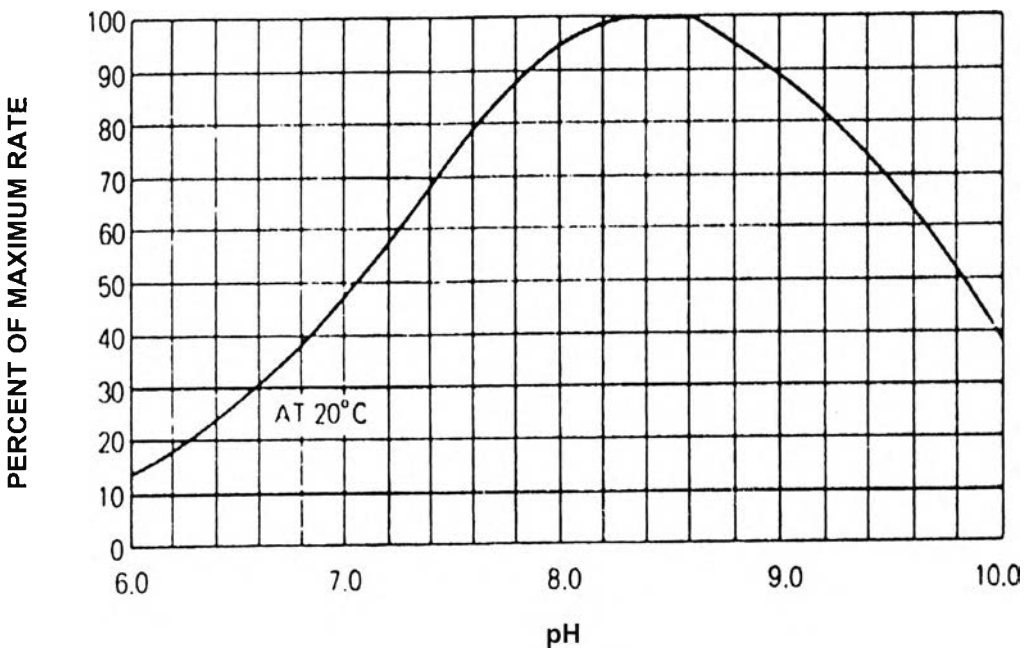


Figure 2.1 Effect of pH on nitrification rate at 20 °C (Wild et al., 1971)

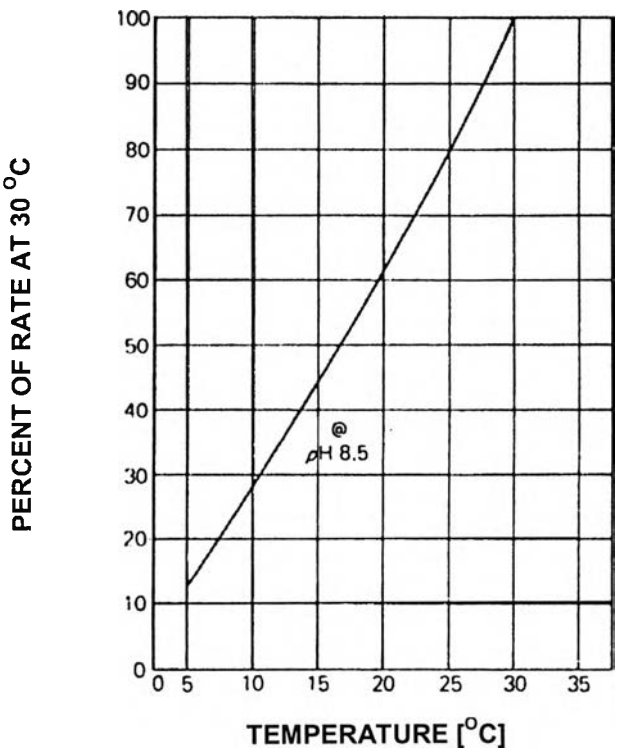


Figure 2.2 Effect of temperature on nitrification rate at 30 °C (Wild et al., 1971)

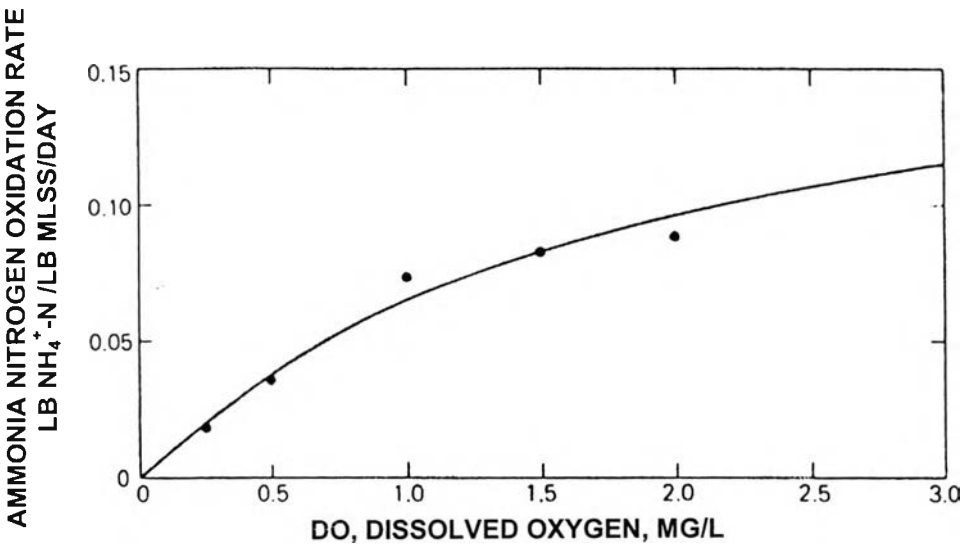


Figure 2.3 Effect of dissolved oxygen on nitrification rate at 30 °C (Nagel, and Hawort, 1969)

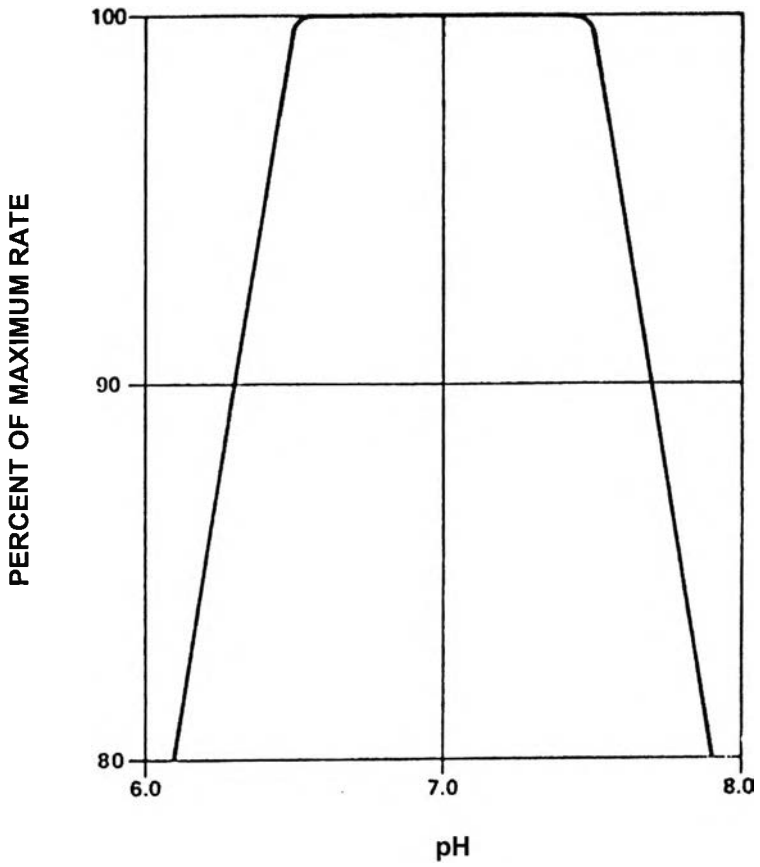


Figure 2.4 Effect of pH on denitrification rate (Sawyer et al., 1973)

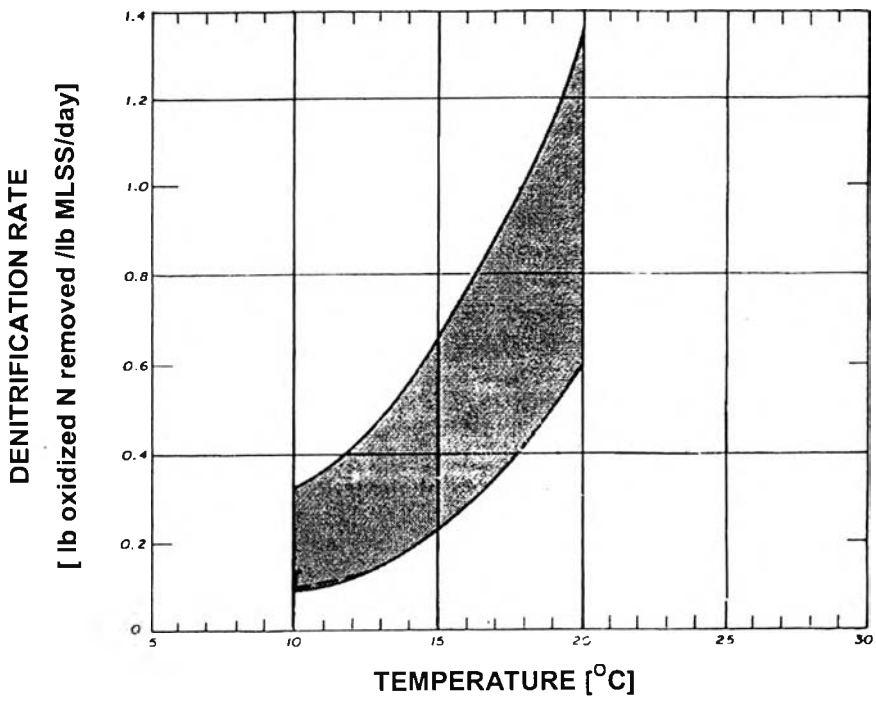


Figure 2.5 Effect of temperature on maximum denitrification rate (US. EPA, 1975)

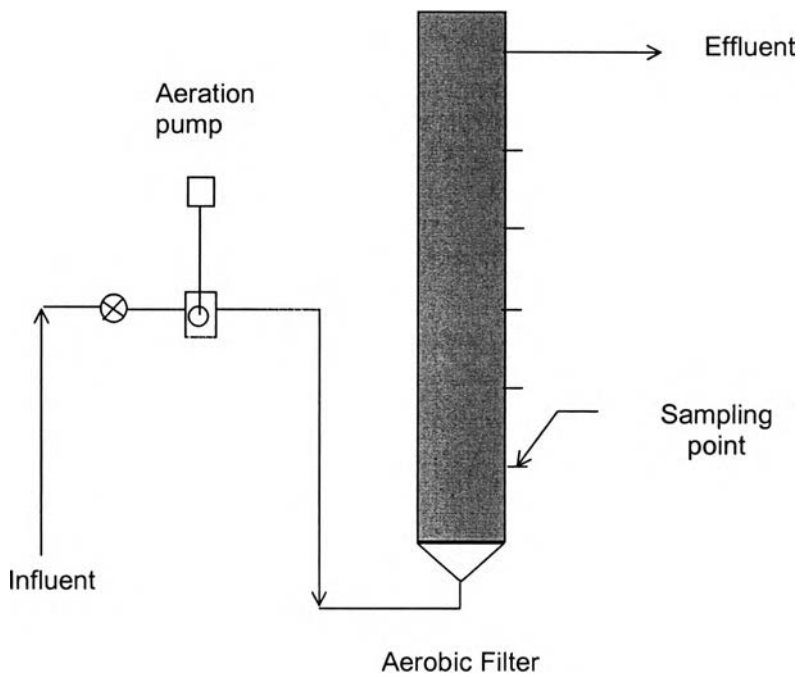


Figure 2.6 Fixed film column (Abeysinghe et al., 1996)

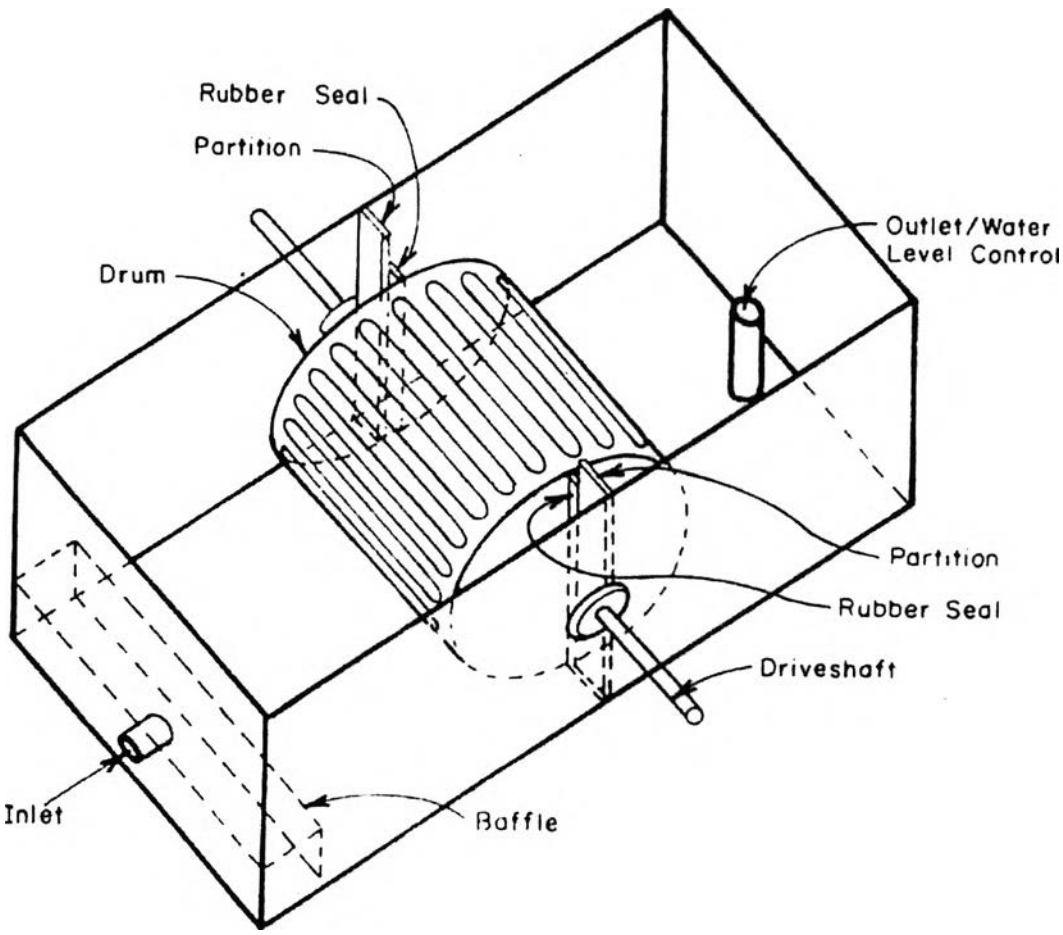
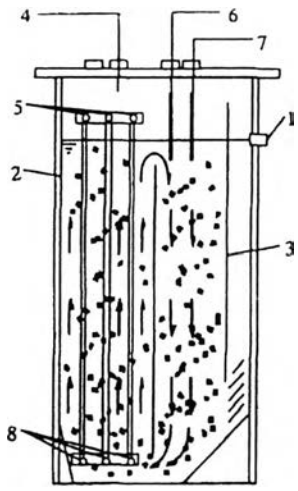


Figure 2.7 Biodrum biofilter (Wortman and Wheaton, 1991)



Working volume 15.7dm^3
Filling ratio 15.0%

1. Overflow drain
2. Water jacket
3. Carrier separator
4. Feed supply
5. Compressed air supply
   (Dissolved oxygen, DO at 5.0±0.4)
6. DO sensor
7. pH sensor
8. Porous resin-made rods

Figure 2.8 Floating bead carriers for nitrification (Sakairi et al., 1996)

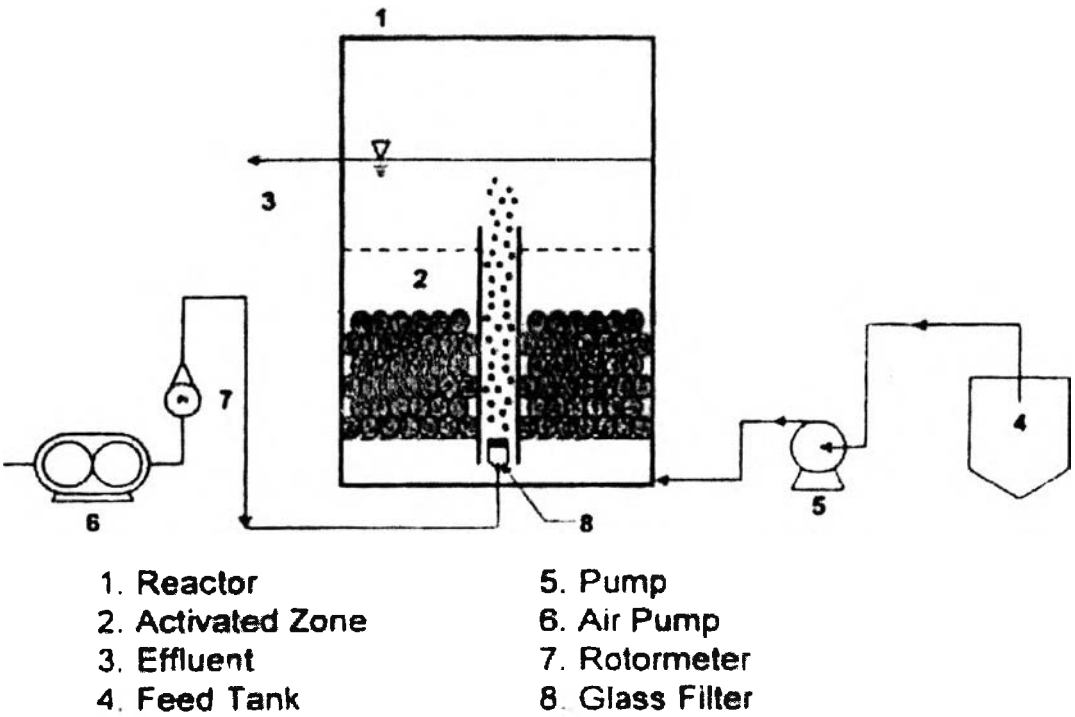


Figure 2.9 Activated sludge for nitrification (Kim et al., 2000)

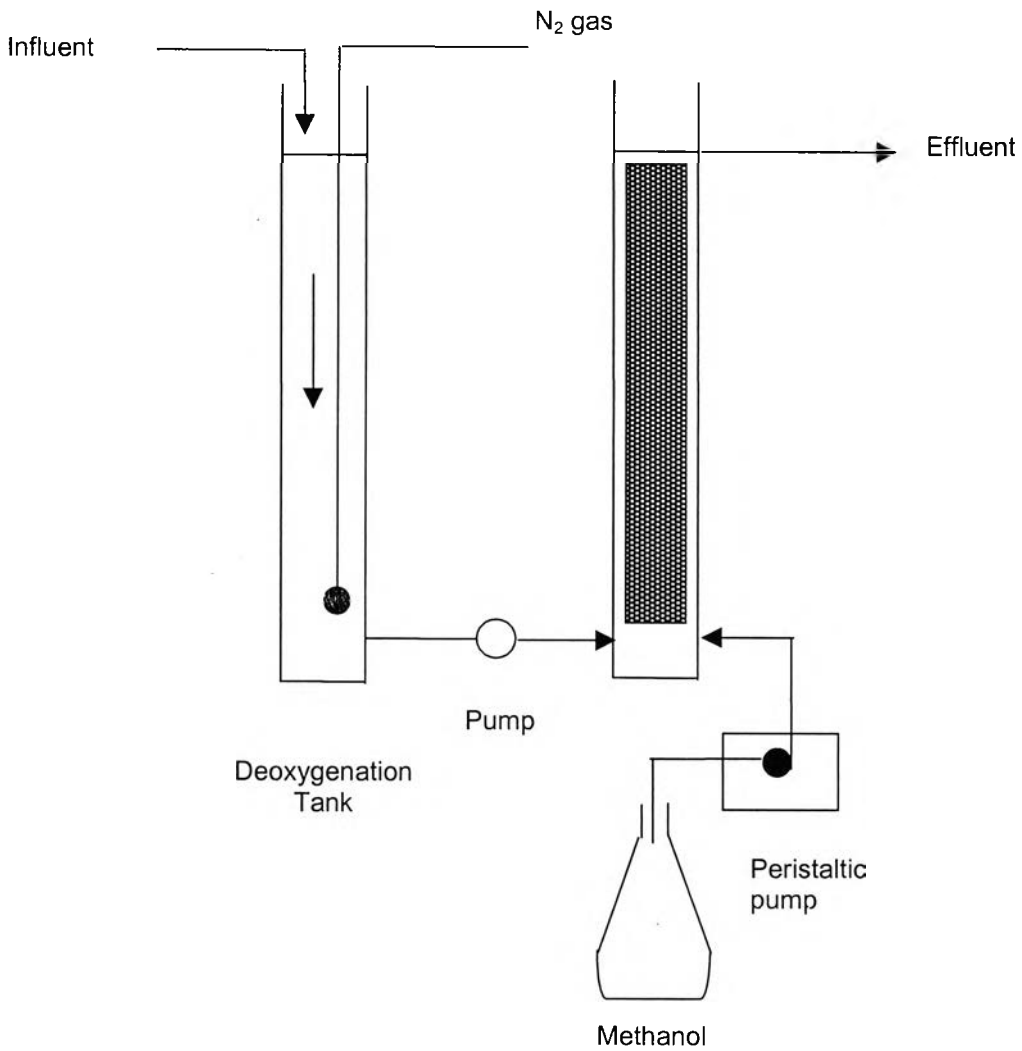


Figure 2.10 Fixed film denitrification column (Menasveta et al., 2001)

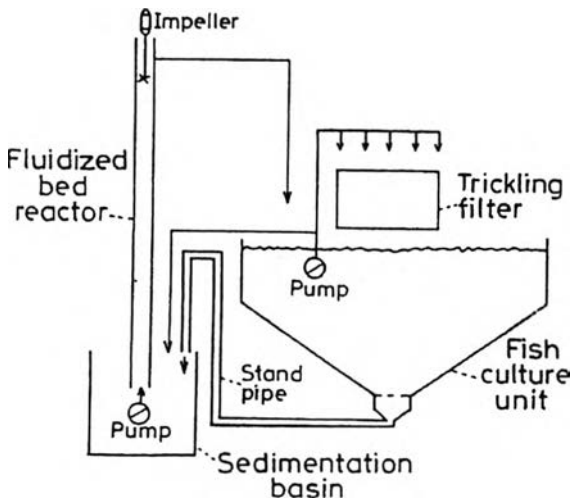


Figure 2.11 Fluidized bed column (van Rijn and Rivera, 1990)

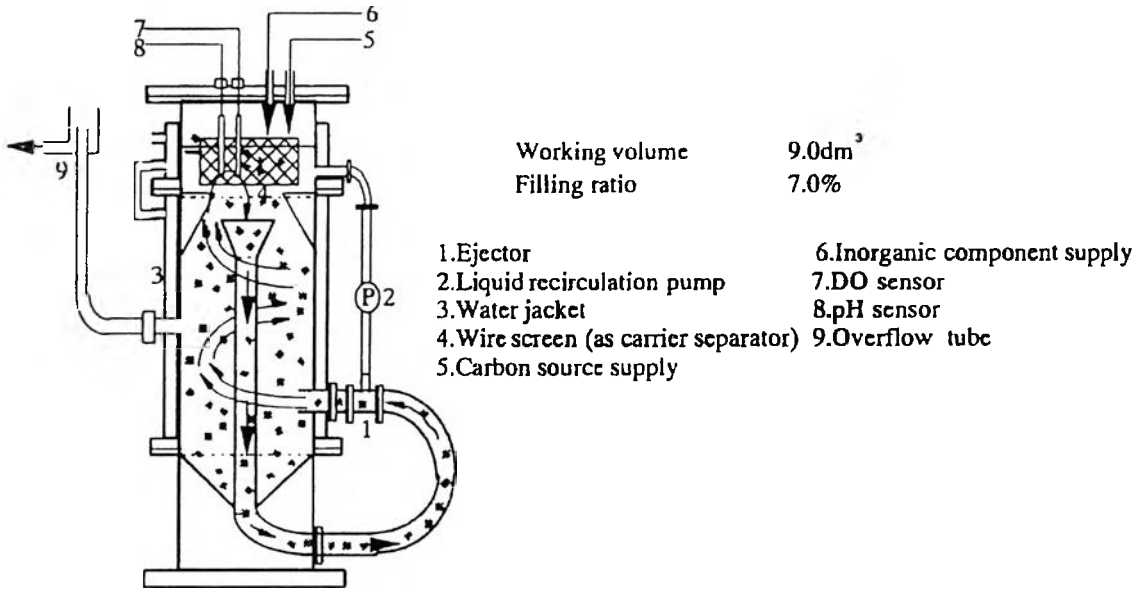


Figure 2.12 Floating bead carriers for denitrification (Sakairi et al., 1996)