2. REVIEW OF LITERATURE

In India, urban and rural areas derive a major component of their domestic water supply from ground water. Advancement of human civilization has put serious questions to the safe supply of ground water for potable purpose. Apart from its depletion due to excessive use, the quality has degraded due to the transport of various organic and inorganic pollutants to the ground water. Nitrate in water supplies has become one of the key environmental issues because of its implications on human health (Jenny et al., 1995; Thind and Kansal, 2002).

Nitrate, the most oxidised species of nitrogen, is a common contaminant in groundwater, which typically originates from human and animal wastes and from agriculture (Aravena et al., 1993; DeSimone and Howes, 1998). Nitrate can also be generated through conversion from atmospheric nitrogen by bacteria (Fetter, 1993), or through the oxidation of the more reduced species of nitrogen, nitrite and ammonia (Postma et al., 1991). Nitrate is generally considered as conservative solute in aerobic ground water environment (DeSimone and Howes, 1998).

2.1 SOURCES OF NITRATE (POINT AND NON-POINT SOURCE)

There are many sources of nitrogen (both natural and anthropogenic) that could potentially lead to the pollution of the groundwater with nitrates. The anthropogenic sources are really the one that most often cause the amount of nitrate to rise to a dangerous level. Waste materials are one of the anthropogenic sources of nitrate contamination of groundwater. Many local sources of potential nitrate contamination of groundwater exist such as, "sites used for disposal of human and animal sewage; industrial wastes related to food processing, munitions, and some polyvinyl facilities; and sites where handling and accidental spillage of nitrogenous materials" (Hallberg and Keeney, 1993).
2.1.1 Non Agricultural Sources

One potentially large source of nitrogen pollution of groundwater is the application of nitrogen-rich fertilizers to turfgrass. This occurs on golf courses and in residential areas. There are five fates for this nitrogen once it is applied to turfgrass. It may be: i) taken up by plants ii) stored in soil iii) lost to atmosphere iv) lost to groundwater and v) lost to runoff. Many studies have shown that most of the nitrogen, about 30 to 50 percent, is taken up by the plant. According to a United States Golfing Association study only one to two percent of the nitrogen is leached beyond the root zone (Bocher and Ward, 1995).

2.1.2 Agricultural Sources (Fertilizers and Animal Wastes)

The main source of nitrate pollution in the groundwater results from the actions of farmers. Approximately 90% of nitrate originates from agricultural sources divided almost equally among legumes, manure and commercial fertilizer. The remaining 10% comes from septage, sludge disposal and other sources (Shaw and Byron, 1994). The farmers first deplete the soil by "excessive, repeat planting" and then try to replenish the resulting less-productive soil by putting more and more nitrogen-based fertilizer on the land to keep crop yields constant (Behm, 1989).

The problem caused by farms, results from the grazed grasslands and feedlots. In grazing pastures animal wastes are concentrated in small pastures, this leads to inefficient use of nitrogen and causes the potential for groundwater contamination by nitrate. This problem is even worse in Europe where grazing pastures are usually more intensively fertilized than in the U.S., therefore there is more nitrate available to be leached to the groundwater (Hallberg and Keeney, 1993). Even small farms can contribute to the problem of excess nitrates because of the high concentrations of manure that they may have in the barnyard or feedlot areas (Hallberg and Keeney, 1993).
The more usage of nitrogen fertilizer, the greater the chance of nitrate pollution of groundwater (De Rao, 1980; Schepers et al., 1984). Apart from nitrate, nitrogen is applied in the form of ammonium ($\text{NH}_4^+$) and amide ($\text{NH}_2$), which generate nitrate in soil system through mineralization. It is fairly rapid in tropical and subtropical soils (Majumdar and Gupta, 2000). Due to its high solubility in water and low retention by soil particles, nitrate is prone to leaching to the subsoil layers and ultimately to the ground water, if not taken up by plants or denitrified to $\text{N}_2\text{O}$ and $\text{N}_2$. "Farmers still consider nitrogen fertilizer 'cheap insurance' against crop failure" (Looker, 1991). Approximately one dollar worth of fertilizer could bring in ten dollars of corn if the soil has a lack of nitrogen. So the farmer would, financially speaking, much rather add too much nitrogen than too little. To add to this problem, it is very difficult to determine exactly how much nitrogen a crop will need before harvest time due to yearly change in yields and weather conditions. Even if farmers cut down on nitrogen fertilizer, there will still be some nitrate leaching. As Dennis Keeney, the Director of the Leopold Center for Sustainable Agriculture at Iowa State University, states, "Even if farmers add no fertilizer to fields, tilling the earth with machinery makes land more susceptible to leaking nitrogen" (Looker, 1991). Although sustainable practices may not eliminate nitrates, it might lower them to a safe level. Obviously, if there is a chance of nitrogen pollution when no fertilizer is applied, the chance of pollution is greatly increased when a large amount of fertilizer is applied.

2.1.3 Natural Sources

Natural sources also contribute a high concentration of nitrate to the groundwater. One example of this is the effect of forested areas on the leaching of nitrate to the groundwater. Natural, mature forests conserve nitrogen but human disturbances can lead to nitrate pollution of the groundwater. However, forests represent a very small source of nitrogen compared to agriculture (Hallberg and Keeney, 1993).
2.1.4 Industrial Sources

Many industries generate high nitrate bearing waste waters. Industries producing basic organic chemicals, fermentation products, fertilizers like calcium, potassium, sodium, nitrates, nuclear power, stainless steel, nitroglycerine, trinitrotoluene etc., generate high nitrate containing wastewaters. Indirect sources are waste water emanating from dairy, slaughter house, food and meat processing etc., giving rise to concentrated organic nitrogenous wastewaters. In the final stage of aerobic stabilization of such waste waters, organic / inorganic nitrogen is ultimately converted into nitrate as end product of metabolism (Deshmukh, 1992).

2.2 NITRATE REMOVAL

With the increasing concern of high concentrations of nitrate in drinking water, many methods have been developed to efficiently remove nitrate. Nitrate is a stable and highly soluble ion and has a low potential for adsorption or coprecipitation (Kapoor and Viraraghavan, 1997). These properties are responsible for difficulty in nitrate removal using conventional water treatment processes including lime softening and filtration. The technology for nitrate removal from drinking water does exist. Reverse osmosis (Rautenbach et al., 1986; Szpyrkowicz and Barnl, 1995; Tannehill et al., 1996), ion exchange (Clifford and Liu, 1993a; Tannehill et al., 1996; Matosic et al., 2000; Kim and Benjamin, 2004), ion exchange membrane bioreactor system (Fonseca et al., 2000; Velizarov et al., 2002 a and b), catalytic reduction (Reddy and Lin, 2000), electrolysis (Elmidaoui et al., 2001), activated carbon process (Sison et al., 1996), land disposal (Deshmukh, 1992), distillation (Mirvish Sidney, 1977), chemical denitrification (Hecke et al., 1986; Warna et al., 1994; Lin and Wu, 1996; Hu et al., 2000; 2001), wetland remediation (Ingersoll and Baker, 1998), phytoremediation (Delgado et al., 1993; Thomas and Kalaroopan, 1994; Min et al., 1998; Sanderson et al., 2001), microbial treatment,
especially anaerobic treatment (Kapoor and Viraraghavan, 1997; Devito et al., 2000; Kesseru et al., 2002; Natcheva and Beschkov, 2003) are the possible methods available.

2.2.1 Physical Methods

2.2.1.1 Reverse Osmosis

Osmosis is a process where a thin semi-permeable membrane separates two solutions of different concentrations and flow occurs naturally from high to low concentration. Reverse osmosis requires induced pressure to create a flow from low to high concentration through a semi-permeable membrane. This pressure must overcome the osmotic pressure to force pure water through the pores, leaving the ions on one side (Szpyrkowicz and Barnl, 1995; Tannehill et al., 1996). Pressures ranging from 300 to 1,500 psi (2,070 kPa - 10,350 kPa) are applied to overcome the normal osmotic flow (Kapoor and Viraraghavan, 1997). The membranes used in this procedure are commonly composed of cellulose acetate and membranes made of polyamides and composite membranes are used to a lesser extent. These membranes do not have a preference for any particular ion, but the amount of salt rejected is proportional to the valence of the ions in solution (Kapoor and Viraraghavan, 1997). There are problems resulting from deposition of soluble materials, organic matter, suspended particles, pH variations and chlorine exposure, therefore, pretreatment is necessary. Pretreatment of water will often extend the life of the membrane and improve efficiency. Typical pretreatment includes adding sodium hexametaphosphate for carbonate and sulfate control, as well as filtration and lowering of pH by the addition of an acid. Post treatment steps are also required and include air stripping for stabilization, pH adjustment to control corrosion and chemical addition for disinfection. The semi-permeable membrane can be expected to remove nitrates efficiently for three to five years (Tannehill et al., 1996).
The major disadvantage of this method is the disposal of the super concentrated ion solution that is produced. The disposal options depend on conditions of federal, state and local regulations in effect. The options include discharge to lakes and streams or oceans, deep well injection, lined evaporation lagoons, irrigation and dilution in wastewater systems (Tannehill et al. 1996). Nitrate separation of sixty-five percent was observed for influent NO$_3^-$ - N concentrations of 18 - 25 mg.l$^{-1}$ (Guter, 1982). It was also found that twenty-five percent of the total influent water became waste brine solution. In comparison to ion exchange reverse osmosis is estimated to be three times more expensive.

2.2.1.2 Ion Exchange

Ion exchange involves exchange of ions in the water and the exchange resin. An anion exchange resin consists of a polymer of positively charged sites that are bonded to negatively charged ions; in this case sodium chloride is generally used to charge the column. When sodium chloride is flushed through the resin column, chloride ions bind to the positively charged sites on the resin. Then water containing nitrate is passed through the column and the nitrate ions, which are negatively charged, displace the chlorine ions on the resin (Tannehill et al., 1996; Matosic et al., 2000; Boumedieene and Achour, 2004).

Clifford and Liu (1993 a) conducted a study to estimate nitrate removal from drinking water by ion exchange. The raw water contained 18 to 25 mg.l$^{-1}$ NO$_3^-$-N, 43 mg.l$^{-1}$ sulfate, and 530 mg.l$^{-1}$ TDS. The process reduced the nitrate level to below 10 mg.l$^{-1}$. The problems associated with ion exchange include disposal of the spent regenerant brine containing nitrate and excess NaCl. This causes corrosion and negative health effects due to the increase in chloride content of the product water (Kapoor and Viraraghavan, 1997).
There are several advantages of ion exchange which include reuse of treated wastewater, utilization of product regeneration for fertilizer and there are no other pollutants (Leakovic et al., 2000). This method also has its disadvantages including unsatisfactory anion resin conditions due to the production of nitric acid and unsatisfactory vacuum evaporation conditions (Leakovic et al., 2000). Another problem noted by Kapoor and Viraraghavan (1997) was that resin often fouled due to the presence of organic matter in the influent. The resin could be restored, however, with the addition of bentonite clay to backwash water. Precipitates of iron and silica reduced the nitrate removal capacity of the resin.

2.2.1.3 Ion Exchange Membrane Bioreactor

This technique combines ion-selective membrane dialysis (ion exchange) and biological remediation. The advantage of this method compared to the individual components is that the bioreactor relies on the use of a nonporous membrane that keeps the water being treated separate from the microbial culture and from the biomedium where the culture is suspended (Fonseca et al., 2000). The probability of the transfer of species between the treated water and the bioreactor depends on the characteristics of the membrane. The membrane can be selected to facilitate the removal of ionic pollutants from the water and to retard the transfer of other inorganic and organic contaminants in the biomedium. An anion-exchange membrane and an anoxic denitrifying bioreactor are used. The removal of nitrate is accompanied by the counter transport across the membrane of a second ion, equivalent in molality, known as the counter ion (Fonseca et al., 2000; Velizarov et al., 2002 a).

The advantages of this combined technique include prevention of secondary pollution of the treated water with particulates and dissolved organic and inorganic substances. Microbial contamination is also prevented due to the segregation in the
bioreactor. High nitrate removal (approximately 98% with a current of 20mA) due to facilitated transport through the membrane was achieved and there was less brine solution to be disposed of (Islam and Suidan, 1998). About 87% nitrate removal was achieved at a nitrate concentration of 150 mg.l\(^{-1}\) in polluted water (Velizarov et al., 2002b). Ion exchange bioreactor is also a simplified water treatment process since it does not require deoxygenation of the treated water (Fonseca et al., 2000).

Disadvantages of this process include a higher hydraulic residence time of the water stream (HRT = 4.4h) compared with other biological treatment processes (Fonseca et al., 2000). In addition, it was observed that a biofilm developed on the biomedium side of the membrane, which hindered the transport of nitrate from the water to the biological compartment. The cause of this biofilm needs to be addressed to clarify its impact on nitrate degradation rate and the transmission of ethanol to the water stream, before this can become a large scale process (Fonseca et al., 2000).

2.2.1.4 Catalytic Reduction

The catalytic process has potential to remove nitrate from groundwater in small agricultural communities to treat drinking water (Reddy and Lin, 2000). Other studies completed in this area discovered that palladium-alumina catalysts were effective in the reduction of nitrate to nitrite and ammonia in the presence of hydrogen (Horold et al., 1993). In addition, the lead copper catalyst showed complete removal of nitrate from water having an initial concentration of 100 mg.l\(^{-1}\) NO\(_3\). This process is still in the development stages and the kinetics and long-term performance of catalysts has not been studied (Kapoor and Viraraghavan, 1997). This technique has potential for field application because rhodium can be coated onto fiberglass mesh and a photovoltaic cell can supply the redox potential. Sunlight could be used as an energy source and therefore, this method could become quite inexpensive.
2.2.1.5 Electrodialysis

Electrodialysis (ED) is similar to reverse osmosis in many ways. In electrodialysis, ions are transferred from a solution of lower concentration to a solution of higher concentration due to application of a direct current. Electrodialysis selectively removes undesired ions as water flows through a semi-permeable membrane. Similar to reverse osmosis, the water must be pretreated. In electrodialysis reversal (EDR) process, the polarity of two electrodes are reversed two to four times an hour to change the direction of ion flow. This process reduces scaling and chemical usage compared to conventional ED and has been used for the production of drinking water from brackish and seawater (Kapoor and Viraraghavan, 1997; Elmidaoui et al., 2001). Nitrate removal efficiency is similar to reverse osmosis, but electrodialysis is limited to treat soft water and requires less acid dosages and has higher water recovery rates. Currently this process is considered too expensive (Kapoor and Viraraghavan, 1997).

2.2.1.6 Activated Carbon Process

Sison et al. (1996) proposed a denitrification process using granular activated carbon (GAC) column and dynamic modes of external carbon addition. This process is suitable for water or wastewater containing NO\textsubscript{3} but insufficient organic matters. It was demonstrated that 87% of nitrate was removed by adding the organic matters once a day with continuous inflow of 20 mg.l\textsuperscript{-1} of nitrate. Sison et al. (1995) also performed high loading denitrification from various concentrations (10, 20, 40, 60 and 80 mg.l\textsuperscript{-1}) of nitrate solution by biological activated carbon process.

2.2.2 Chemical Methods

2.2.2.1 Chemical Denitrification

Reduction of nitrate can also be induced by basic pH conditions according to the following reaction (Kapoor and Viraraghavan, 1997):
It has been demonstrated that a Fe:NO$_3^-$ ratio of 15:1 is needed in the presence of copper catalyst for the reaction to occur. Using iron has many disadvantages such as generation of large quantities of iron sludge and production of ammonia that requires removal by air stripping.

Aluminum has also been used to reduce nitrate, where ammonia was found to be the principal reaction product (60-95%), which could be removed by air stripping. The optimum pH level was found to be 10.25 (Murphy, 1991). Water treatment plants that use lime for water softening, which reduces additional costs of raising the pH, can effectively use the process of reactions. Experimental results showed that only 1.16g of aluminium was required for the reduction of 1g of nitrate, making this process more economical than that with iron. However, this process is still too expensive to implement on a large scale (Kapoor and Viraraghavan, 1997).

Chemical reduction of nitrate (Young et al., 1964; Hecke et al., 1990) and nitrogen oxide (Horold et al., 1993; Warna et al., 1994; Lin and Wu, 1996; Hu et al., 2000 and 2001) using ferrous iron as bulk reducing agent has been investigated. Using Fe$^{2+}$ produces voluminous amount of sludge (Sorg, 1978), since eight moles of ferric hydroxide is generated per mole of nitrate reduced. In recent years, zero valent iron has been intensively studied for its ability to reduce chlorinated solvents (Orth and Gillham, 1996) and has been successfully applied to remediate ground water (Vidic and Pohland, 1996). Huang et al. (1998) have studied the reduction of nitrate from an aqueous solution by metallic iron.
2.2.3 Biological Methods

2.2.3.1 Wetland Remediation

Various biological denitrification technologies have been established in order to solve the problem of nitrate in drinking water and ground water. One method to treat a large quantity of groundwater by denitrification is to use constructed wetlands. Wetlands are efficient in removing nitrate from the water because of two environmental characteristics that promote denitrification, which are anoxic sediments and a healthy supply of carbon from plant growth (Ingersoll and Baker, 1998). Wetlands have been constructed for several different pollutants from various sources, including sewage effluent, mine drainage, and urban runoff (David et al., 1997).

The majority of the organic carbon required for denitrification in wastewater wetlands is supplied by the wastewater itself, however, dissolved organic carbon (DOC) of ground water is very low and thus, plants are needed to supply the carbon required. Therefore, a reasonable postulation would be that carbon supply is the limiting factor in a wetland constructed for nitrate removal of low DOC waters (Ingersoll and Baker, 1998). Ingersoll and Baker, (1998), showed that the limiting carbon : nitrogen ratio is approximately 5:1. It was also discovered that nitrate removal efficiencies increased as the C: N ratio increased to 5:1. A key finding was that the reaction rate constant for nitrate was directly dependent upon carbon addition rate. The importance of these findings suggests that the performance of the nitrate-treatment wetland would be enhanced by increasing plant growth and consequently the carbon supply. This could be completed by optimizing the habitat for maximum plant productivity, fertilizing the plants with phosphorus or cutting the plants and leaving the clippings in place.

Constructed wetlands could be used for remediation of nitrate-polluted aquifers. The wetland would remove the nitrate through denitrification, but as a side effect produce
elevated DOC. This method would be the most cost-effective where the groundwater is shallow, land prices are low and situated in a temperate climate.

2.2.3.2 Nitrate Uptake by Terrestrial Plants

In soils, nitrogen is available as dissolved nitrogen (e.g. amino acids), $\text{NH}_4^+$, $\text{NO}_3^-$ or any combination of these, for absorption by plants (Glass and Siddiqi, 1995). The relative amount of these various forms of N depend upon a number of biotic and abiotic factors which determine the rates of mineralization, nitrification, denitrification, leaching, relative uptake of different N forms by plants and microorganisms etc. (Stark and Hart, 1997; Eviner and Chapin, 1997). Min et al. (1998) have used the terrestrial plants *Trembling aspen* and *Lodgepole pine* for uptake of $\text{NO}_3^-$ and $\text{NH}_4^+$ from aqueous solution.

Barley (Kronzucker et al., 1999), Switchgrass (Sanderson et al., 2001), Duckweed (Jiang et al., 2001) and Bluegrass (Jiang et al., 2001; Jiang and Sullivan, 2004) plants were also used for uptake of $\text{NO}_3^-$ and $\text{NH}_4^+$ from synthetic solution. The use of the *Lemna minor* L. species of duckweed is an emergent technology that may be effective for the removal of nitrogen and phosphorus from enriched waters (Smith et al., 2004).

A few plant species have been noted for their ability to accumulate nitrate in their roots or aboveground parts. The members of the genera *Beta*, *Borago*, *Chenopodium* and *Menyanthes* are nitrate-storing plants (Salisbury and Ross, 1978). *Borago officinalis* (borage) is being increasingly grown since its seed oil is a rich source of gamma-linolenic acid, a compound of rising interest as a natural pharmaceutical (Redden et al., 1995). Borage can also be sown in the autumn and persist over the winter. Swiss chard (*Beta vulgaris*), in addition to being an edible vegetable, it also has the virtue of accumulating very high amounts of nitrate (up to 3.8 g/kg fresh weight) in its leaves (Santamaria et al., 1999).
Several species of grasses have been shown to display increased nitrate reductase activity in the presence of N fertilizers. Timothy (*Phleum pratense*) had increased total N, nitrate N, and amino N in leaves and stems which correlated with the amount of calcium nitrate added (Friedrich *et al.*, 1977). At the highest levels of fertilization, leaf nitrate was at a toxic level. Switchgrass (*Panicum virgatum*) showed a similar response, but to a lesser degree.

### 2.2.3.3 Nitrate Uptake by Aquatic Plants

Presence of aquatic plants in natural or constructed wetlands not only reduces the concentration of problematic nutrients from the waste water, but also alter the physico-chemical environment of the water, rhizosphere and underlying sediments (Reddy and Patrick, 1984). Water hyacinth is a floating aquatic plant, which has been employed for wastewater in many parts of the world. Water hyacinth has been used successfully by the National Aeronautics and Space Administration / National Space Technology laboratories to remove organics and heavy metals from chemical wastes before their discharge (Wolverton *et al.*, 1977). Polprasert and Khatiwada (1998) studied an integrated kinetic model for water hyacinth ponds used for waste water treatment. Water hyacinth ponds have been found effective in the removal of organic matter, suspended soils, nitrogen and phosphorus as well as the reduction of heavy metals like Zn, Cr and Cd, trace organics and pathogens (Reed *et al.*, 1988; USEPA 1988; Metcalf and Eddy, 1991; Delgado *et al.*, 1993).

Orth and Sapkota (1988) found that the COD and suspended solids removal efficiencies increased more than 50% in a facultative pond implanted with water hyacinth when compared with a control facultative pond without water hyacinth plants. Mitchell (1978) have selected aquatic plant species for wetland remediation based on the following criteria:
❖ Rapid and relatively constant growth rate
❖ Ease of propagation
❖ Capacity for absorption of pollutants
❖ Tolerance of hyper eutrophic conditions and
❖ Ease of harvesting and potential usefulness of harvested material

Thomas and Kalaroopan (1994) have used various aquatic macrophytes *Eichornia crassipes* (Water hyacinth), *Spirodela* sp. (Duck weed) and *Salvinia molesta* (Salvinia) for septic tank effluent treatment. These aquatic macrophytes are able to transfer oxygen into the bed, creating aerobic microzones around the plant roots and anaerobic zones away from them. As a result aerobic and anaerobic bacteria will both carry out the breakdown of the organic matter and removal of nitrogenous compound through nitrification and denitrification. Larson et al. (2001) have studied effective nitrate removal (from 350 to 75 mg.L\(^{-1}\)) in aqueous solution using floating aquatic plants, hornwort.

**2.2.4 Microbial Denitrification**

Biological denitrification is used to mark the reduction of oxidized nitrogen compounds to gaseous nitrogen (Chen and Lin, 1993). As a result of the reduction, different intermediate and end products are produced: nitrite (NO\(_2^\cdot\)), nitric oxide (NO), nitrous oxide (N\(_2\)O) or molecular nitrogen (N\(_2\)). In the biological step of a waste water treatment plant, the denitrification process to molecular nitrogen runs usually according to the following reaction (Schlegel, 1992; Shrimali and Singh, 2001).

\[ 4\text{NO}_3^- + 2\text{H}_2\text{O} \longrightarrow 2\text{N}_2 + 5\text{O}_2 + 4\text{OH}^- \]

In nature, riparian zones remove nitrate from groundwater and is believed to be due to microbial denitrification (Devito et al., 2000). Denitrification requires a sufficient supply of nitrate, an energy source that is often organic matter, and anaerobic conditions. It follows the reaction :

\[ \text{NO}_3^- \longrightarrow \text{NO}_2^- \longrightarrow \text{N}_2\text{O} \longrightarrow \text{N}_2 \]
These conditions are often met in riparian zones, therefore, a system analogous to this has been proposed for remediation of contaminated ground water and waste water (Payne, 1973; Blaszczyk et al., 1996; Schipper and Vukovic, 1998). Nitrogen chemistry is complicated due to its numerous oxidation states the element assumes in its compounds such as, NO$_3^-$ (+5), ammonium NH$_4^+$ (-3) and nitrite NO$_2^-$ (+3) (Reddy and Lin, 2000).

Denitrification is generally accepted as the process of reduction of nitrates, via nitrites, to nitrous oxides and nitrogen gas. However, many denitrifying bacteria only have the enzymatic ability to reduce nitrates to nitrites with no further reduction of the nitrites produced (Ketchum, 1988; Cappuccino and Sherman, 1992; Robertson and Kuenen, 1992; Glass et al., 1997). Robertson and Kuenen (1992) reported that most of the denitrifying bacteria in aquatic systems are only capable of incomplete denitrification. The full effect of this on nitrogen removal during activated sludge treatment has, however, not yet been determined (Drysdale et al., 1999).

Schipper and Vukovic (1998) created a porous reactive wall constructed in the polluted plume path to remove the contaminant as the groundwater passed through. Nitrate removal by denitrification is limited by the availability of an energy source, to overcome this, the wall was constructed of twenty-percent sawdust as a carbon source to stimulate denitrification. The wall successfully decreased nitrate concentrations in the groundwater throughout the study period. However, this removal could be due to denitrification or immobilization of nitrogen. Immobilization seemed unlikely to be a major sink as the nitrogen concentration in the wall decreased throughout the study suggesting no net nitrogen accumulation. Denitrification over an extended time depends on nitrate input and a supply of carbon from degrading sawdust. It was predicted that overtime the sawdust would degrade and nitrate removal would consequently decline, therefore, fresh sawdust would need to be added annually.
The main concern with treating drinking water by biological denitrification is that there might be some remnant microbes in the treated water, which then requires post treatment. Therefore, this technique is more widely used for treatment of wastewater. The major limitations of this process include the rate of diffusion of substrate and reaction products through the matrix, loss of cells from the matrix, reduced activity of cells and short life span of the matrix (Kapoor and Viraraghavan, 1997). To overcome these problems, the biofilm reactor was invented, which keeps denitrifying bacteria and the carbon energy source away from the treated water. The cost for a heterotrophic denitrification plant would be about twice that of ion exchange, with operating costs about equivalent. The cost for an autotrophic biological system would cost approximately twelve times that of ion exchange (Kapoor and Viraraghavan, 1997).

2.2.4.1 Denitrifying microorganisms

A variety of facultative and obligate anaerobic bacterial genera are capable of the dissimilatory reduction of nitrate to nitrite. In this anaerobic process, nitrate functions as an acceptor in electron-transport-coupled phosphorylation. The fate of nitrite produced during "nitrate respiration" varies from species to species. Some bacteria reduce nitrate to nitrite and no further reduction, whereas other species convert this substrate to nitrogen gas, other nitrogenous oxides, or ammonia (Schipper and Vukovic, 1998). The reduction of nitrate or nitrite to ammonia is classically identified as assimilatory reduction since the primary function of this transformation is assumed to be generation of physiological useful form of reduced nitrogen. To date, very few ammonia producing dissimilatory nitrate — reducing bacteria have been identified. These organisms, which include *Escherichia coli*, *Veillonella alcalescens* and *Achromobacter fischeri*, at first, appear to have the best of both worlds. They not only can obtain energy from the anaerobic reduction of nitrate but also produce ammonia, which is a useful form of reduced nitrogen.
Bacteria capable of reducing nitrate are easily isolated from aquatic and terrestrial environments. *Pseudomonas* was identified as the most common denitrifying genus isolated from seawater (Baumann *et al.*, 1972), soil (Gamble *et al.*, 1977; Greenberg and Becker, 1977), freshwater (Buchanan and Gibbons, 1974; Heitzer and Ottow, 1976; Carter *et al.*, 1995) and aquatic sediments (Heitzer and Ottow, 1976). Bacterial genus *Halomonas* was isolated from alkali lake (Peyton *et al.*, 2001) and activated sludge (Kargi and Dincer, 1996). *Citrobacter* sp., capable of dissimilatory nitrite reduction to ammonium was isolated from soil (Smith, 1982). *Pseudomonas aeruginosa* is a representative of a wide range of other denitrifying bacteria including *Pseudomonas denitrificans*, *Alcaligenes denitrificans* and *Paracoccus denitrificans* (Koneman *et al.*, 1979). The denitrifying bacterium, *Pseudomonas aeruginosa* was chosen for nitrate removal study because of its commercial availability, denitrification abilities and physiological versality. It is commonly found in terrestrial soil and can grow in a variety of low nutrient conditions (Doudoroff and Palleroni, 1974).

exists regarding the bacteria involved in denitrification as well as the extent to which these bacteria contribute to nitrate and nitrite reduction under anoxic conditions. It is generally presumed that *Pseudomonas* spp. are also being involved in the enhancement of biological phosphate removal (Osborn *et al.*, 1989; Kavanaugh and Randall, 1994; Jorgensen and Pauli, 1995) and are the predominant microorganisms through which denitrification is achieved (Janda *et al.*, 1988; Gray, 1990; Lazarova *et al.*, 1992). According to Otlanabo (1993) various species of *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Bacillus*, *Chromobacterium*, *Flavobacterium*, *Hyphomicrobium*, *Pseudomonas*, *Vibrio* and others are responsible for denitrification in soil. It therefore seems unlikely that only *Pseudomonas* spp. are responsible for denitrification occurring in such an incredibly diverse microbial consortia as that of activated sludge (Drysdale *et al.*, 1999).

A large diversity of organisms was shown to be involved in nitrate reduction. *Acinetobacter* sp., *Aeromonas* sp., *Pseudomaons* sp. and *Shewanella putrefaciens* were isolated from a hydrogenotrophic reactor for the denitrification of drinking water (Liessens *et al.*, 1992). In a hydrogenotrophic denitrification reactor, Selenka and Dressler (1990) classified the causal chemolithotrophic strains as representatives of the genera *Pseudomonas*, *Alcaligenes* and *Achromobacter*. *Aeromonas* is one of the most prevalent nitrate-respiring organisms in estuarine environments (MacFarlane and Herbert, 1982). Bazylinski *et al.* (1986) isolated *Chromobacterium violaceum*, a nitrate utilizing organism from freshwater habitats.

The different bacteria were isolated from various ecological environments. It would therefore be interesting to know which ecosystems contribute most to increase the aerobic denitrifying population and thus to answer the question of whether there is a specific ecological niche for aerobic denitrification. *Thiosphaera pantotropha* (Robertson and Kuenen, 1983) was isolated from a denitrifying/sulfate treatment plant, *Citrobacter*
species (Smith, 1982) from an agricultural field. *Microvirgulc aerodenitrificans* (Patureau *et al.*, 1994) from an upflow anoxic-aerobic filter, strain 1 (Frette *et al.*, 1997) from an alternating activated sludge plant and strain TL1 (Lukow and Diekmann, 1997) from the oxic tank of a nitrification plant. Carter *et al.* (1995) isolated 29 strains from 3 cultivated soils and freshwater sediment, which were completely different. By using two-step PCR amplification, the strain *M. aerodenitrificans* was found in methanogenic and nitrifying/denitrifying ecosystems. To find denitrifiers in a methanogenic microflora may be surprising. However, it is consistent with results obtained by Jorgersen and Tiedje (1993) who reported a high frequency of denitrifiers and isolated species of *Pseudomonas* and *Alcaligenes* in anaerobic, nitrate-free habitats. Aerobic denitrification has been well studied mainly through pure strains such as *Thiosphaera pantotropha* (Robertson and Kuenen, 1983) and *Microvirgula aerodenitrificans* (Patureau *et al.*, 1994). According to some workers (Tiedje, 1981; Garcia, 1982; Tiedje, 1988), assimilatory nitrate reduction to ammonium is the major nitrate reduction pathway in anaerobic digesters, because of the abundance of facultative and obligate anaerobes (ammonium formers).

Many investigators have observed that nitrite can inhibit denitrification, especially at high concentration. Accumulation of extracellular nitrite has been reported during denitrification in pure cultures, implying that, under some conditions, denitrifying bacteria transport the nitrite intermediate out of the cell and later take the extracellular nitrite back into the cell for complete denitrification. Recently, Komaros *et al.* (1996) have shown that nitrite was accumulated in cultures of *Pseudomonas denitrificans*, ATCC 13867, when nitrate was present. Rijn *et al.* (1996) confirmed that nitrate inhibits nitrite reduction in *Pseudomonas stutzeri* isolated from a denitrifying fluidized bed reactor. They observed that for *P. stutzeri* the level of nitrite accumulation was determined by the number of electrons supplied per mole of carbon/energy substrate compound, with relatively oxidized substrates like acetate, compared with butyrate.
When substrate electrons were limited, nitrate seemed to be the preferred electron acceptor, compared to nitrite. Competition for substrate electrons between nitrate and nitrite reductases also has been reported in *Paracoccus denitrificans* and *Pseudomonas flourescens* (Thomsen et al., 1994; Almeida et al., 1995). Gee and Kim (2004) achieved nitrite accumulation by *Nitrobacter* using sequencing batch reactor under aerated and non aerated condition.

Autotrophic bacteria such as *Thiobacillus denitrificans* and *Thiobacillus denitrificans* are capable of reducing nitrate to nitrogen gas and *Micrococcus denitrificans* are reducing nitrate to nitrogen (Claus and Kutzner, 1985). The bacterial species *Bacillus licheniformis* (No 40-2) utilized ammonium and nitrate salts simultaneously under aerobic condition (Konohana et al., 2000). Yang et al. (1995) and Shanthi et al. (2001a) performed nitrate removal using immobilized microbial consortium under high salt conditions. Kesseru et al. (2002) studied denitrification activity by immobilized *Pseudomonas butanovora* in aqueous solution supplemented with different organic substrate. Recently Natcheva and Beschkov, (2003) have used a thermophilic aerobic bacteria *Geobacillus* sp. (NBIMCC 3729) for denitrification process in aqueous solution. The mesophilic (*Chromobacterium violaceum*) and psychrophilic (*C. lividum*) species were reported to differ in their pattern of nitrate dissimilation (Sneath, 1956; Payne, 1981). *Cyanobacteria* (Hu et al., 2000b), *Chlorella kessleri* (Lee et al., 2002) and marine diatom *Skeletonema costatum* (Jochem et al., 2000) were also used for efficient removal and utilization of nitrate from ground water.

The elimination of nitrate in industrial wastewater streams with a relatively high nitrate content such as those resulting from the production of nitroaromatic compounds, the synthesis of nitroorganic compounds in pharmaceuticals, nitrogen containing product in chemical fertilizers, waste waters from nuclear fuel processing, and the cleaning of
tanks in dairy factories has been considered a difficult task for microorganisms (Lawson, 1981; Walker et al., 1989; Clarkson et al., 1991; Francis and Makin, 1991; Smith et al., 1994). Biotechnological process for the removal of high nitrate concentrations will become possible only if microorganisms are able to preferentially tolerate high nitrate concentrations. Microbes from ground of an explosives factory were relatively tolerant to high concentration of nitrate (Ramos et al., 1996). Wang et al. (2004) have reported denitrification process via nitrite and nitrogen removal from high nitrogen soybean waste water.

Several authors have studied nitrification and denitrification under activated sludge process by heterotrophic bacteria. The activated sludge process is the most widely applied biological wastewater treatment process in the world. Originally the process was designed as a single aerobic reactor for the removal of organic matter from wastewater but it has since been significantly developed to enhance its nutrient removal capabilities (Lu and Leslie Grady, 1988; Gray, 1990; Wentzel et al., 1992). These improvements were induced by modifying the process from a single aerobic reactor to multi-reactor processes consisting of anaerobic, anoxic and aerobic zones with inter-reactor recycles thus enabling the process to progressively include nitrification, denitrification and phosphorus removal (Wentzel et al., 1992). Denitrification by heterotrophic bacteria in activated sludge treatment is of particular interest in that nitrate and nitrites are eutrophic (Gray, 1990), hazardous to human health (Terblanche, 1991; Kempster et al., 1997) as well as inhibit phosphorus removal during activated sludge treatment (Gruenebaum and Dorgeloh, 1992; Kuba et al., 1996). During this process under anoxic conditions certain heterotrophic bacteria are stimulated into utilising nitrates and nitrites as final electron acceptors for cellular respiration in place of oxygen (Cappuccino and Sherman, 1992). This results in oxidation of organic matter as well as reduction of the nitrates and nitrites into nitrous oxides and nitrogen gas (Wanner and Grau, 1988). Shanthi et al. (2001 b)
have performed removal of nitrogenous compounds from sewage wastewater by a bench scale activated sludge process.

### 2.2.4.2 Effect of Plant Residues on Denitrification

Animal litters like wheat straw, flax straw, Zeolite stall fress, spruce saw dust and beech saw dust were used to adsorb ammonium and nitrate ion (Ninenya et al., 2000). Few studies concerning about the effect of plant residue on physical and biochemical qualities of the overall rate of denitrification process in water systems have been carried out. Several studies have been conducted on the influence of one or two crop residues on denitrification (Aulakh et al., 1984), but such studies were performed in the soil systems, which somewhat have different characteristics with water systems.

The capacity of several plant residues to support the growth of denitrifying bacteria in the water denitrification has been reported. Soares et al. (1998) have used wheat straw as substrate for water denitrification and found that wheat straw is a suitable carbon source for water denitrification and, at the same time, can serve as the sole support for bacterial growth. Ezeronye and Okerentugba (1999) also observed removal of nitrate from fertilizer plant effluent using cassava peels. Aslan and Turkman (2004) used organic solid substrates such as poplar, hornbeam, pine shavings and wheat straw as a carbon source for denitrification in drinking water treatment.

Volokita et al. (1996 a, b) also demonstrated that cotton and newspapers could effectively support denitrification of nitrate-contaminated water. These workers demonstrated that cotton could be used effectively as the energy source for groundwater denitrification (Volokita et al., 1996a). By using newspapers as the sole carbon source as well as the sole physical support of the bacteria, these workers observed that the treated water was characterized by low DOC and no detectable flavour, color or colour, but washout of bacteria was relatively high, requiring further disinfection of the treated water.
(Volokita et al., 1996b). Nevertheless, in all of those studies using plant residues or newspaper in the water denitrification, the effects of plant residues, on the carbon availability and the effectiveness of bacterial supporting media on the denitrification process have not been determined.

2.2.4.3 Effect of Carbon Source on Nitrate Removal

Bacterial assimilation can be encouraged by providing readily degradable carbon source in a quantity commensurate with nitrate-N (Deshmukh, 1992). For denitrification, readily biodegradable carbon source is mandatory (Bode et al., 1987). It is suspected that poorly degradable carbon source results in nitrate-ammonification instead of nitrate denitrification. It is believed that nitrate ammonium reduction is favoured in carbon - rich (or nitrate - poor) environments (Tiedje, 1981 and 1988).

A variety of carbon sources, such as methanol (Sollo et al., 1976; Werner and Kayser, 1991; Akunna et al., 1993; Christensson et al., 1994; Thalasso et al., 1997; Bilanovic et al., 1999), methane (Thalasso et al., 1997), glycerol and sucrose (Sison et al., 1995; Pinar et al., 1998), lactic acid (Akunna et al., 1993), galactose, fructose and lactose (Pinar et al., 1997), ethanol, acetate and sugars (McCarty et al., 1969; Blaszczyk et al., 1981; Gonzalez et al., 1992; Kitsos et al., 1992; Fenerhake and Jordening, 1993; Almeida et al., 1995; Hasselblad and Hallin, 1998; Oh and Silverstein, 1999) have been used for nitrate removal from wastewater. Different authors have worked on denitrification with ethanol (Hancher et al., 1978; Schugerl, 1989; Chang et al., 1992; Blaszczyk, 1993) and acetic acid (Francis and Hancher, 1986; Wildner et al., 1987) as carbon source, but the studies were carried out with an initial nitrate concentration lower than 10 mM. It was shown that ethanol and acetic acid allow a very high denitrification rate and efficiency. The studies on the influence of two carbon sources (ethanol and acetic acid) for the denitrification rate of high nitrate concentrated industrial wastewater
(115 mM nitrate and 0 mM nitrite) showed that the high specific denitrification rate was observed on acetic acid by the fact that acetic acid is a directly assimilable carbon source. Blaszczyk et al. (1981) observed that acetic acid was found to be a good source of carbon for the denitrification of both type of nitrogen (nitrate and nitrite). Both heterotrophic and autotrophic bacteria can carry out denitrification. Under anoxic conditions, heterotrophic bacteria utilize organic substrates including methanol, ethanol, and acetic acid for the nitrate to nitrogen conversion. In contrast, autotrophic denitrification utilizes substrates including hydrogen or reduced sulfur compounds and carbon dioxide or bicarbonate as the carbon source required for cell synthesis (Kapoor and Viraraghavan, 1997).

Akunna et al. (1993) used batch test to determine the potentials of digested sludge to reduce nitrate and nitrite in the presence of five different carbon sources like glucose, glycerol, acetic acid, lactic acid and methanol. Ammonium accumulation was found in glucose and glycerol media. Dissimilatory reduction to ammonium accounted for up to 50% of reduced nitrate and nitrite. The rest were denitrified. Ammonification was higher than denitrification activities only when glucose and glycerol were present in the media. Several researchers have made comparative studies on the effects of methanol and ethanol as external sources for denitrification (Christensson et al., 1994; Hallin et al., 1996; Nyberg et al., 1996; Hallin and Pell, 1998). Their studies showed that ethanol should be a better external carbon source than methanol, especially when added to the denitrification zone of a single-sludge biological nitrogen removal system. In the latter case, the influent and external carbon is used concomitantly for denitrification and the external one is provided only when the influent COD to N ratio is low.

The aerobic nitrate reductive efficiency of Pseudomonas sp. isolated from domestic sewage wastewater under varying pH, temperature, hardness and heavy metal in water medium under aerobic condition and in the presence of glucose as carbon source
was studied by Sreelatha et al. (1997). Bacterial strains isolated from activated sludge from a swine wastewater treatment system and a standard *Pseudomonas* strain was examined with respect to carbon sources and temperature required for denitrification. The results showed that the nitrite was accumulated under aerobic condition, if nitrate and carbon sources are available. The carbon source used was L-asparagine, sodium acetate, lactic acid, glucose, ethanol and methanol. The ideal carbon sources found suitable for denitrification were L-asparagine and sodium acetate.

### 2.2.4.4 Effect of temperature on nitrate removal

The effect of temperature on the denitrification rate is another important feature in the design of a denitrification process (Orhon et al., 2000; Carrera, 2003). Incubation near 30°C generally, but not always favors denitrification. Thermophilic and psychrophilic denitrifying bacteria are known to have different temperature optima than do the mesophiles (Evans, 1954; Konishi, 1969). Some usually high temperature optima reported are possibly a reflection of nitrobiological reactions (Bremmer and Shaw, 1958). Sreelatha et al. (1997) have reported effect of various factors such as pH, temperature and hardness on the rate of nitrate reduction and the level of nitrite production by *Pseudomonas* sp. Most of the studies of the effect of temperature on denitrification have been carried out with soil in which they appear to be marked temperature dependent. The rate of N$_2$O production is very high during denitrification at high temperature (60 - 70°C) (Keeney et al., 1979; Knowles, 1981), whereas in low temperature (0 - 5°C) the rate of denitrification is decreased (Bailey and Beauchamp, 1973; Smid and Beauchamp, 1976).

### 2.3 Coagulation Process

Coagulation involves the destabilization of colloidal particles such as mineral colloids, microbial colloids and virus particles by coagulants (Williams and Culp, 1986).
The commonly employed coagulants for water and waste water treatment are alum (aluminum sulfate), ferric sulfate, copperas (ferrous sulfate), chlorinated copperas (a mixture of ferric sulfate and chloride), lime (calcium carbonate - precipitating agent), poly aluminium chloride (PAC), ferrous chloride and ferric chloride (Manivasakam, 1987; Lee et al., 2004). Perkowski and Kos (2002) used hydrogen peroxide and ferrous ions as coagulants for treatment of textile dyeing waste water.

Coagulants used for water and waste water treatment are predominantly inorganic salts of iron and aluminum (Jiang and Graham 1998). Some authors have reported the use of alum (aluminum sulfate) as a coagulating agents for treatment of steel mill waste water (Woodrow, 2001), pulping effluents (Dilek and Bese, 2001), phosphorous containing water (Kim et al., 2003), metal contaminated waste water (Fatoki and Ogunfowokan, 2002). Jiang and Graham (1998) have reported alumium sulfate, ferric sulfate and ferric chloride were effective, highly charged, pre polymerized inorganic coagulants used for phosphorous removal. Aluminum sulfate (Alum) is a white crystalline product which is almost insoluble in anhydrous alcohol, but readily soluble in water. Most of the alum produced today is used in the pulp and paper industry as well as water and wastewater treatment. It is inexpensive and effective for a broad range of treatment problems because it can function as a coagulant, flocculant, precipitant and emulsion. As a coagulant and flocculant, alum removes turbidity, suspended solids and colloidal color, reduces biochemical oxygen demand (BOD) and clarifies wastewater.

Lime effectively acts as a precipitant for phosphates, many trace metals, and bacteria, and as a coagulant for the removal of suspended and colloidal materials in municipal wastewater (Grabow, et al., 1978; Fujita and Iwashima, 1989; Dziubek, et al., 1989). Calcium carbonate, calcium sulfate and sodium chloride are used as coagulants for treatment of municipal waste water (Diwani and Rafie, 2003).
Poly aluminium chloride (PAC) is a water soluble polymer. It precipitates insoluble matter of aluminium poly hydroxide that absorbs suspended pollutants, which should be removed from the water. It is used as the coagulant or flocculant during treatment of drinking water and waste water. It is also used in the pH adjustment and is often employed in the place where the pH of the receiving stream is higher than 7.5. PAC is also used in the pulp or paper industry as a size bonding agent, drainage work aid and surface trash scavenger. PAC is effective useful substitute used as coagulant for water and waste water treatment (Malhotra, 1994).

2.4 Disinfection of drinking water

Water used for drinking and cooking should be free of pathogenic (disease causing) microorganisms that cause such illness as typhoid fever, dysentery, cholera and gastroenteritis. Purification of drinking water containing pathogenic microorganisms requires specific treatment called disinfection. Several disinfection methods such as chlorination (Wagenet and Lemley, 1998; Jensen et al., 2003), chloramination (Dice, 1985), ozonation (Singer, 1995), ultraviolet radiation (Whitby et al., 1984; Oppenheimer et al., 1997; Gravetz and Linden, 2004), boiling (Scibilia, 2000; Drinking Water Resources, 2003), pasteurization (Wagenet and Lemley, 1998), solar radiation (Joyce et al., 1996), SODIS (solar water disinfection) and SOPAS (solar pasteurization) disinfection (Wegelin et al., 1994; Wegelin and Sommer, 1996; Sommer et al., 1997) and SOLAIR (sunlight and oxygen) disinfection (Meyer and Reed, 2001) were applied to eliminate disease causing microorganisms in water.

From the over all review physical, chemical and biological processes have been developed for nitrate removal from water. Although physical and chemical process are very effective in removing nitrate from contaminated water but currently they have limited potential for full scale operation and / or their complex process on plant scale. Due to these
limitations for removal of nitrate from ground water, the most versatile and widely used technology is biological denitrification. Several authors have used terrestrial plants for nitrate removal in water. However there were very limited reports on the nitrate removal using aquatic plants. Hence an attempt was made to remove nitrate from synthetic and ground water by aquatic plants such as water hyacinth, water lettuce and salvinia. Similarly aerobic microbial treatment combined with chemical treatment for nitrate removal is not well established. Hence the work was planned to find out whether the aerobic denitrification combined with coagulation will be of successful treatment process for nitrate remediation, followed by various disinfection methods that were effective for the control of microorganisms in water.