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إدارة مصادر المياه

Denitrification of GroundWater Using Sand Filter

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Abstract

Groundwater is the sole source of potable water in Gaza strip. Nitrate concentrations are increasing, in sonic cases rapidly, at rates of up to 10 mg/L per year and generating health hazards especially for babies (Less than six months) and pregnant women.

The quality of the water extracted from the aquifer varies by area and time, but in general, does not satisfy the WHO guideline values for drinking water quality with regard of the concentrations of nitrates. Most municipal wells have nitrate in excess levels of the 50 mg/l. The main sources of nitrates are fertilizers and domestic sewage effluent. The quantities of sewage that infiltrate to the water table on an annual basis through cesspits and septic tanks are significant, about 12 million cubic meters per year.

This research is devoted for the contribution in the improvement of water quality in Gaza Costal Aquifer using sand filters to remove nitrate from drinking water.

Biological removal of nitrate from drinking water was studied in a slow sand filter with using different medium had different particle size. Sand, gravel pack, and granite gravel were used with adding ethanol as source of carbon to enhancing the potential of denitrification. The flow rate, nitrate concentration and pH were studied there impact on nitrate removal through the sand filter.

The denitrification process need start-up period to allow the bacteria to attach to the support particles before it can be able to start removing nitrate.

As a result of this research the slow sand filter was able to provide NO_3 removal up to 95% (110 mg NO_3/liter). The NO_3 removal efficiency dropped when the surface loading rate increased. The efficiency of nitrate removal is more than 90% (60 to 80 g/ m^2.day), 55% (40 to 60 g/ m^2.day), when flow rate velocity 1.2 m/day for reactor with diameters = 50mm and 75mm with gravel media, while 95% (140 to 200g/ m^2.day) for diameter = 75mm for gravel pack media at the same velocity.

The optimum flow rate for each medium depends on its surface area. The material which has more surface area can be able to remove nitrate more than others at the same flow rate.

pH will be affected with the same factors affecting the denitrification process, so when the flow rate velocity was below 0.36 m/day, the pH will be more than 9 and when the surface loading rate was below 30 g NO3/ m^2.day, the pH was more than 9.

ملخص البحث:

المياه الجوفية هي المصدر الأساسي لمياه الشرب في قطاع غزة، و مع زيادة تركيز النترات بصورة كبيرة و متسارعة 10 ملجم/لتر.عام فإن المخاطر الصحية الناتجة و بخاصة عند الأطفال الأقل من ست شهور و النساء الحوامل تزداد بشكل ملحوظ.

المياه المستخرجة من الخزان الجوفي الساحلي تختلف جودتها بحسب المنطقة و الزمن و لكن بشكل عام فإنها لا تتوافق مع معايير منظمة الصحة العالمية لمياه الشرب بالنسبة للنترات (50ملجم/لتر). معظم الآبار في قطاع غزة تتجاوز نسبة النترات بها الحد الأعلى المسموح به.

تعتبر الأسمدة و مياه الصرف الصحي من المصادر الرئيسة لتلوث الخزان الجوفي بالنترات حيث أن كمية مياه الصرف الصحي التى ترشح للمياه الجوفية بشكل سنوي تبلغ حوالي 12 مليون متر مكعب.

هذا البحث يهدف للمشاركة في تحسين جودة المياه في الخزان الجوفي الساحلي باستخدام المرشح الرملي لإزالة النترات من مياه الشرب.

تم دراسة فعالية الإزالة البيولوجية للنترات ممن خلال التدفق البطئ للمياه من خلال معالج يحتوي على انواع و احجام مختلفة من الوسط. الرمل ، الحصى ، و الحصى المستخدم في الآبار استخدموا كوسط تمر من خلاله المياه المراد معالجتها مع اضافة ايثانول كمصدر للكربون و ذلك لدعم عملية ازالة النترات. معدل التدفق، تركيز النترات، و درجة الحموضة تم دراسة تأثيرهم على ازالة النترات من خلال المعالج المستخدم.

عملية ازالة النترات تحتاج الى مدة تحضيرية للسماح للبيكتيريا بالنمو قبل ان تصبح قادرة على ازالة النترات بفعالية.

كنتيجة للبحث فان المعالج قادر على ازالة النترات بنسبة تصل الى 90% (الداخل 110 ملجم/لتر) كما وجدأن كفاءة ازالة النترات تهبط عندما يزداد معدل النترات المراد معالجتها.

كفاءة ازالة النترات تصل الى 90% (60-80 جم/م^2.يوم)، 55 % (40-60 جم/م^2.يوم) عند معدل تدفق 1.2 متر/يوم للمعالجات ذات الأقطار 50 ،75 ملم الممتلئة بالحصى بينما كانت نسبة الإزالة 95% (120-140 جم/م^2.يوم) للمعالج ذا القطر 75 ملم الممتلئ بالحصى المستخدم بالآبار.

معدل التدفق لكل وسط يعتمد على المساحة السطحية لهذا الوسط، حيث أن المواد التى لها مساحة سطحية اكبر تكون قادرة على ازالة النترات بشكل اكبر عند نفس معدل التدفق.

درجة الحموضة تتأثر بنفس العوامل التى تؤثر على عملية ازالة النترات، لذا عندما يكون معدل التدفق أقل من 0.36متر/يوم فإن درجة الحموضة ترتفع لأكثر من 9 ، و عندما يكون معدل التحميل السطحي اقل من 30 جم/م^2.يوم فان درجة الحموضة تكون اعلى من 9 ايضا.

Acknowledgements

I would like to express my sincere thanks to everybody, organizations and institutions whom have contributed to the successful completion of this study and helped to overcome all the difficulties along the way.

The author could not have carried out this study without the active support, guidance and encouragement of both *Dr. Abd el majid Nassar* and *Dr. Jihad Hamad*, hereby, I would like to express my deep thanks to them for their friendly relationships during the study period.

Also, my thanks extend to *Dr. Zeiad Abo Hen* and all staff of environmental and earth science department for their help during samples analysis.

DECIATION

I would like to dedicate this thesis to my parents to whom I owe everything since I was born.

Also, this thesis is dedicated to my wife who supported and encouraged me at all stages of my study, and for my beloved sons: Yehia and Yaser.

Finally, this thesis is dedicated to my brothers, sisters, uncles, and all those believe in richness of learning.

Wassem Y. Haboub

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Chapter 1

Introduction

1.1 Background

Provision of safe water free from contaminants to mankind is a global, regional and national priority. It is estimated that more than 1 billion people in the less developed countries lack access to safe, clean water and an estimated 1.6 million children under the age of 5 die from diarrhoeal diseases each year (Gordan,2004) (WHO/UNICEF,2004). Besides, about 2.6 billion people have no access to improved sanitation facilities (WHO/UNICEF, 2004).

The Gaza Strip is 40 kilometers (km) long and on average 9 km wide located between the Negev desert and the Mediterranean Sea as shown in Figure 1.1. On this narrow band of semi-arid land will reside a population of over two million Palestinians by 2020.(Al Jamal and Yaqubi,2000; Daibes,2003) The Strip is located on the western-most edge of the shallow coastal aquifer that is exploited for municipal and agricultural water supply for Gaza and southwest of the historical Palestine. There is little rainfall arid on reliable riparian flow; hence water for the Gaza Strip is essentially limited to that available from that small portion of the coastal aquifer that underlies its 365-square km (km^2) land area. In 2004, when the population was 1.38 million, an estimated 145 to 149.5 million cubic meters per year (Mm^3/y) of water were pumped from some 4100 wells to supply the people and irrigated agriculture, and an additional 6 to 8.5 Mm^3/y were lost to natural discharge. Natural recharge of the aquifer and return flows in the same period was estimated to be 120 Mm^3/y. That is, about from 30 to 37 Mm^3/y more water was pumped from the aquifer than was recovered. (PWA/USAID, 2000). Its annual safe yield is 60-65 Mm^3. Since 1967, the aquifer has been over pumped by a rate of 90-100 Mm^3/y in order to meet both Israeli settlers and Palestinian water needs.

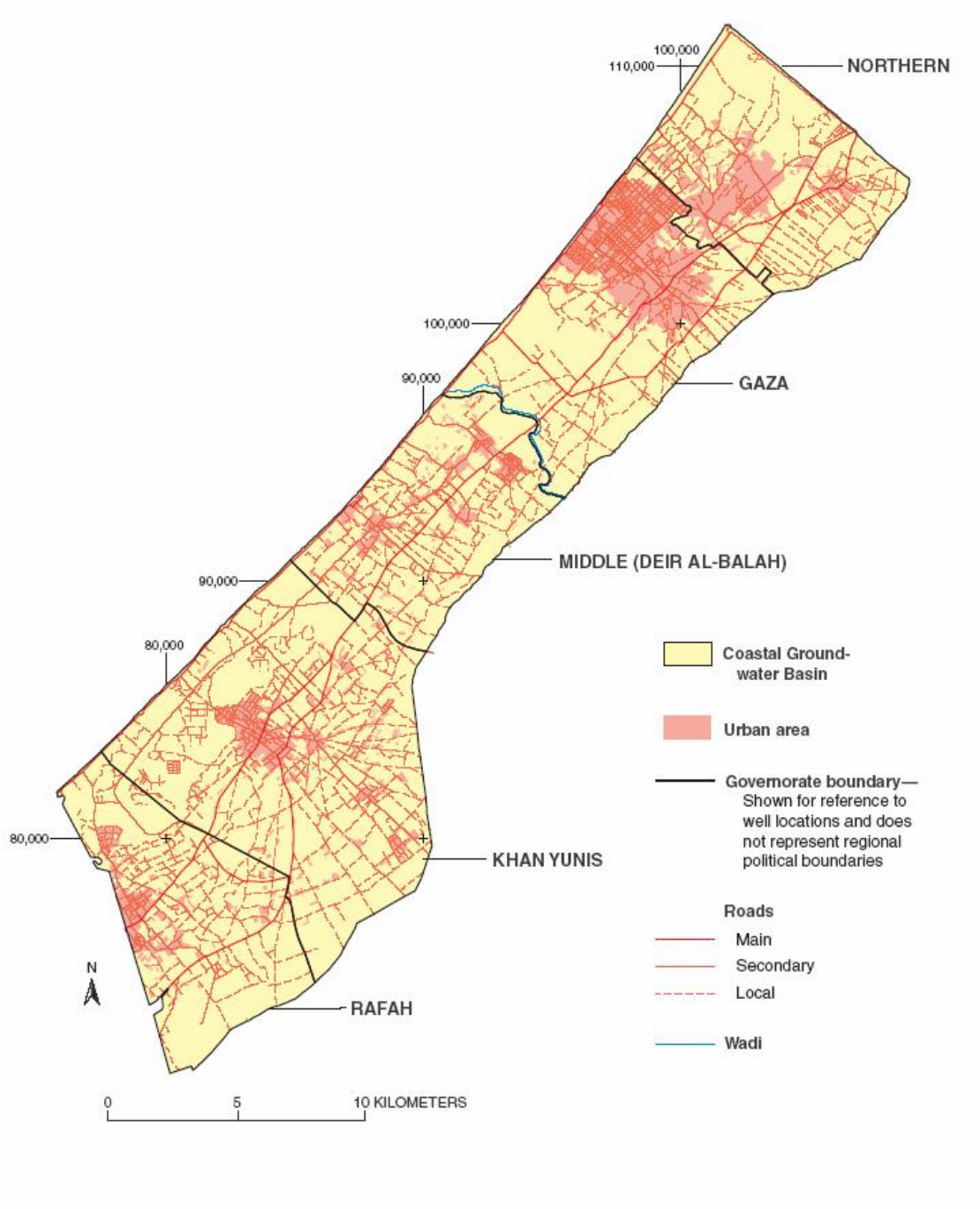


Figure 1. 1Governorates and groundwater basin in Gaza.

1.2 Problem definition

The major documented water quality problems in the Gaza Strip are elevated salinity and nitrate concentrations in the aquifer. The reason for drawing the attention to nitrate is its toxicity to babies (Less than six months) and pregnant women.

Nitrate concentrations in municipal wells in 1998/1999 are shown in Figure 1.2. The world health organization (WHO) drinking water standard for nitrate is 50 mg/L. Most municipal wells in Gaza show nitrate levels in excess of the WHO drinking water standard.

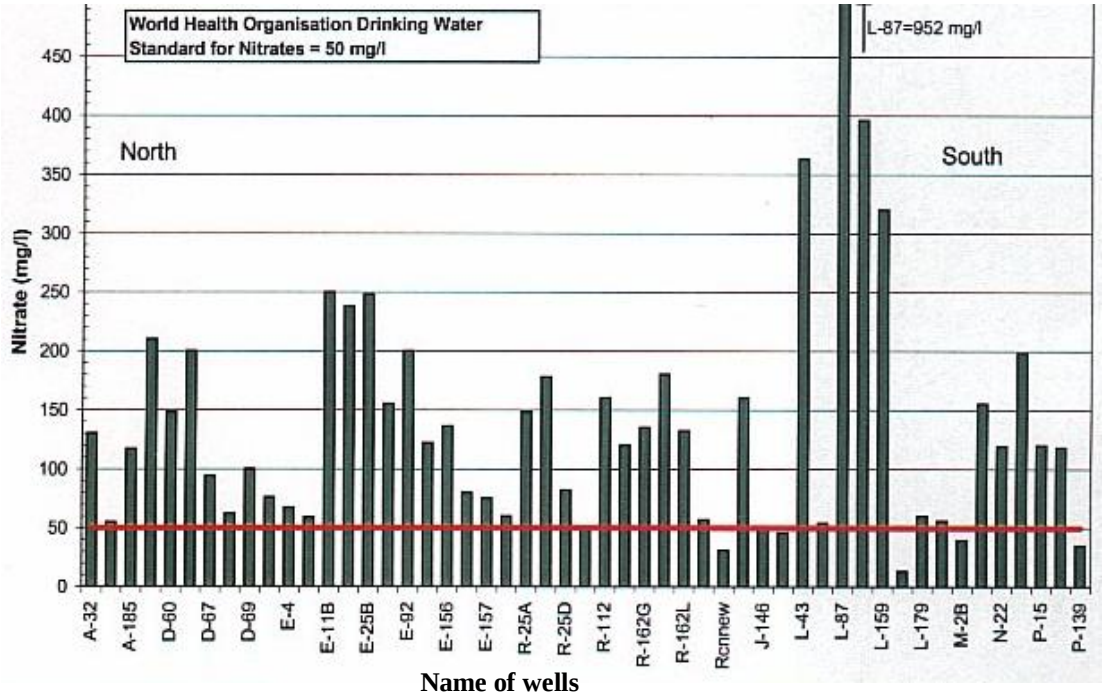


Figure 1. 2 Nitrate concentration in municipal wells in Gaza Strip (1998/1999)

In the worst affected areas (urban centers), nitrate concentrations are increasing, in sonic cases rapidly, at rates of up to 10 mg/L per year (PWA; 2001). Figure 1.3 shows the nitrate concentration in different areas of Gaza strip.

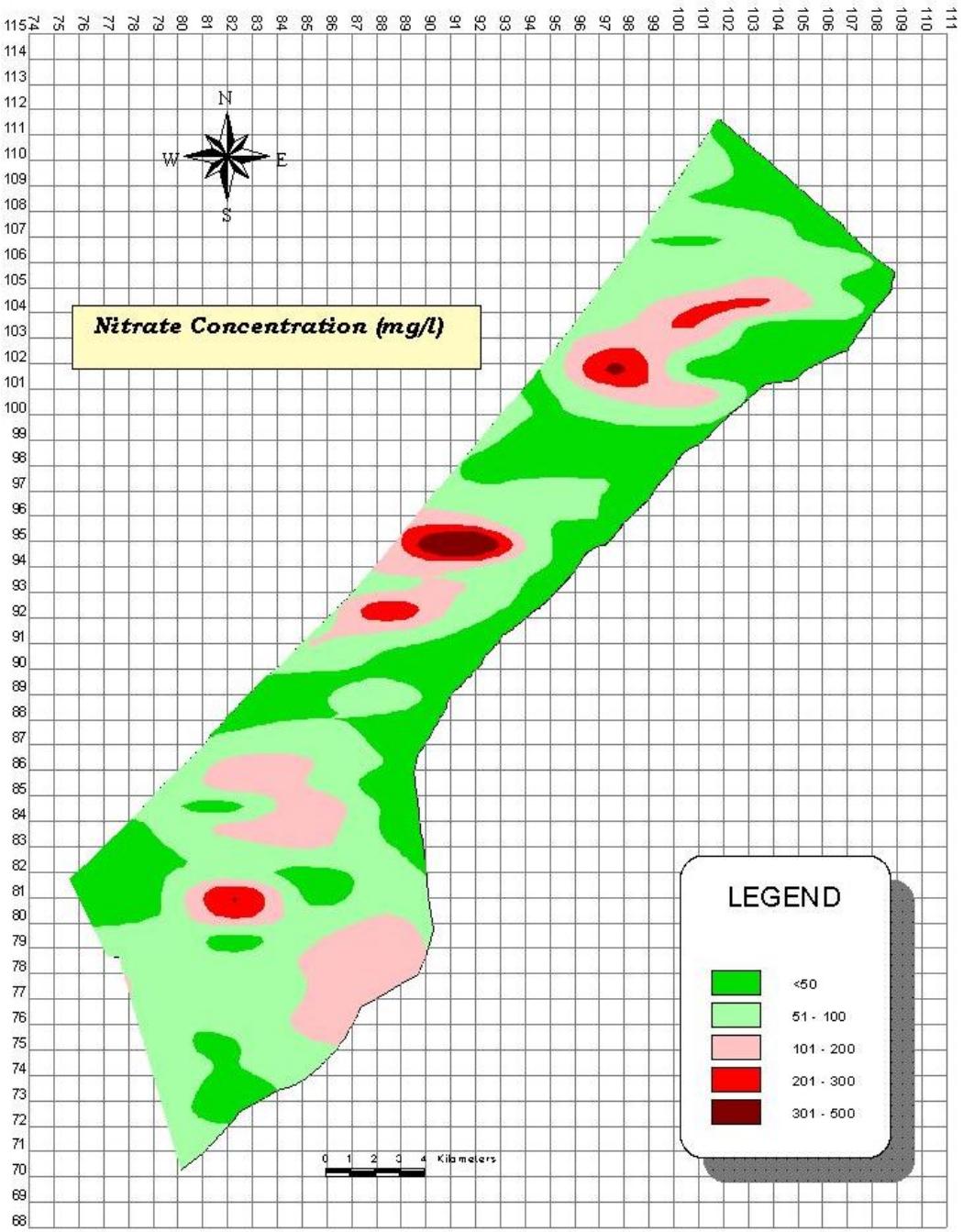


Figure 1. 3 Nitrate Concentration for the year 2002 (mg/l)

(Source: PWA-databank, 2003).

In recent years, the nitrate level in groundwater has increased. This phenomenon has occurred primarily in the coastal area, where the water sources are close to population centers and to industrial and agricultural areas. An increased accumulation of nitrates in groundwater is liable to create a health problem to the population.

The quality of the water extracted from the aquifer varies by area and time, but in general, does not satisfy the WHO guideline values for drinking water quality with regard of the concentrations of nitrates as shown in Table 1.1 (JCT 2006).

Table1. 1 Concentration of nitrate in potable water in Gaza Strip

District	Nitrate, mg/L (WHO value =50)	Mean	Nitrate below 50 mg/L
Northern Area	**13-280**	**101.1**	**10%**
Gaza	**27-224**	**111.6**	**6%**
Middle Area	**17-95**	**49.6**	**10%**
Khan Yunis	**29-380**	**201.0**	**5%**
Rafah	**17-230**	**90.05**	**5%**

The main reasons of high nitrate concentration in ground water in Gaza strip are:

- Domestic and industrial wastewater
- Solid waste dump sites
- Agriculture N-fertilizers.
- High rate of water abstraction.

Until now, no treatment processes in municipal wells are used because of the highly cost of initial, operate, and maintenance of there processes.

1.3 Goal

In Gaza, the water crisis is a function of population growth, an agriculturally intensive economy, a fragile water ecosystem and a highly inequitable distribution of resources.

So the main goal of this research is the contribution in the improvement of water quality in Gaza Costal Aquifer using sand filters to remove nitrate from drinking water.

1.4 Objectives of this thesis

This thesis presents an investigation of Biological denitrification to remove nitrate NO_3^- from ground water.

The specific objectives of this thesis are summarized below.

- To study the characteristics of nitrate removal from groundwater in biological process using a bench scale study.
- To investigate the designing parameters for the sand filter to optimize the removal rate.

1.5 Hypothesis

The denitrification through the sand filter may be the best way for removing nitrate from drinking water.

1.6 Methodology

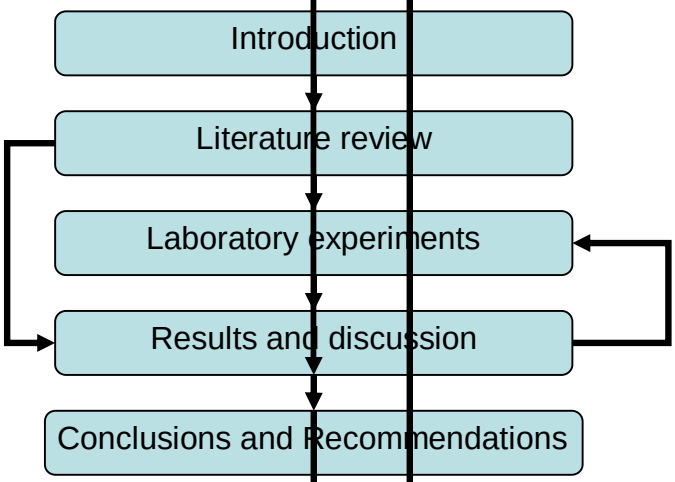
To achieve the objectives of this study, an assessment of the feasibility of removing nitrate from drinking water using sand filters will be investigated.

Bench scale system was fabricated to verify the hypothesis using different size of sand and flow rate.

The system depended on ethanol as source of carbon required to complete the denitrification process.

1.7 Thesis out line

This thesis consists of a general literature study on nitrate problem, conventional biological processes and treatment techniques for removing the nitrate from drinking water. This is followed by a description of the laboratory experiments and rounded off by a discussion and conclusions.



Chapter 2

Literature Review

2.1 Introduction

The quality of drinking water is altered by the use of nitrogen-based fertilizers commonly used in agriculture or products of domestic use. Denitrification of drinking water is needed in this case to reduce the concentrations of nitrites and nitrates present in the water. Therefore it's important to identify the nitrate sources.

In this chapter, a brief review of nitrogen cycle and treatment techniques for nitrate removal is presented.

2.2 The nitrogen cycle

Nitrogen (N), an important constituent of protein and nucleic acids, is the element that required in greatest quantity next to carbon and oxygen for most organisms. Nitrogen in soil and water originates from atmospheric deposition, application of fertilizer, manure, waste material and dead plant and animal tissue. Most of the nitrogen on earth is in the atmosphere, which consists of 78% N_2 gas. The primary forms of inorganic N in water quality management are: ammonia (NH3), nitrite (NO2), and nitrate (NO3) and nitrogen gas (Gale and other, 1993). Before discussing the different nitrate sources the nitrogen cycle is shortly overviewed. The nitrogen cycle is given in Figure 2.1. The illustration in Figure 2.1 shows that nitrogen enters the cycle from several sources electrical discharge (rain cloud), chemical production (industrial fixation and nitrogen fertilizer), and nitrogen fixation (legume fixation and manure). This cycle operates in both natural and cropland ecosystems. In most natural ecosystems, nitrogen is usually in short supply and nitrogen cycling is efficient, with low losses. In some ecosystems, however, nitrogen is abundant and loss potential high, explaining why groundwater under some natural ecosystems can be high in nitrate. In cropland agriculture, especially with irrigated land, greater nitrogen inputs are used for higher crop yields, efficiencies of nitrogen use are lower, and the potential for nitrogen losses to groundwater is greater. Nitrogen not removed through crop harvest can reach groundwater as nitrate.

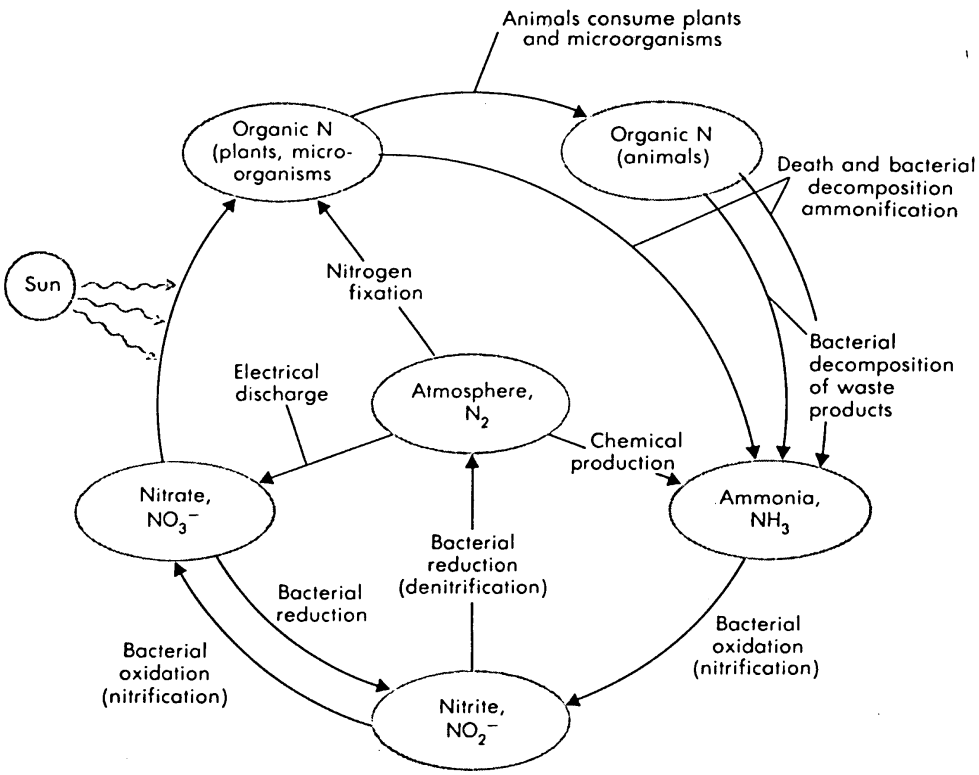


Figure2. 1 Simplified representation of the nitrogen cycle in nature

2.2.1 Mineralization

Mineralization is the decomposition of organic N with the release of ammonium.

$$\textbf{Organic N à } NH_4^+ \qquad (1)$$

This process is either aerobic or anaerobic, but occurs much faster in oxygenated zones (Kathleen, 2000). The rates of mineralization are dependant on temperature, pH (optimum range of 6.5–8.5), the C:N ratio of the residue, available nutrients in the system, and soil conditions such as texture and structure (Reddy and Patrick, 1984). In well saturated soils, pH is buffered around neutrality but under well drained conditions, the pH value of the soil decreases due to nitrate accumulation and the production of protons during nitrification (Patrick and Wyatt, 1964). Organic N (plant detritus, organic sediments and peat) is mineralized to ammonia by a variety of micro-organisms that utilize organic carbon as an energy source.

2.2.2 Nitrification

Nitrification, the biological aerobic oxidation of reduced nitrogen (ammonia) to nitrite by ammonium-oxidizing bacteria (Nitritation) or nitrate (Nitratation) by nitriteoxidizing bacteria is a pivotal chemoautotrophic process in N cycling and regulation of water quality of aquatic environments. (Kowalchuk and Stephen, 2001)

The oxidation of NH_{4+} to NO_{3-} is an exergonic process that yields sufficient energy to synthesize new cells using CO_2 as a carbon source. Nitrification occurs in aerobic regions of the water column, soil-water interface, and root zone (Reddy and D'Angelo, 1997). The oxygen required for the nitrification process is supplied by diffusion from the atmosphere and leakage from macrophyte roots (Armstrong W., Armstrong J., and Beckett PM., 1990). Studies have indicated that DO levels

below 1–2 mg/L in water substantially reduce nitrification (Hammer, and R.L. Knight. 1994) (Lee, Stansbury JS., and Zhang TC., 1999).

Nitritation: $2NH_4^+ + 3O_2 \xrightarrow{\text{Ammonia oxidizing bacteria}} 4H^+ + 2H_20 + 2NO_2^-$ (2)

Nitratation: $2NO_2^- + O_2 \xrightarrow{\text{Nitrite oxidizing bacteria}} 2NO_3^-$ (3)

$2NH_4^+ + 4O_2 \longrightarrow 4H^+ + 2H_2O + 2NO_3^-$ (4)

Nitrification is essentially carried out by two distinct groups of bacteria (ammonium and nitrite-oxidizers respectively) belonging to the family *Nitrobacteriaceae*.

Various heterotrophic and lithotrophic micro-organisms, including bacteria (actinomycetes and planctomycetes), algae and fungi have also been reported to have nitrifying activity. Since autotrophic nitrification usually occurs at higher rates than heterotrophic nitrification it is believed to play a more important role in nature (Focht and Verstraete, 1977).

2.2.3 Denitrification

Denitrification is a biological conservation of nitrate to nitrogen gas, which happens under anaerobic condition in the presence of denitrifying bacteria.

Denitrification is a stepwise enzymatic anoxic reduction process in which nitrite and nitrate are reduced to molecular nitrogen or nitrogen gases by chemoorganotrophic, lithoautotrophic, and phototrophic bacteria (Kadlec, et al. 2000). In this microbial process, the nitrogen oxides irreversibly serve as terminal electron acceptors in the electron transport chain. The electrons are usually but not exclusively transferred from organic compounds through a series of carrier systems to a more oxidized nitrogen form.

$6(CH_2O) + 4NO_3^- \longrightarrow 6CO_2 + 2N_2 + 6H_2O$ (5)

$2NO_3^- \xrightarrow[\text{reductase}]{\text{Nitrate}} 2NO_2 \xrightarrow[\text{reductase}]{\text{Nitrite}} 2NO \xrightarrow[\text{reductase}]{\text{Nitric oxide}} N_2O \xrightarrow[\text{reductase}]{\text{Nitrous oxide}} N_2$ (6)

The resultant free energy conserved as ATP is used by the denitrifying organisms to support respiration (Kadlec et al. 2000).

This process is performed by heterotrophic bacteria under anoxic conditions and uses nitrate as terminal electron acceptor in the presence of a carbon and energy source.

Denitrification is the only process that could reduce NO_3- concentration during downward percolation under cesspits.

2.2.4 Volatilization

Ammonia volatilization is an important process mainly in basic soils. The volatilization of NH_3 is, determined by the percentage of free NH_3 present, which is a direct function of the pH. Under high-pH conditions (pH $\geq$ 7.5), the

concentration of the un-ionised form of ammonia (NH_3) becomes appreciable compared to NH_{4+} and NH_3 is released to the atmosphere.

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \quad (7)$$

Besides pH, other factors affected ammonia. According to Gasser (1963) the most important factor is the cation exchange, while Ivonove(1963) found that the presence of carbonate is the dominant factor for ammonium losses.

According to Yoram and Malka (1977) more ammonia volatilization can occur when ammonia fertilizers are finely and evenly spread on the soil compared to spreading of granular or large droplets of the same fertilizer. The reason for this could be that in the granular form, part of the ammonia will volatilize. Measurement of NH_3 volatilization in soil could be done through the following equation:

$$\mathbf{1/[NH_3] = - B/[K_w/K_b] + [[H+]_0 + B[NH_4+]_0/\{[K_w/K_b] *[NH_4+]_t\}]} \quad (8)$$

Where B is the buffering capacity factor for the soil.

K_w ,K_b are conjugate acidic-base pair ($K_w = 1*10^{-14}$, $K_b = 1.8*10^{-5}$)

2.2.5 Adsorption

Part of NH_4^+ ions is absorbed by the negatively charged clay and organic collides in the soil by the cation-exchange complex.

The cation exchange capacity of soil depends upon the amount and type of clay and the amount of organic matter. The fraction of the cation exchange capacity that may be used to absorb NH_4^+ depend upon the concentration of other cations in the water applied because these cations complete with NH_4^+ for exchange sites.

The NH_4^+ adsorbed by the soil cation exchange capacity is only temporarily immobilized because it can be readily oxidized to NO_3^- when oxygen is available. However, this adsorption is extremely important because its retains nitrogen within the root zone for a times. (Gabriel and Charles, 1990)

2.3 Sources of Nitrate

In most naturally occurring environments, nitrate concentrations in groundwater are less than 3 mg/L (Smith et al.,1987). Nitrogen losses due to denitrification help to maintain relatively low nitrate concentrations in ground- and surface waters. National standards have been established for drinking water at 10 mg/L nitrate-nitrogen (WHO 1996).

This standard applies to all public supply systems. To provide a higher margin of health safety, Germany and South Africa have lowered their nitrate-nitrogen drinking water standards to 4.4 mg/L (Kross 1995). Groundwater concentrations of nitrate larger than10 mg/L are attributed to the sources listed below.

2.3.1 Human and Animal Wastes

Waste produced by humans and animals are major sources of nitrate in any area characterized by significant human or animal populations. Nitrates from such waste can exhibit the characteristics of either point or nonpoint source pollution. Point sources occur at or near the actual waste facility involved and typically exhibit high levels of nitrate or ammonia in a limited area. Diffuse sources are spread over large areas (eg. in agricultural fertilizations), and impacted aquifers are often characterized by lower (but ≥10 mg/L) levels of nitrate-nitrogen.

Nitrate from human waste originates mostly from individual septic systems or municipal wastewater treatment facilities. Typically, effluent from such septic systems is in the order of 30 to 60 mg/L of total nitrogen, with ammonia making up the majority of the nitrogen. The nitrogen content of this effluent varies widely depending upon the condition of the individual system and the type of waste being introduced. The majority of the population is served by municipal wastewater treatment systems. Nitrogen content of effluent from municipal systems will vary according to the nature of the incoming waste stream and the type and condition of the system. However, after primary treatment with activated sludge, the effluent typically still contains about 15 to 35 mg/L of total nitrogen.

Waste from dairies, open feedlots, confined feeding operations, stockyards and other facilities for raising and holding animals is also a potential source of nitrate and other forms of nitrogen. While public concern over animal waste includes such issues as odour, flies and surface water impacts, these facilities represent a massive source of nitrogen and other nutrient inputs to groundwater. For example, the University of Nebraska Cooperative Extension (1998) estimates that waste from animal stock typically contains from about 0.1 to 0.4 kg of nitrogen per kilogram of animal weight.

Typically, total nitrogen concentrations of dairy wastewater range from 150 to 500 mg/L.

2.3.2 Fertilizers

Nitrogen is the major component of fertilizer for agricultural, turf and garden use.

Nitrogen fertilizer normally takes one of two forms:

- inorganic fertilizer;
- animal waste.

Inorganic fertilizer usage in Gaza has become common place in the last half of the twentieth century with the advent of anhydrous ammonia, and similar formulations that have greatly increased crop yields. In some cases, fertilizer has been over-applied, either from a lack of understanding of its impacts or crop nutrient requirements.

Animal waste has been applied to cropland for generations, both as a means of fertilization and waste disposal.

Nitrate's high solubility and low sorptivity allows infiltration beyond the root zone when over-applied or over-watered. Thus, infiltration via precipitation or irrigation water easily transports nitrate, which is not taken up by plants, downward to groundwater. As a result of this process, elevated groundwater nitrate levels have occurred in heavily farmed areas. Recent attempts to reduce nonpoint nitrate contamination in groundwater have focused on proper timing of application and reduced amounts of fertilizer and irrigation water.

2.3.3 Industrial Uses of Nitrate

Nitrogen compounds are used extensively in industrial settings. Some of the predominant nitrogen compounds used in industry are:

- anhydrous ammonia,
- nitric acid,

- ammonium nitrate,
- urea.

A few of the industrial uses for nitrate include:

- manufacturing of plastic,
- metal processing,
- raw material in the textile industry,
- pulp, paper and rubber production and
- household cleaners.

Nitrate contamination may result from improper handling, disposal and use of these compounds, and levels of contamination will depend on the source.

2.3.4 Naturally Occurring Nitrates

It is unusual for pristine groundwater systems to accumulate more than 3 mg/L nitrate (Madison and Brunnet 1985). However, some naturally occurring processes may occasionally cause nitrate contamination in groundwater.

During lightning storms, atmospheric nitrogen is converted to nitrate and deposited to the soil through rain. In arid conditions, high nitrate concentrations may be caused by evapotranspiration of infiltrating rainwater in the shallow subsurface aquifer. During storm events, this high nitrate concentration may be transported to the shallow aquifer where nitrate concentrations can be up to 60 mg/L (McQuillan 1995).

Nitrate concentrations larger than or equal 10 mg/L in groundwater may also be attributed to geologic formations. Sedimentary deposits with high organic matter may release nitrogen. In New Mexico, two limestone formations have been identified, with naturally occurring groundwater nitrate concentrations between 12 and 15 mg/L (Titus 1980).

Table 2.1 summarizes the maximum total nitrogen concentrations found in groundwater related to a variety of common sources.

Table 2. 1 Maximum total nitrogen concentration (mg/L) in discharging water from typical nitrogen sources.

Source	Total Nitrogen (Max. Conc. mg/)
Human Waste (septic system effluent)	100
Dairies (wastewater)	500
Animal Feedlots (runoff, wastewater)	500
Fertilizer Manufacturer (groundwater)	10,000
Over Fertilized Croplands (groundwater)	100

2.4 Properties of Nitrate

Nitrate is a major anion that is primarily in the aqueous phase in both the vadose and saturated zones of the subsurface. Nitrate is nonsorptive and for the most part does not exchange on sediment surfaces in the vadose zone or groundwater. It has a very low probability of retardation onto soil colloids. Nitrate solutions tend to move through soils at virtually the same speed as the wetting front in the vadose zone or with groundwater flow. Nitrate tends to move unhindered and unchanged through a soil profile or aquifer matrix (Bohn, et al., 1979).

There are three major forms of nitrogen in the soil and vadose zone that may cause nitrate contaminated groundwater: organic nitrogen, ammonia nitrogen, or nitrate aqueous in pore water.

2.5 Effects of Nitrate

Nitrate concentrations larger than or equal 10 mg/L in groundwater have many adverse effects on human and animal health and on the environment. These effects are described below.

2.5.1 Human Health Effects

Methemoglobinemia, also known as Blue Baby Syndrome, is a disease generally resulting from the ingestion of high concentrations of nitrate in its inorganic form (Burt *et al.* 1993). In the stomach and small intestine of individuals with very low stomach acidity, indigenous bacteria chemically reduce the nitrate (NO_3-) to nitrite (NO2-), a more reactive form of the compound. Nitrite is absorbed through the walls of the small intestine into the blood stream where it combines with haemoglobin to form methemoglobin. This process blocks the oxygen-carrying capability of the blood. When the concentration of methemoglobin becomes too high, the victim becomes cyanotic and can die of asphyxiation. The body does not have the capability to naturally change the methemoglobin back to effective haemoglobin.

The cause of Blue Baby Syndrome is generally the mixing of infant formula with water containing greater than 10 mg/L nitrate as nitrogen. Infants are not the only susceptible population, however (Winneberger, 1982). Children and adults suffering from maladies or treatments that lower the levels of stomach acid are also vulnerable to methemoglobinemia.

Although methemoglobinemia is the only disease that is currently directly attributable to elevated nitrate concentrations, there are other suspected health effects. Important amongst these is the possibility of spontaneous abortions in women of childbearing age. A small study of these occurrences was carried out in Indiana, USA in 1993 (Centres for Disease Control and Prevention). Four women, living in residences served by private wells contaminated with nitrate ranging from 19 to 29 mg/L nitrate as nitrogen, experienced a total of eight spontaneous abortions. Three of the women lived within two kilometres of a point source of nitrate contamination. One of the women had four spontaneous abortions within the first 8 to 11 weeks of her pregnancies. At least one of these women had previously carried a child to term. The fourth woman resided approximately 16 kilometres from the first three. She had previously carried four babies to healthy births but had two spontaneous abortions in 1994. The home's water supply contained an average nitrate concentration of 29 mg/L. After switching to nitrate-free drinking water, all four women carried babies to term.

Another health concern, which has been under study for many years, is nitrate contaminated drinking water's link to non-Hodgkin's lymphoma and stomach cancer (Tannenbaum and Green, 1985; WHO, 2003). Although this link is very tenuous and controversial, research and surveys are ongoing in an attempt to document the connection.

2.5.2 Animal Health Effects

Nitrate-contaminated water consumed by livestock has resulted in nitrate poisoning.

At high enough nitrate concentrations (>300 mg/L), nitrate poisoning may result in animal death. At lower concentrations, nitrate poisoning can increase the incidence of still born calves, abortions, retained placenta, cystic ovaries, lower milk production, reduced weight gains, and vitamin A deficiency.

Livestock may be harmed at nitrate-nitrogen concentrations between 100 to 300 mg/L, and nitrate poisoning in cattle, sheep, and horses may occur at concentrations greater than 300 mg/L nitrate-nitrogen. Recommended limits of nitrate in drinking water for livestock and poultry should not exceed 100 mg/L. Accurate assessment of the source of nitrate poisoning in stock is difficult because of the potential of nitrate accumulation in crops which may further cause nitrate accumulation in the animal (Kvasnicka and Krysl 1990, Faries et al.. 1991).

2.5.3 Environmental Effects

Nitrogen concentrations exceeding background levels (~3 mg/L) in surface waters reflect pollution from domestic, industrial or agricultural sources (Smith et al.. 1987).

Since the early 1970s, trends show an increase in nitrate concentrations in rivers and streams. Nitrogen is one of the most important nutrients that regularly limits primary productivity. Excess input of nitrogen to the environment results in eutrophication in fresh and marine waters (Cole 1983).

The effects of nutrient loading on water quality and productivity are particularly important for natural water bodies, which are often sources for municipal water supplies and water-based recreation (Kimmel 1991). Levels of nitrate much lower than the maximum contaminant level for drinking water contribute to increased rates of eutrophication in surface waters (Cole 1983).

Runoff from cropped agricultural fields and feedlots is significantly higher than from pastureland (Beaulac and Reckhow 1982). In a study by Smith et al. (1987), increased nitrogen loading to runoff from cropped lands was associated with increased nitrogen fertilization rates, which amounted to a 68% increase on cultivated lands from 1970 to 1981 (Smith et al.., 1987). Runoff from animal feedlots provides high concentrations of nitrate and ammonium (Beaulac and Reckhow 1982).

Wetlands and forested areas are our prime defenses for trapping and purifying nutrients in runoff before they enter streams (Fennessy and Cronk 1997). When there is nitrate loading to coastal streams and rivers, it generally stimulates algal blooms in salt-water estuaries and bays. In the Gulf of Mexico, nitrate runoff from the Mississippi River has resulted in up to 7,032 sq. miles of hypoxia (Rabalais et al..2001). In Chesapeake Bay rivers, animal waste nitrogen is believed to be the

cause of a deadly *Pfisteria* bloom in the summer of 1998 (Burkholder and Glasgow Jr. 1997).

2.6 Nitrate transport mechanism

The mechanism of interactions between contaminants and soil are greatly influenced by the chemistry of the soil constituents, the pH of the system, the specific contaminants and the carrying capacity of the soil. Movement of any dissolved ion such as NO_3 through the soil is governed by two mechanisms: convection and diffusion.

2.6.1 Convection

The simplest representation of the mass transport of solute be convection is given in the following equation:

$$J_{sc} = J_w C \qquad (9)$$

Where

J_{sc}: the mass of the solute per unit area per unit time transported by convection.

J_w: the water or soil solution flux.

C: the solute concentration in mass per solution volume.

2.6.2 Diffusion

Solutes dissolved in solution spread out under the influence of molecular-scale collisions, a process known as molecular diffusion.

The diffusion flux of solute J_{sd} in one dimention is described by Fick's law of diffusion, which in soil is written as:

$$J_{sd} = - \acute{\varepsilon} (\theta) D_{sw} \ \partial c/\partial z \qquad (10)$$

Where;

D_{sw} : the binary diffusion coefficient of the solute in water.

$\acute{\varepsilon} (\theta)$: tortuosity factor

When water from the unsaturated zone joins underlying groundwater, it tends to stay at the top of the aquifer. If this water reaches an aquifer with good quality water, it could sink deeper into the aquifer and eventually reach the lower boundary. This would cause more complete mixing with the original ground water.

2.7 Factors affecting nitrification

The occurrence of nitrification is significantly influenced by temperature, pH, alkalinity, inorganic C source, the microbial population and concentration of NH_4–N, dissolved oxygen and inimical pollutant compounds. Whereas nitrification occurs over a wide temperature range of 4–40^oC, the optimum temperature in pure cultures ranges from 25–30^oC, and 30–40^oC in soils. A narrow optimum pH (7.2–8.6) exists (Gerardi MH., 2002) but acclimatized systems can be operated to nitrify at a much lower pH value. Nitrification is obligatorily coupled to oxygen consumption and has an effect on the decrease in wastewater alkalinity. Such a decrease in wastewater alkalinity might cause a decrease in its pH when the alkalinity of the wastewater is low or when its ammonia content is relatively high (Kadlec et al..; 2000). During ammonium

oxidation, the wastewater alkalinity increases slightly due to CO_2 consumption for autotrophic growth whereas acidic nitrite formation results in a drop in wastewater pH.

Thus if the buffer capacity of the system wastewater is weak, the pH might drop well below 6.7 preventing further autotrophic nitrification. Although effective nitrification has been reported in systems with residual oxygen as low as 0.5 ml/L, DO concentrations below 1.5 mg/L are reported to limit the nitrification process. (Gerardi MH, 2002; Hammer et al.. 1994)

Nitrifying bacteria are sensitive organisms that are extremely susceptible to a wide range of inhibitors present in wastewaters. Such inhibitory pollutants include phenolic compounds, cyanide, thiourea, anilines and heavy metals primarily originating from industrial processes. Extremely high concentrations of ammonical nitrogen and nitrous acid are reported to be inhibitory (substrate inhibition) to the nitrification process.(Gerardi MH, 2002) Similarly, high organic loading inhibits nitrification by promoting heterotrophic growth and activity which culminate in limited nitrifier growth and activity as a result of strong competition for the available oxygen and ammonia.

2.8 Traditional Treatment of Nitrate Contaminated Groundwater

Remediation of nitrate plumes has not been as common or extensive as other contaminants of concern. However, when a groundwater nitrate plume has been identified, certain corrective remediation activities have been employed. Site-specific conditions determine which remediation option to use. Most common remediation options involve the pumping of contaminated groundwater before undergoing treatment.

2.8.1 Monitored Natural Attenuation

Monitored natural attenuation refers to prohibiting further groundwater contamination and allowing natural advection, dispersion and chemical and biological degradation of the plume.

For various reasons, no remediation action for nitrate-contaminated ground water has been a common approach and perhaps the option most often chosen. Some reasons for monitored natural attenuation are:

- . public awareness
- . extent of contamination
- . inconsistent regulatory enforcement
- . economic issues and
- . responsible parties who are unable to pay for remediation.

When a supply well is impacted with nitrate contamination, certain institutional actions are taken to provide clean water without addressing the contamination. Examples of this are deepening the supply well to find clean water, blending the contaminated water with clean water to meet standards, or supplying an alternate water supply. If no action is taken, groundwater nitrate plumes remain and may continue to increase in concentration and size, posing a continued or greater threat.

2.8.2 Pump with Beneficial Use

Pumping and using nitrate-contaminated groundwater has been the most common remediation technique employed after no action. This remediation usually entails pumping large volumes of contaminated water and directly applying it onto croplands.

Crops remove nitrates from the root zone for growth. The crops are then harvested, removing nitrogen from the environment. However, there are numerous disadvantages to this remediation technique. These include:

- high costs
- considerable engineering and planning to extract and deliver the contaminated water
- possibility of further nitrate contamination
- developing appropriate land use for crop application
- regulatory permitting

2.8.3 Pump and Treat

Pumping and treating nitrate-contaminated groundwater is another remediation technique that is often employed. This option has been used at public supply wellheads and may not address treating of the nitrate plume. The treatment of the nitrate-contaminated groundwater may be through:

- wastewater treatment plants,
- reverse osmosis,
- ion exchange, or
- electrodialysis.

Nitrate-contaminated groundwater is pumped and discharged to existing wastewater treatment plants for nitrate removal, or specific treatment plants are constructed to address the nitrate contamination. This treatment may be expensive, and existing treatment plants may not be able to handle the increased volume.

2.8.3.1 Ion exchange

The USEPA has identified the Best Available Technologies that are capable of removing regulated contaminants from drinking water are anion exchange and reverse osmosis.

Nitrate can be removed by an anion exchanger. Mostly synthetic exchangers such as polystyrene resin are used, for nitrate removal. Ion exchange introduces another substance, normally chloride or hydrogen carbonate, to trade places with nitrate in water. In the exhaustion cycle of the resin, the ion with the lowest affinity would "break through" first and the ion with the highest affinity would appear in the effluent last. The expected order of affinity would be as follows:

Sulfate > Nitrate > Nitrite > Chloride > Bicarbonate

An ion exchange unit operates much like a household water softener. For nitrate removal, the resin exchanges chloride ions for nitrate and sulfate ions in the water.

After treating many gallons of water, the resin will "run out" of chloride. Regenerating the resin with a concentrated solution of sodium chloride recharges it for further treatment. Water high in sulfates hinders the nitrate exchange and reduces system effectiveness. If the resin becomes saturated, it releases the nitrates in place of sulfates, resulting in an increased nitrate concentration in the treated water. Also, nitrate ion exchange can make the water corrosive. Finally, ion exchange can be expensive and requires maintenance. Since the backwash brine will be high in nitrates, care must be given to its disposal.

2.8.3.2 Reverse osmosis

Reverse osmosis forces water under pressure through a membrane to filter out contaminants. As illustrated in Figure 2.2 water enters the unit under pressure, it pushes against a cellophane-like plastic sheet or cellulose—also called a semi permeable membrane. The membrane acts like a sieve, leaving ions like nitrates on one side and allowing ion-free water to pass through the membrane. The membrane filters reject around 83 to 92 percent of the incoming nitrate. If nitrate-nitrogen levels are extremely high (greater than 110 mg/l) up to 90 percent may be removed. Reverse osmosis requires a sediment filter, storage tanks, a membrane, and an activated carbon filter. Many factors like water pressure and temperature, membrane selection, and proper maintenance influence performance. While reverse osmosis can be an effective nitrate remover, it has disadvantages. Reverse osmosis is expensive. Reverse osmosis is also a slow inefficient process, sometimes producing only a few cubic meter a day of purified water, while wasting up to 90 percent of the incoming water. This is especially true for low pressure systems. Reverse osmosis is economically more feasible for treating water with high TDS (5000-35000mg/l).

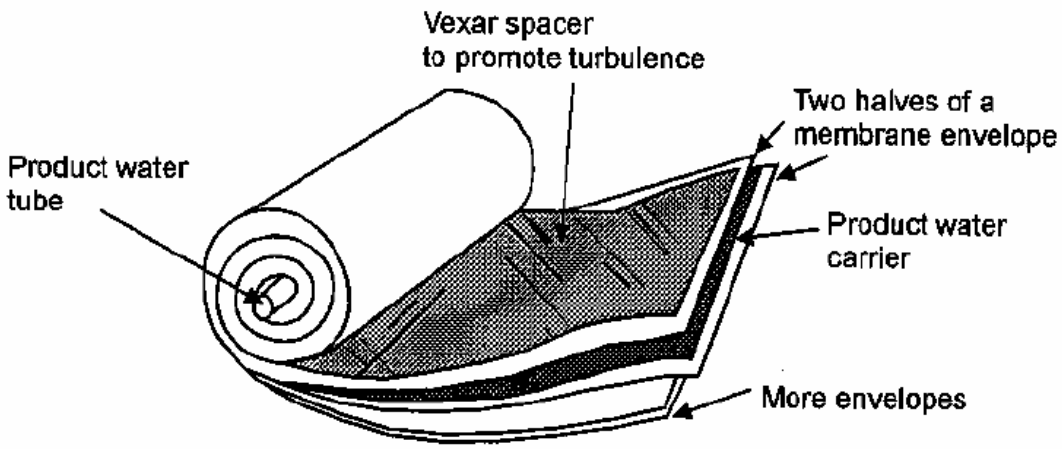


Figure2. 2 Spiral wound reverse-osmosis element

2.8.3.3 Electrodialysis

Electrodialysis reversal EDR is an electrochemical process in which ions migrate through an ion-selective semi permeable membrane as a result of their attraction to the electrically charged membrane surface. A positive electrode (anode) and a negative electrode (cathode) are used to charge the membrane surfaces and to separate contaminant molecules into ions. The process relies on the fact that electrical charges are attracted to opposite poles. As a result of the removal process, reduction in ions (or TDS) is obtained. A common EDR system includes

a membrane stack which layers several cell pairs, each consisting of a cation transfer membrane, a demineralized flow spacer, an anion transfer membrane, and a concentrate flow spacer as shown in Figure2.3. A single-stage EDR system usually removes 50 percent of the TDS. The main disadvantage of EDR that its not suitable for higher TDS sources, not suitable for high levels of Fe and Mn, H2S, chlorine, or hardness, and limited current density; current leakage; back diffusion.

In electrodialysis, ions are transferred through membranes from a less concentrated to a more concentrated solution due to the passage of a direct electric current. This process is expensive and requires close monitoring (Kappor, 1997).

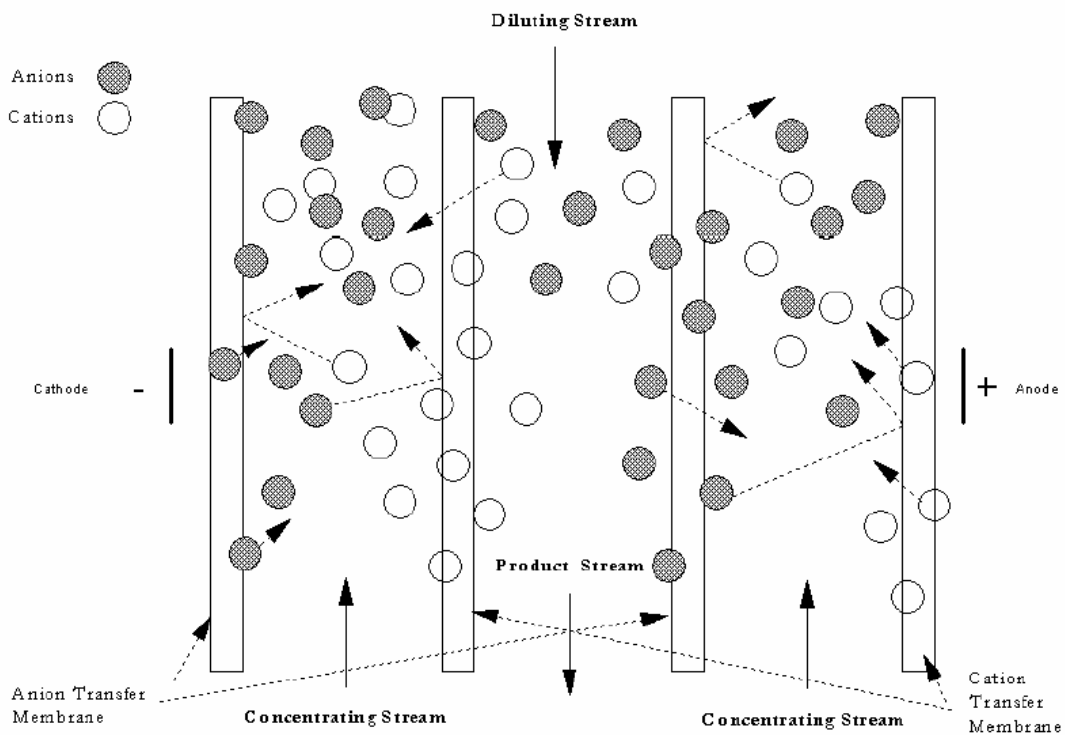


Figure2. 3 Transfer of ions within the electrodialysis stack

2.8.4 Pump and Waste

Pumping nitrate-contaminated groundwater to waste has also been employed. The nitrate-rich water may be discharged to a contained evaporation system or injected into a deep saline aquifer or geologic unit. Groundwater resources are lost to evaporation or injection. Disposal of the residue from evaporation may be a problem if improperly managed. It is not environmentally sustainable to move a contaminant source to an uncontaminated location.

2.8.5 Phytoremediation

Phytoremediation is a means of removing, transforming, or binding contaminants in soil and groundwater through the use of plants, both as active and passive remediation tools. Plants can remediate contaminants through several processes (Schnoor 1997):

- . phytotransformation,
- . phytoextraction,

- . phytostabilisation, and
- . rhizofiltration.

Of these, phyto-transformation is the process most active in plant removal of nitrogen compounds. In addition to their ability to transform nitrogen compounds, some plants transpire great quantities of water acting as groundwater extraction and flow control structures. Phytoremediation techniques generally meet with public acceptance due to the ease of understanding and a desire to see living things transform a contaminated site.

In 1987, Licht and Schnoor (1993) effectively demonstrated the potential of phytoremediation for nitrate removal. They planted a buffer strip of poplar trees between a stream and a cornfield from which nitrate was leaching into a stream. By 1990, when the trees were three years old, they were effectively reducing nitratenitrogen from 35 mg/L to 3 mg/L in groundwater leaving the cornfield.

While this technique is a highly effective means of dealing with fertilizer and other nitrogen compound contamination, there are limits to its application. High concentrations of nitrate and/or ammonium can result in plant toxicity, either overall or at certain developmental stages of the plant. Alkaline or saline soils may also prove toxic, as may the presence of other contaminants.

Depth of contamination may exceed the rooting depth of plants, thus also limiting the application, though some sites show that nitrogen uptake and transpiration can dramatically alter contaminant patterns at depths up to 10 meters below ground (ITRC 2000). Poorly drained soil conditions and heavy, tight soils may limit rooting depth, even with species that are normally deep rooted. Traffic patterns, property boundaries, right-of-ways, building proximity, and regulatory restrictions may also prove to be limiting issues. Another potentially limiting factor in the decision to employ phytoremediation is the length of time it takes plantings to mature sufficiently to become effective at nitrogen removal. Sites that demand immediate action to protect drinking water supplies may not be able to wait for maturation of a planting.

Table 2.2 summarizes the basic methodology, benefits and concerns about the traditional methods for dealing with nitrate-contaminated groundwater that have been discussed above.

Table 2.2: Summary of Traditional Methods in Dealing with Groundwater Nitrate Contamination.

Remediation Technology	Basic Methodology	Benefits	Concerns
Monitored Natural Attenuation	Monitoring of groundwater	• no equipment cost • no clean up cost or efforts • dilution to meet standard	• increase in plume size • impact on receptors • violation of standard
Pump and Use	Impacted groundwater pumped and used	• plume containment • mass removal • beneficial use of extracted water	• long-term engineering cost • regulatory permitting
Pump and Treat	Impacted groundwater pumped and treated	• plume containment • mass removal • re-use of clean water	• long-term engineering cost • treatment system potentially expensive • hazardous concentrated waste stream
Pump and Waste	Impacted groundwater pumped and wasted	• plume containment • mass removal	• long-term engineering cost • potential plume migration • regulatory issues
Phytoremediation	Impacted groundwater treated by plant uptake	• plume containment • low cost • aesthetically pleasing	• depth to water is a limiting factor • land requirements • property rights • long-term management of plants

2.9 New Technologies of Nitrate Contaminated Groundwater

New remediation technologies that address nitrate contamination rely on microbial denitrification.

2.9.1 Microbial denitrification

In the natural environment nitrogen is cycled through plants and animals in a complex cycle of biological and chemical processes as shown in Figure 2.1. Denitrification refers to a microbial process where nitrate is reduced to nitrite, gaseous oxides of nitrogen which are then further reduced to nitrogen gas. Within the nitrogen cycle, denitrification represents a loss of nitrogen.

Denitrifying microorganisms use nitrate dissimilatively, as a terminal electron acceptor for respiration. Approximately 45 genera of bacteria and fungi can reduce nitrate dissimilatively to nitrite (Payne 1973), but reduction to these products does not result in a loss of fixed nitrogen. The denitrifying bacteria are capable of reducing nitrate to the gaseous forms of molecular nitrogen (N_2) and nitrous oxide (N_2O), which may be easily lost from the ecosystem (NRC 1978).

Some denitrifying microorganisms are also facultative, meaning they have the ability to replace aerobic respiration with anaerobic respiration when oxygen in

limited. This means that oxygen is replaced by an alternative electron acceptor, namely nitrate.

Many denitrifying bacteria are also heterotrophic meaning they utilize carbon from organic compounds rather than from carbon dioxide. They are able to utilize a wide range of carbon compounds (sugars, organic acids, amino acids) as sources of electrons.

The genus *Pseudomo nas* most probably represents the most active and abundant denitrifiers in the natural environment (Riley, 2002). Other important groups are the *Alcaligenes* and *Flavobacterium* (Hiscock et al.. 1991).

As described by the ecological redox sequence in Figure2.4, nitrate is the first compound to be reduced after oxygen depletion.

Nitrate reduction is described by the following half-reaction:

$$NO_3^- + 6H^+ + 5e^- \text{ ó } 1/2\ N_{2\,(g)} + 3\ H_2O \qquad (11)$$

As previously stated, denitrification is usually catalyzed by heterotrophic bacteria that derive their energy requirements from the oxidation of organic material. For example, the oxidation half-reaction of carbohydrate is:

$$CH_2O + H_2O \text{ ó } CO_{2\,(g)} + 4\ H^+ + 4e^- \qquad (12)$$

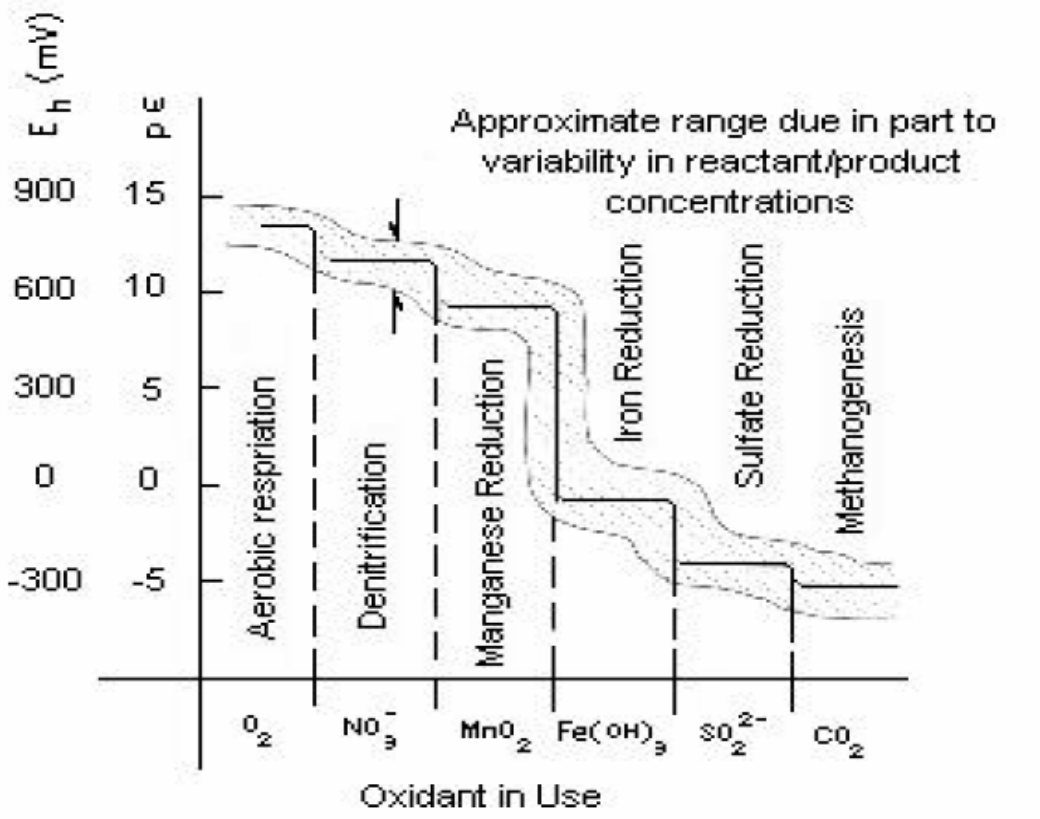


Figure2. 4 The ecological redox sequence showing that denitrification preferentially occurs when E_h < 750 mV or pe <12 (from Hemond and Fechner-Levy 2000).

Combining equations (11) and (12) for heterotrophic denitrification yields:

$$5CH_2O + 4NO_3^- + 4H^+ \rightarrow 2\,N_{2\,(g)} + 5\,CO_{2\,(g)} + 7H_2O \qquad (13)$$

Since many bacteria are only capable of performing one of the steps in the complete nitrate reduction to nitrogen gas, the denitrifying microorganisms must be considered as a group of complimentary microorganisms able to carry out the conversion of nitrate to gaseous nitrogen in its entirety.

2.9.2 Factors controlling microbial denitrification

There are a number of factors that influence microbial denitrification. These will be discussed below.

2.9.2.1 Oxygen

Oxygen, which competes with nitrate as an electron acceptor in the energy metabolism of cells, is important in microbial denitrification. The gradual depletion of oxygen or provision of semi-anaerobic conditions favors denitrification. It is generally accepted that an anaerobic environment is required for microbial denitrification to take place.

The magnitude of oxygen inhibition and the response of denitrification rate to oxygen concentration is illustrated by two sets of data in Figure 2.5. The pattern in each data set is similar even though the data from two very different experimental systems: a soil core and a wheat-*Azospirillum* rhizosphere association grown in soft agar. Note the dramatic drop in denitrification rate with a slight increase in oxygen concentration.

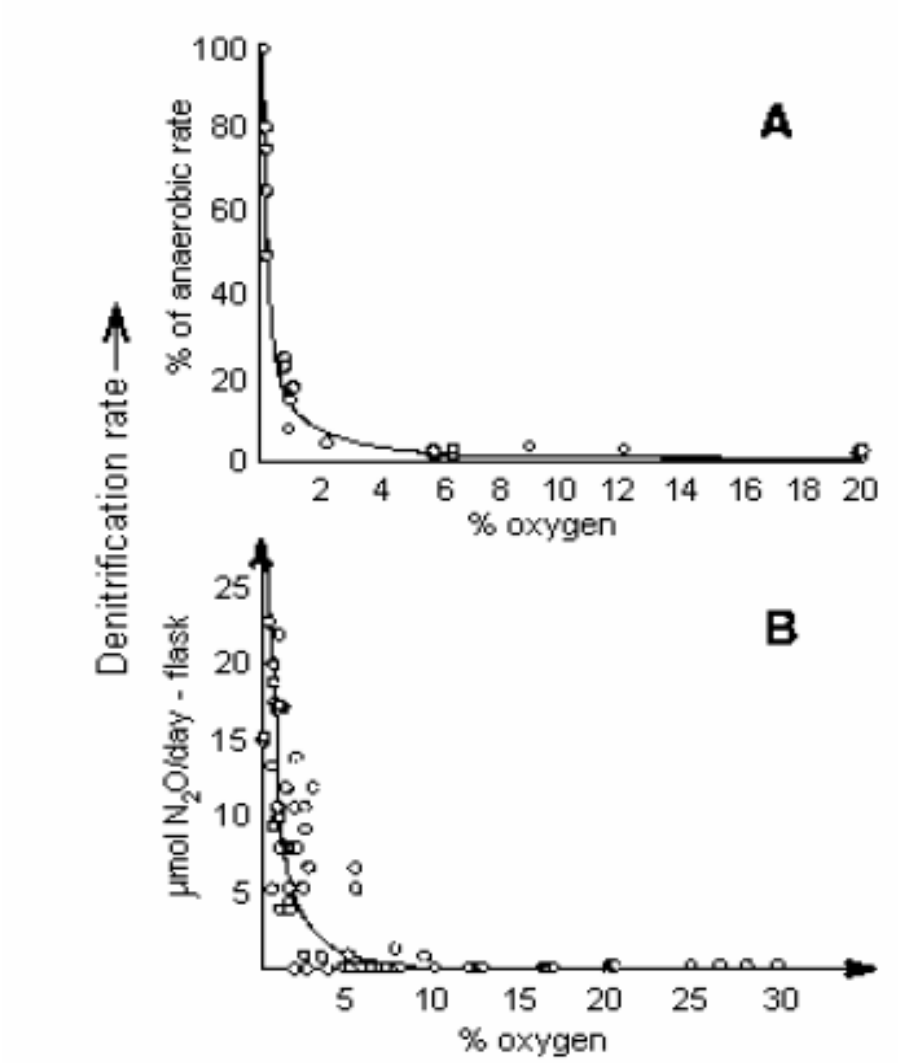


Figure2. 5 Effect of O2 concentration on denitrification in (A) a soil core and (B) a wheat -*Azospirillum* rhizosphere association (from Tiedje 1988).

From Figure 2.5 and other studies on oxygen concentration effects on denitrification

(Skerman and MacRae 1957; Knowles 1982), it can be concluded that the active denitrifying microorganisms of groundwater and soils environments have very low thresholds for oxygen. However, Knowles (1982) explains that in soils there are frequently inter-aggregate air-filled pores surrounding intra-aggregate water filled pores, which become anaerobic permitting denitrification to occur.

Thus, the microenvironment inhabited by denitrifying microorganisms may be anaerobic, while measurable oxygen concentrations in the subsurface environment around these sites reveal oxygen concentrations greater than would normally be expected to support microbial denitrification.

2.9.2.2 Nitrate Concentrations

Broadbent and Clarke (1965) state that the rate of denitrification is independent of the concentration of nitrate, but at some concentration, diffusion or enzymatic affinity clearly will begin to affect the rate of reaction. The lack of correlation between the concentration of nitrate and the rate of denitrification in many studies

probably reflects the unrealistically high additions of nitrate that are commonly made in such studies (larger than100 mg/L nitrate-nitrogen). Starr and Parlange (1975) and Vanderborght and Billen (1975) found that denitrification kinetics appear to be first-order when concentrations of 40 mg/L nitrogen or greater were employed.

These differences come about because of the difficulty in predicting the kinetic parameters for denitrification. Bowman and Focht (1974) recognized that the kinetics of denitrification must reflect both carbon and nitrate availability. For example, multiple-Monod kinetics is appropriate for biodegradation reaction processes that involve several solutes (Kinzelbach et al..1991). The kinetic equation for denitrification has the following form:

$$r = \mu_{\max}^{denit} X \left[\frac{k_b}{k_b + X}\right]\left[\frac{k_{O_2}}{k_{O_2} + O_2}\right]\left[\frac{CH_2O}{K_{CH_2O} + CH_2O}\right]\left[\frac{NO_3}{K_{NO_3} + NO_3}\right]$$

where r is the substrate utilization rate by denitrification (mg/L/day); $\mu^{denit}_{\max}$ is the maximum substrate utilization rate for denitrification whereby nitrate-nitrogen is 24 reduced to nitrogen gas (1/day); K_{CH2O} and K_{NO3} are the half-saturation constants for CH_2O and NO_3 (mg/L); X is the heterotrophic biomass concentration (mg/L) and k_b and k_{O2} are the heterotrophic biomass and oxygen inhibition constants (mg/L).

Accurately predicting and measuring all these parameters and then assessing goodness to fit the proposed model proves very difficult in a system as complex as the subsurface. Thus, there is no universal agreement on whether nitrate concentration affects the rate of denitrification.

2.9.2.3 pH

Denitrification is related to pH, with an optimum in the range 6.0-8.0, which is similar to that for heterotrophic organisms generally (Firestone 1982). There is general consensus that denitrification rates decrease when pH drops below 6.0 (Bremner and Shaw 1958; Broadbent and Clarke 1965), but several studies have reported that significant denitrification can occur in soils of pH less than 5.0 (Van Cleemput and Patrick 1974; Gilliam and Gambrell 1978). It is not known whether the limited ability of denitrifiers to function in acid soils results from a direct effect of soil solution pH or from pH-induced deficiencies or toxicities.

2.9.2.4 Temperature

Temperature is also a significant controlling factor on denitrification. At low temperatures, denitrification decreases markedly but is measurable between 0 and 5ºC (NRC 1978). Maximum temperatures for denitrification seem to be about 75ºC (Bremer and Shaw 1958).

A synergistic effect of temperature and oxygen upon denitrification can be noted: at a high temperature, oxygen solubility is less, thus increasing the biological rate, and *vice versa*. Generally, a doubling of denitrification rate is possible with every 10ºC increase in temperature (Gauntlett and Craft 1979).

While the optimum temperature range for denitrification depends on the denitrifying species, it is generally accepted that denitrification occurs at

significant rates in the temperature range 15 to 60°C (Nommik 1956; Goering and Dugdale 1966; Konishi 1969).

2.9.2.5 Nutrients

The availability of nutrients is an important requirement in sustaining biological cell growth. According to Champ et al.(1979), the nutrients necessary for biosynthesis consist of those elements required in large amounts (C, H, O, N, P and S), the various minerals required in minor amounts (K, Na, Mg, Ca and Fe), and trace amounts of certain metals (Mn, Zn, Cu, Co and Mo). Most groundwater contains adequate concentrations of the necessary minerals and trace metals to support biosynthesis (Champ et al., 1979).

2.9.2.6 Carbon

Organic carbon availability is one of the most important factors that affect denitrifying activity in soil. The organic carbon acts as both a source of cellular material for biological respiration and an electron donor for dissimilatory nitrate reduction. The presence of ample carbon substrate can cause rapid oxygen consumption in soil microenvironments, depleting oxygen concentrations, thus indirectly enhancing the potential for denitrification. Once anaerobic microsites have formed, carbon is also required in anaerobic respiration by denitrifiers.

Organic carbon required for denitrification is found naturally in soils. Plant tissues, manure and soil organic matters are all sources of carbon for denitrifiers. The response of denitrifiers to these sources is complex, mainly owing to the fact that decomposition products and populations of microorganisms involved are not well understood (Beauchamp et al., 1989). The kind of decomposition products and their rate of production will vary depending on the oxygen status of the soil.

Many authors have attributed significant quantities of unrecovered nitrogen in manured soils to denitrification losses (Olsen et al., 1970; Guenzi et al., 1978) since the application of manure increases the soluble carbon content of the soil. However, it has also been suggested that since manure contains mostly the undigested remains of animal feed and a high microbial population. It would not be expected that compounds which have resisted decomposition, in the animal gut, be readily available carbon sources (Beauchamp et al., 1989). The change in microbial environment after manure application to the soil may, however, change the population dynamics, killing off some microorganisms and thus making microbial carbon available to denitrifying bacteria. This supports observations of enhanced denitrification under manure application.

While denitrification is enhanced by carbon found in the natural environment, it can also be increased with external additions of carbon. These can be natural carbon sources supplying quantities of carbon greater than those found naturally, for instance, straw, mulch, sawdust and woodchips, or forms that are not usually found in the natural environment, like methanol, ethanol and acetate. There are two different approaches to enhancing denitrification by adding external sources of carbon. The first is the formation of an *in situ* reactive zone. This is where carbon is actively added to the groundwater system, usually in a liquid form, like ethanol or molasses. The second involves passive bioremediation where carbon is added to the system in solid form, usually constructed as a wall of material so that contaminated groundwater can flow under natural gradients through the carbon source.

2.9.3 Heterotrophic biological denitrification

Heterotrophic biological denitrification is a well-established process in the realm of wastewater treatment. However, this process has not been used on a full-scale basis in the field of water treatment in the U.S., but there are several full-scale plants being operated in Europe (Dahab and Woodbury, 1998; Gayle, et al., 1989). The primary reason behind the slow transfer of technology from the wastewater treatment to potable water treatment is the obvious concern over potential contamination of the treated water by bacteria and residual organics from the bio-denitrification process. This is a legitimate concern that must be kept in mind when designing such treatment processes for water treatment.

Numerous studies (Dahab and Woodbury, 1998 and Dahab and Kalagiri, 1996) reported on the potential of using biological denitrification for nitrate reduction in groundwater supplies in laboratory-scale experiments. The results indicated that fixed-film denitrification can be expected to reduce the nitrate concentration in the influent water supply from as high as 100 mg/L (as N) to levels within the 1.0 mg/L (as N) range. These removals translate into an efficiency of nearly 100 percent, which is generally not matched by other processes available for nitrate reduction. However, some residual soluble as well as insoluble organic matter should be expected in the denitrified water supply. Further treatment can reduce these solids to levels sufficient to meet prevailing drinking water standards.

In heterotrophic biological denitrification, facultative microorganisms are contacted with the water supply containing nitrates and an added carbon source in an anoxic (oxygen-free) environment. Under these conditions, the bacteria utilize nitrates as a terminal electron acceptor in lieu of molecular oxygen. In the process, nitrates are reduced to nitrogen gas, which is harmless and can be directly discharged to the atmosphere. The extraneous carbon source is necessary since it supplies the energy required by the microorganisms for respiration and synthesis while serving as an electron donor. Most denitrification studies have used methanol (CH_3OH) as the carbon source. If a simple carbon source is chosen such as ethanol or acetic acid, then the biomass produced during the process should be correspondingly low; a useful characteristic in that the overall excess biomass production is minimized.

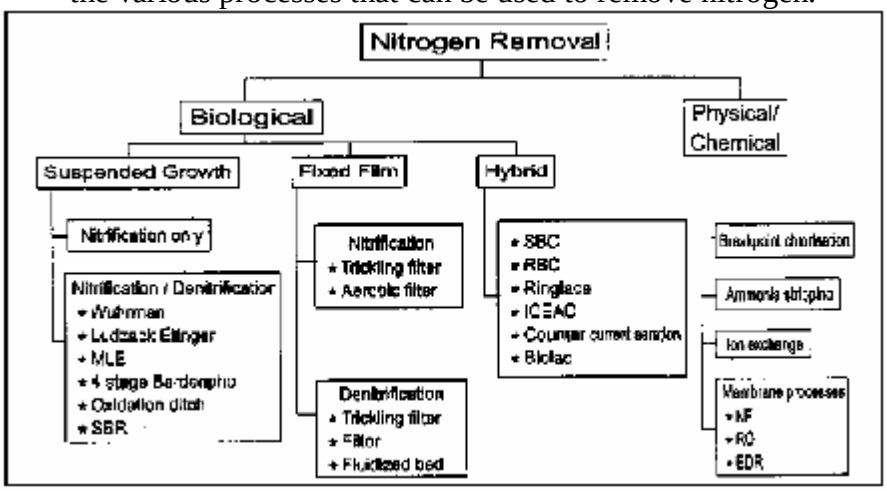
Since heterotrophic denitrifying bacteria require an organic carbon source for their respiration and growth, a wide variety of organic compounds have been used. These organics include methanol, ethanol, acetic acid, glucose, and other more complex organics. While the types of organic compounds may affect the biomass yield, the choice is generally based on economic comparison. The availability of ethyl alcohol from agricultural sources could make this carbon source a strong candidate for denitrification systems. It should be noted that methanol toxicity is such that it is not recommended as electron donor and carbon source for drinking water denitrification.

Another important factor in heterotrophic biological denitrification is the presence of dissolved oxygen in the waters and its inhibiting effects. To effect denitrification, the oxygen concentration must be reduced to a level low enough to avoid inhibition or repression of nitrate reductase. Unless dissolved oxygen is removed by chemical addition, the amount of electron donor (organic carbon) added must be equal to that needed to remove the oxygen as well as the nitrate.

Biological denitrification can be carried out in suspended or attached growth systems. In suspended growth systems, the bacterial culture is "suspended" within the contents of the reactor vessel by constant mixing or agitation. In these systems, sedimentation is required to settle out the bacterial biomass so it can be returned to the reactor vessel, or otherwise removed by wasting. Such systems are common in wastewater treatment applications. The principal advantages of suspended growth systems include the ability of constant return to biomass into the system and small tankage requirements. However, suspended growth systems are subject to damage or washout by hydraulic transients and influent shock loads. They are generally not suited for handling periods of extended shutdown.

In fixed-film (also known as biofilm) systems, the bacterial biomass is physically attached to a solid matrix, which serves to support the bacterial mass by providing surface area on which the bacteria can grow in a film-like layer. Attached growth systems can be of the static media type or the expanded-bed (i.e. fluidized) type. In static media systems, the solid matrix typically is made up of synthetic modules that are stacked in some fashion (or simply dumped, depending on their size and configuration) in the reactor vessel. These media can have high porosity, light weight (when synthetic materials are used) and high specific surface area (i.e. surface area per unit volume of medium). Static media attached growth systems are operated in either down flow or up flow regimes although up flow systems are more common due to the reduced chance of plugging associated with their operation and the fact that the bacterial biomass is constantly submerged.

Fluidized-bed systems are operated in an up flow manner so that the bacterial growth matrix bed is expanded hydraulically as the water is pumped from the bottom to the top of the reactor. In expanded-bed systems, the support media are generally of the granular type (both natural and synthetic) to facilitate expansion of the bed. As the bed is expanded the entire surface of the granular material is made available for bacterial support. Because of this fact, expanded-bed systems have been reported to be loaded at rates exceeding static-bed systems. However, the additional costs associated with pumping to maintain bed expansion or fluidization must also be considered during design evaluation. With no known exceptions, all full-scale biological denitrification systems designed for potable water treatment have been of the static-bed fixed film type. Figure2.6 summarizes the various processes that can be used to remove nitrogen.



(Source: WEF 1998.)

Figure2. 6 Processes used nitrogen removal

Chapter 3

Methodology

3.1 Experimental description

The objective of this study is to present experimental data on the biological removal of NO_3–N through different filter media at various filtration rates under laboratory conditions.

A lab-scale packed bed reactor was used in this study as shown in Figure 3.1. The lab-scale plant consisted of two cylindrical PVC-U columns filed with 80 cm height of three different media in each stage with up-flow operating system.

In the first stage, sand was used with diameter = 1.0 mm as media. Ethanol was used as source of carbon with ratio 2:1 (ethanol wt./nitrate nitrogen wt.) after the flow reaches a steady-state flow. The flow rate was varied through the operation.

Second and third stage was the same as the first stage but the media was granite gravel with diameter =2 to 9.5 mm, and quartz gravel pack with diameter =1.18 to 4.75mm respectively.

Flow rate, nitrate load and pH the main factors were observed during operation to illustrate the relationship between them and the removal efficiency.

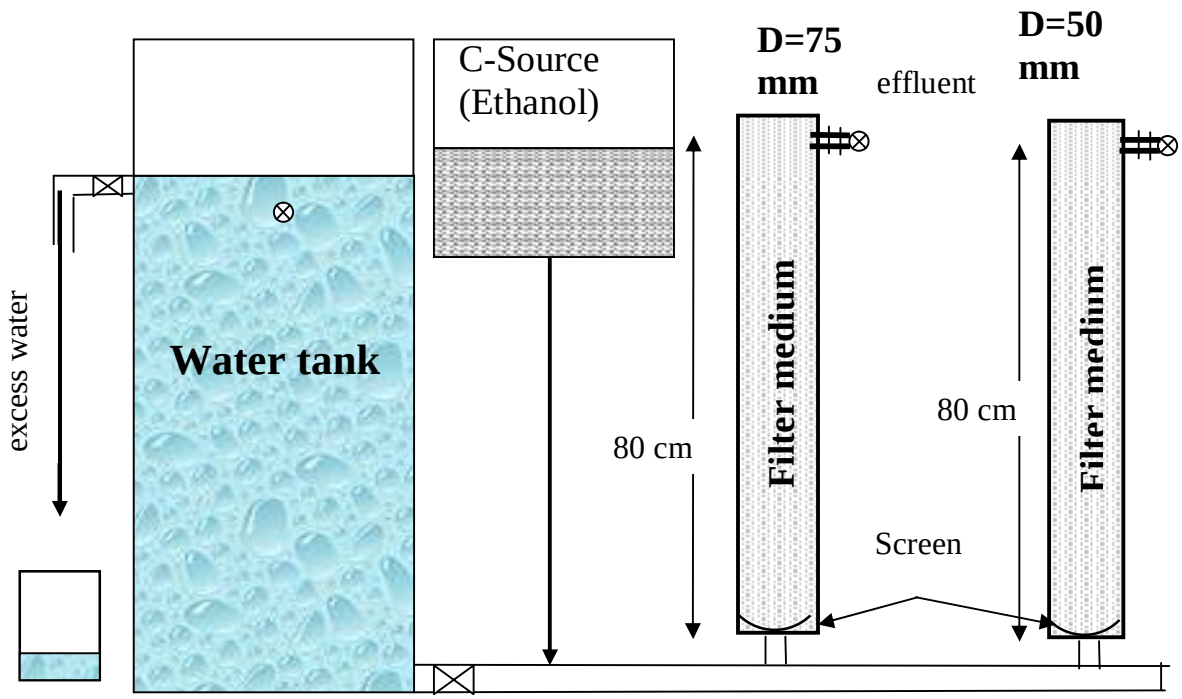


Figure3. 1 Schematic diagram of the system

3.2 Materials

Filter material with different grain size used in the testing program such as sand, gravel pack and gravel. All filter materials mainly consist of silica.

Several tests have been conducted on these filter materials such as sieve analysis; constant head test and dry density.

3.2.1 Sieve analysis

Filter material was sieved through a stainless steel sieves with the mesh size shown in table 3.1.

Table 3. 1 Sieves size were used

Sieve Size	Sieve Size	Sieve No.
mm	in /1000	
16	629.92	
9.5	374.02	3/8"
4.75	187.01	4
2	78.74	10
1.18	46.46	16
0.6	23.62	30
0.3	11.81	50
0.15	5.91	100
0.075	2.95	200

Grain size determination was conducted in according to a standard sieving procedure.

3.2.2 Permeability test

A permeability test was conducted in filter material to measure the coefficient of permeability, it was determined with the help of Darcy's law.

A typical arrangement of a constant head permeability test is shown in Figure 3.2. In this type of laboratory setup, water supply at inlet is adjusted in such a way that the difference in head between inlet and outlet remains constant during the period of test. After a constant rate of flow is established, water is collected in a graduated flask for known duration.

Calculations: The coefficient of permeability is given by:

$$K = QL/Aht \qquad (16)$$

Where

K = coefficient of permeability, cm/sec.

Q = volume of water collected cc.

L = length of specimen, cm

h = pressure head, cm of water

A = area of specimen (cross section in cm^2)

t = duration of collection water

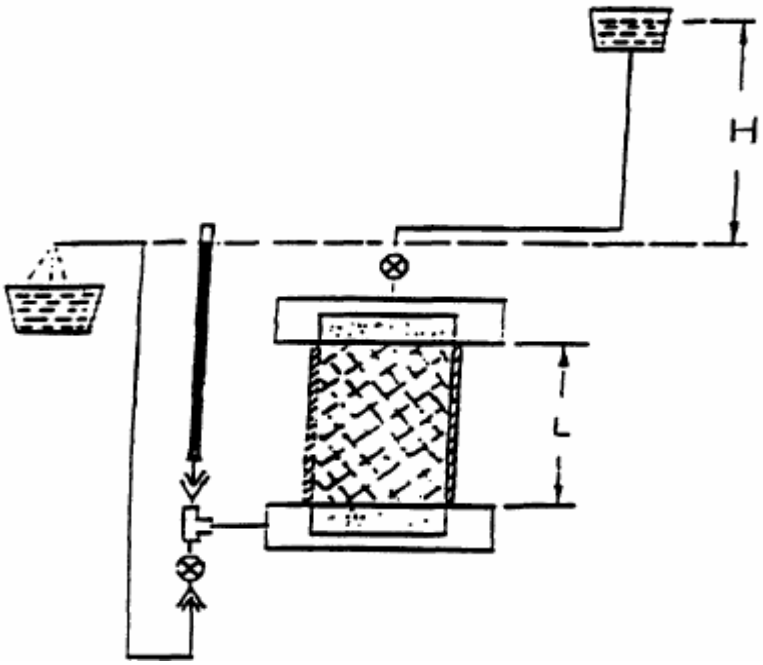


Figure3. 2 Constant head permeability test

3.3 Experimental apparatus

The experimental apparatus was composed of:

- Water tank (500 liter).
- Small tank 20 liter contained the source of carbon.
- Two reactors with the same height 95cm and different diameters (75mm and 50mm). It was filled with 80cm of three different medium, fine sand, gravel and gravel pack with grain size of 0.15 to 0.6mm, 1.18to 9.5mm, and 1.18 to 4.75 respectively.
- Valves for each reactor and for the inlet of water, controlled to the flow rate.
- Manometers before the inlet of reactors to measure head loss.

The heterotrophic denitrification reactor was made of PVC-U pipe. A schematic diagram of the system shown in Figure 3.1

The system was designed to work under different head of water in up flow mode.

3.4 Chemical Use

Successful biological denitrification requires the use of chemicals that can facilitate the vitality of the biological culture that removes nitrate from water as well as re-

condition the treated water quality to meet prevailing drinking water quality. Typical chemical additives are listed in Table 3.2 below.

Table 3. 2 Typical Chemical Additives Required for Biological Denitrification.

Use/Addition	Purpose	Chemical
Pre-denitrification	Organic Carbon Source	Organic Carbon sources, Ethanol (CH_3CH_2OH) C/N : 2/1
Pre-denitrification	increase concentration of NO_3	Potassium nitrate KNO_3

3.5 Water sampling and analysis

Every 24 h, water samples (200 ml) were collected from the inlet and the outlet of the column. Nitrate, pH, and temperature were routinely monitored in all samples.

3.5.1 Measurement of NO_3 as N by spectrophotometer Instrumentation

The concentration of nitrate was determined by Hach DR4000U UV/Visible Spectrophotometers. The instrument was turned on and warmed up for 20 min before starting any sample measurement. The cuvette was cleaned every time before the use by rubbing the inner wall with a detergent-saturated cotton-tipped stick.

Nitrate Determination Method

The method was a modified type of the "Ultraviolet Spectrophotometric Screening Method" [Arnold, et al., 1992]. In this method, wavelength settings were 275 nm, 220 nm (275 nm can be eliminated in the nitrate detection; however, it was useful to detect nitrite). According to this standard method, common interfering material, such as bacterial cells, resulted in predictable absorption or scattering at 275 nm and produced absorption at 218 nm two times as much as that at 275 nm.

NO_{2^-} had no absorption at 275 nm but equivalent absorption at 220 nm; dissolved NO_{3^-} had no absorption at 275 nm but significant absorbance at both 220 nm. Using these data the detection of NO_{3^-} and NO_{2^-} was carried out as follows:

(1) Deionized water produced a reproducible background absorbance at the wavelengths used. In the experiment, deionized water was used as blank; signals at those three fixed wavelengths were taken.

Table 3. 3 Blank (Deionized Water) Absorbance

A_{275}	Absorbance at 275 nm
A_{220}	Absorbance at 200 nm
$A1 = A_{220} - 2*A_{275}$	corrected absorption at 220 nm

(2) Standards consisting of deionized water, NO_{3^-} and NO_{2^-} (with concentrations simulating those of realistic samples) were measured at those three wavelengths, and the variables described in the above table were used to get the corrected nitrate absorbance.

(3) Samples consisting of deionized water, NO_3^- and/or NO_2^- were used as standards. The highlight was that after these calculation processes, NO_2^- signal was eliminated and only NO_3^- signal was left (part of NO_3^- signal was also subtracted).

Stock Solution for Nitrate Determination: Potassium nitrate was dried at 105 °C for 24h. A mass of 0.7218 g was dissolved in 100 mL sterilized deionized water and diluted to the 1000 mL scale in a volumetric flask to prepare stock solution I which contained 100 mg NO_3^-N /L.

Standard Solution Series: Nitrate-containing standards were prepared by the following:

0, 1.00, 2.00, 4.00, 6.00, 8.00, 10.00 mL of stock I were separately added to 100 mL volumetric flask and diluted to 100.0 mL. The resulting concentration range was: 0, 1, 2, 4, 6, 8 and 10 mg NO_3^-N/L.

The signals of those standard solution series, used to establish the linear calibration line.

Sample Preparation: Ten milliliter of water was taken out of the test tube after the bacterial growth in experimental and diluted to 50 mL with deionized water (5 dilution times). The diluted sample was filled in a cuvette to full volume. Absorption was measured separately and recorded at 275 nm, 220 nm.

3.5.2 Measurement of pH

pH is a logarithmic notation used to measure hydrogen activity (i.e., whether a solution is acid or basic).

$$pH = - \log [H^+]$$

As a simplification, it is assumed that pH is a function of the hydrogen ion concentration {[H+]} when in reality it is related to the hydrogen ion activity H^+. Since pure water is slightly ionized, it is expressed as an equilibrium equation termed the ion product constant of water. The concentration of these two ions is relatively small and is expressed as a simple logarithmic notation. pH is the negative log of the hydrogen ion (Bailar, 1978).

The pH was measured with HANNA H8314 membrane pH meter

Characteristics of influent water

Influent to biofilter was tap water with characteristics shown in table 3.4.

Table 3. 4 Characteristics of influent water

Nitrate concentration	Rang 80 to 120 mg NO_3/l
pH	7.5 - 8.5
Temperature	15– 22 C^o

3.6 Calculation

According to daily measurement some items were calculated:

The nitrate removal (mg/liter) =

nitrate concentration in influent (mg/liter) – nitrate concentration in effluent (mg/liter)

Flow rate velocity (m/day) is an average value of velocity which was measured during three hours three times at least

$$\text{Flow rate velocity} = \text{Flow rate} / \text{Area}$$

$$(\text{m/day}) = (\text{m}^3/\text{day}) / (\text{m}^2)$$

The surface loading rate was calculated by multiply the concentration of nitrate with the flow rate velocity.

$$\text{Surface loading rate} = \text{Nitrate concentration} * \text{Flow rate velocity}$$

$$(\text{g/m}^2.\text{day}) = (\text{g/m}^3 \text{ or mg/liter}) * (\text{m/day})$$

3.7 Start-Up And Initial Performance

After the apparatus had assembled, a permeability test had been done for every reactor to determine the coefficient of permeability K.

For the first stage the reactors filled with compacted sand, then system was operated for 10 days without any external addition to reach at steady state flow.

In the first 2 weeks, inlet nitrate concentration was constant and the hydraulic gradient was regulated to be form 0.6 to 0.85. During these 2 weeks the system was operated without inoculating the column as the control run. As expected, no denitrification occurred. On Day 10 (steady state flow), ethanol was added to the columns to encourage denitrification.

Ethanol added to system after it was diluted with the same water quality used as influent with continuous rate 8.6 l/day.

3.8 System Operation

For sand media, the system was filled with fine sand filter of an average diameter of 1mm. The sand was washed several times to remove impurities before packing the filter.

The microorganisms was lifted to grow naturally without any inoculation. The nitrate levels were measured in the inlet and the outlet of both the reactors. Furthermore, pH, and temperature were monitored in the inlet and outlet reactors.

Water flow rate velocities (V) (V=Q/A, where Q is the measured flow rate and A is the cross section of the column) were calculated in m day^{-1}.

After fourteen days of adding ethanol, effluent NO_3 concentration started to decrease in reactor with diameter =75 mm. After the 75 mm reactor was treated all influent nitrate, the denitrification process was started in the next reactor 50mm in diameter.

When the flow rate through the filter could not be maintained, the media was washed out through increasing the differences of the inlet and outlet height.

It took three days for the filter to function normally after the backwash. After that the filter was able to remove nitrate from influent with concentration of 80 to 160 mg NO_3/L.

For gravel media, the filter was filled with gravel ranging from 2 to 9.5 mm in diameter. The gravel was washed several times to remove impurities before packing the filter. Flow rate was measured daily in the second stage to determined the relation between the flow rate and the removal of nitrate. The way of input ethanol was also changed from dropped through the pipe to be added directly to the tank.

The filter was operated at filtration rate between 0.36-6.5 and 1-4 m/day for reactors with diameter 50mm and 75mm respectively.

The nitrogen loading rate was varied by changing the influent flow rate. The flow rate was adjusted by a valve at the influent.

When the flow rate velocity was less than 0.36 m/day the reactor was almost clogged and needed to be wash.

After washing out the filter was operated for at least 8 days until achieving higher than 90% NO_3 removal.

For gravel pack (quartz) media, the filter was filled with quartz ranging from 1.18 to 4.75 mm in diameter. The quartz was washed several times to remove impurities before packing the filter. Filter with diameter 75 mm was only used to avoid fluctuation of input and to be more controlled. Flow rate was measured daily in this stage also. The way of input ethanol was also the same as in the previous stage.

The filter was operated at filtration rate between 0.36-6 m/day for reactor.

The nitrogen loading rate was varied by changing the influent flow rate. The flow rate was adjusted by a valve at the influent.

After washing out the filter was maintained its efficiency higher than 90% NO_3 removal. But the flow rate was decreasing to cloggy rapidly.

The stainless steel mesh which was in the bottom of reactor was tore to prevent the collection of microorganisms on its surface which lead to stop the flow rate.

Chapter 4

Results and Discussion

In Gaza, the water crisis is a function of population growth, an agriculturally intensive economy, a fragile water ecosystem, and a highly inequitable distribution of resources

So the basic problem is the deterioration of Gaza Costal Aquifer.

This research aims to investigate the ability of biological denitrification process on the removal of nitrate from groundwater using reactors with three different media, and determined parameters which optimize the efficiency.

The investigation of feasibility and performance of denitrification process was done be using reactors made from PVC-U pipe filed with 80 cm height of three different media: quartz sand with diameter = 1.0 mm, granite gravel with diameter =2 to 9.5 mm, quartz gravel pack with diameter =1.18 to 4.75mm with up flow operating system. The system feed with ethanol as source of carbon with ratio 2:1 C/N.

Flow rate, nitrate load and pH the main factors were observed during operation to illustrate the relation between them and efficiency removal.

4.1 Material Used

Three different materials were selected to investigate the feasibility and performance of denitrification.

Sieve analysis for bio filter's media

4.2.1 Sand Filter

Local sand has been used. The sand used had small particle size. As illustrate in Figure 4.1 the average size of particle is in the range of 0.6 to 0.15 mm.

The permeability of the sand was $9*10^{-3}$ cm/sec measured using a constant head permeability test.

4.2.2 Granite Gravel Filter

The granite gravel used had large particle size comparing with sand. As illustrate in Figure 4.1 the average size of particle is ranged from 2 to 9.5 mm.

At a steady state, the permeability of the granite gravel in reactors was 0.1315, 0.1382 cm/sec. for reactor 50mm and 75 mm respectively.

4.2.3 Gravel pack Filter

The gravel pack used had uniform particle size with an average particle size is in the range of 1.18 to 4.75 mm.

The permeability of the gravel pack in reactor with diameter 75mm was 0.738 cm/sec.

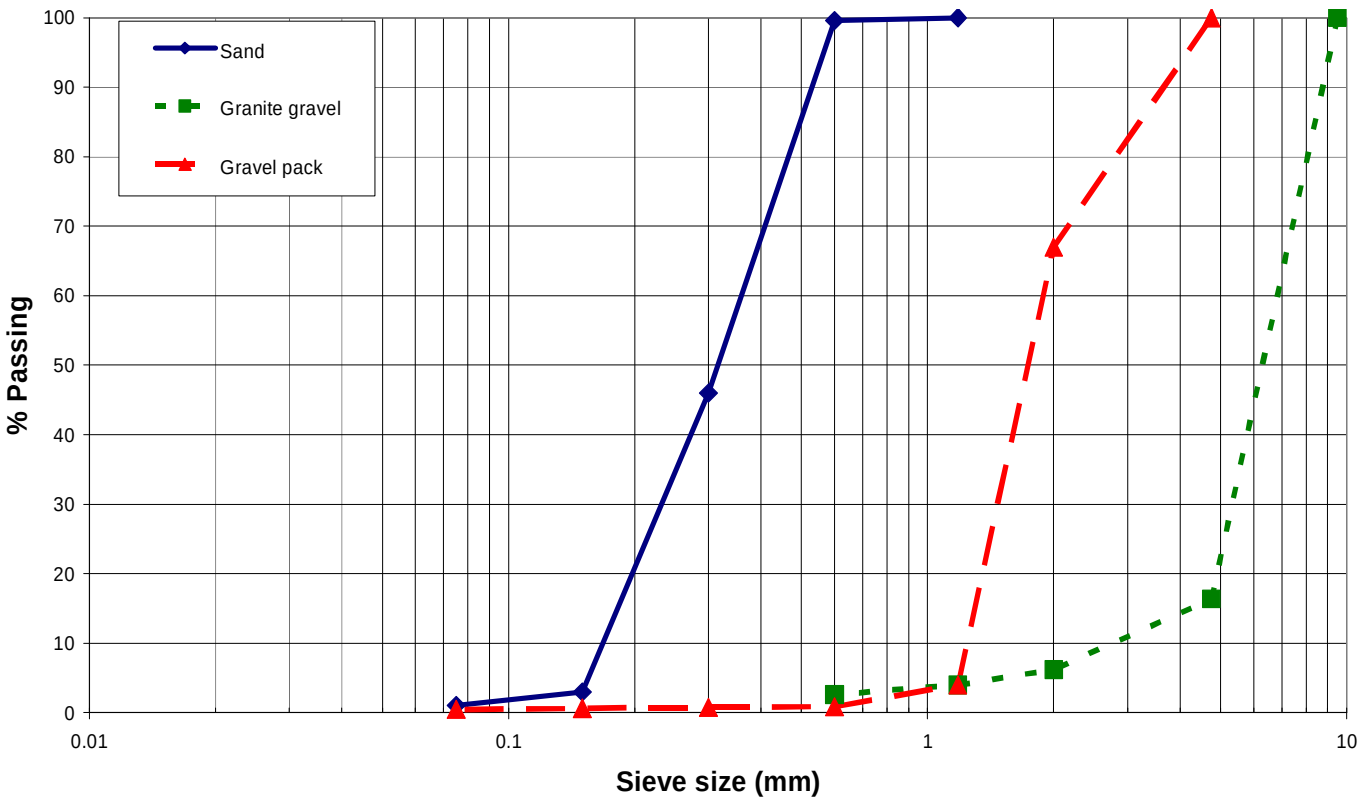


Figure4. 1 Grain size distribution of filter materials

4.2 Removal efficiency versus time

The sand filter was operated at low velocity at the beginning of experiments to promote microbial growth through the filter bed and NO_3 concentration, pH, and temperature in the effluent was measured during this stage.

As illustrate in Figure 4.2 with first two weeks reactor with diameter 50mm with ethanol addition show no removal of nitrate was detected. In the third week the rates of denitrification increased slowly. After about 18 days enough biomass was attached to degrade the Ethanol completely.

The nitrate removal increases rapidly from 9% in day 14 to 96% in day 17. After that the reactor was clogged at day 22. The reactor was wash out by pressurized water for 5 minute at day 23 which causes moving out of the sand particle out the reactor. After that reactor was able to recover its efficiency of removing nitrate during 2 days (100% removal of nitrate at flow rate 4.3 L per day)

On day 25 the system has been washed again to reach the flow rate of 14.73 L per day. In spite of the washing of reactor, it is still able to achieve 100% of removal rate.

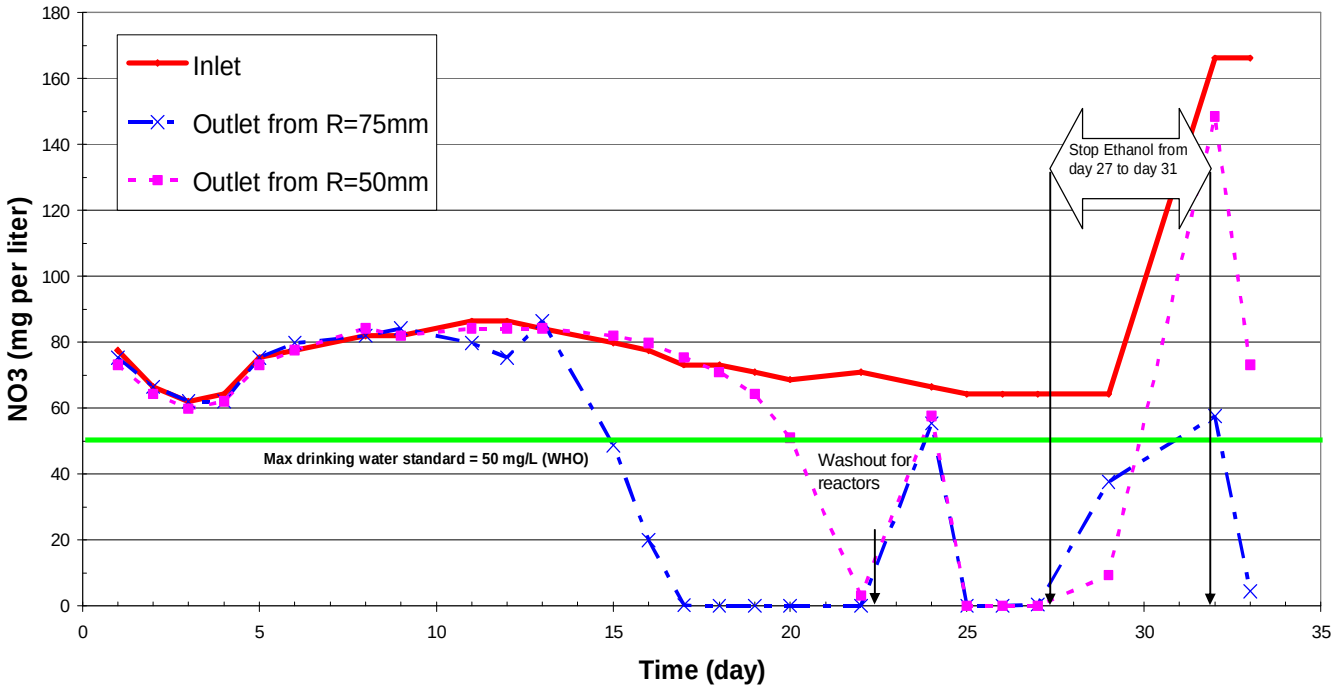


Figure4. 2 Time course of nitrate removal in sand column reactor d=50mm and d=75 mm during the operational period (33 d).

For the other reactor with 75 mm diameter denitrification process starts after two weeks (before the reactor 50mm). The reactor was able to reach 100 percent of efficiency for influent of 70 mg NO_3/liter. When the ethanol had been stopped the flow rate increased, nitrate removal efficiency decrease as a result of decreasing the source of carbon which means stop of growing of heterotrophic bacteria.

In spite of increase nitrate load in influent (166mg/liter), the reactor can remove the nitrate with efficiency 97%.

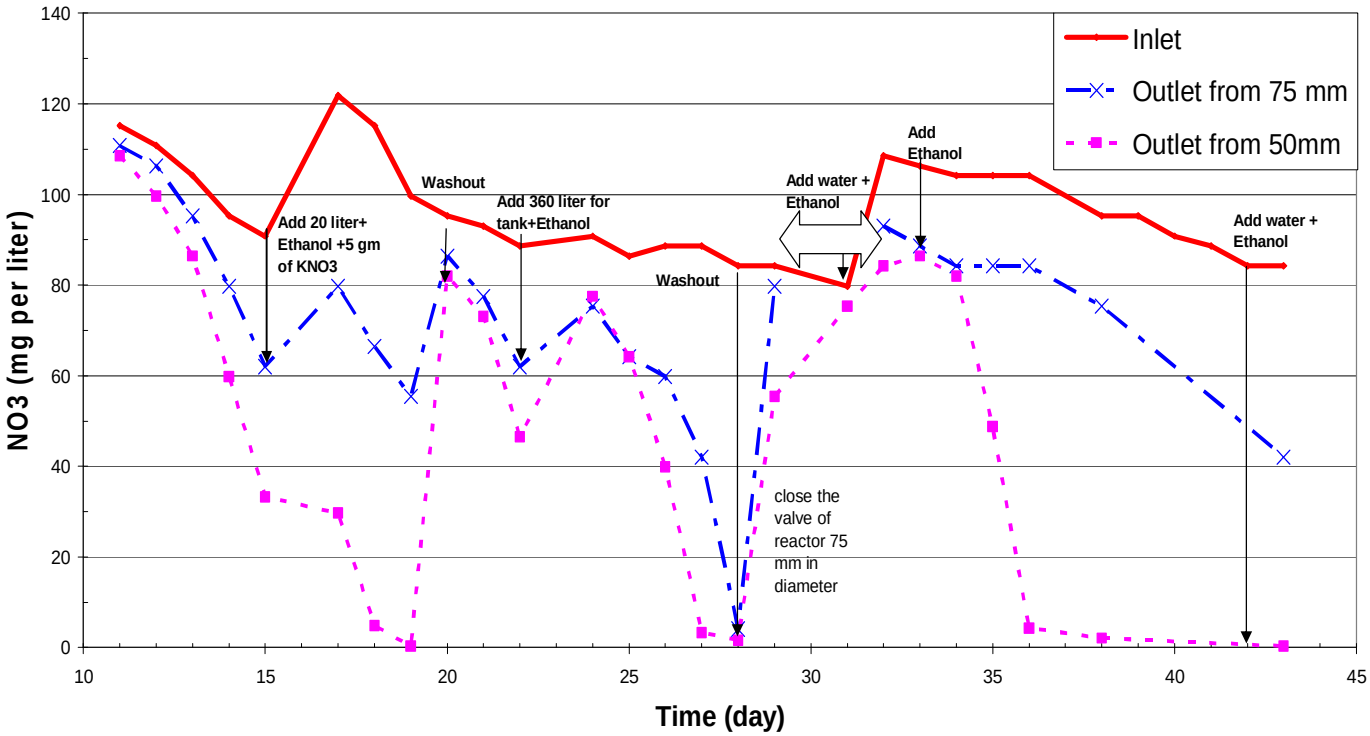


Figure4. 3 Time course of nitrate removal in Gravel column reactor d=50mm and d=75 mm during the operational period (43 d).

The gravel media filter was operated at higher velocity than sand filter. The denitrification process increases rapidly after 10days to reach of 100% efficiency at 100mg NO_3/liter at 19 day. From period (10-24 day) the denitrification process fluctuate because of adding water and washout more than once in this period.

The increasing of nitrate removal has a linear function as it clear in Figure 4.3 in periods from 11 to 18, 24 to 27, and 34 to 43.

The reactor with 75mm diameter was more sensitive to changing in any parameter because it is closer to the source of water. Its position allow to take most of source of carbon under laminar flow.

There is no need for high pressure to wash out the reactors but the efficiency of removing nitrate would highly affected by the washing.

Because stainless steel wire mesh was used (50mesh /inch, wire dia= 0.22 mm) after valve and before media the flow during reactor was slow. And because of growing of bacteria on the mesh the flow stopped in the reactor for some times.

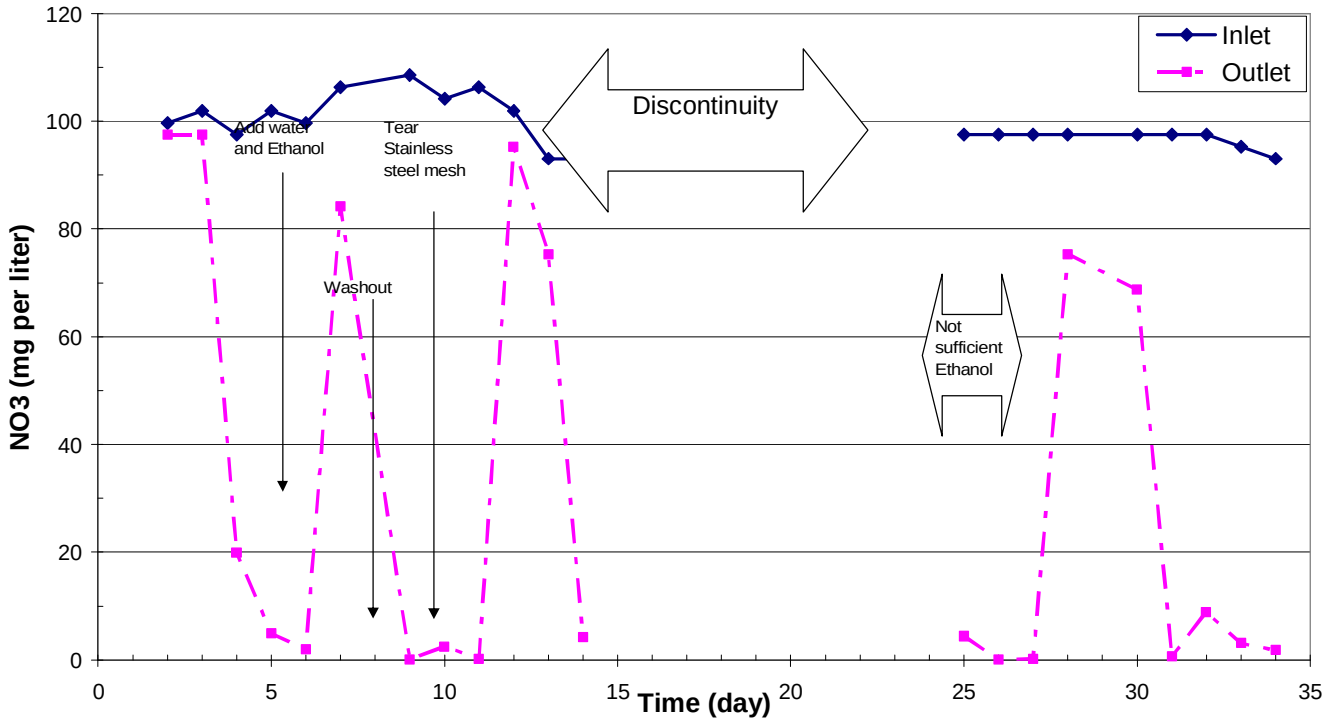


Figure4. 4 Time course of nitrate removal in gravel pack column reactor d=75 mm during the operational period (35 d).

Comparing with previous media (sand and gravel), the denitrification process in gravel pack was started rapidly reaching to efficiency of 100% during one week.

Because of stainless steel wire mesh were used (50mesh /inch, wire dia= 0.22 mm) after valve and before media the flow during reactor was slow. And because of growing of bacteria on the mesh the flow stopped in the reactor some times so the mesh has tear at 11 day. As shown in Figure 4.4 the filter can recover its efficiency in shorter time.

From above the denitrification process need a start-up period to allow the bacteria to attach to the support particles before it can be able to start removing nitrate. The sand filter system was capable of achieving good nitrate removal larger than 90% in drinking water.

The system recovered quickly from the upsets regained typical nitrogen removal.

As the nitrate concentration increased, more time was needed to achieve a high percentage of removal on the other hand total suspended solids increased with time.

The denitrification performance of the system is affected by quantitative changes of the carbon source.

4.3 Removal efficiency versus flow rate velocity

As illustrate in Figure.4.5 there are a relationship between the flow rate velocity and nitrate removal rate:-

$R^2 = 0.7737$ for reactor d= 50mm with media granite gravel

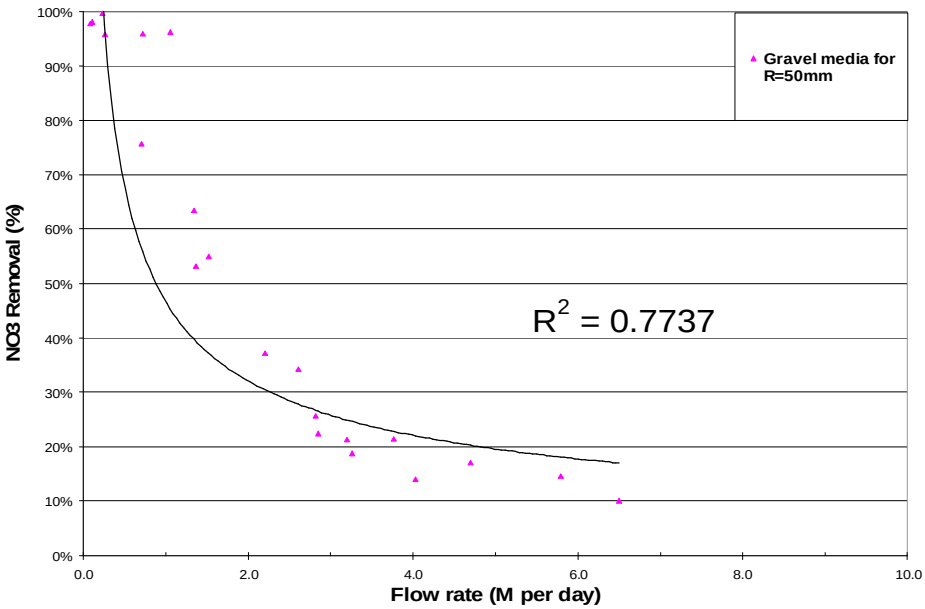
$R^2 = 0.9237$ for reactor d= 75mm with media granite gravel

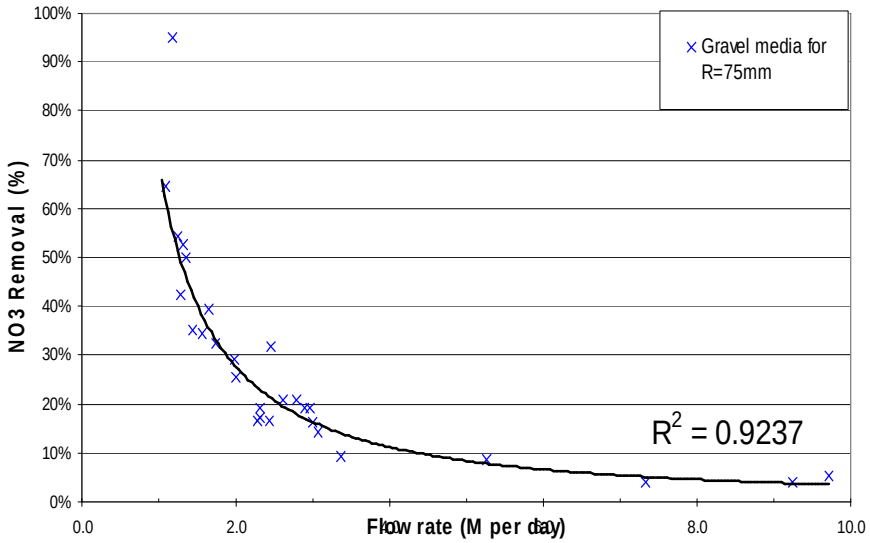
$R^2 = 0.8143$ for reactor d= 75mm with media gravel pack.

The water velocity was gradually lowered leading to almost complete clogging according to continuous denitrification process.

For gravel media the reactor was able to remove 50 mg NO_3/liter when it had flow rate velocity 1.3 m/day. The lower NO_3 concentration was observed for the filtration rates before clogging 0.72 m/day. Increasing filtration rates, causes decreased the NO_3 removal. The difference between reactors with diameter 75mm, and 50mm for the same media can be explained by the way of assemble the system which causes inequitable distribution for ethanol per reactors and different pressure per reactors.

For gravel pack media which has more surface area than natural gravel and operated with one reactor with diameter 75mm to avoid any misunderstanding according to the way of flow the filter able to remove up of 90 mg NO_3/liter when it had flow rate velocity 2 m/day.





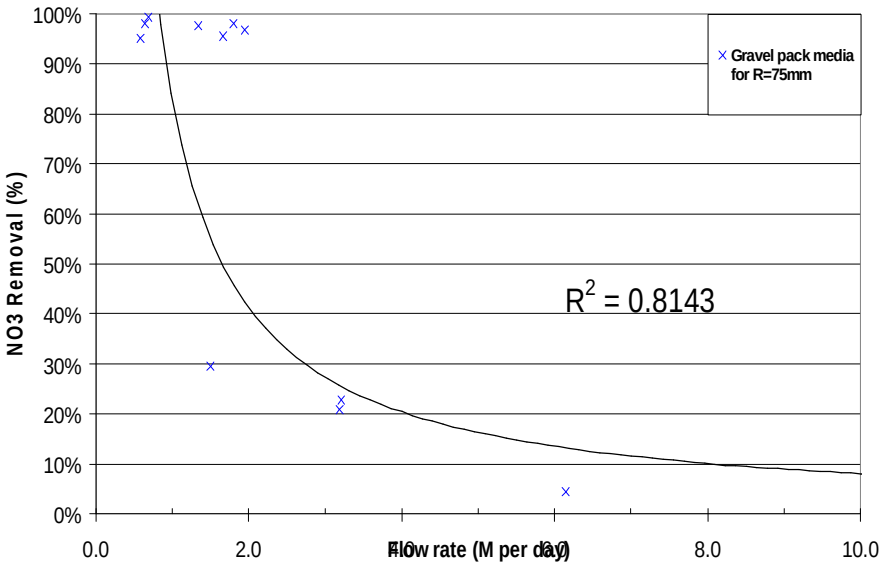


Figure4. 5 Relationship between nitrate removal and flow rate velocity

Figure 4.6 show that the reactor filled with gravel decrease the removal rate of nitrate g/m^2.day when the velocity below 1 m/day.

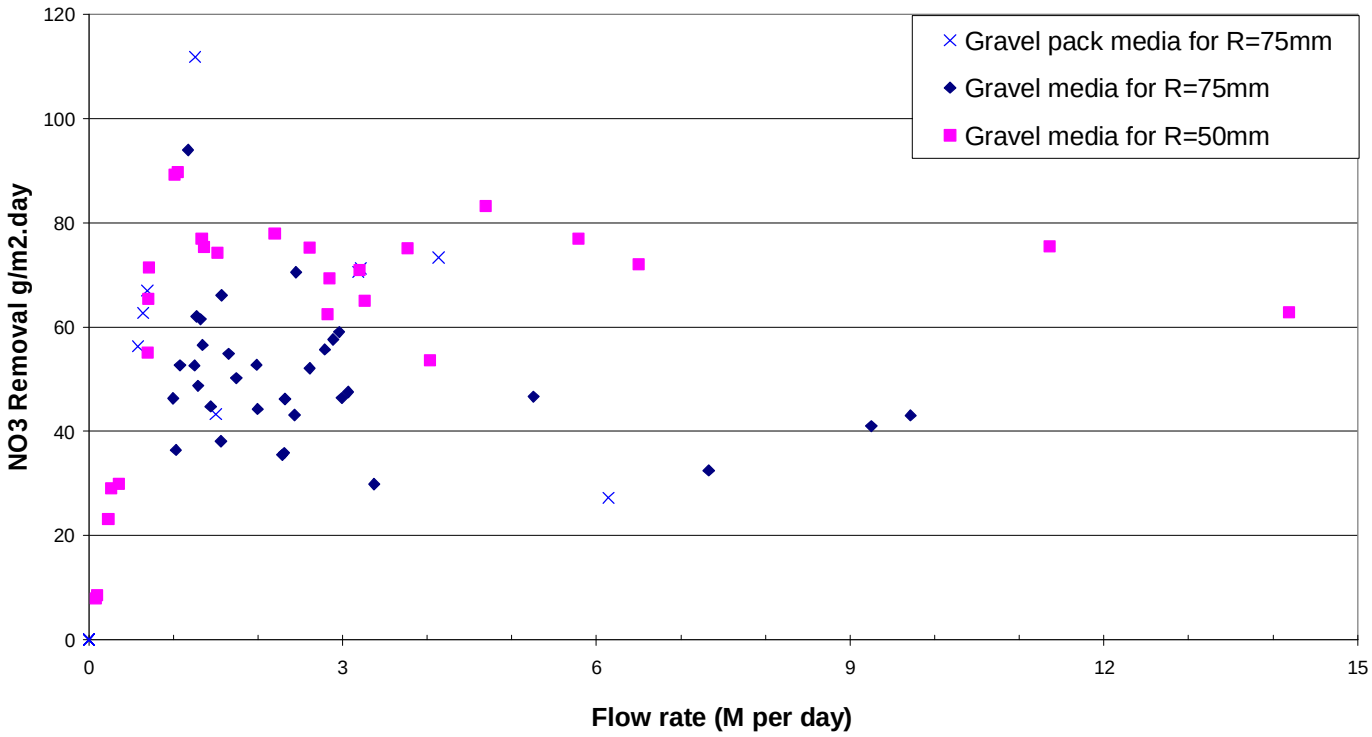


Figure4. 6 Relationship between surface load removal mg.m-2.day-1 and flow rate velocity

Aslan and cakici, 2007 studied the biological removal of nitrate in slow sand filter in rang from 0.36 to 1.44 m/day. They found NO_3 removal efficiency was 100%, 99%, and 94% -inlet NO_3 was 100mg/liter- at the filtration rate of 1.44, 1.2, 0.96 m/day at 80 cm filter depth, respectively.

Nakhla and Farooq, 2005 studied the impact of filtration rates in the range of 0.15–0.38 m/hour, on nitrogen elimination in slow sand filter. Nakhla and Farooq achieved about 80% denitrification efficiency in raw wastewater including average 3.2 mg TKN/l at the same depth of 80 cm.

Soares and abeliovich, 1997 studied in up flow laboratory reactors using wheat straw as source of carbon. The highest rates of denitrification (235 g NO_3 removed /m^3.day) were observed in fresh reactors during their first week of operation and the efficiency of the process declined thereafter. The lowest rates of denitrification (approximately 140 g NO_3 removed /m3.day at 2.208 m /day. The rate of denitrification was affected by the water velocity and decreased at velocities above 0.054 m /day.

Table 4.1 summarized the relationship between removing efficiency and flow rate found in this study.

Table 4. 1 Relationship between removing efficiency and flow rate

Velocity flow rate (m/day)	% Nitrate removal	Nitrate removal (mg/liter)	Surface loading rate removal ($g/m^2.day$)
0.3 to 1	93 to 100	80 to 110	0 to 90
1 to 1.5	65 to 35	25 to 60	35 to 95
1.5 to 3	40 to 15	15 to 35	35 to 80
3 to 4	25 to 15	10 to 25	30 to 80
> 4	< 20	<15	30 to 80

Thus, the water velocity plays an important role in the denitrification performance of the system and the reasons for the sharp decrease in efficiency at the higher velocities may include wash-out of bacteria, wash-out of extracellular enzymes and wash-out of solubilized substrate.

So water velocity has a marked effect on the denitrification performance of the system. The optimum flow rate for each media depends on its surface area. The media which has more surface area can be able to remove nitrate more than others at the same flow rate. The system was unable to provide nitrate removal rate of more than specific value for each media per square meter per day.

4.4 Removing efficiency and Nitrate load

It was clear that the process was able to provide NO_3 removal up to 95 % (110 mg NO_3/liter) as shown in Figure 4.5.

The range of removing nitrate is more than 90% (60 to 80 g/ m^2.day), 55% (40 to 60 g/ m^2.day), when flow rate velocity 1.2 m/day for reactor with diameters = 50mm and 75mm with gravel media, while 95% (140 to 200g/ m^2.day) for diameter = 75mm for gravel pack media at the same velocity.

The NO_3 removal efficiency dropped when the surface loading rate increased as shown in Figure 4.7.

Table 4.2 summarized the relationship between surface loading rate and removing efficiency.

Table 4. 2 Relationship between removing efficiency and surface loading rate

Surface loading rate ($g/m^2.day$)	% Nitrate removal
0 to 50	95 to 100
50 to 100	55 to 100
100 to 200	20 to 65
200 to 250	15 to 40
> 250	< 20

Aslan and cakici, 2007 evident that the process was unable to provide NO_3 removal rate of more than 120 g/m^2.day (1.2 m/day flow rate velocity). They showed NO_3 removal performances with daily removal being between 36 and 130 gNO_3/m^3 at filtration rates between 0.36 and 1.44 m/day (nitrogen loadings were 36 and 144 g/m^2 day), respectively.

Rocca, Belgiorno,and Meriç, 2005 reported Nitrate removal efficiency of the Heterotrophic denitrification reactor was over 90% for 85 mg/ℓ of inlet nitrate concentration. The process maintained its high performance up to 358 mg of daily nitrate inlet with a maximum specific volumetric ratio of 108 gNO_3/m^3·day.

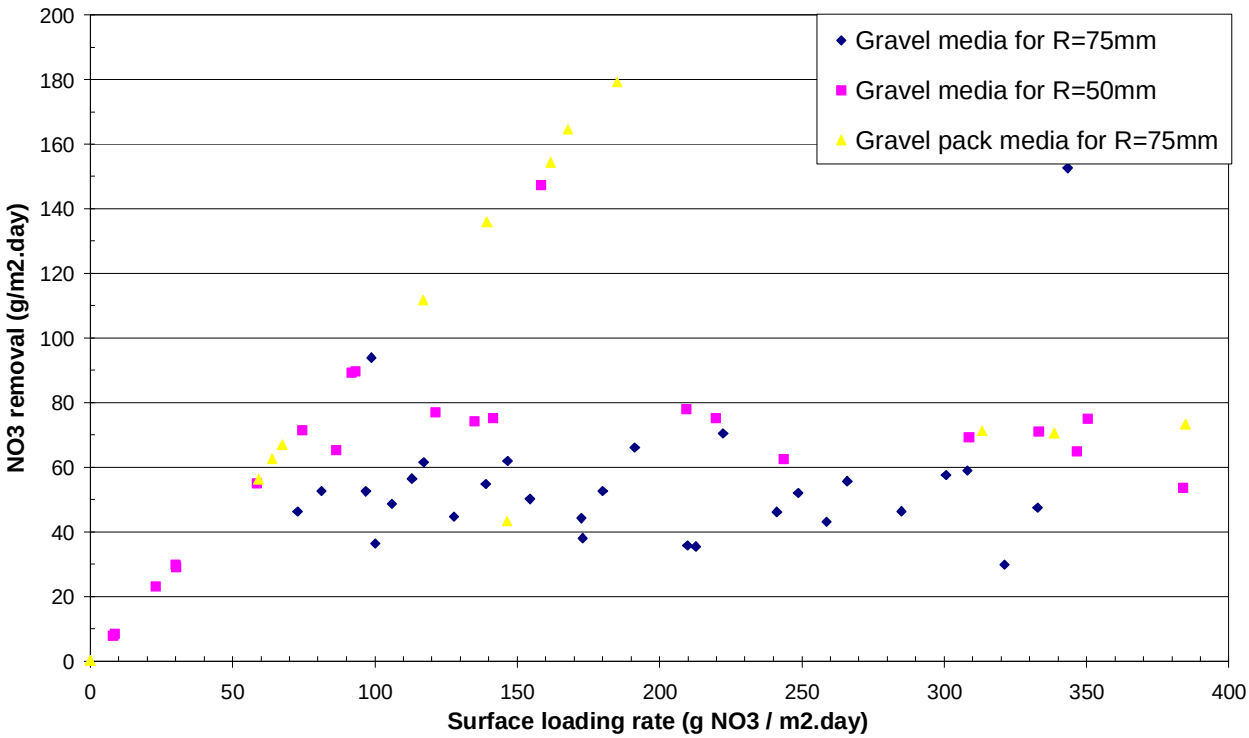


Figure4. 7 Relationship among surface loading rate $g.m^{-2}.d^{-1}$ inlet and outlet

4.5 pH

Alkalinity is produced during the conversion of NO_3 to nitrogen gas resulting in an increase in effluent pH. Throughout the experimental study, because of the denitrification process the final pH at the effluent was higher than initial pH and in the range of 7.6–9.0.

When the flow rate velocity was below 0.36 m/day, the pH will be more than 9 as shown in Figure 4.8.

Chung and Bae, 2002 investigated both transformations separately in column reactors in a pH range of 7 to 9. In this study were the same numbers

The pH-level determined in the filter bed at first sight seemed not to be in agreement with the generally observed gain of acid binding capacity by denitrification accompanied by a pH increase. No significantly lower pH-levels were found at operation.

As outlined by McCarty et al. 1969, the stoichiometric equations of denitrification with different carbon sources showed acetate to yield twice the amount of CO_2 as methanol or ethanol for the same amount of nitrate. Thus, a high dosage of acetate

might cause a pH drop. On the other hand, ethanol was shown to yield a higher biomass concentration than does acetate or methanol. A high amount of biomass generally promotes clogging of the filter bed and might lower stripping rates of CO_2 and concomitantly lower pH-levels.

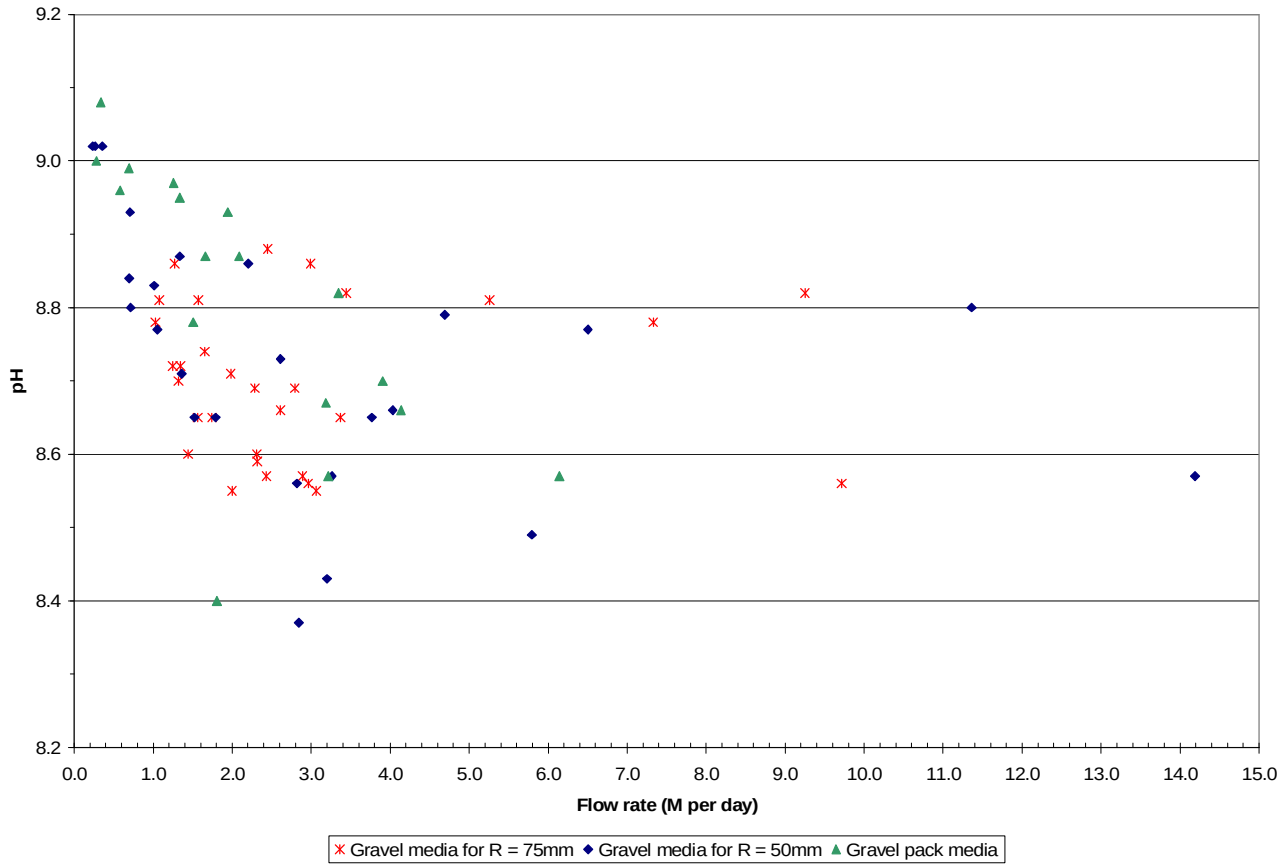


Figure4. 8 pH and flow rate velocity Relationship

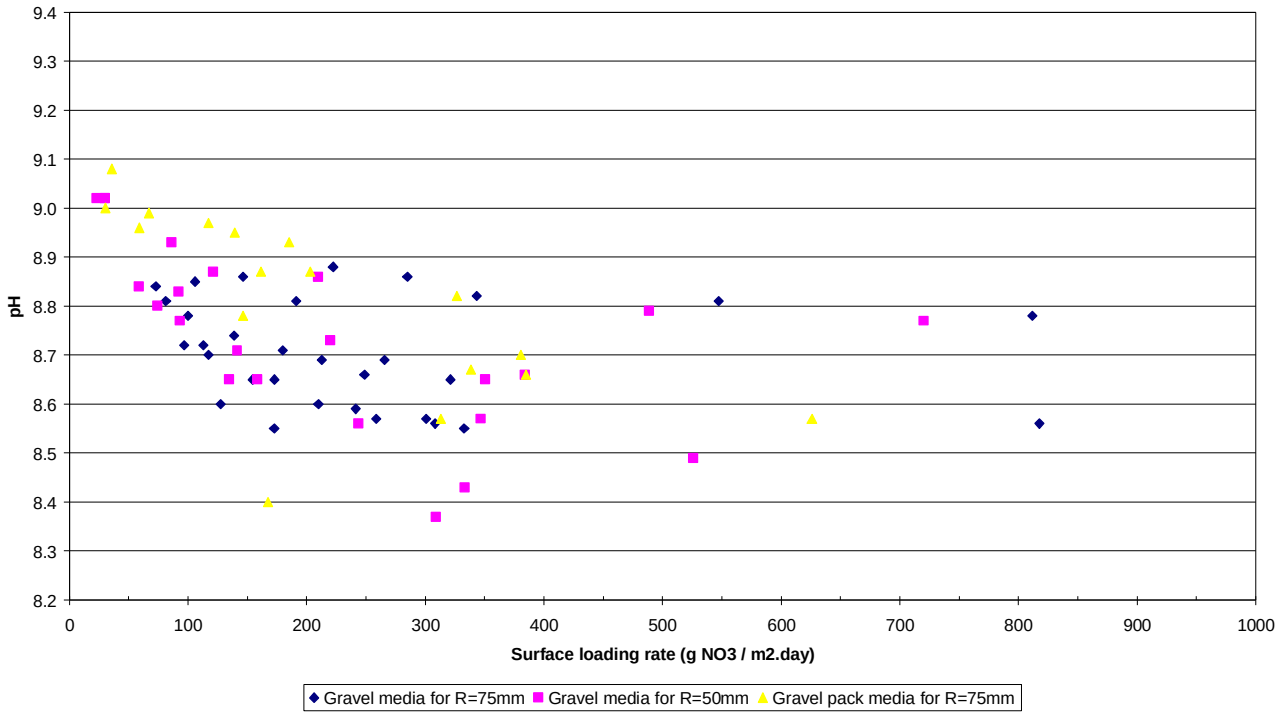


Figure4. 9 pH and surface loading rate Relationship

So pH will affected by the same factors affecting the denitrification process. So when the flow rate velocity was below 0.36 m/day, the pH will be more than 9.

When the surface loading rate was below 30 g NO_3/ m^2.day, the pH will be more than 9 as shown in Figure 4.9.

4.6 System operation

4.6.1 Clogging of the filter bed and wash-out of floating carrier material

Biomass yield in methanol-fed denitrification was determined by Nyberg et al. (1992) as 0.2-0.3 g volatile solids to gram of methanol added. Thus, a high dosage of methanol will lead to high cell yields. Carbon sources other than methanol also may provide high cell yields, as mentioned above, and thus clog up the carrier material. In the system studied here, in particular at a ethanol dosage exceeding the 2.5:1 ratio, thick biofilms developed on the grains. These may entrap gas bubbles and float the carrier material. This phenomenon was observed to cause occasional losses of the compacted fine sand. No such losses were found in the quartz system, presumably because of its higher density and thinner biofilms.

4.6.2 Relationship between ethanol consumption and nitrate removal

The consumed ethanol expressed by the formula (CH_3CH_2OH) was calculated as around 15 % on the basis of simultaneous oxygen consumption, nitrate reduction

[Ethanol] (mg/L) = 1.78 [NO_3-N] + 0.67 [DO].

The optimum C/N ratio was assumed to be 2:1 ethanol/N achieved in this study.

When ethanol was stopped during the day 27 to day 32 of test the flow rate of the reactor increase and the denitrification process was decreased because of absent of the substrate.

For optimum operation, the system should be evaluated to allow the nitrogen bubbles

to leave the system to avoid clogging which decreasing permeability of the reactors.

A system of parallel reactors with the three different media from large to small grain size could be employed without clogging.

Table 4.3 shows the main parameters affected the sand filter to optimize the removal rate according to this study.

Table 4. 3 Designing parameters for sand filter to optimize the removal rate

Item	Designing parameters
1	Source of carbon: type and its ratio
2	Filter materials
3	Flow rate
4	Nitrate load

Chapter 5

Conclusion and Recommendation

As a result of this research project the following points can be concluded:

5.1 Conclusions

1. The denitrification process need start-up period to allow the bacteria to be attached to the support particles before it can be able to start removing nitrate.
2. The sand filter system was capable of achieving good nitrate removal larger than 90% in drinking water.
3. The system recovers quickly from the upsets (clogging of bacteria) and regained its removing efficiency.
4. As the nitrate concentration increased, more time was needed to achieve a high percentage of removal, on the other hand, the total suspended solids increased with time.
5. The denitrification performance of the system is affected by quantitative changes of the carbon source.
6. Water velocity has a marked effect on the denitrification performance of the system. The optimum flow rate for each media depends on its surface area. The media which have more surface area will to remove nitrate more than others at the same flow rate.
7. The ethanol is a suitable source of carbon for the denitrification process, the ratio 2:1 C/N was enough to complete the denitrification process.
8. pH will be affected with the flow rate. So when the flow rate velocity was below 0.36 m/day, the pH will be more than 9.
9. For optimum operation, the system should be evaluated to allow the nitrogen bubbles to leave the system to avoid clogging which decreasing permeability of the reactors.
10. Clogging may be an operational problem for packed bed reactor due to the accumulation of gas, excess cell growth. Regular back-washing may alleviate the clogging problem.

5.2 Recommendation

Following this study there are a number of recommendations that can be made for further work in the study of biological reactor.

These include:

- Now that the laboratory column method has been established in this study, it would be highly recommended that research continue on the factors which limit denitrification. This would be beneficial to further formulate design guidelines. A number of factors could be studied in the laboratory. These include:
  - Nitrate concentrations – to determine maximum concentration which a biological water treatment can effectively treat before levels become toxic to microorganisms, or before reductions are so small that they require parallel treatment of another kind.
  - Carbon type – to determine if it suitable for microbial denitrification.
  - Carbon Nitrate ratio – to determine the optimum ratio of the carbon to the nitrate
  - Hydraulic residence time – to determine how long the contaminated water must be in contact with the media to ensure optimal denitrification. This would vary depending on the concentration of the contaminant source.
  - Distance from source – to determine whether there is some minimum distance the reactor must be located from the source.
- Research on the effect of combining different media would be beneficial to reduce clogging in the reactor.

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§ Appendix – A: Permeability test result

For Sand

When h = 80.5-16.5 = 64 cm

Table A. 1 results of constant head for sand media

t (sec)	Q (cc)	K (cm/sec)
60	213.3	0.00919
60	208.4	0.00898
60	208	0.00897
60	205.2	0.00885
60	205.8	0.00887
K average		**0.00897**

When h = 144.5-16.5 = 128 cm

Table A. 2 results of constant head for sand media

t (sec)	Q (cc)	K (cm/sec)
60	420.2	0.00906
120	836.4	0.00901
180	1242.4	0.00893
240	1652.3	0.00890
300	2063.8	0.00890
K average		**0.00896**

Water Content, w% = [(W3-W2)/(W3-W1)] x 100

Water Content, w% = 9.13%

Wt. of soil + Mold = 2860 g

Wt. of Mold = 966 g

Wt. of soil = 1894 g

Wet unit Wt. = 1.86 g/cm^3

Dry unit Wt. = 1.7 g/cm^3

For Gravel pack

When h = 60.3-16.5 = 43.8 cm

Table A. 5 results of constant head for gravel pack media

t (sec)	Q (cc)	K (cm/sec)
60	465.1	0.02929
60	465.9	0.02934
60	463.9	0.02922
60	462.9	0.02916
60	462.8	0.02915
K average		**0.02923**

Appendix – B: Sieve analysis test result

Table B. 4 Sieve analysis results for sand media

Sieve Size mm	Sieve Size in /1000	Sieve No.	Weight Retained	% Retained	Total Cumulative Retained
4.75	187.01	4			
2	78.74	10			
1.18	46.46	16	0	0.00	0.00
0.6	23.62	30	1.07	0.37	0.37
0.3	11.81	50	154	53.61	53.98
0.15	5.91	100	123.73	43.07	97.06
0.075	2.95	200	5.35	1.86	98.92
0	0.00	Pan	1.4	0.49	99.41

Table B. 5 Sieve analysis results for granite gravel media

Sieve Size mm	Sieve Size in /1000	Sieve No.	Weight Retained	% Retained	Total Comulative Retained
16	629.92		0	0.00	0.00
9.5	374.02	3/8"	52	0.00	0.00
4.75	187.01	4"	1728	83.64	83.64
2	78.74	10"	210	10.16	93.80
1.18	46.46	16"	46	2.23	96.03
0.6	23.62	30"	28	1.36	97.39
0	0.00	Pan	2	0.10	97.48

Table B. 6 Sieve analysis results for gravel pack media

Sieve Size mm	Sieve Size in /1000	Sieve No.	Weight Retained	% Retained	Total Comulative Retained
4.75	187.01	4			0.00
2	78.74	10	42.1	32.99	32.99
1.18	46.46	16	80.4	63.01	96.00
0.6	23.62	30	4	3.13	99.14
0.3	11.81	50	0.1	0.08	99.22
0.15	5.91	100	0.2	0.16	99.37
0.075	2.95	200	0.2	0.16	99.53
0	0.00	Pan	0.1	0.08	99.61

Appendix – C: Itinerary of laboratory field work

Stage one (Fine sand filter)

- **Reactor 50 mm in diameter**

Day	NO_3 Concentration (mg NO_3 / Liter)		pH	
	Inlet	Outlet	Inlet	Outlet
1	77.5	73.1	7.95	8.6
2	66.5	64.2	8.5	8.6
3	62.0	59.8	8.35	8.75
4	64.2	62.0	8.57	8.35
5	75.3	73.1	8.59	8.73
6	77.5	77.5	8.64	8.65
8	82.0	84.2	8.69	8.72
9	82.0	82.0	8.7	8.76
11	86.4	84.2	8.74	8.79
12	86.4	84.2	8.7	8.81
13	84.2	84.2	8.86	8.82
15	79.7	82.0	8.75	8.82
16	77.5	79.7	8.88	8.82
17	73.1	75.3	8.79	8.82
18	73.1	70.9	8.83	8.86
19	70.9	64.2	8.88	8.84
20	68.7	50.9	8.9	9.08
22	70.9	3.1	8.95	9.18
24	66.5	57.6		8.79
25	64.2	0.0	8.73	8.91
26	64.2	0.0	8.68	8.91
27	64.2	0.0		
29	64.2	9.3		
32	166.1	148.4	8.61	8.78
33	166.1	73.1	8.62	8.98

- **Reactor 75 mm in diameter**

Day	NO_3 Concentration (mg NO_3 / Liter)		pH	
	Inlet	Outlet	Inlet	Outlet
1	77.5	75.3	7.95	8.4
2	66.5	66.5	8.5	8.44
3	62.0	62.0	8.35	8.4
4	64.2	62.0	8.57	8.5
5	75.3	75.3	8.59	8.52
6	77.5	79.7	8.64	7.93
8	82.0	82.0	8.69	8.6
9	82.0	84.2	8.7	8.66
11	86.4	79.7	8.74	8.43
12	86.4	75.3	8.7	8.45
13	84.2	86.4	8.86	8.55
15	79.7	48.7	8.75	8.61
16	77.5	19.9	8.88	8.73
17	73.1	0.2	8.79	8.8
18	73.1	0.0	8.83	8.72
19	70.9	0.0	8.88	8.8
20	68.7	0.0	8.9	8.85
22	70.9	0.0	8.95	8.91
24	66.5	55.4		8.59
25	64.2	0.0	8.73	8.85
26	64.2	0.0	8.68	8.84
27	64.2	0.4		
29	64.2	37.7		
32	166.1	57.6	8.61	8.86
33	166.1	4.4	8.62	8.81

Stage two (Granite gravel filter)

- **Reactor 50 mm in diameter**

Day	Flow rate (Liter/day)	NO_3 Concentration (mg NO_3 / Liter)		pH	
		Inlet	Outlet	Inlet	Outlet
4	8.81	126.3	99.7	8.39	8.39
7	23.90	119.6	48.7	8.68	8.47
10	9.12	106.3	68.7	8.94	8.94
*	22.17				
11	22.30	115.2	108.5	8.92	8.8
12	12.77	110.8	99.7	8.9	8.77
13	9.22	104.1	86.4	8.95	8.79
14	4.32	95.2	59.8	8.91	8.86
15	2.62	90.8	33.2	8.95	8.87
17	1.39	121.8	29.7	8.84	8.93
18	0.52	115.2	4.9	8.9	9.02
19	0.46	99.7	0.3	8.68	9.02
20	7.92	95.2	82.0	8.65	8.66
21	7.40	93.0	73.1	8.64	8.65
22		88.6	46.5	8.69	8.72
24	11.37	90.8	77.5	8.48	8.49
25	5.54	86.4	64.2	8.38	8.56
26	2.99	88.6	39.9	8.45	8.65
27	2.07	88.6	3.3	8.44	8.77
28	0.20	84.2	1.6		
29	5.13	84.2	55.4	8.46	8.73
31	27.86	79.7	75.3	8.57	8.57
32	5.59	108.5	84.2	8.39	8.37
33	6.40	106.3	86.4	8.42	8.57
34	6.29	104.1	82.0	8.52	8.43
35	2.67	104.1	48.7	8.42	8.71
36	1.40	104.1	4.3	8.38	8.8
38	0.17	95.2	2.1	8.49	
39	0.00	95.2		8.43	
40	1.99	90.8	2.7	8.46	8.83
41	3.51	88.6	6.2	8.5	8.65
42	1.37	84.2	5.1	8.5	8.84
43	0.70	84.2	0.3	8.56	9.02
45	0.00	77.5		8.59	
46	0.00	75.3		8.62	

- **Reactor 75 mm in diameter**

Day	Flow rate (Liter/day)	NO_3 Concentration (mg NO_3 / Liter)		pH	
		Inlet	Outlet	Inlet	Outlet
4	188.93	126.3	126.3	8.39	8.22
7	89.86	119.6	115.2	8.68	8.48
10	25.54	106.3	106.3	8.94	8.62
10*	52.42				
11	40.87	115.2	110.8	8.92	8.82
12	32.38	110.8	106.3	8.9	8.78
13	23.23	104.1	95.2	8.95	8.81
14	13.22	95.2	79.7	8.91	8.86
15	10.82	90.8	62.0	8.95	8.88
17	6.94	121.8	79.7	8.84	8.81
18	5.62	115.2	66.5	8.9	8.86
19	15.22	99.7	55.4	8.68	8.82
20	14.90	95.2	86.4	8.65	8.65
21	10.10	93.0	77.5	8.64	8.69
22		88.6	62.0	8.69	8.64
24	10.20	90.8	75.3	8.48	8.6
25	8.82	86.4	64.2	8.38	8.55
26	7.70	88.6	59.8	8.45	8.65
27	5.84	88.6	42.1	8.44	8.7
28	5.18	84.2	4.1		
29	42.92	84.2	79.7	8.46	8.56
31		79.7		8.57	
32	13.54	108.5	93.0	8.39	8.55
33	10.75	106.3	88.6	8.42	8.57
34	10.24	104.1	84.2	8.52	8.59
35	13.08	104.1	84.2	8.42	8.56
36	12.76	104.1	84.2	8.38	8.57
38	12.34	95.2	75.3	8.49	8.69
39	11.54	95.2	75.3	8.43	8.66
40	8.76	90.8	64.2	8.46	8.71
41	6.37	88.6	57.6	8.5	8.6
42	7.29	84.2	50.9	8.5	8.74
43	5.93	84.2	42.1	8.56	8.72
45	5.52	77.5	35.4	8.59	8.72
46	4.77	75.3	26.6	8.62	8.81
47	4.54	97.5	62.0	8.67	8.78
48	6.90	110.8	86.4	8.44	8.65
52	5.71	82.0	44.3	8.71	8.85
53	4.40	73.1	26.6	8.7	8.84

Stage Three (Gravel pack filter)

- **Reactor 75 mm in diameter**

Day	Flow rate (Liter/day)	NO_3 Concentration (mg NO_3 / Liter)		pH	
		Inlet	Outlet	Inlet	Outlet
2	124.0	99.7	97.5	8.37	8.47
3	27.1	101.9	97.5	8.38	8.57
4	17.2	97.5	19.9	8.4	8.7
5	2.6	101.9	4.9	8.4	8.96
5*	12.7				
6	2.8	99.7	1.9		
6*	123.6				
7	14.1	106.3	84.2	8.45	8.67
9	1.23	108.5	0.1	8.76	9
9*	24.40				
10	5.91	104.1	2.5	8.37	8.95
10*	19.44				
11	1.49	106.3	0.2	8.4	9.08
11*	232.75				
12	90.20	101.9	95.2	8.42	8.51
13	18.27	93.0	75.3	8.55	8.66
14	5.56	93.0	4.2	8.61	8.97
23	607.82				
25	7.33	97.5	4.4	8.45	8.87
26	9.22	97.5	0.1	8.46	8.87
27	2.21	97.5	0.4		
27*	20.74				
28	14.21	97.5	75.3	8.38	8.57
30	6.64	97.5	68.7	8.36	8.78
31	3.06	97.5	0.6	8.33	8.99
31*	15.31				
32	14.79	97.5	8.8	8.38	8.82
33	8.59	95.2	3.1	8.43	8.93
33*	10.30				
34	7.97	93.0	1.8	8.34	8.4

Appendix – D: Pilot biological reactor

Figure D. 1 Up-flow Reactors were used

Figure D. 2 Assembly way of system

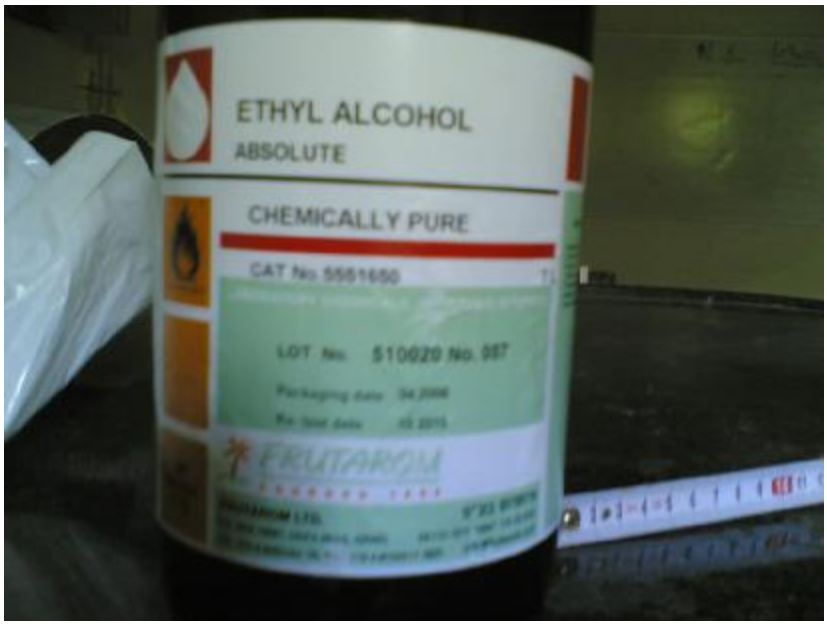

Figure D. 3 Ethanol was used as source of carbon

Figure D. 4 Stainless steel wire mesh was used between filter material and valve

50 mesh/inch , wire diameter = 0.22 mm

Figure D. 5 Tank was contained ethanol

Figure D. 6 Fine sand material

Figure D. 7 Granite gravel material

Figure D. 8 Spectrophotometer (Hach DR 4000U UV) was used to measure nitrate

Figure D. 9 Biofilm was formed on gravel material

Figure D. 10 Bacterial layer was growth surrounded the pipe