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THE EFFECT OF PESTICIDE ADSORPTION ON
BIOLOGICAL DENITRIFICATION OF
DRINKING WATER
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ÖZET
PESTİSİT ADSORPSİYONUNUN BİYOLOJİK DENİTRİFİKASYON
ÜZERİNE ETKİSİ

Böke, Nuran

Doktora Tezi, Kimya Mühendisliği Bölümü

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Hızlı şehirleşme, endüstriyel ve tarımsal faaliyetlerin artışı doğal çevreye birçok kirleticinin salınmasına yol açmıştır. İçme suyu kaynaklarındaki ve atıksulardaki fazla nitrat insan ve hayvan sağlığına zararlı olduğu için endişe uyandırmıştır. Nitratı içme suyu kaynaklarından uzaklaştırmak için iyon değişimi, ters osmoz, elektrodializ, katalitik denitrifikasyon ve biyolojik denitrifikasyon gibi yöntemler kullanılmaya başlanmıştır. İnorganik nitratı zararsız azot gazına dönüştüren biyolojik denitrifikasyon yöntemi bu teknikler arasında umut verici olanıdır.

Tarım kimyasalları tarımda verimliliği artırmakla birlikte gereğinden fazla ve dikkatsizce kullanıldıklarında çevreye zararlı olan maddelerdir. Fenoksi asit herbisitler tarımda geniş yapraklı yabancı otların kontrolünde yaygın olarak kullanılır. Bu bileşikler zehirli olup doğal çevrede aylarca bozunmadan kalabilirler. Bu maddelerin su kaynaklarından uzaklaştırılması dünyanın birçok ülkesinde çözülmesi gereken bir sorun haline gelmiştir.

Nitratlı gübre ve pestisitler tarımda birlikte kullanıldıklarından su kaynaklarında bu iki kirliliğe çoğunlukla birlikte rastlanmaktadır. Bu nedenle nitrat ve pestisitın sudan eşanlı olarak uzaklaştırılması elverişli bir yöntem gibi görünmektedir. Biyolojik denitrifikasyonda pestisit için adsorplayıcı görevi de yapacak bir katı substrat kullanarak bu tür bir eşzamanlı uzaklaştırma işlemini gerçekleştirmek mümkün olabilir.

Bu tezde sürekli biyolojik denitrifikasyon yöntemiyle model içme suyundan nitrat uzaklaştırılması ve eşanlı olarak pestisitın adsorpsiyon yoluyla giderilmesi incelenmiştir. Temel amaç poly(ϵ -kaprolakton) (PCL)'in electron verici olarak biyolojik denitrifikasyonda kullanılabilirliğini ve verimini araştırmak olmuştur. *Acidovorax avenae subsp. Avenae* LMG 17238 adlı saf bakteri kültürü kullanılarak kesikli ve sürekli biyolojik denitrifikasyon deneyleri gerçekleştirilmiştir. Çeşitli bakteri kaynaklarının ve pestisit kirliliğinin biyolojik denitrifikasyona etkisinin incelendiği deneyler de gerçekleştirilmiştir.

Deneyler karbon kaynağı olarak poly(ϵ -kaprolakton) ve bakteri kaynağı olarak LMG 17238 kullanımı ile biyolojik denitrifikasyonun geçerliliğini göstermiştir. 2,4-D adsorpsiyon denge verileri incelenen konsantrasyon aralığında Freundlich ve Langmuir modellerine uymuştur. Nitrat ve 2,4-D'nin eşzamanlı olarak sürekli biyolojik denitrifikasyon işlemi ile uzaklaştırılmasının mümkün olduğu belirlenmiştir.

Anahtar kelimeler: Biyolojik denitrifikasyon, içme suyu, biyolojik olarak parçalanabilen polimer, poly(ϵ -kaprolakton) (PCL); 2,4-D adsorpsiyonu.

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ABSTRACT

THE EFFECT OF PESTICIDE ADSORPTION ON BIOLOGICAL DENITRIFICATION OF DRINKING WATER

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The fast movement of human towards urbanization, industrialization and agricultural activities introduced various contaminants into the environment. Excessive nitrate in drinking water resources and wastewater streams raised concerns due to severe impacts on human and animal health. Diverse methods are reported to remove nitrate from drinking water resources, such as ion exchange, reverse osmosis, electrodialysis, catalytic denitrification, and biological denitrification. Among these techniques biological denitrification, capable of reducing inorganic nitrate compounds to harmless nitrogen gas is the most promising.

Agricultural chemicals, while often benefiting farming productivity can have detrimental environmental effects when applied improperly. Phenoxyacid herbicides are widely used to control broad-leaved weeds in agriculture. However, these compounds are toxic and may persist in the environment for many months. Removal of these potentially harmful compounds from water resources has emerged as an important issue of environmental protection.

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Because of the application of fertilizers and pesticides in agriculture the combined occurrence of nitrate and pesticides in groundwater is frequently encountered. As a consequence, the development of processes which allow a simultaneous elimination of both kinds of contaminants seems advantageous. Such a simultaneous elimination seems possible in the framework of a biological denitrification process with a solid substrate that also acts as an adsorbent for the pesticide.

In this thesis removal of nitrate from model drinking water by continuous biological denitrification and simultaneous pesticide adsorption was studied. The main objective was to investigate the feasibility and the efficiency of poly(ϵ -caprolactone) (PCL) as electron donor in biological denitrification. Batch and continuous experiments were carried out using bacteria culture *Acidovorax avenae subsp. Avenae* LMG 17238. The effect of different bacteria and pesticide contamination on biological denitrification were also investigated.

The experiments show that the combination of poly (ϵ -caprolactone) with LMG 17238 is a feasible denitrification procedure. Equilibrium data fitted very well to the Freundlich and the Langmuir equilibrium model in the studied concentration range and it has been determined that simultaneously 2,4-D and nitrate removal is possible in continuous biological denitrification.

Key words: biological denitrification, drinking water, biodegradable polymer, poly(ϵ -caprolacton (PCL), adsorption of 2,4-D.

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NOMENCLATURE

b	bonding energy of adsorption (L/mg)
C	nitrate concentration (mg NO ₃ ⁻ -N/L) (Michaelis-Menten Eq.)
C _{eq}	equilibrium concentration of 2,4-D in the solution (mg/L)
DR	denitrification rate (mg NO ₃ ⁻ -N/L*d)
F	flow rate (mL/day)
k	the maximum denitrification rate (mg NO ₃ ⁻ -N/L*h)
K _s	half - saturation constant (NO ₃ ⁻ -N/L).
l	length of column (m)
L	nitrate load [NO ₃ ⁻ -N/(m ² * d)]
P _{wt}	the water vapor pressure at the time t
q _{eq}	the adsorbed amount of 2,4-D by adsorbent (mg/g)
Q _o	Langmuir constants related to maximum adsorption capacity or monolayer capacity (mg/g)
S	concentration of growth limiting substance in solution (mass/unit volume) (Monod equation)
S	nitrate concentration (mg NO ₃ ⁻ -N/L) (Monod Eq.)
S _a	cross sectional area of denitrification column (m ²)
SV	space velocity (h ⁻¹)
T _t	the temperature at the time t of reading in (K)
V _{bed}	volume of packed bed (L)
V _m	the maximum denitrification rate (mg NO ₃ ⁻ -N/L*d)
V _{RVST}	the amount of gas in reaction vessel at the start (mL)
V _{Sample}	the amount of produced gas at the time t (mL)

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VSS	volume specific surface area (m^2/L)
μ	specific growth rate , (time^{-1})
μ_m	maximum specific growth rate, (time^{-1})
η	removal ratio of $\text{NO}_3^- \text{-N}$ /L (%)
τ	the empty bed residence time (h)

1 Introduction

Water resources include surface waters (i.e. coastal bays, lakes, rivers, and streams) and groundwater. These water resources may be used for drinking water, industrial processes, agriculture, and irrigation. They also provide opportunities for recreation, such as fishing, boating and swimming. But water resources have been in danger (EPA, 2008, <http://www.epa.gov/climatechange/basicinfo.html>).

World's climate is changing: The phrase 'climate change' is growing in preferred use to 'global warming' because it helps convey that there are changes in addition to rising temperatures. Over the past 200 years, the burning of fossil fuels, such as coal and oil, and deforestation have caused the concentrations of heat-trapping "greenhouse gases" to increase significantly in our atmosphere. These gases prevent heat from escaping to space, somewhat like the glass panels of a greenhouse. Greenhouse gases are necessary to life as we know it, because they keep the planet's surface warmer than it otherwise would be. But, as the concentrations of these gases continue to increase in the atmosphere, the Earth's temperature is climbing above past levels. If greenhouse gases continue to increase, climate models predict that the average temperature at the Earth's surface could increase from 1,8 to 4,0 °C above 1990 levels by the end of this century. Rising average temperatures are already affecting the environment. Some observed changes include shrinking of glaciers, thawing of permafrost, later freezing and earlier break-up of ice on rivers

and lakes, lengthening of growing seasons, shifts in plant and animal ranges and earlier flowering of trees.

All regions of the world show an overall net negative impact of climate change on water resources and freshwater ecosystems. Areas in which runoff is projected to decline are likely to face a reduction in the value of the services provided by water resources. The beneficial impacts of increased annual runoff in other areas are likely to be tempered in some areas by negative effects of increased precipitation variability and seasonal runoff shifts on water supply, water quality and flood risk.

Water resources have also been under the threat of contamination from human activities: leakage from storage tanks, if not handled properly from septic systems, landfills, injection wells, waste piles; misuse of pesticides and fertilizers, industrial activities are important factors to contamination in water sources.

In this thesis it was attempted to make a modest contribute to the studies of removing nitrate and pesticide contamination from drinking water.

2 Literature Survey

2.1 Drinking Water

Water fit for human consumption is called drinking water or potable water. Water that is not potable can be made potable by filtration or

distillation (heating it until it becomes water vapor, and then capturing the vapor without any of the impurities it leaves behind), or by other methods (chemical or heat treatment that kills bacteria). Sometimes the term safe water is applied to potable water of a lower quality threshold (i.e., it is used effectively for nutrition in humans that have weak access to water cleaning processes, and does more good than harm). Water that is not fit for drinking but is not harmful for humans when used for swimming or bathing is called by various names other than potable or drinking water, and is sometimes called safe water, or "safe for bathing". The United States (U.S.) Environmental Protection Agency (EPA), World Health Organization (WHO) and Turkish Standards Institution (Türk Standardları Enstitüsü) (TSE) have set some standard values for drinking water, Drinking water is usually supplied by a combination of surface water and underground water (groundwater) sources. Surface water includes rivers, lakes, and reservoirs. Groundwater lies under the surface of the land, where it travels through and fills openings in rocks.

2.2 Drinking Water Standards

The Safe Drinking Water Act (SDWA) of EPA passed in 1974 and amended in 1986 and 1996 (EPA, 1996).

The WHO set up the latest guidelines for drinking-water quality in 1993, which are the international reference point for setting standards of drinking water (WHO, 1993).

The EU drew up the Council Directive 98/83/EC on the quality of water intended for human consumption by reviewing the parametric values of the old Drinking Water Directive of 1980. The EU standards are more recent, complete and strict (EU Drinking Water Directive, 1998), than the WHO standards (WHO, 1993).

Standards for some contaminants in drinking water according to WHO, EU (<http://www.lenntech.com/WHO-EU-water-standards.htm>) and TSE [www.rainline.com.tr, TS 2005 (TS 266)] were listed in Table 2.1.

2.3 Nitrate

In this study it was tried to remove nitrate contamination from drinking water. The nitrate ion is a polyatomic ion with the empirical formula NO_3^- and a molecular mass of 62,0049. It is the conjugate base of nitric acid, consisting of one central nitrogen atom surrounded by three identical oxygen atoms in a trigonal planar arrangement. The nitrate ion carries a formal charge of negative one, where each of oxygen carries a $-2/3$ charge while the nitrogen carries a $+1$ charge, and is commonly used as an example of resonance. The three canonical structures of the nitrate ion are shown resonating in Fig. 2.1.

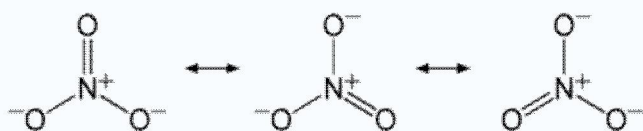


Figure 2.1 Structure of nitrate ion

Table 2.1 Drinking water standards for some contaminants

Contaminants	WHO standards (1993)	EU standards (1998)	TSE standards (2005)
Aluminium (Al)	0,2 mg/L	0,2 mg/L	0,2 mg/L
Ammonia (NH ₄ ⁺)	No guideline	0,50 mg/L	0,05 mg/L as NH ₃
Antimony (Sb)	0,005 mg/L	0,005 mg/L	0,005 mg/L
Arsenic (As)	0,01 mg/L	0,01 mg/L	0,01 mg/L
Barium (Ba)	0,3 mg/L	Not mentioned	0,3 mg/L
Boron (B)	0,3 mg/L	1,00 mg/L	1,00 mg/L
Bromate (Br)	Not mentioned	0,01 mg/L	0,01 mg/L
Cadmium (Cd)	0,003 mg/L	0,005 mg/L	0,005 mg/L
Chromium (Cr)	0,05 mg/L	0,05 mg/L	0,05 mg/L
Copper (Cu)	2 mg/L	2,0 mg/L	2,0 mg/L
Iron (Fe)	No guideline	0,2 mg/L	0,3 mg/L
Lead (Pb)	0,01 mg/L	0,01 mg/L	0,01 mg/L
Manganese (Mn)	0,5 mg/L	0,05 mg/L	0,05 mg/L
Mercury (Hg)	0,001 mg/L	0,001 mg/L	0,001 mg/L
Molibdenum (Mo)	0,07 mg/L	Not mentioned	
Nickel (Ni)	0,02 mg/L	0,02 mg/L	0,02 mg/L
Nitrogen (total N)	50 mg/L	Not mentioned	
Selenium (Se)	0,01 mg/L	0,01 mg/L	
Silver (Ag)	No guideline	Not mentioned	Not mentioned
Sodium (Na)	200 mg/L	200 mg/L	200 mg/L
Uranium (U)	1,4 mg/L	Not mentioned	
Zinc (Zn)	3 mg/L	Not mentioned	3 mg/L
Chloride (Cl ⁻)	250 mg/L	250 mg/L	250 mg/L
Cyanide (CN)	0,07 mg/L	0,05 mg/L	0,05 mg/L
Fluoride (F)	1,5 mg/L	1,5 mg/L	1,5 mg/L
Sulfate (SO ₄ ²⁻)	500 mg/L	250 mg/L	250 mg/L
Nitrate (NO ₃ ⁻)	(See Nitrogen)	50 mg/L	50mg/L
Nitrite (NO ₂ ⁻)	(See Nitrogen)	0,50 mg/L	0,5 mg/L
2,4-D	30 µg/L	0,1 µg/L for one pesticide and 0,5 µg/L for total of all pesticides present	0,1 µg/L
MCPA	2 µg/L		
Simazine	2 µg/L		
2,4-DB	90 µg/L		
Dichlorprop	100 µg/L		
Fenoprop	9 µg/L		
Mecoprop	10 µg/L		
2,4,5-T	9 µg/L		

2.3.1 Nitrate Contamination in Drinking Water

Groundwater is a significant source of water for both domestic and agricultural use; it is the source of domestic water for 50 percent of the total US population and for nearly 90 percent of the rural population. Groundwater comprises 75-80 percent of the water used for agricultural irrigation (Bouchard et al., 1992). About two-thirds of the drinking water in The Netherlands originates from groundwater (Van der Hoek and Klapwijk, 1986) and the coastal aquifer of Israel supplies about 25% of the total water consumption (Mercado et al., 1988); (Özgen, 2004).

Nitrate is the most ubiquitous chemical contaminant in the world's aquifers and the levels of the contamination are increasing. Increased degradation of drinking waters in European Community are consequences of steadily increasing NO_3 levels in surface, ground, and coastal waters (Spalding and Exner, 1993). Increased nitrate contamination of available and future drinking water sources constitutes an important and rapidly growing environmental problem in many countries (Vолоkita et al., 1996). Estimates based on data collected in the US Environmental Protection Agency (USEPA) National Pesticides Survey indicates that 5 percent of both public and private drinking water wells have nitrate concentrations greater than the USEPA maximum contaminant level (MCL) of 10 mg nitrate-N/L. Also a survey carried out by the French Department of Health in 1982 showed that more than one million people were using water with a nitrate content higher than the European standard for drinking waters (Chevron et al., 1997) and it is

reported that about 50% coastal wells in Israel exceeds the 45 mg/L nitrate concentration and in the 18% of the wells nitrate concentration is above the maximum concentration of the Israeli standard – 70 mg/L (Mercado et al., 1988). In The Netherlands it is estimated that about 25% of the groundwater water well-fields exploited by waterworks may experience problems, either with nitrate itself or with the reaction products of nitrate reduction (Van der Hoek and Klapwijk, 1986). Eight percent of the public waterworks in Denmark and 5% of the those in the former Federal Republic of Germany supply groundwater that exceeds the EC limit for NO_3^- -N. In eastern and central England, NO_3^- concentrations are increasing in groundwater, which provides 30 to 50% of the drinking water. Nitrate contamination of groundwater is also a growing problem in the Caribbean, Africa, the Middle East, Australia, and New Zealand (Spalding and Exner, 1993).

Mapping of NO_3^- concentrations in more than 87000 wells in the USA was the first comprehensive, nation-wide evaluation of the areal distribution of NO_3^- in groundwater (Spalding and Exner, 1993). Their database was the 25-yr record of NO_3^- analyses in the U. S. Geological Survey's Water Storage and Retrieval System (WATSTORE). The studies of Madison and Brunett showed that nationally the presence and the predicted occurrence of NO_3^- in groundwater are non-uniform and are skewed toward the central and western USA (Spalding and Exner, 1993).

National Alachlor Well Water Survey (NAWWS), and USEPA's National Pesticide Survey (NPS) was to estimate with statistical accuracy

the proportion of the population served by drinking water wells that exceed the nitrate MCL. In the NAWWS 1,430 domestic rural wells in 89 counties in 26 states were sampled, whereas 783 rural domestic wells in 90 counties in 38 states and 566 community water systems in 50 states were sampled in the NPS. There are nearly 10.5 million rural domestic wells and nearly 38,300 community water systems in the USA. In the NPS, the estimated incidence of contamination, which is defined as the $\text{NO}_3\text{-N}$ levels in the excess of 10 mg/L MCL, was lower in the high capacity community water systems (1.2%) than in the domestic wells (2.4%).

This is to be expected. High capacity wells pump groundwater from a larger vertical interval of the saturated zone than do domestic wells; consequently, concentrations of contaminants, which usually are higher at the top of the aquifer, are diluted. The discrepancies in the results for the rural domestic wells in the two surveys are related to land use. Because sampling sites in the NPS were selected without regard to pesticide or land use, inclusion of nonagricultural areas significantly lowered the number of wells that exceeded the MCL. The NAWWS, on the contrary, was restricted to regions of alachlor use. Because these areas are that are intensively cropped to corn (*Zea mays*) and soybean (*Glycine max*) N-fertilizer, livestock wastes, and mineralized N from legumes and other organic matter are potential sources of NO_3^- contamination. Consequently, the results of the NAWWS are a “worst-case” estimate of the incidence of NO_3^- -contaminated wells. From the NPS, USEPA (1992) estimates 4.5 million people including 66,000

infants under 1 yr of age are served by the community water systems or rural domestic wells that exceed the 10 mg/L NO_3^- -N MCL (Spalding and Exner, 1993).

In Turkey Nif Mountain karstic aquifer constitutes a significant portion of Izmir's water resources. Researchers aimed at making a seasonal assessment of nitrate contamination extent in this region. For this purpose 25 wells and 34 springs were selected in the study area and water samples representing steady-state winter ground water flow conditions and dry summer conditions in April and September 2006, respectively, were collected. In order to spatially and temporally assess the nitrate analysis results of the samples, maps showing nitrate concentration distribution and seasonal changes were generated. As a result nitrate concentrations exceeding 50 mg/L were observed west of the Nif Mountain for both sampling periods. Furthermore, the spatial distribution of seasonal changes of nitrate contamination appeared to be heterogeneous. It is also observed that the expected decrease in dilution due to less precipitation and ground water recharge in the summer months was not reflected in the regions nitrate concentrations (Polat et al., 2007)

2.3.2 Adverse Health Effects of Excessive Nitrate Intake in Human Body

Elevated nitrate concentrations in drinking water sources present a potential risk to public health. Nitrate levels have been increasing due to

increased usage of nitrogeous fertilizers, changes in land-use patterns from pasture to arable, and increased recycling of domestic wastewater in low-land River. Concentrations greater than 10 mg NO_3^- -N/L can be fatal to infants under six months of age. In infants, NO_3^- is reduced to NO_2^- , which combines with hemoglobin in the blood to form methaemoglobinaemia, and leads to a condition commonly known as “blue baby syndrome”. Upper gastrointestinal cancers related to consuming drinking water which has high level of nitrate was reported in the literature (Türkdoğan, M. K. et al., 2003).

2.4 Processes for Removing of Nitrate Contamination from Drinking Water

Nitrate is a stable and highly soluble ion with low potential for coprecipitation or adsorption. These properties make it difficult to remove nitrate using conventional water treatment technologies, such as lime softening and filtration (Kapoor and Viraraghavan, 1997). More sophisticated technologies such as ion exchange, reverse osmosis, electrodialysis, catalytic denitrification and biological denitrification are required for lowering the concentration of nitrate to acceptable levels.

2.4.1 Ion Exchange

The ion exchange process involves passage of nitrate-laden water through a resin bed containing strong base anion exchange resins on which nitrate ions are exchanged for chloride or bicarbonate ions until

the resins exchange capacity is exhausted. The exhausted resin is regenerated using a concentrated solution of sodium chloride or sodium bicarbonate.

The anion exchange resins used for the production of demineralised water (conventional resins) are capable of removing nitrate but suffer from the following disadvantages:

- Ø Increased chloride in the treated water as a result of regeneration with sodium chloride, coupled with reduced bicarbonate, a situation which could lead to the corrosion of brass fittings in the distribution system.
- Ø Low nitrate removal capacity for water high in sulfate because of higher selectivity for sulfate rather over nitrate shown by the conventional resins; lower nitrate capacity results in greater salt requirements and larger volumes of waste regenerant.
- Ø Displacement of nitrate from the resin by sulfate (because of the sulfate selectivity) if the run is continued past the point at which nitrate leaving the bed is equal to the inlet concentration.

Nitrate selective ion exchange resins, such as alkyl phosphonium anion exchange resin which has pendant alkyl groups of C₃ or larger, Purolite A 520 E are now available. These resins have a higher selectivity for nitrate over sulfate and have a lower total capacity for high sulfate waters. The lower total capacity results in lower chloride concentrations in the treated water, and the nitrate selectivity prevents the displacement of nitrate by sulfate.

2.4.2 Reverse Osmosis (RO)

In a reverse osmosis process, ionic species present in water are removed by forcing the water across a semi-permeable membrane and leaving nitrate and another ionic species behind. Removal of nitrates is achieved by subjecting water in reverse osmosis cells to pressures exceeding its corresponding osmotic pressure. Pressure ranging from 2070-10350 kPa is applied to reverse the normal osmotic flow of water.

Membranes commonly used are made of cellulose acetate, while membranes made of polyamides and composite membranes are also available. These membranes do not show preference for any ion, but the degree of salt rejection is found to be proportional to the valence of ions present in the water supply.

Common problems associated with RO membranes include fouling, compaction, and deterioration with time. These problems result from the deposition of soluble materials, organic matter, suspended and colloidal particles and other contaminants, pH variations, and chlorine exposure; thus the RO process requires pretreatment.

Cost analysis showed that the operating cost of the RO process was lower in comparison to a fluidized bed reactor, though the investment cost of RO was indicated to be higher than for FBR process (Soares, 2000).

2.4.3 Electrodialysis (ED)

In ED ions are transferred through membranes from a less-concentrated solution due to the passage of a direct electric current. ED treats the water by selective removal of undesirable ions through a semi permeable membrane. An electrodialysis system requires a supply of pressurized water (345-578kPa), a membrane stack and a direct current power source. The water requires pretreatment systems similar to those used for reverse osmosis.

The nitrate removal efficiency of ED and RO processes is more or less the same. The ED process is limited to treating soft waters; ED requires lesser acid dosages in comparison to RO and has higher water recovery rates. Presently nitrate removal by ED is considered an expensive process, requiring close monitoring (Soares, 2000).

2.4.4 Catalytic Denitrification

The process can be easily automated and operated, and may be useful for small water treatment systems. The process is still in the development stage and kinetic parameters and long term performance of catalysts is yet to be studied (Soares, 2000).

2.4.5 Biological Denitrification

Biological denitrification is commonly used for the treatment of municipal and industrial wastewater. However, increasing knowledge

and experience indicates that biological denitrification treatment may also be effective for removing nitrates from drinking water (Chevron et al, 1997). Biological denitrification has been studied both at laboratory and full scale plants in Europe and the process has been evaluated to a limited extent in North America (Kapoor and Viraraghavan, 1997). It is not surprising that most of the work is being done in Europe, where nitrate contamination is more extensive (Gayle et al., 1989). The first European full-scale denitrification plant was constructed in Bucklesham, Great Britain, in 1982.,

A wide range of biological denitrification processes have been developed throughout Europe. They vary in the type of food/energy sources used and the nature of the support bed. The principle variations are:

- Ø Process using autotrophic bacteria which obtain energy from oxidation of inorganic chemicals (hydrogen) or sulfur and carbon from CO₂/bicarbonate
- Ø Process using heterotrophic bacteria which obtain energy and carbon require for growth from organic compounds – the carbon sources normally used for denitrification are methanol, ethanol or acetic acid.

2.4.5.1 Autotrophic Biological Denitrification

In operating the denitrification process it is important to minimize the amount of food/energy sources entering the water supply system in order

to prevent any problems of bacterial growth in the distribution system. In heterotrophic processes this is done by controlling the carbon sources dose in response to the dissolved oxygen and nitrate concentration in the feedwater. The autotrophic process offer advantages in this respect. Excess hydrogen can be easily removed by aeration of the water. Autotrophic denitrification by sulfur – oxidizing bacteria uses beds of sulfur granules upon which the bacteria grow; the energy sources (sulfur) is utilized directly by the bacteria, and does not enter the water being treated. A disadvantage of the autotrophic processes arises from the relatively low reaction rates compared with heterotrophic denitrification. This results in the need for larger autotrophic plants for the same degree of treatment and, therefore, higher capital costs.

2.4.5.2 Heterotrophic Biological denitrification

Heterotrophic denitrification is carried out by bacteria using an external carbon source in an anoxic environment. Heterotrophic denitrifiers derive electrons and protons required for nitrate reduction to elemental nitrogen from organic carbon compounds. Such compounds include carbohydrates, organic alcohols, amino acids and fatty acids. The C/N ratio required for complete nitrate reduction to nitrogen gas by denitrifying bacteria depends on the nature of the carbon source and the bacterial species. For most readily available organic carbon sources, a COD/NO₃⁻-N (w/w) ratio from 3.0 to 6.0 enables complete nitrate reduction to elemental nitrogen, where COD stands for chemical oxidation demand and is expressed as mg O₂/L. As noted above, carbon

limitation will result in the accumulation of intermediate products, such as NO_2 and N_2O , while excess carbon will promote dissimilatory nitrate reduction to ammonia. In addition, denitrification rates depend on the type of carbon source.

From this time on “biological denitrification” term will be used instead of “heterotrophic biological denitrification” for abbreviation.

2.4.6 Bacteria in Biological Denitrification

All microorganisms require nitrogen for protein synthesis and growth. Many may use either ammonium-N or nitrate-N, although ammonium is the preferred since it is in the most easily used form for protein synthesis. The reduction of nitrate to ammonium to synthesize protein is called assimilatory nitrate reduction. The microbial reduction of nitrate to gaseous nitrogen products is termed dissimilatory denitrification or nitrate respiration. (Gayle et al., 1989) This respiratory process, in which nitrates or nitrites serve as terminal electron acceptors instead of oxygen, results in generation of ATP (Intra-cellular unit used to exchange energy between cellular functions). When, electrons are transferred from the donor to acceptor, the organism gains energy which can be applied for the synthesis of a new cell mass and the maintenance of the existing cell mass (Mateju et al., 1992). Since facultative heterotrophs prefer to respire using dissolved oxygen (DO), the denitrifiers will continue to respire aerobically as long as DO is available. It is only when the DO is

depleted that denitrifiers begin using nitrate for respiration, which begins the denitrification reaction.

Biological denitrification occurs naturally in sediments of rivers, lakes and reservoirs where, in the absence of dissolved oxygen (anoxic conditions), certain widely occurring bacterial species can use nitrate for respiration, converting it to nitrogen gas (Eq. 2.1).



To use this natural reaction in a water-treatment process requires the establishment of high bacterial concentrations, achieved by providing a food/energy source for bacterial growth and a large surface area for bacterial attachment in biological reactors. Generally denitrification is considered to be an anoxic process, occurring in the presence of nitrate and in the absence of molecular oxygen. Nitrate is slightly less thermodynamically favorable as an electron acceptor than is oxygen. Surprisingly, most of the organisms to denitrify are not strict anaerobes, but rather are facultative organisms, which under anoxic conditions use nitrate as a final electron acceptor (Gayle et al., 1989). Mixed or pure bacteria culture can make denitrification. But repeatable results are obtained with pure bacteria culture.

2.4.7 Carbon Sources in Biological Denitrification

Without organic carbon as the food supply, facultative heterotrophs cannot continue to grow and multiply. Organic carbon can be supplied to the denitrification phase of biological treatment using either exogenous sources (such as methanol, ethanol and acetic acid) or internal sources (influent wastewater).

2.4.7.1 Water Soluble Carbon Sources

The objective in denitrification is the removal of nitrate (electron acceptor) by adding a sufficient amount of carbon substrate (electron donor). This requires knowledge of both the kinetic and stoichiometric requirements of the system. The stoichiometry of biological denitrification is dependent on the type of external carbon source used. Some soluble carbon sources used in denitrification processes are ethanol, methanol, acetic acid and glucose (Mohseni et al, 1998). Stoichiometric equations can be developed by both theoretical and laboratory investigations. Once the stoichiometric equation is known, then the amount of carbon required to destroy the nitrate ion can be determined. Water soluble carbon sources and their stoichiometric relationship in denitrification are shown in the Table 2.2 (Gayle et al, 1989). Biological denitrification with these carbon sources in several types of reactors are listed in Table 2.3.

Table 2.2 Stoichiometric relationships of heterotrophic denitrification with important carbonaceous substrates

Carbon Source	Stoichiometric Equation
Methanol	$6CH_3OH + 6NO_3^- + 1.304H^+ \rightarrow 2.870N_2 + 0.261C_5H_7O_2N + 4.696HCO_3^- + 9.391H_2O$
Ethanol	$5C_2H_5OH + 12NO_3^- \rightarrow 10HCO_3^- + 2OH^- + 9H_2O + 6N_2$ $0.6C_2H_5OH + NO_3^- \rightarrow 0.102C_5H_7NO_2 + 0.714CO_2 + 0.286OH^- + 0.98H_2O + 0.449N_2$
Acetic Acid	$5CH_3COOH + 8NO_3^- \rightarrow 8HCO_3^- + 2CO_2 + 6H_2O + N_2$ $0.819CH_3COOH + NO_3^- \rightarrow 0.068C_5H_7NO_2 + HCO_3^- + 0.301CO_2 + 0.902H_2O + 0.466N_2$
Glucose	$C_6H_{12}O_6 + 2.8NO_3^- + 0.5NH_4^- + 2.3H^+ \rightarrow 0.5C_5H_7NO_2 + 1.4N_2 + 3.5CO_2 + 6.4H_2O$

Table 2.3 Biological denitrification of drinking water with soluble carbon sources

Substance	Column	Packing material	Nitrate Removal	Reference
Acetic Acid	Chateau Landon, France, full-scale, named as Nitrazur, up-flow fixed bed reactor, 50 m ³ /h capacity.	Ceramics	From 80 to 30 mg/L as NO ₃ ⁻	Gayle et al. (1989)
Ethanol	Eragny, France, full-scale, named as Biodenit, down-flow anaerobic bioreactor, 80 m ³ /h capacity.	Expanded clay with grain size between 2 and 5 mm	From 68 to 25 mg/L as NO ₃ ⁻	Gayle et al. (1989); Mateju et al. (1992); Kapoor and Viraraghavan, (1997)
Methanol or ethanol	Pilot-scale, packed bioreactor	Polystyrene beads	From 55 to 3 mg/L as NO ₃ ⁻	Frank and Dott (1985)
Ethanol	Bench-scale packed columns	Immobilized P. denitrificans encapsulated in alginate gel.	From 101 to 0.3 mg/L as NO ₃ ⁻	Gayle et al. (1988); Mateju et al. (1992)
Ethanol	Packed bioreactor, 2.5 cm diameter, 100 cm high (only 75 cm is packed)	273 µm mean particle size, 151 g active carbon	90% removal efficiency (Flows up to 273 mL/h)	Pekdemir (1998)
Ethanol	Cyclically operated fixed-film Plexiglass reactors, 70 mm diameter, 460 mm high	16 mm in diameter, 0.44 m ² /m ³ surface area, cylindrical Pall rings.	98 % (HRT=1 h) 99.5% (HRT=0.5h)	Dahab and Kalagiri (1996)

2.4.7.2 Biodegradable Polymer Poly(ϵ -caprolactone) (PCL) as Carbon Source

The American Society for Testing and Materials (ASTM) definition for a biodegradable plastic is used, which states, “a plastic designed to undergo a significant change in its chemical structure under specific environmental conditions resulting in a change of properties that may vary as measured by standard test methods appropriate to the plastic and the application in a period of time that determines its classification, in which the degradation results from the action of micro-organisms occurring naturally such as bacteria, fungi, and algae (Goldberg, 1995).

Biodegradable polymer poly(ϵ -caprolactone) (PCL) was used as carbon source in this study (Fig. 2.2). Synthesis of PCL, an aliphatic polyester; was explained based on literature (Ray, 2005).

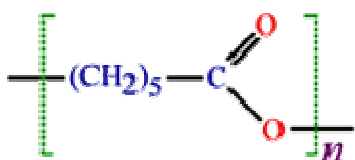


Figure 2.2 Structure of poly(ϵ -caprolactone)

Aliphatic polyesters: The broad range of biodegradable and biocompatible aliphatic polyesters have been prepared over the last forty years. Among them, poly(ϵ -caprolactone) (PCL) and poly(lactide) (PLA)

are produced on an industrial scale. PCL, a semi-crystalline polyester commercialised under the tradenames CelgreenTM, TONETTM and CAPATTM respectively by Daicel, Dow Chemicals and Solvay, has a low glass transition temperature of -62 °C and a melting temperature of 60 °C. It has good adhesive properties, and is highly miscible with many pigments, fillers and polymers, including poly(vinyl chloride) (Ray, 2005).

Preparation of aliphatic polyesters by step-growth: Aliphatic polyesters can be prepared by two distinct processes, i. e., step-growth polymerisation and ring-opening polymerisation. The step-growth polymerisation process relies on condensation of hydroxy-acids or mixtures of diacids and diols. The major drawbacks of polyadditions have been known for a long time. Any departure from the reaction stoichiometry has a deleterious effect on the chain length. Condensation at high temperature and usually for a long reaction time is favourable to side reactions. Finally, the reaction is equilibrated, and water has to be removed from the polymerisation medium for increasing conversion and molecular weight.

Formation of polycaprolacton, which is an aliphatic polyester, with a step-growth mechanism can be seen in Fig. 2.3.

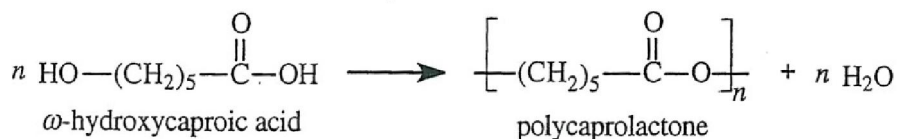


Figure 2.3 Polymerisation of ω -hydroxycaproic acid to form polycaprolacton.

Preparation of aliphatic polyesters by ring-opening polymerisation: The second process, ring-opening polymerisation of lactones, lactides and glycolide is free from these limitations. High molecular weight polyesters can be easily prepared under mild conditions from lactones of different ring size, substituted or not by functional groups. A broad range of anionic, cationic and coordinative initiators/ catalysts have been reported in the scientific literature. Fig. 2.4 shows the production of polycaprolactones by a ring opening addition polymerisation carried out by SOLVAY.

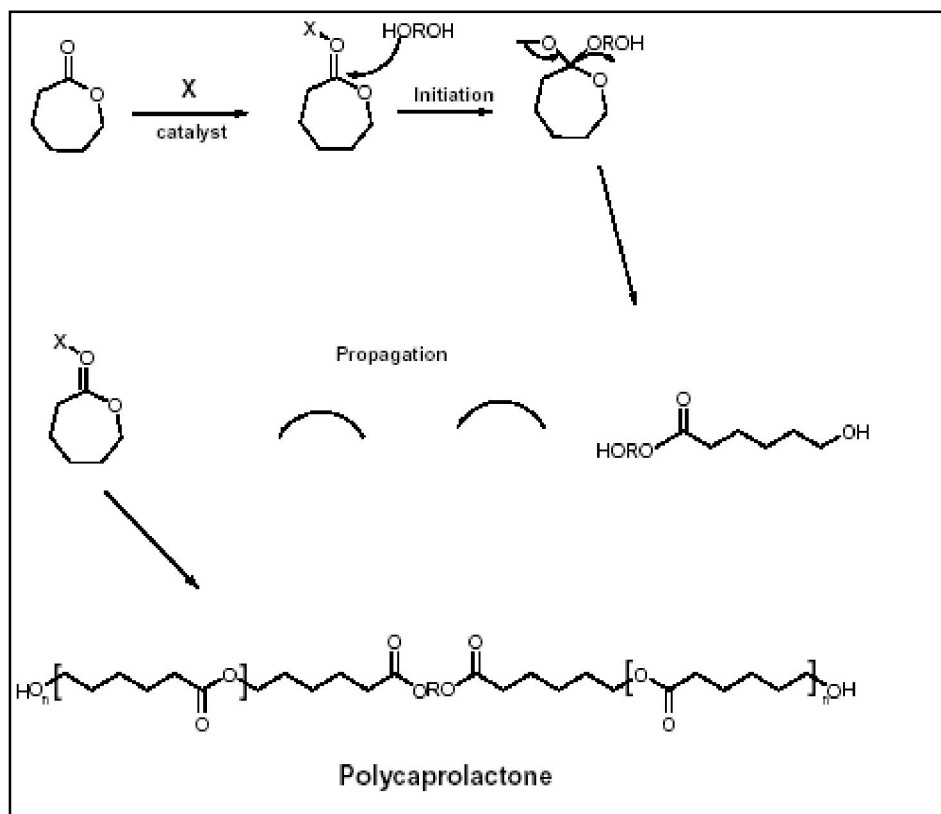


Figure 2.4 Ring opening polymerisation of caprolactones

Poly(caprolactone) (PCL) is recognized as one of the few synthetic polymers which are biodegradable. Like any polymer, the biodegradability is dependent on the chemical structure and not on the raw material source. The polymer backbone is hydrolyzable at the ester linkages, allowing for degradation by microorganisms. A proposed degradation scheme is presented in Fig. 2.5 (Goldberg, 1995). The inherent biodegradability of poly(caprolactone) was originally established in a series of fungal growth studies. These studies indicated that PCL could be utilized by microorganisms as an energy source. In

subsequent studies, the biodegradability of PCL has been confirmed in aerobic soil burial, compost, and respirometry studies. Furthermore, the ability of this polymer to be fabricated into useful articles has been demonstrated (Goldberg, 1995).

The PCL used as carbon source in this study was Dow Chemicals TONE P767. TONE polymers have desirable thermogravimetric and electrical properties. They have strong light stability and, unlike most commercial polymers, offer the unique property of being truly biodegradable when composted. TONE polymers available as pellets and can be fabricated by conventional thermoplastic polymer processing techniques (Producer's data sheet, DOW Chemicals). PCL and nitrate consumption by bacteria in denitrification are as follows.

$$+ 6 \quad \rightarrow 3 \quad + 6 \quad + 2 \quad + 6 \quad + \quad (2.2)$$

26

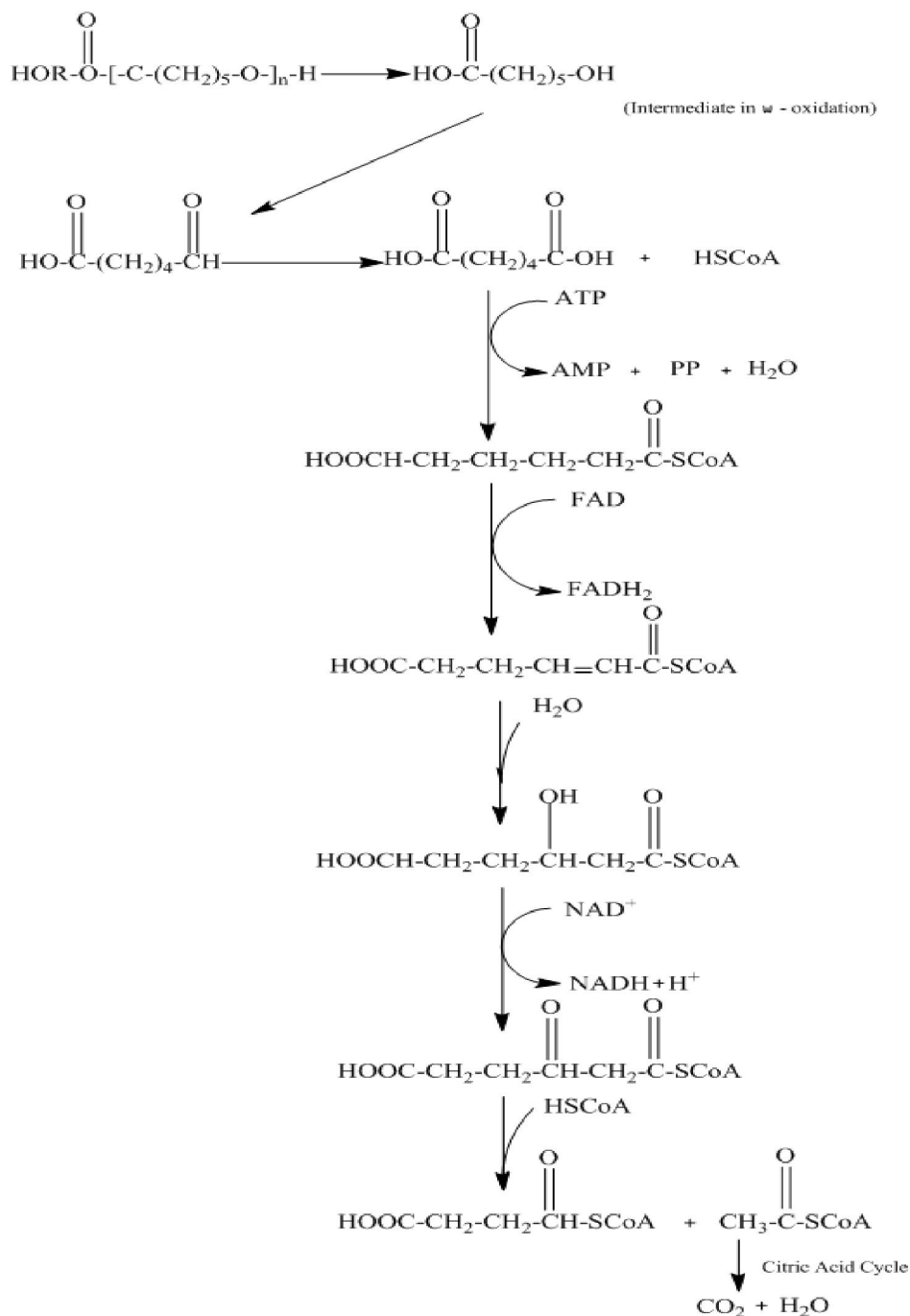


Figure 2.5 Proposed scheme for the biodegradability of poly(caprolactone).

2.5 Pesticides

A pesticide may be a chemical substance or biological agent (such as a virus or bacteria) used against pests including insects, plant pathogens, weeds, mollusks, birds, mammals, fish, nematodes and microbes that compete with humans for food, destroy property, spread disease. Pesticides are usually, but not always, poisonous to humans (WHO, 1985).

2.5.1 Pesticide Contamination in Drinking Water

Pesticides are among the major organic compounds encountered in wastewater effluents of pesticide industry and domestic activities. These compounds are also commonly used in agriculture to protect crops from pest organisms. Since most pesticides are used in open environment such as ploughed fields, residues of them have become one of the major diffuse pollution risks to the world drinking water supplies. Numerous cases of pesticide residue have been reported in the literature (Moore and Ramamoorthy, 1984). Residual concentrations of pesticides in the food products are alarming especially in the developing countries. Leafy vegetables, cereals, fruits, rice, meat, milk, fish intestine and edible portions and even human milk have been reported to be contaminated by various pesticides in a range of concentrations 0,1 mg/L – 25,7 mg/ L.

The contamination of water of ponds, rivers and ground water sources with various pesticides and herbicides is posing a direct threat to human

health. They are considered dangerous and harmful to living systems because of their toxicity and carcinogenicity even at low concentrations.

Environmental regulations in developed countries have become very strict for drinking water treatment over the last few years, especially regarding pesticide compounds.

To protect consumers from the adverse effects associated with high contamination intake, the United States, Canada, the European Union (EU), and the World Health Organization (WHO, 1985) have set standards to regulate contamination in water (Sayre, 1988; Deininger et al., 1998; Nalcaci et al., 2006).

2.5.2 Pesticide Usage

The agricultural area in the current 15 member states of the EU countries comprises over 130 million hectare. Almost 7 million farmholdings exist. By the sales data taken from Eurostat (the statistical agency of the European Union) and Oskam et al. (1997), the highest usage of pesticides was in Italy, followed by the United Kingdom and the lowest consumption of pesticides was in Finland between 1993-2000 as seen in Table 2.4 (Şirin, 2007).

The pesticide usage in Turkey can be shown in Table 2.5 (Delen et al., 2004). In Izmir amount of widely used pesticides between 2000 and 2004 is given in Table 2.6. It is seen that 2,4-dichlorophenoxyacetic acid

(2,4-D) which accounted for approximately 20% of total consumption between 2000 and 2004 is the second more common used pesticide after Trifluralin, an anilin group herbicide (Izmir Agriculture Management, 2004).

Table 2.4 Pesticide usage in EU countries

Country	1993-1995 (kg/ha)	1996 (tons)	1997 (tons)	1998 (tons)	1999 (tons)	2000 (tons)
Belgium	1,2	4.787	4.715	4.718	4.724	4.124
Denmark	1,7	2.632	2.685	3.283	2.915	-
Germany	2,6	15.941	17.790	15.394	12.696	14.834
Greece	13,5	-	2.252	2.442	2.579	-
France	5,6	-	-	27.419	36.052	-
Italy	9,3	24.468	25.848	25.921	24.913	24.943
Austria	4,0	1.873	1.549	1.607	1.536	
UK	6,4	2.0885	22.733	22.753	24.131	24.118
Sweden	4,4	1.097	1.507	975	1.235	
Finland	1,2	841	915	815	674	734

Table 2.5 Pesticide usage in Turkey (kg or L)

Pesticide Groups	1979	1987	1994	1996	2002
Insecticides	2.287.658	3.303.446	2.064.991	3.027.380	2.250.898
Acaricide	203.107	240.360	192.279	223.857	296.809
Lipids	1.594.526	2.147.106	2.147.106	2.871.160	2.428.238
Fumigant& Nematicides	315.665	322.227	530.738	1.076.661	1.559.489
Rodenticides &Molluscides	5.600	2.124	2.509	3.268	1.794
Fungicides	1.537.315	2.611.960	2.201.406	2.951.191	1.964.292
Herbicides	2.451.977	3.495.044	3.902.588	3.643.971	3.697.397
TOTAL	8.395.848	12.112.267	10.871.792	13.797.488	12.198.917

Table 2.6 Mostly used herbicides in Izmir during 2000-2004 (kg)

Herbicide	Herbicide Group	2000	2001	2002	2003	2004	%
2,4-D	Phenoxy Herbicide	25.340	17.801	13.281	14.752	24.632	17,41
Trifluralin	Anilin	26.280	32.875	30.382	113.073	122.740	59,12
Glyphosate isopropyla min	Aminophos phonates	12.852	15.814	28.942	27.656	34.163	21,70
Metalochlor	Amides and Anilides	3.100	1.543	515	500	4.090	1,77
TOTAL		67.572	68.033	73.120	155.981	185.625	100

2.5.3 Pesticide Regulations

The WHO guidelines for drinking-water quality on 1993 set standard for various pesticides from 0.03 µg/L to 100 µg/L. The level for 2,4-D is set as 30 µg/L.

In the EU Drinking Water Directive [Council Directive 98/83/EC] regarding to the quality of water intended for human consumption the values for individual pesticides and for total pesticides were retained 0.1 µg/L and 0.5 µg/L respectively, and more stringent values such as 0.03 µg/L were introduced for certain pesticides.

EPA National Primary Drinking Water Regulations set some non-enforceable levels, based solely on possible health risks and exposure, are called Maximum Contaminant Level Goals (MCLG). The directory of MCLG values of contaminants are tabulated in EPA Drinking Water Regulation sheets on web pages. For example, the MCLG for 2,4-D has been set at 70 µg/L because this level of protection would not cause any of the potential health problems . Based on MCLG, EPA has set an enforceable standard called a Maximum Contaminant Level (MCL). MCLs are set as close to the MCLGs as possible, considering the ability of public water systems to detect and remove contaminants using suitable treatment technologies. The MCL has been set at 70 µg/L for 2,4-D because of the given present technology and resources, this is the lowest level to which water systems can reasonably be required to remove this contaminant should it occur in drinking water (www.epa.gov/safewater/contaminants/index.html#listmcl).

2.5.4 Classification of Pesticides

Pesticides are classified according to the type of pest they control. Some type of pesticides were given in Table 2.7.

Table 2.7 Some pesticides

Type of pesticide	For the control of
Bactericides	bacteria
Herbicides	weeds
Fungicides	fungi
Insecticides	insects
Miticides	mites

2.5.5 Herbicides

Herbicide is a chemical compound that kills plants or inhibits their normal growth. A herbicide in a particular formulation and application can be described as selective or nonselective. Selective herbicides kill specific targets while leaving the desired crop relatively unharmed. Some of these act by interfering with the growth of the weed and are often based on plant hormones. Herbicides used to clear waste ground are nonselective and kill all plant material with which they come into contact.

2.5.6 Phenoxyalkanoic Acid Herbicides

Phenoxyalkanoic acid herbicides are widely used in agriculture to control the growth of broad-leaf weeds in crops (Fig. 2.6). These compounds are considered potential pollutants owing to their high soil mobility, long halflives and large scale of application. Due to their persistence, polar nature and water solubility, they are present in several environmental compartments including water, soil, cereals and vegetables. Hence, there is increasing interest in methods for the determination of phenoxyalkanoic acid herbicide residues as well as their phenolic metabolites in natural samples (Biesaga et al., 2005).

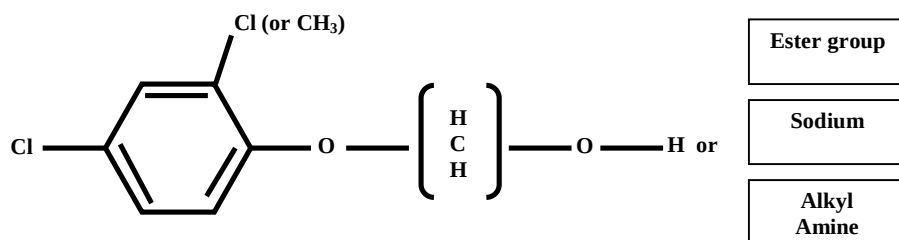


Figure 2.6 Chemical structure of phenoxyalkanoic acid herbicides

One class of phenoxy herbicides, the phenoxyacetic acids [2-methyl-4-chlorophenoxyacetic acid (MCPA), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5 trichlorophenoxyacetic acid (2,4,5-T); (2) the phenoxypropionic acids 2-(4-chloro-2 methylphenoxy)- propionic acid (MCP), 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP), 2-(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP); (3) the phenoxybutyric acids 4-(4-chloro-2-methylphenoxy)butanoic acid (MCPB) and 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB)] are listed in Fig. 2.7 (Argese et al., 2005).

Phenoxy herbicides are known as synthetic auxins and bring to cellular proliferation of components of the plants, which is rapid and out of control and causes their death. The phenoxy herbicides have low to moderate toxicity toward humans and animals. Some of them, such as 2,4-D and 2,4,5-T, have been extensively studied; however, their mechanism of action towards non-target organisms is not fully elucidated yet (Zychlinski and Zolnierowicz, 1990; Suwalsky et al., 1996; Palmeira et al., 1994).

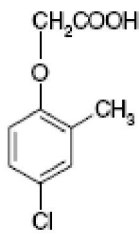
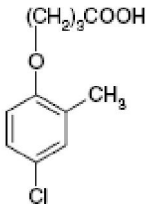
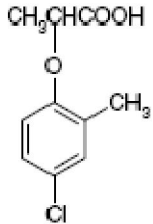
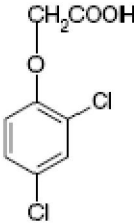
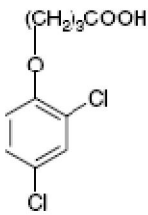
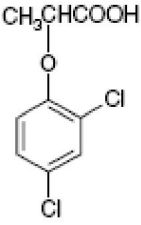
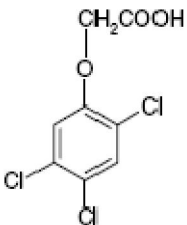
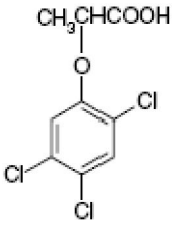
MCPA 	MCPB 
MCPP 	2,4-D 
2,4-DB 	2,4-DP 
2,4,5-T 	2,4,5-TP 

Figure 2.7 Structural formulas of the some phenoxyalkonic acid herbicides

2.5.7 Chemical Properties and Synthesis of 2,4-dichlorophenoxyacetic acid (2,4-D)

Chemical Properties: The basic form of 2,4-D is 2,4-dichlorophenoxyacetic acid (CAS 94-75-7) but is often formulated as an inorganic salt, amine or ester (WHO, 1984). The Chemical properties of 2, 4-D are given in Table 2.8 (Walters, 1999; EPA Ground water & drinking water, Technical Fact sheet on: 2,4-D, 2002).

Table 2.8 Chemical properties of 2, 4-D

Molecular weight	221,04
Melting point	138 °C
Boiling point (at 4 mmHg)	160 °C
Water solubility (average of 2 values near 25°C at pH=7)	0.5 g/L of water at 20 °C; Slightly soluble in water
Vapor pressure(at 25°C)	53 Pa at 160 °C
Hydrolysis half-life (average of two values at 25°C, pH=7)	39 days
Aqueous photolysis half-life(at 25°C)	13,0 days
Anaerobic aquatic half-life	312 days
Aqueous aerobic half –life	15,0 days
Aerobic half-life	66,0 days
Soil photolysis half-life	393 days
Field dissipation half-life	59,3 days
Henry's Law Coefficient	$1,02 \cdot 10^{-8}$ atm-cu m/mole
Octonal/water coefficient (Kow)	Log Kow = 2.81
Soil adsorption coefficient (Koc)	19.6 to 109.1; low to moderate mobility in soil

Synthesis of 2, 4-dichlorophenoxyacetic Acid: 2,4-D is commonly prepared by the condensation of 2,4-dichlorophenol with monochloroacetic acid in a strongly alkaline medium at moderate temperatures. The synthesis of Dow 2,4-dichlorophenoxyacetic acid is shown in Fig. 2.8.

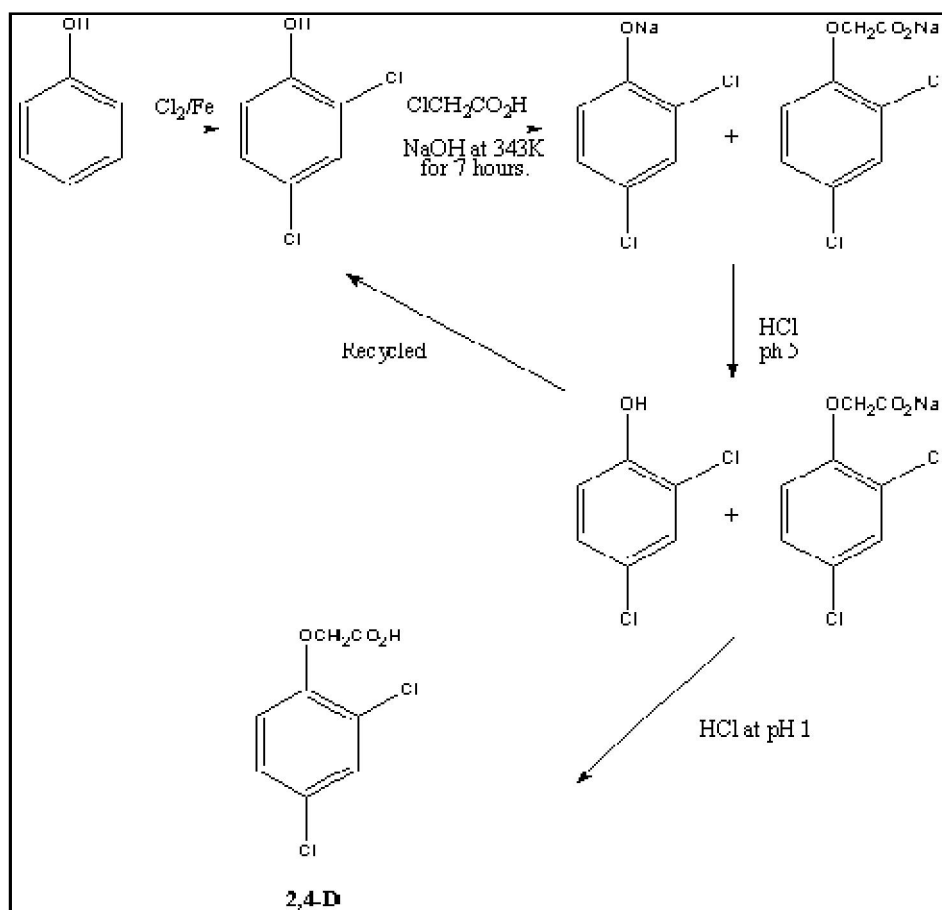


Figure 2.8 Synthesis of Dow 2,4-dichlorophenoxyacetic acid

2.5.8 Adverse Health Effects of Excessive 2,4-D Intake in Human Body

2,4-D can be absorbed through the skin or through the lungs if inhaled. Once in the body, 2,4-D is distributed rapidly with the greatest concentrations appearing in the kidneys and liver. The majority of the compound is excreted unmetabolized. Due to its solubility in water, 2,4-D is not believed to accumulate in tissues, but is excreted in the urine in less than a week (Ibrahim et al., 1991). Nevertheless, some agricultural workers and other applicators have experienced long term complications including pain and paralysis following exposure to 2,4-D.

In 1991, the case-control studies showed evidence of a relationship between 2,4-D exposure and non-Hodgkins lymphoma in humans, with some studies showing an increased risk with increased exposure level. In another study of human exposure, female applicators were found to have a significant increase in cervical cancer associated with 2,4-D application.

Due to the many confounding factors that make identification of cause and effect mechanisms difficult, other expert review panels including the EPA, Agriculture and Agri-food Canada, and the World Health Organization concluded that 2,4-D alone is not carcinogenic (Mullison and Bond, 1991).

2.6 Processes for Removing of Pesticide Contamination from Drinking Water

Powdered activated carbon (PAC) filtration, granulated activated carbon (GAC) filtration, and reverse osmosis have been demonstrated to be highly effective water treatment processes for removal of organic chemicals, including certain pesticides (primarily acetanilide herbicides), but specific removal data on most pesticides are not available. Among these treatment processes, PAC is the more common method because it can be used in concert with conventional water treatment systems with no significant additional capital investment. Available data suggest that about 46% of large community water systems (those that serve > 100,000 people) use PAC at some time during the year, and that most of these systems are surface water-based systems. Air stripping is an effective water treatment for volatile pesticides, but this method is used at less than 1% of the community water systems.

2.6.1 Adsorption of 2,4-D from drinking water

The best method for removal of 2,4-D from water is the adsorption as explained above. This operation can be carried out several kinds of adsorbents. Some adsorbents used in 2,4-D removal were tabulated in Table 2.9.

Table 2.9 Different adsorbents used in the adsorption of 2,4-D from water

Adsorbate	Material Type	Adsorbent Type	Adsorbent	References
2,4-D	Inorganic	Alumina	γ -Alumina	Clausen et al., 2001
			Alumina	Goyne et al., 2004
			Zinc-aluminium-chloride layered double hydroxides	Legrouri et al., 2005
		Silica	Silica-supported β -cyclo dextrin derivatives	Phan et al., 2002
			Chemically modified silica gel surface	Prado and Airoidi, 2001
			Silica	Goyne et al., 2004
		Zeolites	Zeolite-supported TiO_2	Shankar et al., 2006
		Hydrotalcite	Calcinated Hydrotalcite	Pavlovic, 2005

2,4-D			Sepiolite and DAS	Akçay et al., 2005
	Organic	Polymer	Modified hypercrosslinked polystyrene (NDA-99)	Qiu et al., 2005
			Crosslinked Poly(N-vinyl pyrrolidone)	Saraydin and Karadag, 1996
			Waste tyre rubber granules	Alam et al., 2006
			Rubber granules,	Alam et al., 2000
		Carbon	GAC SLS103, WWL	Cho et al., 2003
			H- type Gac 6-16 mesh size	Aksu and Kabasakal, 2004
			PAC	Aksu and Kabasakal, 2005
			GAC F400	Chingombe et al., 2006
				Cho et al., 2003

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2,4-D				Kim et al., 2002
			Viz.wood charcoal, bottom ash	Alam et al., 2000
			CH supplied PAC & (CL)(grad 20070D)	Belmouden et al., 2000
		Clay	Organo-clays	Akçay and Yurdakoç, 2000
		Soil	Alfic Dystric Eutrocrept	Morillo et al., 2001
			Sonning Soil I-II-III	Spark and Swift, 2002
			Thirteen Constrasting Field Soils	Boivin et al., 2005
			Sepiolite and DAS	Akçay et al., 2005
	Biological	Macro fungi sajor caju and florida		Alam et al., 2000
		Fertilizer and Steel Industry Wastes		Gupta et al., 2006

2.6.2 Adsorption equilibrium modeling in a batch system

The adsorption capacity can be correlated with the variation of surface area and porosity of the adsorbent. Higher surface area and pore volume will result in higher adsorption capacity. Equilibrium data, commonly known as adsorption isotherms, are basic requirements for the design of adsorption systems used for the removal of organic pollutants. The Langmuir, Freundlich are the most frequently used two-parameter models in the literature describing the non-linear equilibrium between adsorbed organic pollutant (adsorbate) on the adsorbent (q_{eq}) and organic pollutant in solution (C_{eq}) at a constant temperature.

2.6.3 The Langmuir Model

The Langmuir equation which is valid for monolayer sorption onto a surface with a finite number of identical sites is given by Eq.(2.3).

$$q_{eq} = \frac{Q_o b C_{eq}}{1 + b C_{eq}} \quad (2.3)$$

where q_{eq} is the amount adsorbed (mg/g), C_{eq} is the equilibrium concentration of the adsorbate (mg/L), and parameters Q_o and b are Langmuir constants related to maximum adsorption capacity (mg/g) (monolayer capacity) and bonding energy of adsorption (L/mg), respectively, which are functions of the characteristics of the system as well as time (Langmuir, 1918).

The equation $\left[\frac{1}{q_{eq}} = \frac{1}{Q_o} + \frac{1}{bQ_o C_{eq}} \right]$ is the linearized form of the

Langmuir equation, for $1/q_{eq}$ versus $1/C_{eq}$, for the adsorption of pesticides showing the applicability of the equation. The values of monolayer capacity (Q_o) and Langmuir constant (b) have been evaluated from the intercept and the slope of this plot.

The adsorption capacity can be correlated with the variation of surface area and porosity of the adsorbent. Higher surface area and pore volume will result in higher adsorption capacity. The Langmuir equation is used for homogeneous surfaces.

2.6.4 The Freundlich Model

The Freundlich model assumes neither homogeneous site energies nor limited levels of sorption. The Freundlich equation has the general form:

$$q_{eq} = K_F C_{eq}^{1/n} \quad (2.4)$$

where q_{eq} is the amount adsorbed (mg/g), C_{eq} is the equilibrium concentration of the adsorbate (mg/L), K_F ((mg/g)*(mg/L)ⁿ) and n are the Freundlich constants related to adsorption capacity and adsorption intensity, respectively (Freundlich, 1906). Eq. (2.4) can be linearized in logarithmic form.

The linearized form of Freundlich equation is in the form of

$\left[\ln q_{eq} = \ln K_F + \frac{1}{n} \ln C_{eq} \right]$. A plot of $\ln q_{eq}$ versus $\ln C_{eq}$ would give a

straight line, slope of which is $1/n$ and intercept is $\ln K_F$. The value of K_F can be used as alternative measure of adsorption capacity, while $1/n$ determines the adsorption intensity.

2.7 Processes for Simultaneous Removal of nitrate and pesticides from drinking water

Simultaneous removal of nitrate and pesticide from drinking water was investigated by several authors in literature. Berbar investigated separation of nitrate from water in the presence and in the absence of a commercial pesticide commonly used in the region by ion exchange process (Berbar et al., 2008). Centi used catalysts to eliminate nitrate and pesticides from contaminated water. It is reported that using a Pd-Cu-based catalytic membrane, a reaction temperature below 15 °C, a mixed 4:1 CO₂:H₂ feed and controlling bulk solution pH by HCl addition, it is possible to obtain a nitrate conversion higher than 80% even with ammonium ion formation below 0.5 mg/L, i.e. the maximum concentration allowed to meet the requirements for drinking water quality (Centi, 2003). Combined removal of nitrate and pesticide was also investigated in fixed bed (Urbain, 1996), bioelectrochemical/adsorption (Feleke, 2001), and fluidized bed systems (Katz, 2000). Van der Bruggen used nanofiltration removing pesticides, hardness and nitrates (Van der Bruggen et al., 2001).

Table 2.10 shows some studies conducted for simultaneously removing of nitrate and pesticides from water.

Table 2.10 Simultaneous removal of nitrate and pesticides from water

Technique	Pesticide	Investigated Parameter	Nitrate Removal		Pesticide Removal			Reference
Anion exchange resin (Amberlite IRA 900)	Malathion (commercial pesticide)	Chloride/Nitrate ratio, Contact time, Fixation rate, Nitrate and Pesticide removal	Initial Conc (mol/L)	Fixation Rate for 15 min Contact (%)	the more concentration of chloride anion, the more influence of the pesticide on the fixation process			Berbar, et al. (2008)
			10^{-4}	75,8				
			10^{-3}	70,6				
			10^{-2}	68,9				
Anion Exchange Resins (MIEX®, DOWEX-11®, DOWEX-MSA® and IRA-938®)	Atrazine and Isoproturon	Mineral ion (nitrate, sulfate, bromide) removal, Dissolved organic carbon levels, Pesticide removal	Nitrate concentration, respectively, dropped from 13,5 to 8,7 mg/L initially to less than 0,5 mg/L after 3 min of contact time.		limited sorption of atrazine and isoproturon	Atrazine	Iso proturon	Humbert, et al. (2005)

Nanofiltration (membranes DESAL 51 HL, DESAL 5 DL, UTC-20 and UTC-60)	atrazine and simazine	Rejection of pesticides, Pore size, Pesticide removal, pH, Nitrate concentration, Conductivity	The rejection of nitrate from river water is lower than from distilled water and the nitrate rejection from tap water was similar to the rejection from distilled water.	Rejection of atrazine and simazine from tap water and river water is generally higher than those from distilled water.					Zhang et al (2004)	
Nanofiltration (membranes NF70, NF45, UTC-20 and UTC-60)	atrazine, simazine, diuron and isoproturon	Water flux as a function of time, Pressure, Rejection of pesticides, Hardness and Nitrates	With a nitrate inlet of 44 mg/L		µg/L	NF 70	NF 45	UTC 20	UTC 60	Van der Bruggen et al. (2001)
			Membrane	Outlet (mg/L)	Atrazine	0,07	0,14	0,17	0,18	
			NF70	11	simazine	0,1	0,36	0,29	0,29	
			NF45	37						
			UTC-20	30	Diuron	0,08	0,49	0,5	0,51	
			UTC-60	39	iso- proturon	0,10	0,25	0,27	0,21	

2.8 Mathematical Modeling of Biological Denitrification

Michaelis-Menten Kinetics: A set of experimental rate data shown in Fig.2.6 can be represented mathematically. From information of the type of curve shown in Fig 2.9, the following qualitative features often emerge (Bailey, 1983).

- Ø The rate of reaction is first order in concentration of substrate at relatively low values of concentration. (Recall that if $v = (\text{const}) \cdot (S^n)$, n is the order of reaction rate.)
- Ø As the substrate concentration is continually increased, the reaction order in substrate diminishes continuously from one to zero.
- Ø The rate of reaction is proportional to the total amount of enzyme present.

Henri observed this behavior, and in 1902 proposed the rate equation.

$$v = \frac{V_{\max} S}{K_m + S} \quad (2.5)$$

which exhibits all three features listed above. Notice that $v = V_{\max}/2$ when s is equal to K_m , s is the concentration of the free substrate in the reaction mixture, while e_0 is the concentration of the total amount of enzyme present both the free and combined forms.

Although Henri provided a theoretical explanation for Eq.2.5 using a hypothesized reaction mechanism, his derivation and the similar one offered in 1913 by Michaelis and Menten are now recognized as not rigorous in general. However the general methodology of the Michaelis-Menten treatment will find repeated useful (although still not generally rigorously justified) application in derivation of more complicated kinetic models.

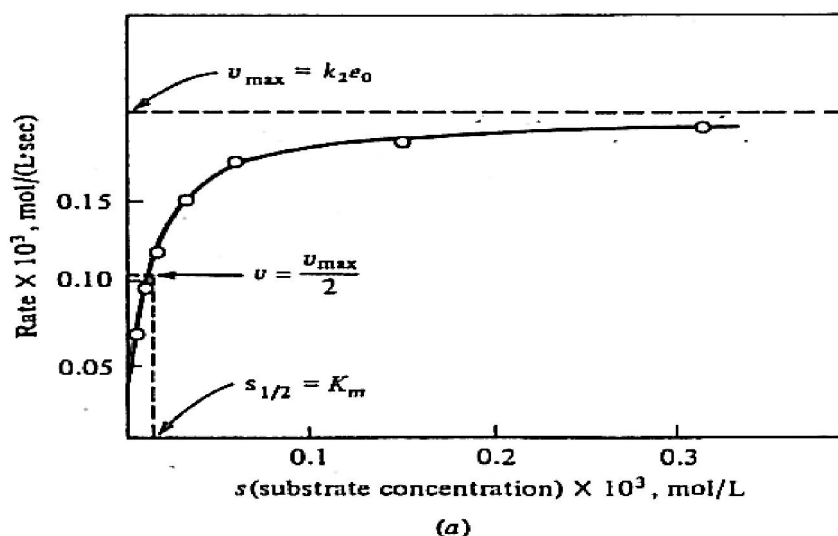


Figure 2.9 Kinetic data for enzyme-catalyzed reactions (Bailey, 1983)

Monod Kinetics: In a batch culture, if one of the essential requirements (substrate and nutrients) for growth were present in only limited amounts, it would be depleted first and growth would cease. In a continuous culture, growth is limited. Experimentally, it has been found that the effect of a limiting substrate or nutrient can often be defined adequately

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using the following expression proposed by Monod (Tchobanoglous, 1991)

$$\mu = \mu_m \frac{S}{K_s + S} \quad (2.6)$$

where μ is specific growth rate, time^{-1} , μ_m maximum specific growth rate, time^{-1} , S concentration of growth limiting substance in solution, (mass/unit volume) K_s half velocity constant, substrate concentration at one half the maximum growth rate, (mass/unit volume). The effect of substrate concentration on the specific growth rate is shown in Fig. 2.10.

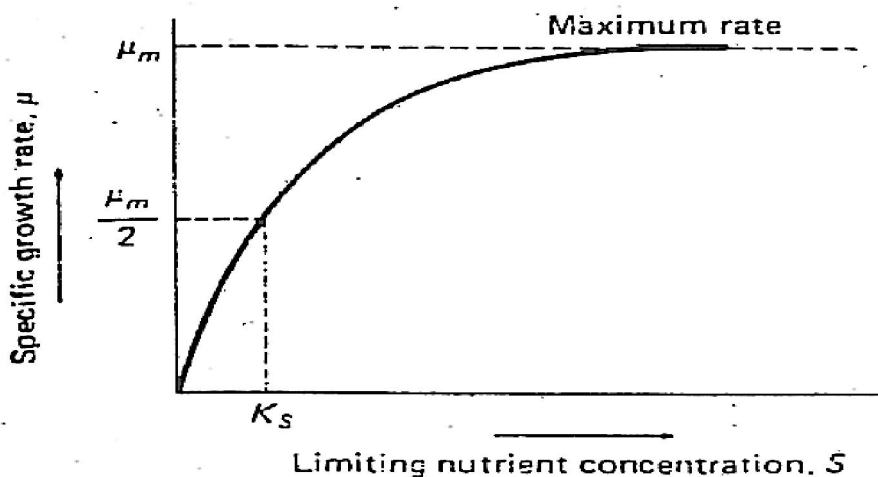


Figure 2.10 Plot showing the effect of a limiting nutrient on specific growth rate.

3 Material and Methods

3.1 Materials and Equipments

The chemicals used in the experiments were TSA-Tryptic Soy Agar (Merck), TSB-Tryptic Soy Broth (Criterion), ammonium iron (III) citrate (Sigma), 2,4-dichlorophenoxy acetic acid (2,4-D) (Sigma-Aldrich); , pH=4 and pH=7 buffer (WTW), C₂H₅OH (Kimetsan); KH₂PO₄ (J.T.Baker); MgSO₄*7H₂O (Riedel); NaCl (Merck); NH₄Cl (Uparc); NaNO₃ (Merck); Na₂HPO₄*2H₂O (J.T. Baker); Na₂Mo₄.2H₂O (Merck); Poy(ε-caprolacton) (PCL Tone P767) (Dow Chemicals).

The devices used in the experiments were, shaker (Memmert WNB 7-45), peristaltic pump (Watson-Marlow 403 UL2, 403-VM4 (10 rpm)), ,tubing (Watson–Marlow marprene grey 1.6, 3.2 mm), micropipette (Socorex Acura Manual 825), pH meter (WTW pH 330/set), autoclave (HMC HIRAYAMA Hiclave HV-50L), magnetic stirrer (IKAMAG RO5 power, Selecta multimatic-5N), scale (AND HM-200), glass Vacuum Filter Holder (Sartorius), cellulose filter with a pore size of 0.45 µm (Sartorius), vortex (Bio Vortex V-1 plus), microwave oven (Bosch 812-c), incubator (Nuve), petri dishes (Isolab disposal), spectrophotometer (Jasco Model 7800 UV/VIS Spectrophotometer), platinum wire loop, Liquid chromatography/Mass Spectrometry (LC/MS-MS) (API 4000 LC/MS/MS system), photometer (PhotoLab, WTW), Sartorius minisart-plus filter with a pore size of 0.45 µm,

3.2 Methods

The methods applied to carry out experiments are briefly discussed below.

3.2.1 Mixed bacteria Culture in Denitrification

Activated sludge: The activated sludge was waited for a night to obtain a much more dense mixture. The supernatant is removed and the sludge is used in the batch denitrification experiments.

Soil suspension: Soil samples were sieved so as to have approximately 1 mm particle size. 0.18 % of sodium pyrophosphate solution ($\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$) of 90 mL was added to 10 gram of sieved soil sample. This mixture was shaken for one hour then the supernatant was used as the bacterial source for the denitrification reaction (Lindahl, 1996).

3.2.2 Reviving the strain LMG 17238

For this project *Acidovorax avenae* subsp. *avenae* LMG 17238 provided from Public Collection curator of BCCM/LMG Bacteria Collection, Ghent University, Faculty of Sciences, Belgium was used. Schematic view of the reviving of LMG 17238 can be seen in Fig. 3.1. Transferring bacteria in Tryptic Soy Broth (TSB) from one tube to another is shown in Fig. 3.2.

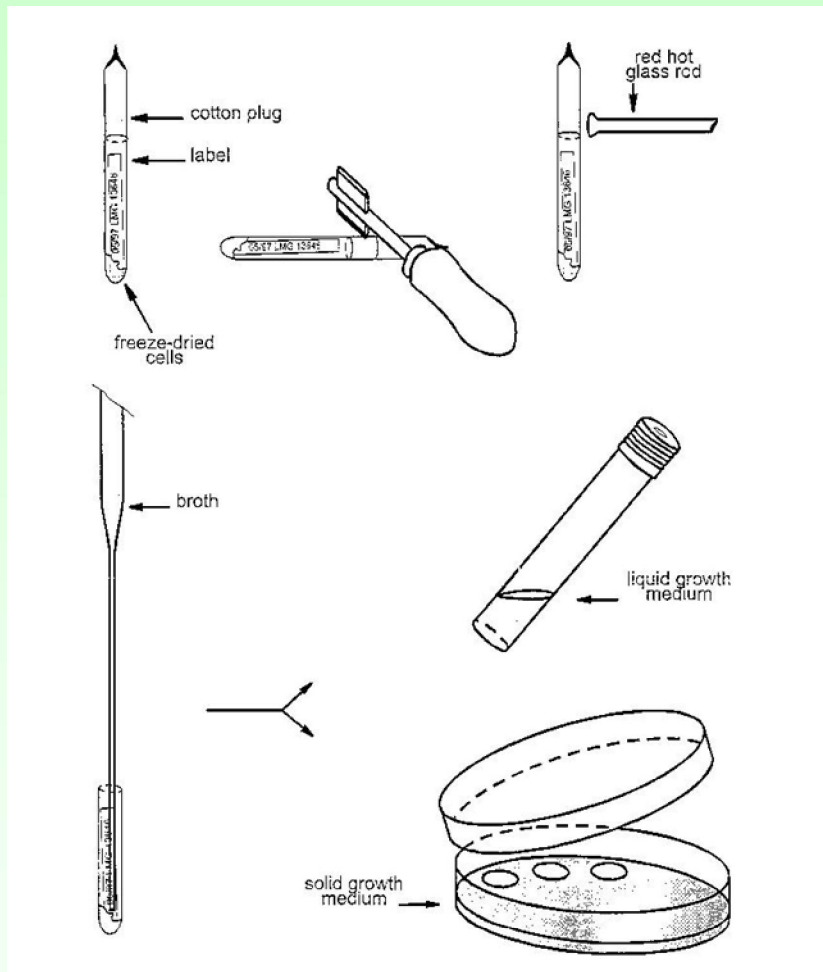


Figure 3.1 Reviving of the pure bacteria culture *LMG 17238*.



Figure 3.2 Transferring of bacteria in TSB from one tube to another

3.2.3 Preparation of *LMG 17238* for using in experiments

A primary bacteria culture on the Tryptic Soy Agar (TSA) slope was prepared and waited in the incubator for 48 hours. At the end of this time 0.86% NaCl was added to the tube, vortex applied. The bacteria culture collected in a capped tube.

3.2.4 Enriched Bacteria Culture and Bacteria Attached PCL

1 g of NH_4Cl , 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.05 g of ferric ammonium citrate are dissolved in 1 liter of 33 mM KH_2PO_4 - $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ buffer, (pH = 6.8) and sterilized (Delafield, et al., 1965). PCL and the bacteria culture (Sec. 3.2.3) were added to this solution and shaken for three weeks obtaining enriched bacteria culture and bacteria attached PCL (Fig. 3.3).

3.2.5 Recycle solution

Recycle solution of 50 mg/L NO_3^- -N, 5 mg/L PO_4^{3-} -P, 5 mL SL6 was prepared. Mixing of recycle solution and enriched bacteria culture was used for inoculation of fixed bed (Mergaert et al., 2001).

3.2.6 Feed solution

The feed solution composition was (NO_3^- -N=23 mg/L, PO_4^{3-} -P = 3 mg/L, MoO_4^{2-} = 0.1 mg/L).

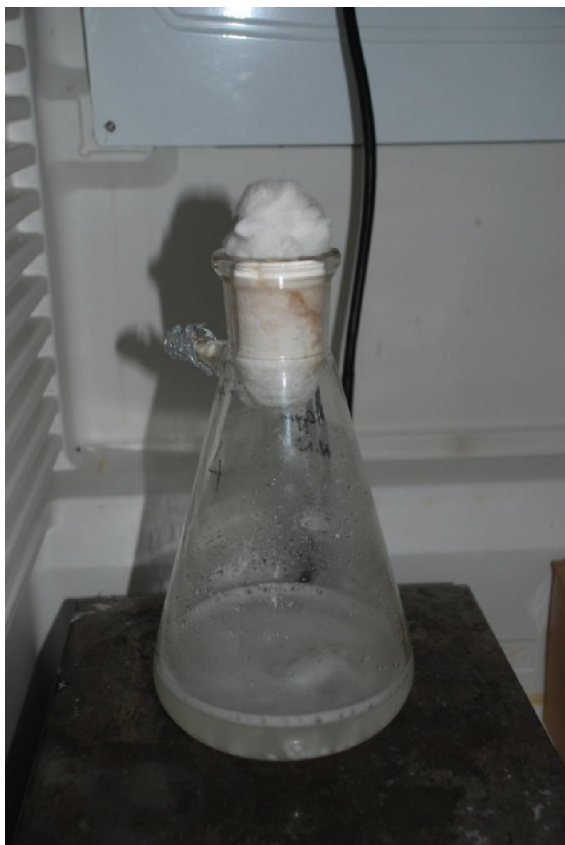


Figure 3.3 Preparation of Enriched Bacteria Culture and bacteria attached PCL.

3.2.7 Sterilized Heavy Metal Ion Solution

Since heavy metal ions (ferric ammonium citrate solution, SL6, sodium molybdate dehydrate) can not be sterilized by autoclaving they are filtered through Sartorius cellulose filtered nitrate with vacuum filtration and stored in capped tubes and placed in the refrigerator.

3.2.8. Viable Count for the Bacteria Culture

Nine milliliter of solutions from a stock solution of 0,86% of NaCl (physiological salt water) were pipetted into sterilized test tubes with plastic caps. These are dilution blanks. The tubes were labeled as 1, 2, 3, 4, 5, 6, 7, and 8 for the 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , and 10^8 dilutions. One milliliter of the sample bacteria suspension was pipetted into the first dilution blank and applied vortex. Then one milliliter of solution from the first tube was pipetted into the second dilution tube and applied vortex. This procedure was continued from the first tube to the eighth tube.

Twin petri dishes were labeled from 10^{-4} to 10^{-8} . One milliliter of the diluted bacteria solution from each tube placed into the center of petri dishes. Fifteen milliliter of melted TSA (50°C) was poured into the each of petri dishes and the petri dishes mixed as follows: The dish was gently moved six times in a clockwise circle of diameter of 15 cm. This movement was repeated counter-clockwise. The dish was moved back and forth six times with an excursion of about 15 cm. To-and-fro movements were repeated. The medium was allowed to settle, inverted, and placed in the incubator at 30°C . After 48 hours colonies in twin petri dishes were counted and average counts between 30 to 300 were evaluated as the result of viable count in cfu/mL (Collins, 1976). Flowsheet and photographs of the procedure can be seen in Fig. 3.4 and Fig. 3.5, respectively. In Fig. 3.5 steps of the viable count procedure were shown in alphabetical order.

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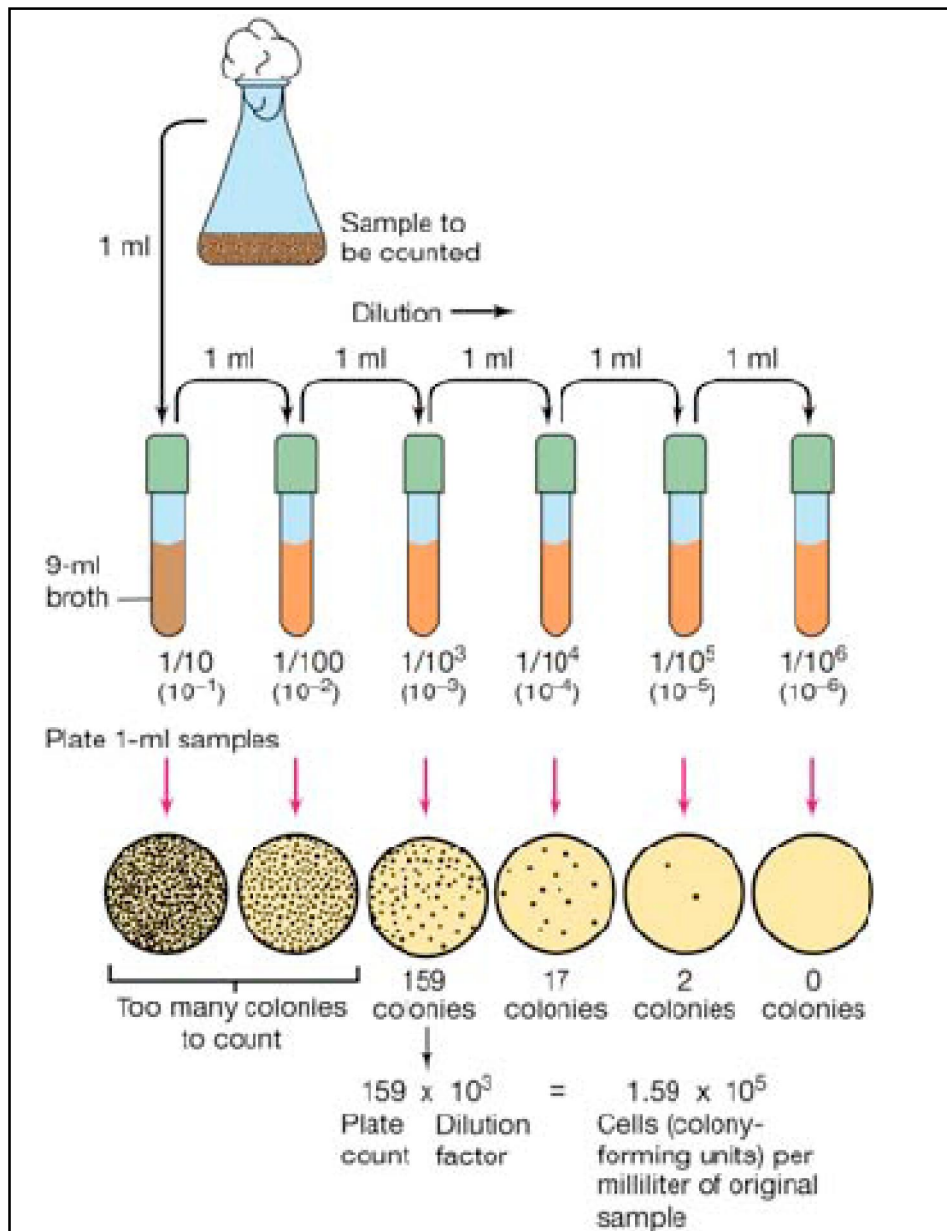


Figure 3.4 Schematic view of viable count technique.

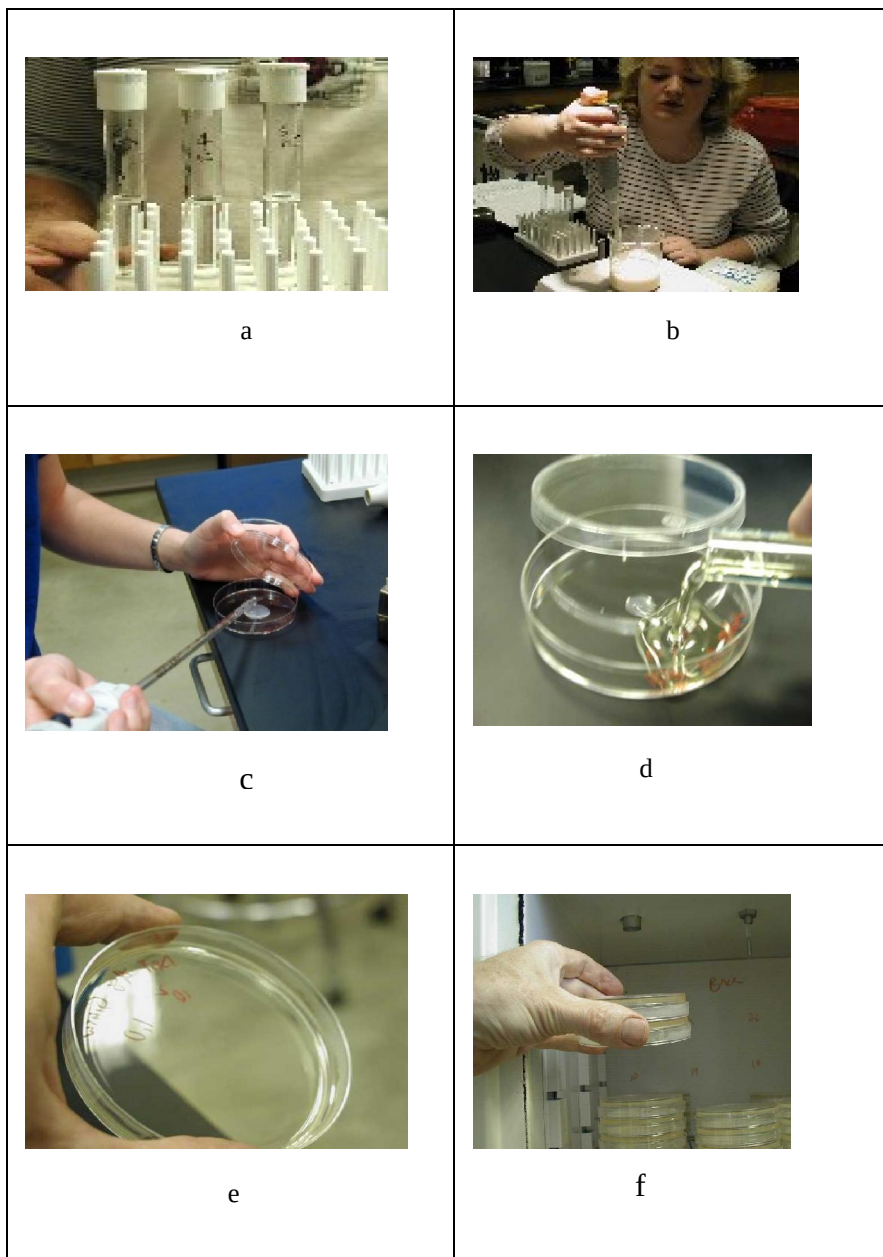


Figure 3.5 Photographs of viable plate count technique

3.2.9 Nitrate Measurements

Denitrification reactor inlet and outlet nitrate contents were measured in WTW PhotoLab instrument. The photometer possesses AQA (Analytical Quality Assurance) functions to assure the quality of the measurement.

3.2.10 UV/VIS Spectrometry Measurements

Concentration of aqueous solutions of 2,4-D were measured in Jasco Spectrophotometer, 7800 UV/VIS. Absorbance of standard solutions (2.0, 4.0, 6.0, 8.0, 10.0 and 20 mg/L 2,4-D), blank and samples were measured at $\lambda_{\max} = 228$ nm. Sample concentrations were calculated via standard line.

3.2.11 Liquid Chromatography-Mass Spectrometry (LC-MS-MS) Measurements

Denitrification inlet and outlet streams were bottled according to standards (filtering through Sartorius Minisart-plus filter, pore size of 0.45 μm). 2,4-D concentration of samples were determined in API 4000 LC-MS-MS device at Ege Üniversitesi İlaç Geliştirme ve Farmakokinetik Araştırma-Uygulama Merkezi (ARGEFAR). Flow rate in the chromatography column was 0.4 mL/min. Chromatographic separation was carried out in a Synergi 2.5 μm Fusion-RP column. Column mobile phase consists of A (aqueous 0.05 % aqueous ammonium

acetate) and B (0.05 % ammonium acetate solution in methanol) solutions.

MS-MS is triple quadrupole type. After purification in LC the sample is turned to molecular ion in electrospray part of the device. This ion was determined in the first mass spectrometer (MS1) and sent to collision cell. The molecular ion was fragmented in collision cell and these fragments were determined when passing the second spectrometer (MS2).

Fig. 3.6 shows photograph of the LC-MS-MS. The mass spectrum of 2,4-D obtained from one of measurements can be seen in Fig. 3.7.



Figure 3.6 Photograph of the LC/MS-MS

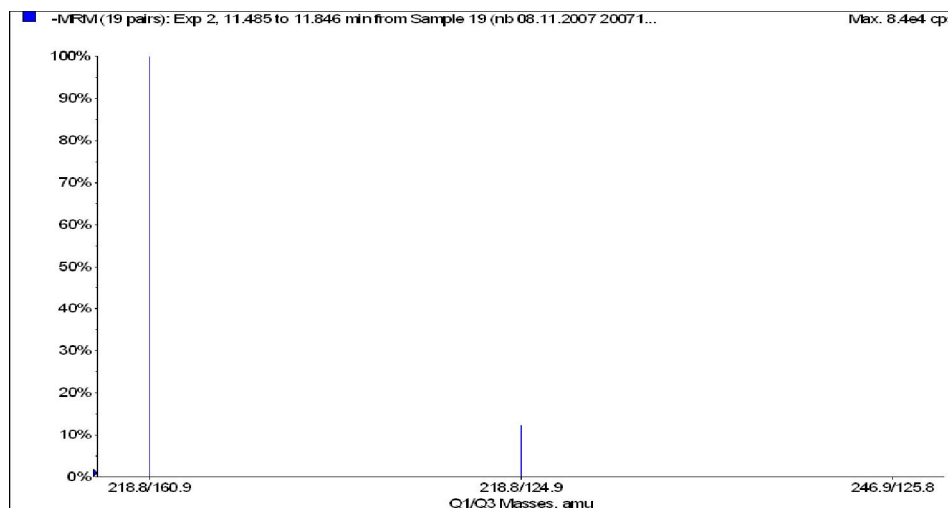


Figure 3.7 The mass spectrum of 2,4-D.

3.2.12 Preparation bacteria attached PCL for Scanning Electron Microscope (SEM)

Samples were prepared twice rinsing the biofilm covered PCL granules with cacodylate (0.1 M), waiting half an hour in glutaraldehyde (2.5 %) in 0.1.M cacodylate buffer, rinsing with cacodylate and ethanol, and finally covering with gold for SEM measurement. SEM measurements were carried out with the environmental scanning electron microscope (ESEM) (Philips XL 30 FEG) in Karlsruhe, Germany.

3.2.13 The Batch Biological Denitrification Experiment

Reactors: The denitrification experiments were done in 500 mL glass reaction vessels (anoxic Eudiometer) with a water medium (250 mL) in

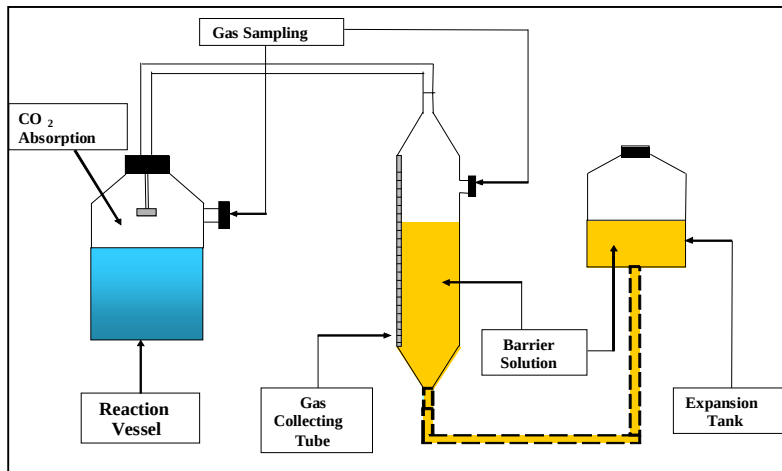
each reactor. PCL was placed in the reactor as the substrate. Reaction vessels were connected via gas-tight tubes to a graduated gas-collecting glass tube (Fig 3.8) (Ovez, 2006).

The glass reaction vessel has a small side outlet for taking water samples. Nitrogen stripping was used to eliminate the initial oxygen and further inlet was prevented with the use of gas-tight tubes and fittings. The gas-tight tube is connected to an expansion tank that can be moved up and down to equalize the levels of the barrier solution (aqueous solution of 20% NaCl and 0.5% citric acid) inside the gas-tight tube and in the expansion tank. In the reaction vessel, connected to the lid over the liquid surface, there is a container that is filled with soda-lime to adsorb CO₂. In order to achieve reproducibility, the experiment was duplicated. The tests were performed in total darkness to obtain the condition suitable for natural denitrification. The volume of N₂ gas (mL) in the collecting tube, atmospheric pressure and temperature were recorded daily. At the end of the experiment, the nitrate concentrations of the water media in the reactors were measured.

Water Medium: In this study, the synthetic standard water medium in the reaction vessels was based upon the medium in DIN V 54900-2 or ISO 14851 with the addition of a 0.5% inoculum, a trace element solution (per L: ZnSO₄·7H₂O (0.2 mg), MnCl₂·4 H₂O (0.06 mg), H₃BO₄ (0.6 mg), CoCl₂·6H₂O (0.6 mg), CuCl₂·6 H₂O (0.02 mg), NiCl₂·6 H₂O (0.04 mg), and Na₂MoO₄·2H₂O (0.06 mg)), and also NaNO₃.

3.2.14 The Batch Adsorption Experiment

Stock solution (100 mg/L) of 2,4-D was prepared by dissolving accurately weighted amounts of material in pure water. Solutions for the experiment were prepared by diluting the stock solution. Batch adsorption experiments were carried out shaking the 2 g of PCL in 50 mL of 2,4 at a constant shaking rate (90 rpm) for 10 days to ensure equilibrium. Concentration of solutions were in the range of 20 to 100 mg/L. The progression of adsorption was determined from the difference between the initial and actual 2,4-D concentration in the supernatant solution. Duplicate experiments were performed and average values were used in the evaluation of adsorption of 2,4-D on PCL (Ovez et al., 2006).



(a)



(b)

Figure 3.8 Flowsheet (a) and photographic view (b) of the batch biological denitrification.

3.2.15 The Continuous Biological Denitrification Experiment

Glass plastic tapped columns were used in carrying out denitrification experiments. Columns were sterilized by recycling 70% ethanol by volume and sterilized pure water. Column volume was measured by filling the column with water. PCL was weighted, filled in the column and water added to fill the column; dead volume was also measured. Using these values packed bed volume and void fraction were calculated (Table 3.1). Volume specific surface and mass specific surface values of the PCL bed can also be seen in this table.

The continuous flow reactor for denitrification was set up as an upflow fixed bed. The aim was to determine the denitrification capacity of PCL as carbon source in continuous denitrification.

The continuous biological denitrification system consisted of a glass bottle of stock feed solution, peristaltic pump, glass denitrification column and a closed bottle for collecting outlet stream. Flowsheet and photographs of the biological denitrification column can be seen in Fig. 3.9 and Fig. 3.10, respectively. Inlet flow, entering into the bottom of the column, exits top of the column with a reduced NO_3^- -N content. The outlet liquid, sent to top up of a sand column exited the bottom having no nitrate.

In order to obtain an efficient denitrification, bacteria must be attached to the PCL granules of fixed bed before denitrification operation started.

Table 3.1 Specifications of the continuous denitrification columns

PCL in the column (gram)	300
Column diameter (m)	0,038
Column area ($\pi D^2/4$) (cm ²)	11,32
Column volume (V _c) (mL)	520
Water discharged from the column filled with PCL (mL)	205
Dead volume (mL)	90
Total particle volume (N _p *V _p) (mL)	520-205= 315
Bed Volume (V _{bed}) (V _{Bed} = V _c - V _{dead}) (mL)	520-90 = 430
Void volume (V _{Void}) (V _{Void} = V _{Bed} - N _p *V _p) (mL)	430-315= 115
Void fraction (V _{Void} / V _{Bed}) (ε)	0,27
Fixed bed height (L) (cm)	38
Mean diameter of a PCL particle (mm)	3,78
Volume of a PCL particle (V _p) (V _p = (4/3*π*r ³) (m ³)	2,83*10 ⁻⁸
Calculated surface area of a PCL particle (A _p) (A _p = (4*π*r ²) (m ²)	4,49*10 ⁻⁵
Fixed bed particle number (N _p) (N _p = V _{Bed} * (1-ε) / V _p)	10976
Flow area (πD ² /4*ε) (cm ²)	3,39
Volume Specific Surface (VSS) (m ² /L)	1,56
Mass Specific Surface (MSS) (m ² /kg)	1,64

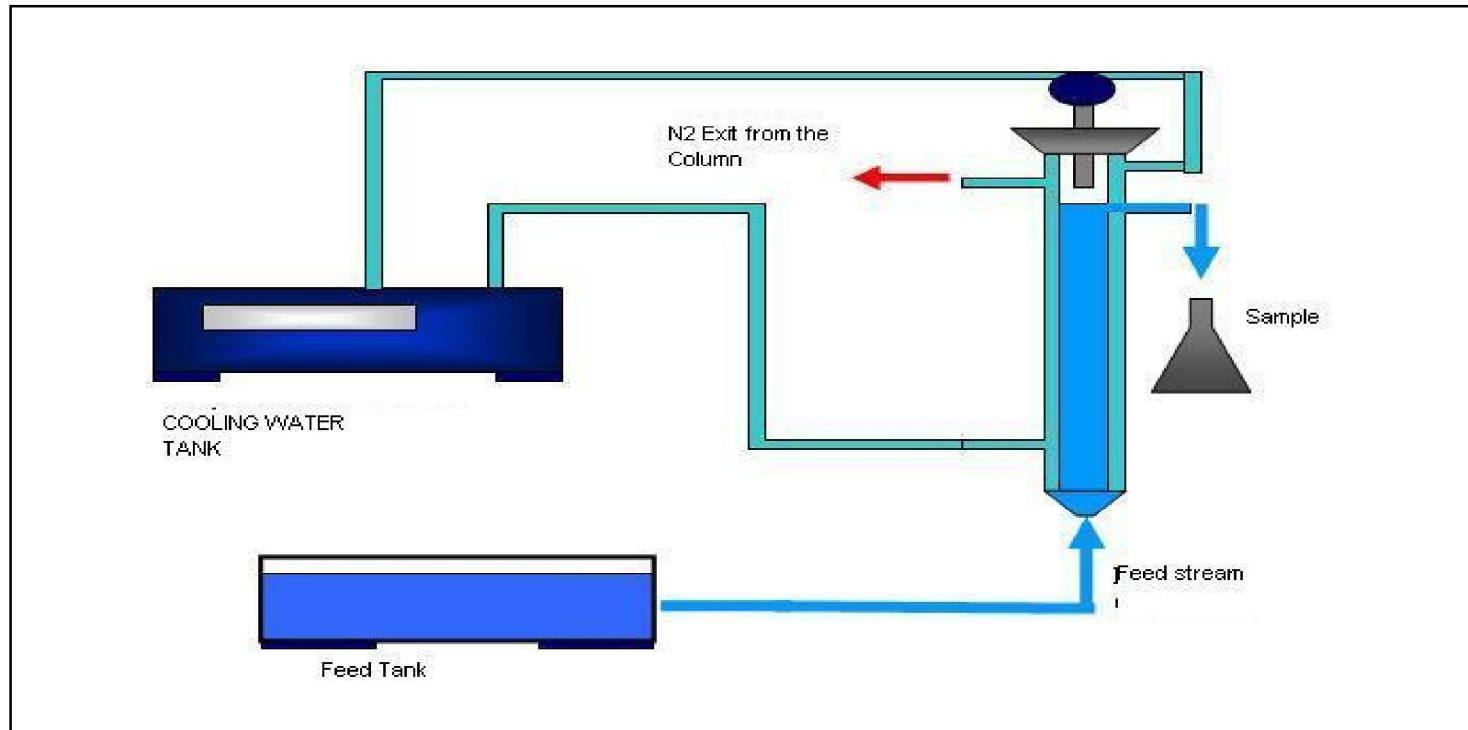


Figure 3.9 Flowsheet of the Continuous Biological Denitrification.

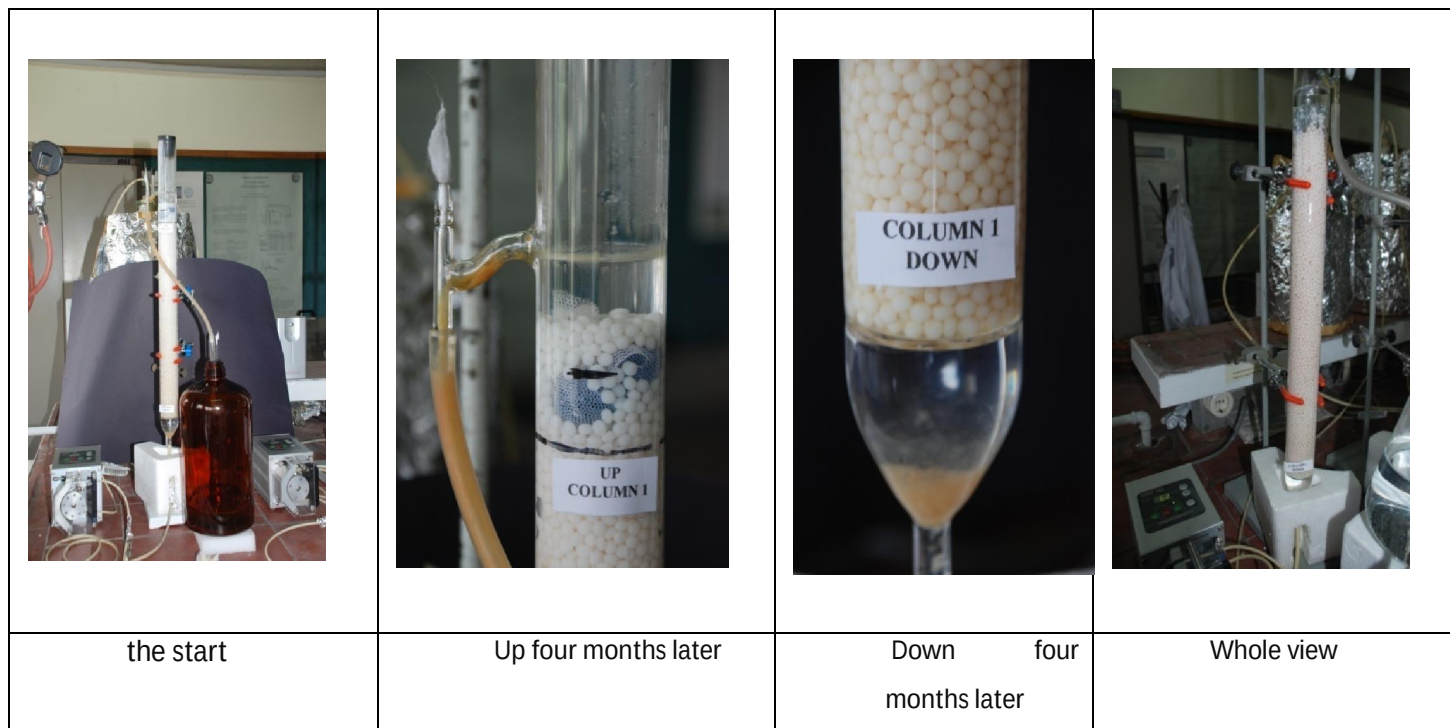


Figure 3.10 Photographs of the denitrification column.

For this purpose fresh PCL granules were mixed with bacteria attached PCL granules taken from enriched bacteria culture and the fixed bed inoculated with a mixture of enriched bacteria culture / recycle solution (Mergaert at al., 2001)

The denitrification column was run in recirculation mode, until reduction of nitrate to nitrite could be detected using test strips (Merck) and the operation was switched to continuous mode.

The column was fed with deionized drinking water which was enriched by the addition of nitrate and – to enhance denitrification performance – phosphate and molybdate. Parameters of the influent water to the reactors (feed solution) were given in Sec. 3.2.6.

3.2.16 The pesticide contamination in the Continuous Biological Denitrification

These studies were carried out to see the effect of pesticide adsorption on performance of the denitrification process. A hundred mg/L aqueous 2,4-D stock solution is prepared in a volumetric flask. The pesticide stock solution to provide the desired pesticide concentration in the inlet flow was added to the glass bottle containing feed solution. Solution in the bottle was mixed with a magnet for homogenous pesticide distribution. Fig.3.11 shows the denitrification column with pesticide contamination and Fig.3.12 shows a general view of denitrification columns.

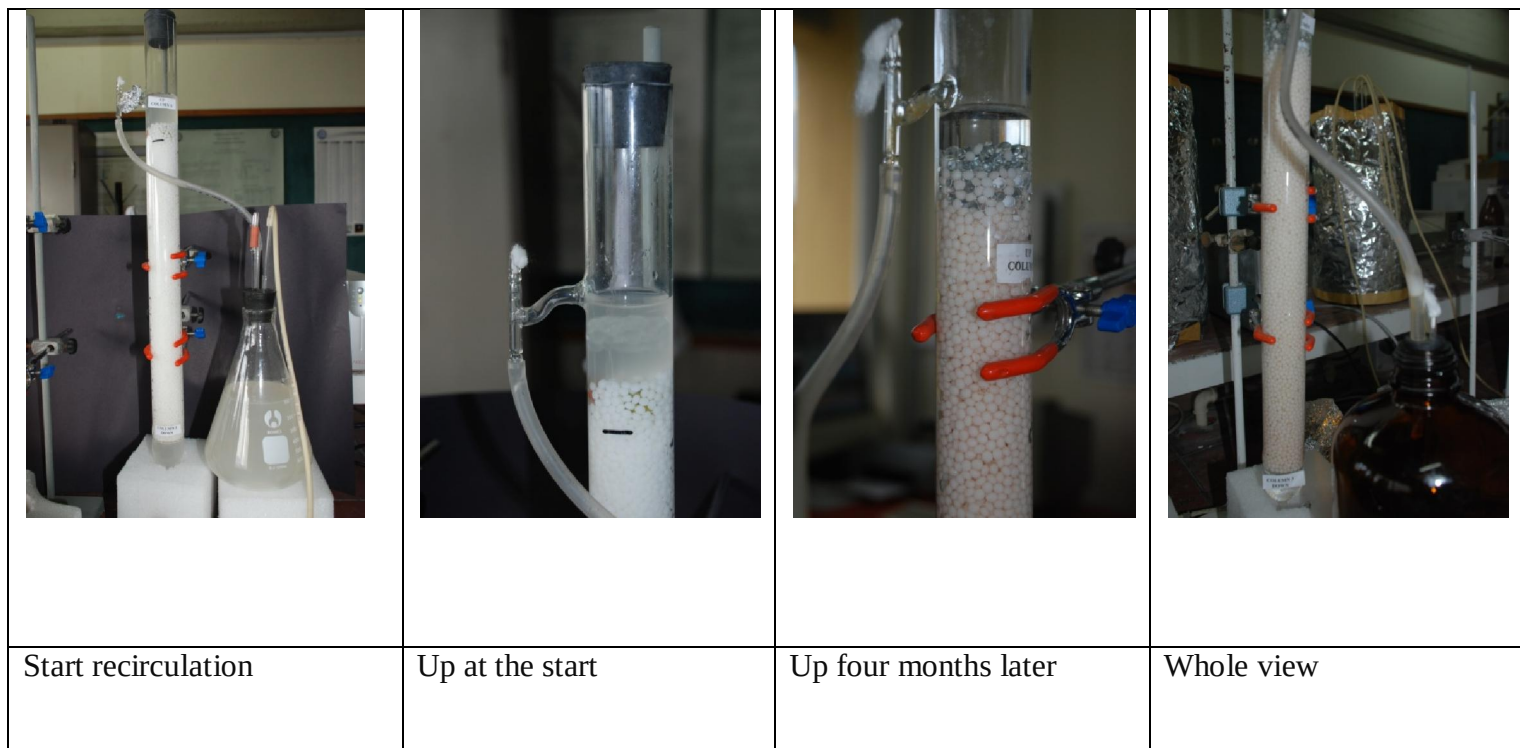


Figure 3.11 Photographs of the biological denitrification column with pesticide contamination.



Figure 3.12. The general view of the the biological denitrification columns.

4 Results and Discussion

4.1 Poly(ϵ -caprolactone) (PCL)

Conventional techniques for heterothropic denitrification of drinking water consist of using fixed or fluidized bed reactors often with a sand packing as a biofilm carrier and soluble organic carbon sources, such as methanol, ethanol, dosed to the raw water stream. An alternative approach is to use natural organic solid substrates that act as biofilm carriers as well as organic carbon sources. The development of water-insoluble biodegradable polymers led to the idea to use such materials in denitrification reactors. Denitrification processes were developed that used the biodegradable bacterial polyester poly(3-hydroxybutyrate-co-hydroxy-valerate) (PHBV) (Müller et al., 1992) as source of carbon for denitrification and electron donor. In this study it was decided to use PCL (TONE P767) as a carbon source because of its property of being biodegradable and also serve as a support for bacteria during denitrification. The specifications of TONE P767 were given in Table 4.1 (Ovez and Höll, 2008)

Table 4.1. Manufacturers data for PCL polymer TONE P-767

Quantity	Value
Molecular weight (Dalton)	~40000
Density (g/mL)	1,14
Water absorption in 24 h (%)	0,35
Melting point (°C)	60

4.2 Bacteria (Pure and Mixed Bacteria Cultures)

The effect of mixed (soil suspension and activated sludge from Çiğli Waste Water Treatment Plant and from a Yeast Factory, both in the city of Izmir, Turkey) and pure (*Acidovorax avenae* subsp. *avenae* LMG 17238) inoculums on denitrification experiments were studied in the batch system. In the batch experiments mixed bacteria culture and LMG 17238 were added as 5% and 1 mL, respectively, into the 250 mL of reaction volume. In the continuous denitrification experiments only the pure bacteria culture LMG 17238 was used.

Growth of bacteria can be evaluated performing viable counts and by physical methods, such as measuring their dry weight, the opacity of the suspension, and how much protein they have incorporated from the medium (Snell, 1981). In the thesis the viable count (Collins, 1976) and modified Lowry protein test (Sperandio, 1993) were applied for determining amount of bacteria in the experiments.

The pure culture LMG 17238 was transferred into sterilized triptic soy broth (TSB), waited for 48 hours for the growing of bacteria at 30 °C, and viable count plus protein tests were conducted. Both tests gave repeatable and high results (Table 4.2).

Amount of protein was determined at the beginning ($t=0$) and at the end ($t=t$) of the batch denitrification experiments. Protein test results of bacteria obtained from different batch denitrification processes were

presented in Table 4.3. In this table blank protein concentrations always smaller than end values, indicating that an increase in the amount of bacteria because of denitrification reaction. Protein amounts of bacteria in Table 4.3 are smaller than those listed in the first column of Table 4.2 because in batch denitrification bacteria were grown in water with nutrient while in the first case growth occurred in a rich medium such as TSB. It can also be seen in Table 4.3 that PCL gave a positive response to the mixed bacteria from Yeast Factory anaerobic sludge but did not work with other bacteria sources.

Table 4.2 Protein test and viable count results of *LMG 17238*

Number of viable cells (cfu/mL)	Amount of total protein (mg/L)
$8.10 \cdot 10^8$	2042
$8.25 \cdot 10^8$	2567
$1.35 \cdot 10^9$	2396
$1.16 \cdot 10^9$	2393
$1.18 \cdot 10^9$	2368
$1.19 \cdot 10^9$	2172
$3.30 \cdot 10^8$	2042

Table 4.3 Protein test results of bacteria obtained from different batch denitrification processes

Soil suspension	Blank t=0	Blank t=t end	Glucose	Sodium Acetate	Methanol	Ethanol	PCL
Total Protein (mg/L)	200	353	500	382	508	463	213
pH	7.0	7.09	7.41	7.44	7.68	7.76	7,00

Çiğli Waste Water	Blank t=0	Blank t=t end	Glucose	Sodium Acetate	Methanol	Ethanol	PCL
Total Protein (mg/L)	116	500	26	0	526	596	120
pH	7.0	7.04	7.42	7,41	7,73	7,64	7,00

Table 4.3 Protein test results of bacteria obtained from different batch denitrification processes (continued)

<i>LMG 17238</i>	Blank t=0	Blank t=t end	Glucose	Sodium Acetate	Methanol	Ethanol	PCL
Total Protein (mg/L)	90	110	57.3	51.33	134.67	94.67	85
pH	7.0	7.09	7.41	7.44	7.68	7.76	7.00

<i>LMG 17238</i>	Blank t=0	Blank t=t end	Yeast Factory sludge
Total Protein (mg/L)	200	235	913
pH	7.0	7.0	7.8

Protein test results of the continuous biological denitrification columns were listed in Table 4.4. For the continuous biological denitrification column protein values were high at the start of the experiment and then remained almost constant because the system reached a steady state. For the continuous biological denitrification column with pesticide contamination, the protein results were high at the start, then remained almost at the same value except month three when pesticide passed through the column. It can be seen that pesticide had a negative effect on protein value.

Table 4.4 Protein test results of bacteria obtained from continuous denitrification experiments

Time (months)	Total Protein (µg protein/g PCL)	
	The continuous biological denitrification column	The continuous biological denitrification column with pesticide contamination
Start	1751	1587
1	767	746
2	700	650
3	680	500
4	690	600
5	700	590
6	705	580

4.3 The Batch Biological Denitrification

Batch biological denitrification experiments were conducted with conventional carbon sources (methanol, ethanol, sodium acetate, and glucose) and with PCL using different bacteria cultures. The aim was to see the effectiveness of PCL in different bacteria environments and to observe the behavior of *LMG 17238* with different carbon sources (Tübitak-Ebitem Project Report, No: 03, 2007).

The denitrification experiments were done in 500 mL glass reaction vessels (anoxic Eudiometer) with a water medium (250 mL) in each reactor. Reaction vessels were connected via gas-tight tubes to a graduated gas-collecting glass tube. The flowsheet and photograph of the system are shown in Fig. 3.8.

The denitrification experiments were conducted with four types of bacteria and different carbon sources. Experiments with ethanol, glucose, and sodium acetate lasted for 10 days, including 3 days of adaptation time of bacteria. Experiments with methanol lasted for 20 days because using of methanol by bacteria takes a longer time, 12 days, than other soluble carbon sources. Batch denitrification experiment with PCL and Yeast Factory bacteria lasted for 54 days, including an adaptation time of 10 days.

The batch denitrification test is based on measuring nitrogen gas production during denitrification, by tracking atmospheric pressure,

temperature and the gas volume in the headspace of a sealed bottle (Ovez, 2006).

Measured nitrogen gas volumes were converted to standard conditions (1013.25 mbar, 273.15 K).

$$= \frac{(\quad - \quad) (\quad + \quad) 273,15}{1013,25} \quad (4.1)$$

The produced N₂ gas volume (equal concentration in water solved) is therefore:

$$\text{---} = \frac{28,014}{22,41} \text{---} \quad (4.2)$$

where V_{Sample} is the amount of produced gas at the time t in mL, P_t the atmospheric pressure at the time t of reading in mbar, P_{wt} the water vapor pressure at the time t of reading at given temperature T_t in mbar, T_t the temperature at the time t of reading in K, V_t the amount of gas at the time t , read at the gas collecting tube in mL, V_{RVST} the amount of gas in reaction vessel at the start in mL, E the weighted portion of natural organic substance in g, and VR is the solution volume in reaction vessel in L.

Fig.4.1. shows the temporal change of nitrogen gas volume produced in denitrification with PCL and Yeast Factory bacteria. Denitrification rate was found to be 1 mg/(L*d).

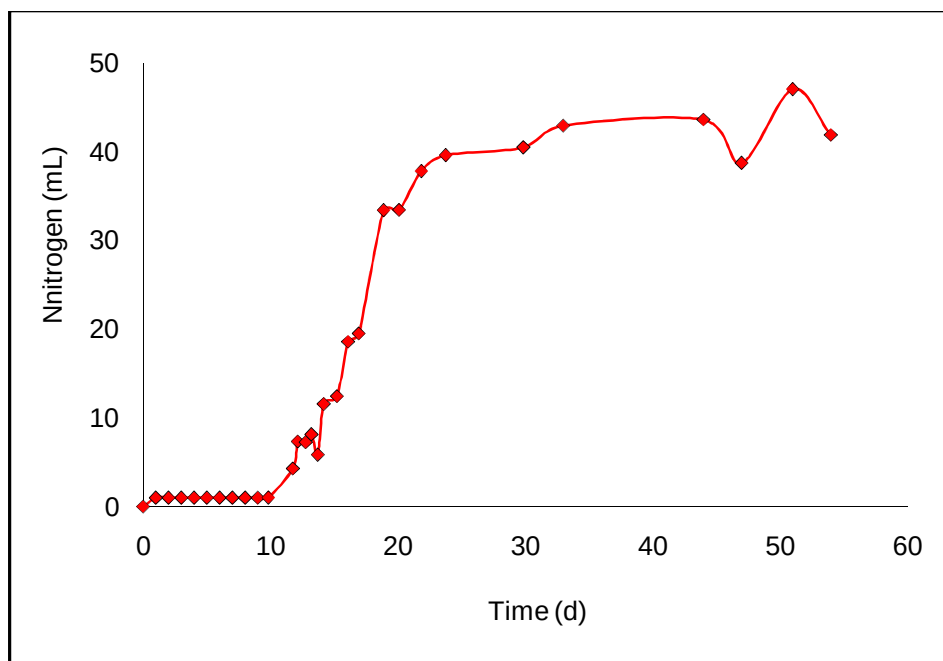


Figure 4.1 Batch biological denitrification test with PCL.

Table 4.5 shows the nitrogen gas volume produced in denitrification obtained with different carbon sources and bacteria. Methanol and ethanol gave good responses to denitrification with mixed culture (soil suspension and Çiğli Wastewater sludge) and pure bacteria culture (*LMG 17238*). Glucose and sodium acetate worked well with bacteria sources of soil suspension and *LMG 17238*. PCL gave a positive response only in the denitrification experiments where Yeast Factory Sludge used as bacterial source.

Table 4.5 The nitrogen gas volume produced at the end of the biological denitrification experiments with different carbon sources. (mg/(L* g_{carbon source})

	NV (mg/L*g)				
Bacteria	Methanol	Ethanol	Glucose	Sodyum Acetate	PCL
Soil Suspension	771,63	982,58	535,11	200,23	-
Çiğli Waste Water sludge	873,39	709,40	0,00	0,00	-
LMG 17238	1161,68	1844,87	673,58	343,92	-
Yeast Factory Sludge	-	-	-	-	15,31

Denitrification studies based on measurement of released nitrogen carried out by several researchers. Sanchez investigated denitrification using a denitrifying sludge and synthetic feed in glass vials of 60 mL. A simple method based on the displacement of an alkaline solution by the N₂ generated during denitrification was used. In the 0.35-0.73 g volatile solid substance (VSS)/L range, the specific denitrification activity (SDA) was strongly affected by variable VSS, but not in the range 1.5-3.0 g VSS/L (Sanchez et al., 2000). Buys developed a test based on measuring the rate of gas production during denitrification, by tracking both the pressure and the gas composition in the headspace of a sealed bottle. The minimum COD/N ratio required for linear gas production curves with acetate/nitrate was 3.5 g COD/g N. A significant increase in specific activity at low solids concentrations (<2 g VSS/L) was observed.

(Buys et al., 2000). Ovez observed nitrogen gas release in a batch denitrification system with synthetic medium and natural organic substances as carbon substrate and biofilm carrier. Complete nitrate removal was achieved in 13, 24, and 20 days in *G. verrucosa*, liquorice, and giant reed, respectively, and their calculated denitrification velocities are respectively given as: 13.125, 6.20, 3.33 mg/L NO_3^- -N/(g d) (Ovez, 2006). Beaubien aimed to determine the impact of biological parameters on the overall performance of the biological denitrification process. A method for evaluating the metabolic activity of the microorganisms was developed based on measuring nitrogen production rate. The method was found to be particularly valuable in monitoring batch and continuous-flow processes and confirmed the existence of several phases in denitrification (Beaubien, et al., 1995).

4.4 The Batch Adsorption of 2,4-D

The batch adsorption test was conducted to determine the adsorption properties of PCL with respect to 2,4-D in addition to the properties given in Table 4.1.

The adsorption characteristics of 2,4-D on PCL was investigated and the relative isotherm was presented in Fig. 4.2.

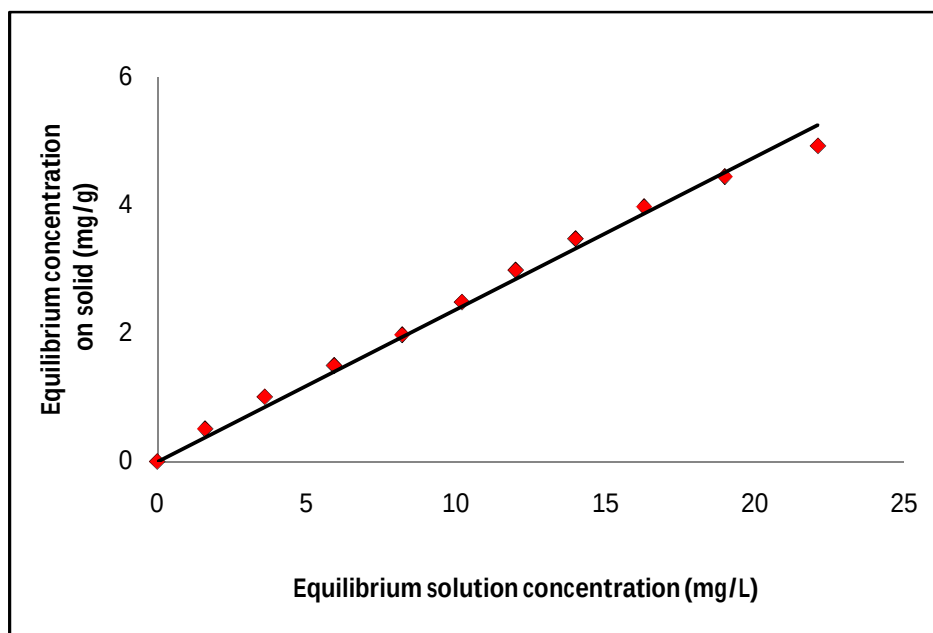


Figure 4.2 Adsorption isotherm of 2,4-D (PCL as adsorbent).

The two most common adsorption models, i.e., Langmuir and Freundlich, were used to describe the equilibrium between adsorbed 2,4-D onto PCL and 2,4-D in solution. The Langmuir isotherm is mainly applied to monolayer adsorption while the Freundlich isotherm is widely employed for sorption surfaces with non-uniform energy distributions. The linearized Langmuir and Freundlich adsorption isotherms of the adsorption of 2,4-D are given in Fig. 4.3. The Langmuir and Freundlich adsorption constants evaluated from the isotherms with the correlation coefficients. Langmuir and Freundlich correlation coefficients (R^2) were found to be 0,995 and 0,998, respectively.

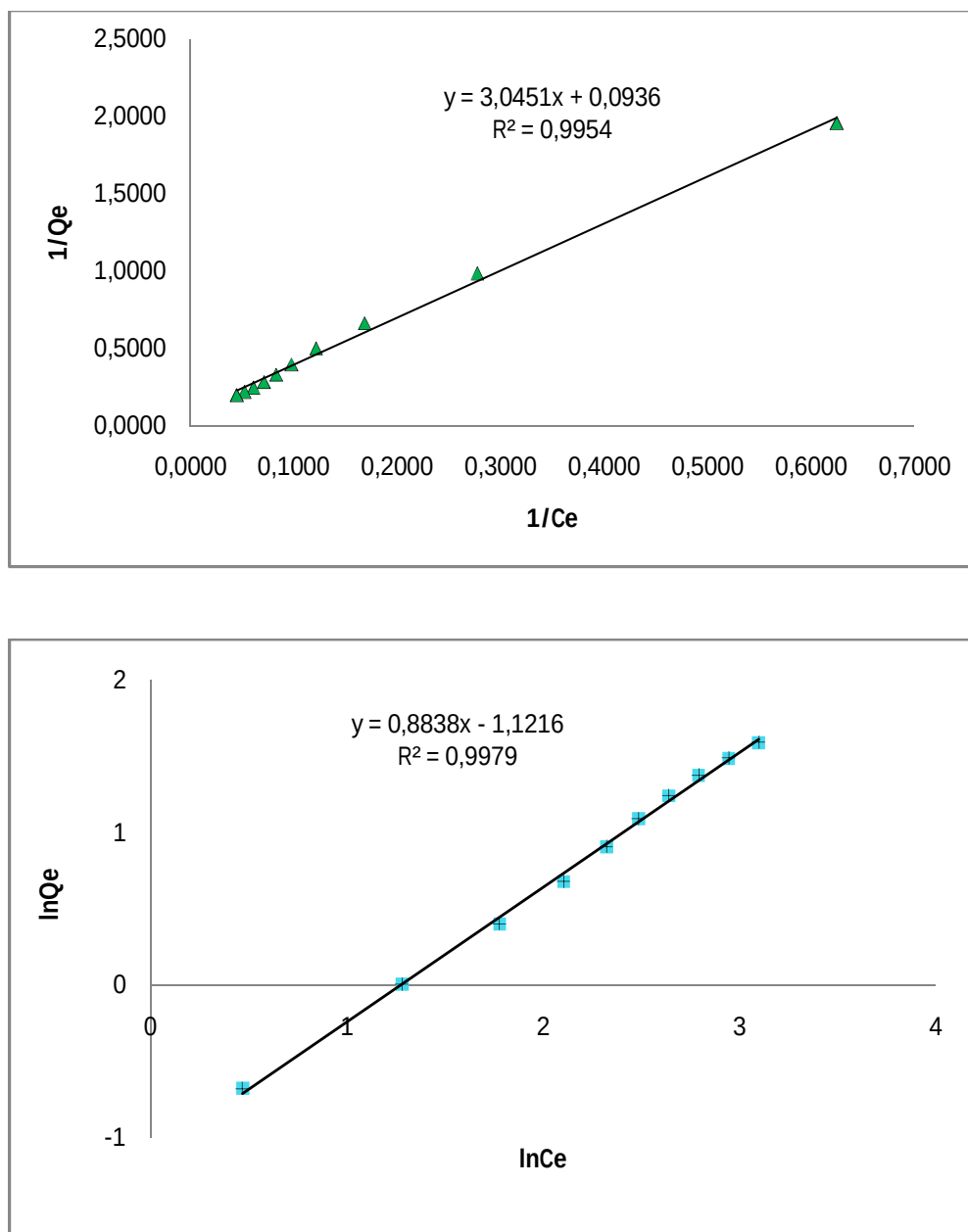


Figure 4.3 Linearized isotherms of the adsorption of 2,4-D . Top: Langmuir linearization, bottom: Freundlich linearization.

The values of the Langmuir constants, Q_0 and b , have been calculated from the plots in Fig. 4.3. The maximum capacity, Q_0 , 10,68 mg/g, defines the total capacities of the adsorbent for 2,4-D as shown in Table 4.6. The value of K_F determined from Freundlich plot from Fig. 4.3 and listed in Table 4.6. Higher values of n indicate stronger adsorption intensity. In the table it can be seen that the n is greater than unity for PCL, indicating that 2,4-D was favorably adsorbed by PCL.

When experimental isotherm and two model adsorption isotherms were written in Fig. 4.4 it has been seen that Freundlich model was more convenient to represent the adsorption of 2,4-D on PCL in the studied range of adsorption.

A comparison of Langmuir and Freundlich constants for pesticide adsorption with different adsorbents were listed in Table 4.6. It can be seen from the table that adsorption capacity of PCL is low according to powdered activated carbon (PAC) and granular activated carbon (GAC), substances that have great surface areas.

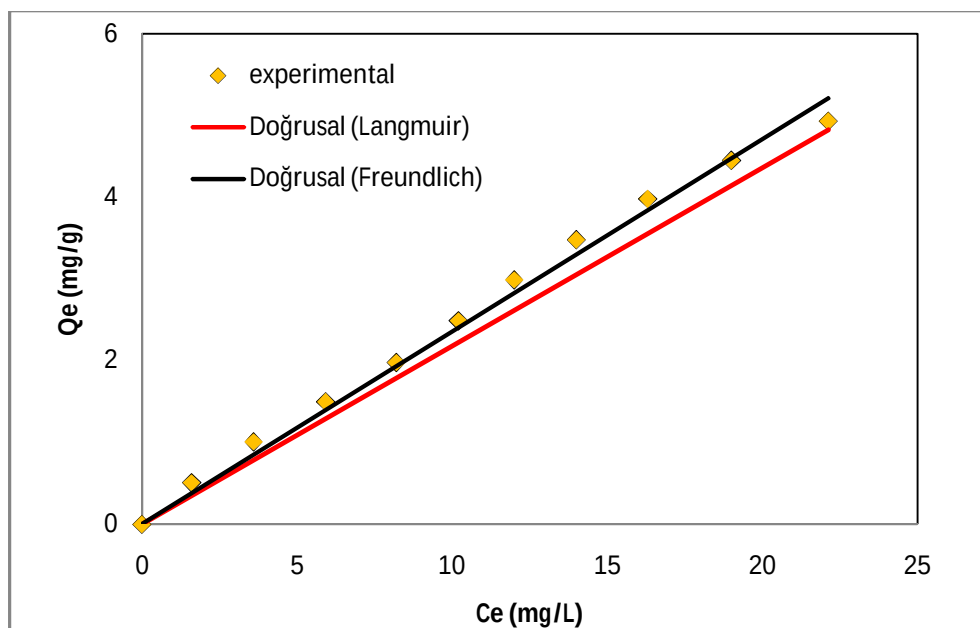


Fig.4.4 Experimental and model equilibrium curves of 2,4-D adsorption.

Table 4.6 Langmuir and Freundlich constants for pesticide adsorption of PCL and other adsorbents

Langmuir					Freundlich		
pH	Adsorbent	Adsorbate	Q _o (mg/g)	b (L/mg)	K _F	n	Reference
2,0	GAC (1,19-3,36 mm)	2,4-D	555,56	0,028	42,48 [(mg/g)* (mg/L) ⁿ]	2,06	Aksu and Kabasakal, 2004
2,0	PAC (<0.15mm)	2,4-D	333,300	0,029	43,930 [(mg/g)* (mg/L) ⁿ]	2,910	Aksu and Kabasakal, 2005
6,5	GAC (1-2 mm)	2,4-D	263, 158	0,031	30,760 [(mg/g)* (mg/L) ⁿ]	2,540	Şirin,S., 2007
6.5	PCL	atrazine	2,008	0,028	0,054 (L/mg)	0,884	Övez and Höll, 2008
6.5	PCL	simazine	0,688	0,043	0,041 (L/mg)	1,428	Övez and Höll, 2008
6.5	PCL	2,4-D	10,680	0,031	0,326 [(mg/g)* (mg/L) ⁿ]	1,133	PhD Thesis

4.5 The Continuous Biological Denitrification

The use of biofilms for water and wastewater treatments reported in the early 1980s (Akinson, 1981). However, it is only during the past few decades that biofilm reactors have become a focus of interest for researchers in the field of removal of contaminants via biological processes (Singh, 2006).

Richard investigated removal of nitrate from drinking water in a semi-industrial pilot using ethanol or acetic acid as carbon source for two years. Depending on the water's rising velocity and the support material's characteristics, fixed or suspended bed was used. (Richard, 1980). Nurizzo carried out groundwater biodenitrification on sand fixed film reactor using sugars as organic carbon source (Nurizzo, 1992). Jang monitored an anoxic biofilm involved in continuous denitrification process to investigate the effect of different concentrations of influent dissolved oxygen (DO) or nitrite on the biofilm (Jang, 2003). Ueda carried out a continuous denitrification for 77 days using sugar-industry wastes, namely final molasses as a carbon source and bagasse charcoal pellets as supporting media for denitrifying bacteria (Ueda, 2006). In the thesis continuous biological denitrification was conducted using packed-bed of PCL granules (Table 3.1). At the beginning of the experiment a recycle operation was applied for attaching bacteria to granular PCL bed and forming of a biofilm.

Inlet and outlet concentrations and flow rates obtained from denitrification reactor were plotted according to time in Fig.4.5. In the first period of the experiment flow rates were gradually increased to determine the optimum flow rate which provides outlet concentrations under 10 mg NO_3^- -N/L. It was observed that outlet nitrate concentrations were up to 10 mg NO_3^- -N/mL in the high flow rates region and remained below 10 mg NO_3^- -N/mL in the optimum flow rate region of 350 mL/day. So it was decided the system to be operated in a flow rate of 350 mL/day.

Removal ratio (η) of NO_3^- -N was plotted together with flow rate according to time in Fig. 4.6. Removal ratio (η) of NO_3^- -N was calculated by Eq. 4.3 using inlet (C_o) and outlet (C_e) NO_3^- -N concentrations.

$$= 1 - \frac{C_e}{C_o} \times 100 \quad (\%) \quad (4.3)$$

Very high removal ratios were observed in the first days of the experiment when flow rates were low as shown in Fig. 4.6. After this period flow rates were increased so removal ratios decreased. For a flow rate of 1.8 L/day removal ratio was observed as 20%. So the flow rate was decreased to an optimum value of 350 mL/d. In the optimum flow rate region the removal ratio remained between 80 and 85%.

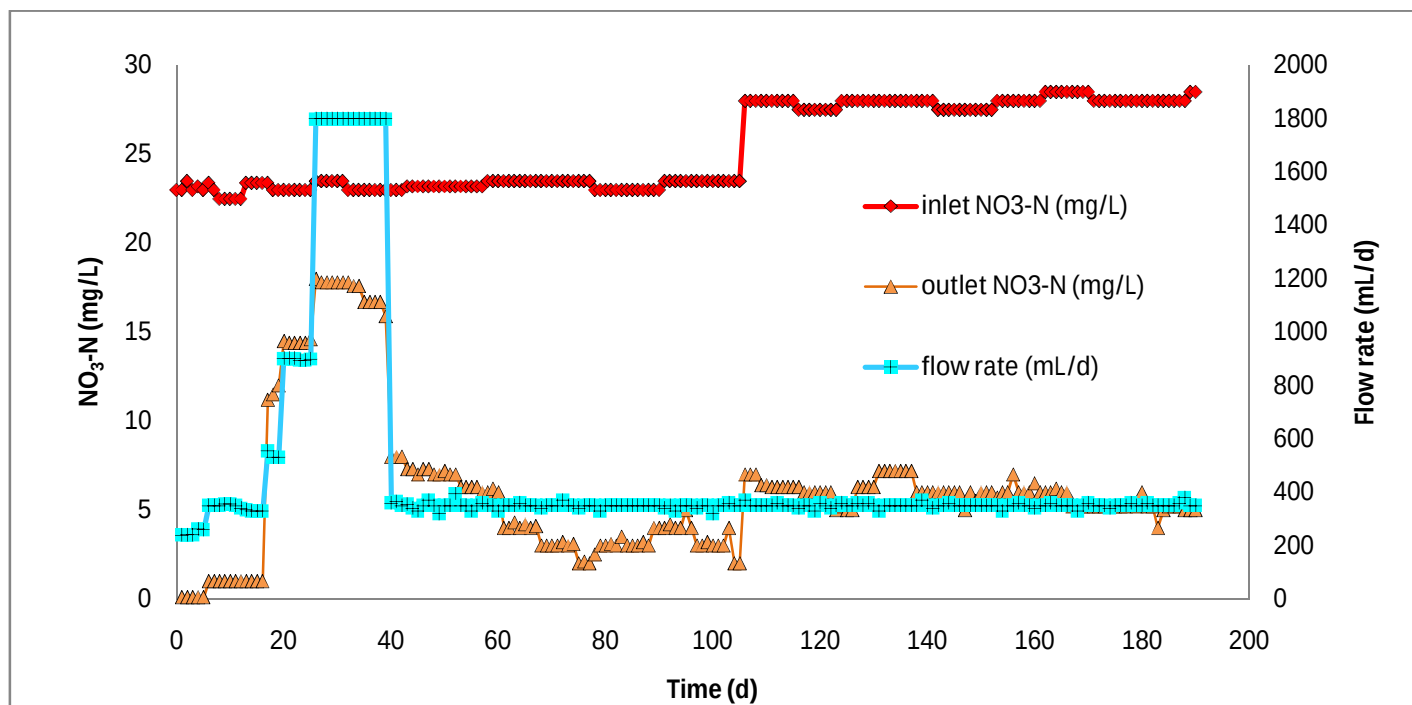


Figure 4.5 Continuous biological denitrification of drinking water by packed-bed of PCL with *LMG 17238* bacteria culture.

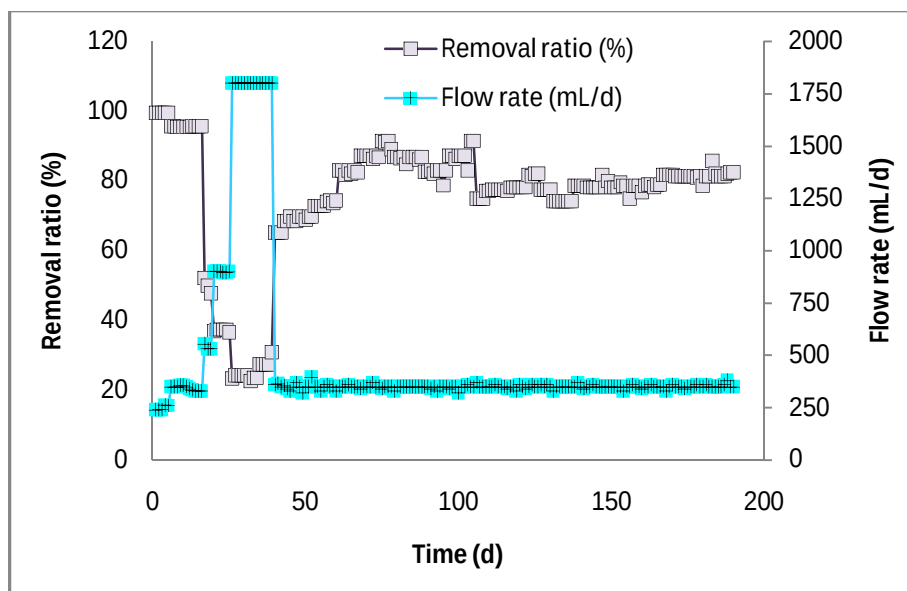


Figure 4.6 Removal ratio of nitrate according to time for the denitrification column.

Nitrate load ($\bar{L}$) of NO_3^- -N was plotted together with flow rate according to time in Fig. 4.7. Nitrate load ($\bar{L}$) was calculated by Eq.4.4 using flow rate (F), inlet concentration of NO_3^- -N (C_o), bed volume (V_{bed}), and volume specific surface (VSS) of the bed. VSS value was taken from Table 3.1. The maximum flow rate to assure effluent concentrations below 10 mg NO_3^- -N /L was 0.35 L/day, as shown in Fig. 4.7, thus corresponding to a maximum nitrate load of 15 mg NO_3^- -N/($\text{m}^2 \cdot \text{d}$) .

$$\bar{L} = (F \cdot C_o) / (V_{\text{bed}} \cdot \text{VSS} \cdot 24) \quad (4.4)$$

Space velocity (SV) was plotted together with flow rate according to time in Fig. 4.8. Space velocity (SV) was calculated by Eq. 4.5 using flow rate (F) and bed volume (V_{bed}). Increased flow rates led to increased space velocity values. Optimum space velocity corresponding to 350 mL/day of flow rate was 0.03 h^{-1} .

$$SV = \text{flow rate/bed volume} \quad (4.5)$$

In Fig. 4.9 denitrification rates (DR) were plotted according to the nitrate load for the experimental data in Fig.4.5. In this graph the diagonal corresponds to removal efficiency of 100%. Denitrification rate (DR) was calculated by Eq. 4.6 using flow rate (F), the difference of inlet and outlet $\text{NO}_3\text{-N}$ concentrations, bed volume (V_{bed}) and volume specific surface (VSS). (VSS value taken from Table 3.1).

$$DR = (F * (C_o - C_e)) / (V_{bed} * VSS * 24) \quad (4.6)$$

As can be seen in Fig. 4.9, under low load conditions, the denitrification rate essentially equals to the load, with removal efficiencies close to 100%. When all data were plotted in this graph, the denitrification rate exhibited a linear relationship up to an input load of $10 \text{ mg NO}_3\text{-N/m}^2\text{*d}$, which is the complete removal capacity, guaranteeing 100% removal by this system. Beyond that load, removal capacity leveled off and the maximum denitrification rate of $12 \text{ mg NO}_3\text{-N/m}^2\text{*d}$ was achieved at a nitrate load of $15 \text{ mg NO}_3\text{-N/m}^2\text{*d}$.

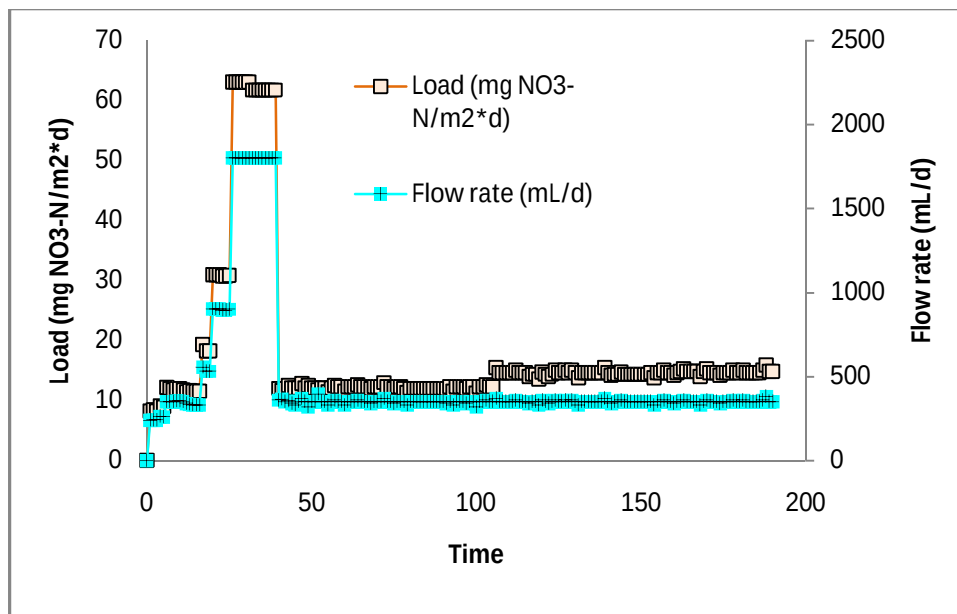


Figure 4.7 Nitrate load according to time for the denitrification column.

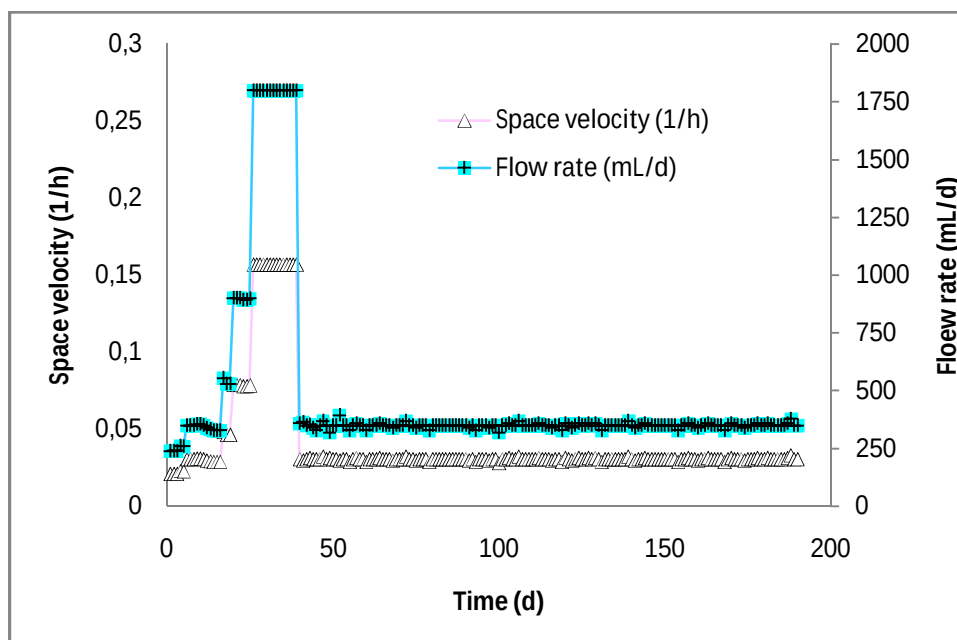


Figure 4.8 Space velocity according to time for the denitrification column.

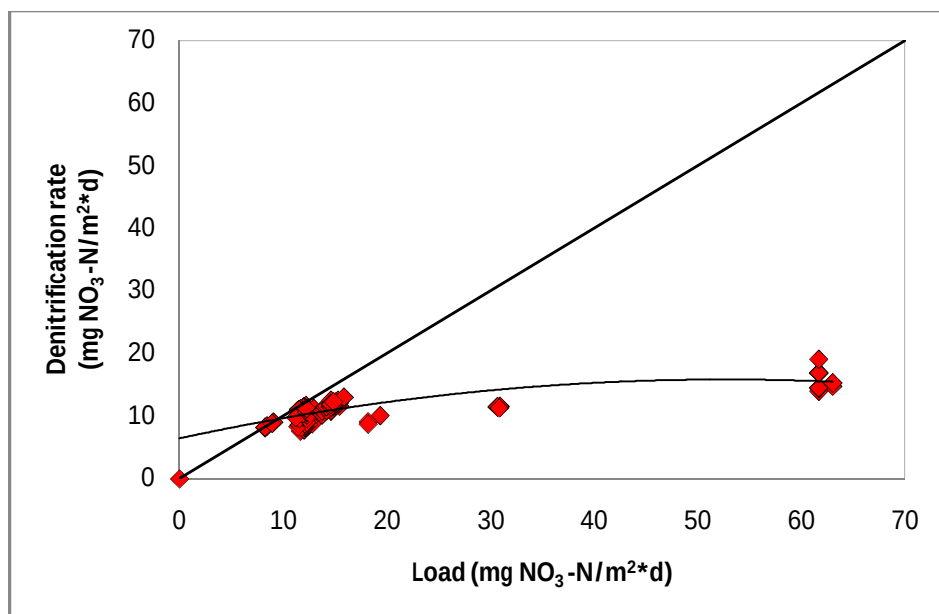


Figure 4.9 Denitrification rate according to NO₃⁻-N load for continuous denitrification of model drinking water.

Table 4.7 shows comparison of denitrification rates of fixed bed biofilm systems using different carbon sources. As shown in the table maximum volumetric denitrification rates were obtained with easy to use carbon sources by bacteria, such as methanol and ethanol.

At the end of the continuous biological denitrification experiment biofilm on PCL granules was photographed at Scanning Electron Microscope (SEM). Sample preparation for SEM was explained in Sec. 3.2.12. PCL granules in the SEM device and biofilm on PCL granule can be seen in Fig. 4.10.

Table 4.7 Denitrification rates in different fixed bed reactor systems using different carbon sources

Carrier	Substrate	Volumetric denitrification Rate (mg NO ₃ ⁻ -N)/(L*d)	Surface related denitrification rate (mg NO ₃ ⁻ -N)/m ² *d	Surface area (m ² /L)	Reference
Sand	Methanol	2700	-	-	Mateju et al., 1992
Polystyrene	Ethanol	1500	-	-	Mateju et al., 1992
Clay	Acetic acid	400	-	-	Mateju, et al., 1992
Silicate	sugar	1150	-	-	Mateju et al., 1992
<i>Liquorice</i>	<i>Liquorice</i>	123,12	57,84	2,13	(Ovez et al.,2006)
<i>Giant reed</i>	<i>Giant reed</i>	86,14	65,76	1,31	(Ovez et al.,2006)
PCL	PCL	18,72	12	1,56	PhD Thesis

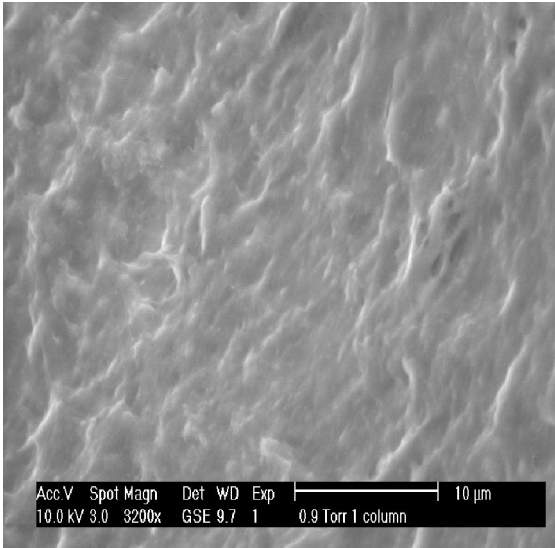
 Acc.V Spot Magn Det WD Exp 0 V 7 PAC 24DB	 Acc.V Spot Magn Det WD Exp 10.0 kV 3.0 3200x GSE 9.7 1 0.9 Torr 1 column 10 µm
Scanning Electron Microscope	A view of biofilm on the PCL granule X 3200

Figure 4.10 Photographs of PCL from scanning electron microscope (SEM).

4.6 The Continuous Biological Denitrification with 2,4-D Contamination

Removal of pesticides with biofilm was widely investigated in literature. Oh completely removed the phenoxy herbicides CPP and 2,4-D in a granular activated biofilm reactor using mixed culture of herbicide degrading bacteria (Oh, 1994). Scott et al. used biofilm covered granular activated carbon for decontamination of streams containing heavy metals and organic chemicals. Electron micrographs illustrate an open film that provides a high surface area for biosorption of metal ions such as cadmium, copper, zinc and nickel. This system has been demonstrated to both entrap metals and also adsorb other contaminants, including an organic residue (atrazine) (Scott et al., 1995).

Removal of pesticides together with nitrate in biofilm operation was attractive for removing these two most common contaminants from water in a single operation. Urbain developed an industrial scale process that combines membrane bioreactor (MBR) technology and powdered activated carbon (PAC). This process denitrifies, removes natural organic matter (NOM) and pesticides, and disinfects (Urbain et al., 1996). Katz studied the simultaneous removal of atrazine and nitrate by atrazine degrading bacterium *Pseudomonas* sp. strain ADP in a continuous fluidized bed biofilm reactor for four months (Katz et al., 2000). In this operation bacteria were used for combined nitrate reduction and atrazine degradation. Feleke used bio-electrochemical reactor coupled with adsorber for combined removal of nitrate and

pesticide (Feleke and Sakakibara, 2001). In the thesis nitrate was removed by a biofilm of denitrifying bacteria attached on PCL granules; simultaneously pesticide removed by adsorption on PCL granules. A continuous reactor with a packed-bed of 300 g PCL was started for investigating removal of both pesticide and nitrate in biological denitrification.

The column inlet and outlet 2,4-D concentrations measurements were carried out at Ege Üniversitesi İlaç Geliştirme ve Farmakokinetik Araştırma-Uygulama Merkezi (ARGEFAR) in the API 4000 LC-MS-MS device.

Fig. 4.11 shows inlet and outlet nitrate concentrations, flow rate values and pesticide concentrations of the continuous denitrification experiment with 2,4-D contamination according to time.

The first pesticide addition to the reactor was started at day 38 when the outlet nitrate concentrations were around 10 mg NO_3^- -N/L (Fig. 4.11). 2,4-D concentration in the feed solution was chosen as 320 $\mu\text{g/L}$ because of high pesticide concentrations can lead to poisoning of bacteria cells. During the passing of the pesticide outlet nitrate concentrations were slightly increased above 10 mg/L so flow rate was decreased in day 51 for recovering of the outlet nitrate concentration. In the decreased flow rate period outlet nitrate concentrations remained below 10 mg NO_3^- -N/L. In this period outlet pesticide concentrations decreased steadily until zero concentration at day 90. That day pesticide flow was stopped

because of the observation of a decrease in monthly protein measurements, carried out on PCL samples taken from the column. The amount of total protein was measured as 500 $\mu\text{g/g}$ PCL, a low value comparing to the previous measurement of 650 $\mu\text{g/g}$ PCL (Table 4.4).

The second period of pesticide passing was started when total protein value was determined as 600 $\mu\text{g/g}$ PCL in day 120 at a flow rate of 250 mL/d (Fig 4.11). Passing of the pesticide lasted in days 128 to day 178. In this period outlet nitrate concentrations were slightly increased above 10 mg/L although outlet pesticide concentrations decreased steadily until zero as shown in Fig. 4.11. As a result passing of pesticide has no major effect on outlet nitrate concentrations in the investigated range of flow rates.

Total amount of pesticide adsorbed by PCL during denitrification was found as 20 μg 2,4-D/g PCL from pesticide concentration measurements. There was no loss of pesticide during denitrification since amounts of input and output pesticide values coincide with each other. So it was decided that biodegradation of 2,4-D did not take place in the biological denitrification operation. Removal ratios of 2,4-D according to time can be shown in Fig. 4.12.

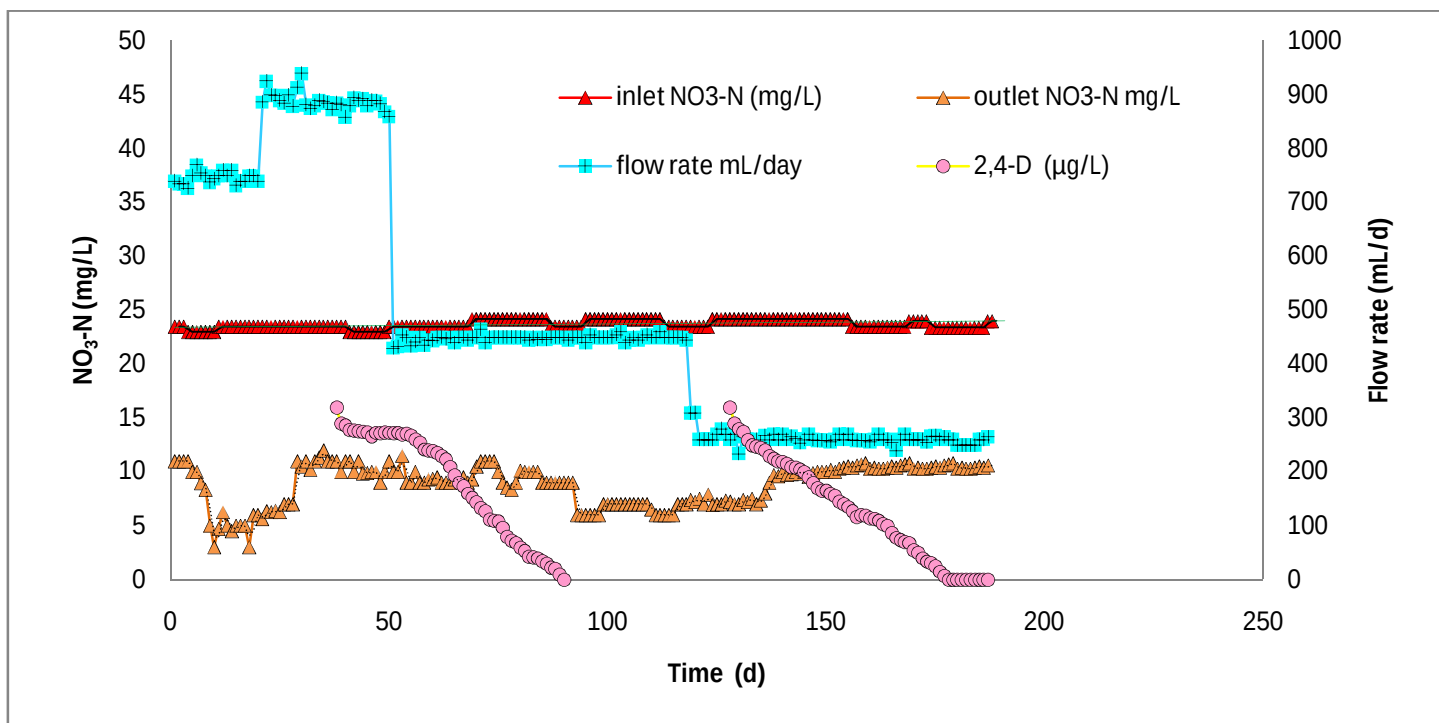


Figure 4.11 Continuous biological denitrification of drinking water by fixed bed of PCL with *LMG 17238* bacteria culture in the presence of 2,4-D contamination.

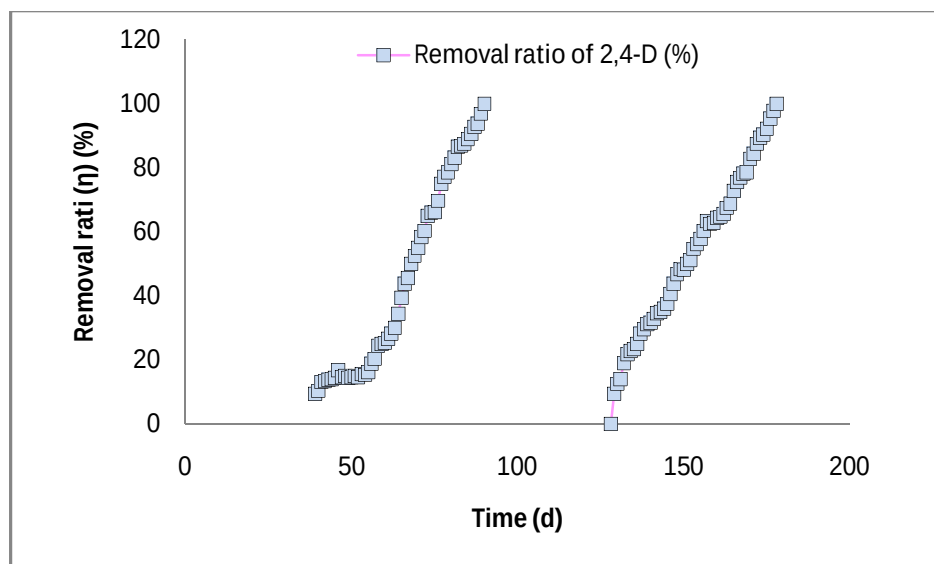


Figure 4.12 Removal ratio of 2,4-D in the biological denitrification reactor.

Removal ratio (η) of NO_3^- -N was plotted together with flow rate according to time in Fig. 4.13. Removal ratio (η) of NO_3^- -N was calculated by Eq. 4.1. As can be seen in Fig. 4.13 removal ratio was high in the first month of the experiment, then remained between 60 and 70%.

Nitrate load (L) was plotted together with flow rate according to time in Fig. 4.14. Nitrate load (L) was calculated by Eq.4.2. In Fig. 4.14 load values decreased gradually in relation to decreasing flow rates.

Space velocity (SV) was plotted together with flow rate according to time in Fig.4.15. Space velocity (SV) values were calculated by Eq. 4.3. Space velocity changed according to changing flow rate between 0,078 and 0,023 h^{-1} .

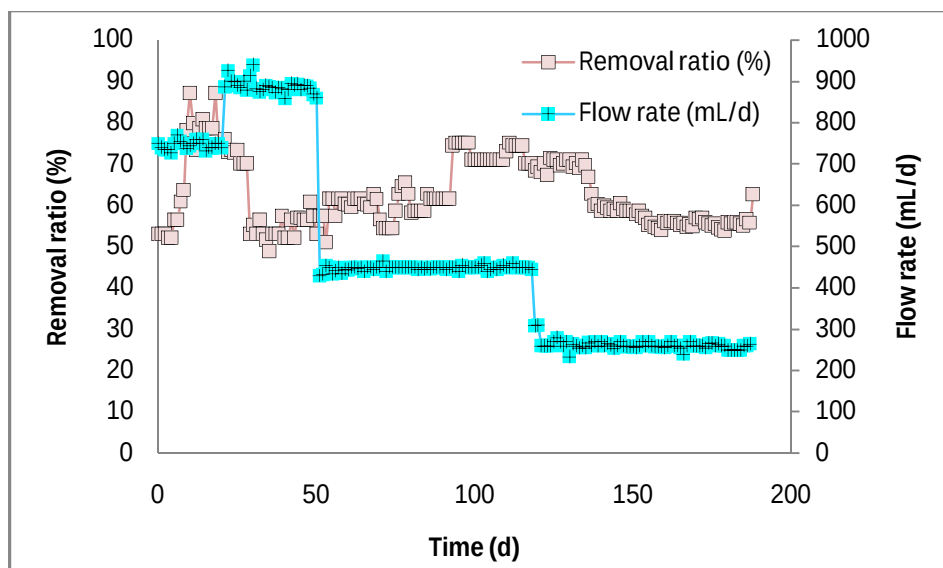


Figure 4.13 Removal ratio of nitrate according to time for the denitrification reactor with 2,4-D contamination.

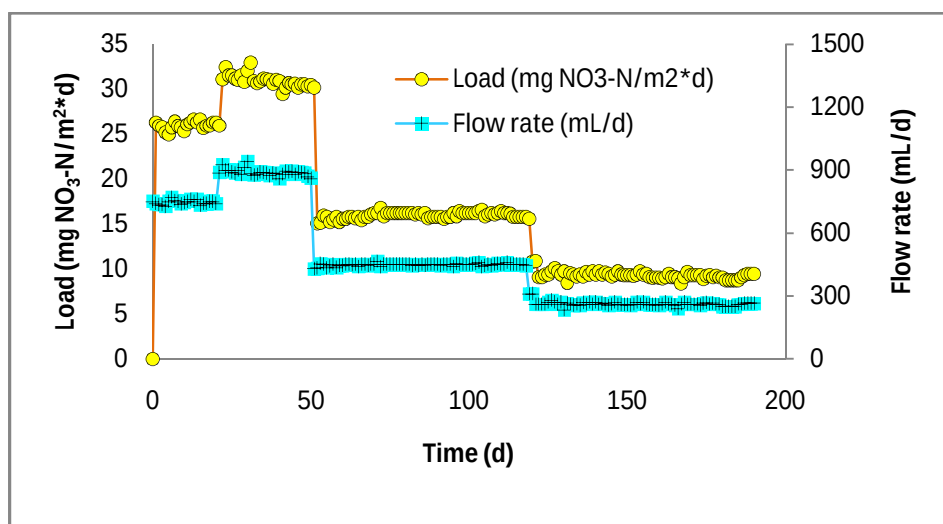


Figure 4.14 Nitrate load according to time time for the denitrification reactor with 2,4-D contamination.

Figure 4.16 shows the denitrification rate according to load. In this graph the diagonal corresponds to removal efficiency of %100. Denitrification rate (DR) was calculated by Eq. 4.6. As shown in Fig 4.16 the maximum removal capacity of $23 \text{ mg NO}_3^- \text{-N/m}^2 \cdot \text{d}$ was achieved at a nitrate load of $33 \text{ mg NO}_3^- \text{-N /m}^2 \cdot \text{d}$.

Nitrate and pesticides removal in different reactors were summarized in Table 4.8. As can be seen from Table 4.8 removals efficiencies changed according to system properties.

Biofilm on the PCL granule taken from the denitrification reactor with pesticide contamination was photographed in SEM (Fig. 4.17). It can be seen from the photograph that the surface of PCL granule completely covered with biofilm. The same photo was taken in two different scale and mother cells can be detectable in the second photograph.

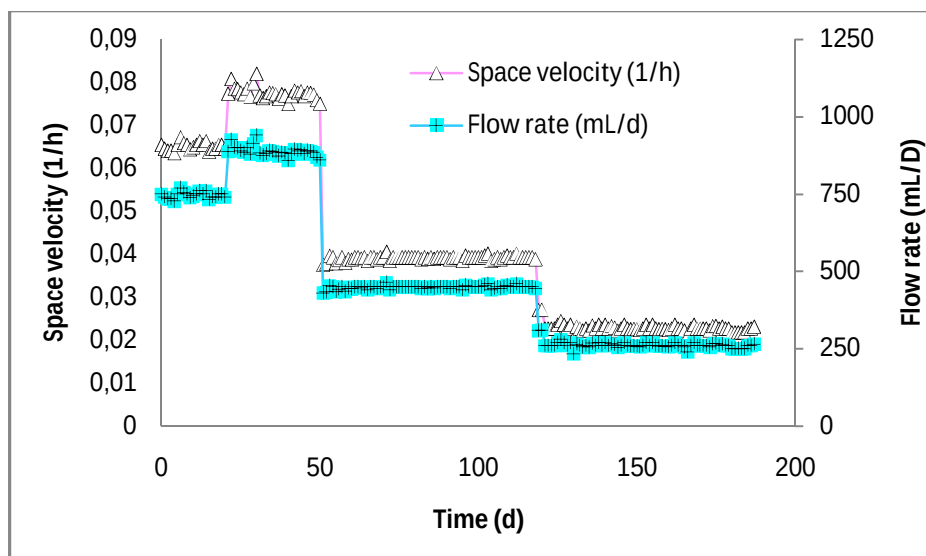


Figure 4.15 Space velocity according to time time for the denitrification reactor with 2,4-D contamination.

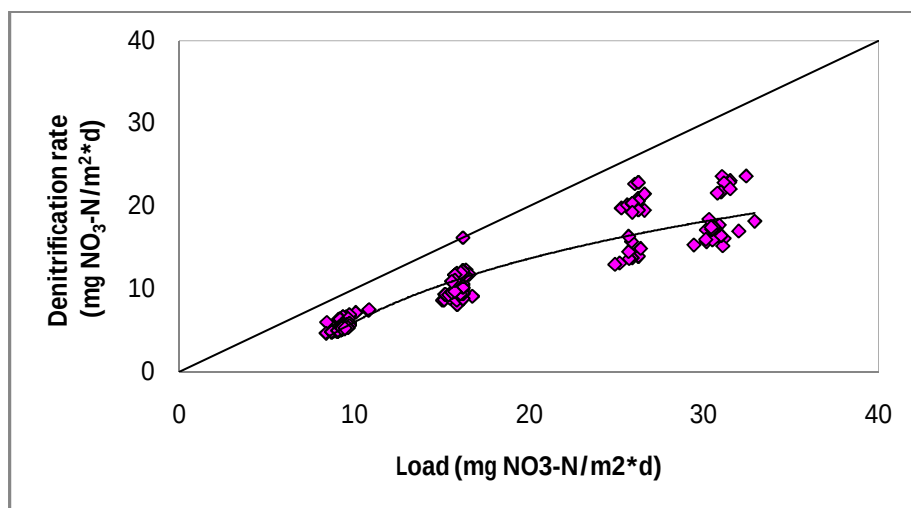


Figure 4.16 Nitrate removal capacity of the denitrification reactor with 2,4-D contamination.

Table 4.8 Nitrate and pesticide removal in different reactor systems

Carrier	Type of reactor	Bacteria	Pesticide	Denitrification results	Pesticide removal results	Time (days)	Reference
	Membrane bioreactor	heterothropic	several	Efficient removal	Activated carbon adsorption 100%		Urbain, 1996
Glass beads	Fixed bed	<i>M91-3</i>	atrazine	Efficient removal	the ratio of nitrate to atrazine utilized, approx. 8:1	50 % atrazine depletion in 6 days	Crawford et al, 1998.
Sintered glass	Fluidized bed Biofilm reactor	<i>Pseudomonas</i> ADP	atrazine	Efficient removal	first 3-5 weeks: 95% later :10-25%	120	Katz, 2000
	bioelectrochemical/adsorption process	-	Iso-prothiolane	Stable removal	97% 10 µg/L <	260	Feleke and Sakakibara 2001
PCL pellets	Fixed bed	<i>LMG 17238</i>	2,4-D	Stable removal	In 52 days 100%	190	PhD Thesis

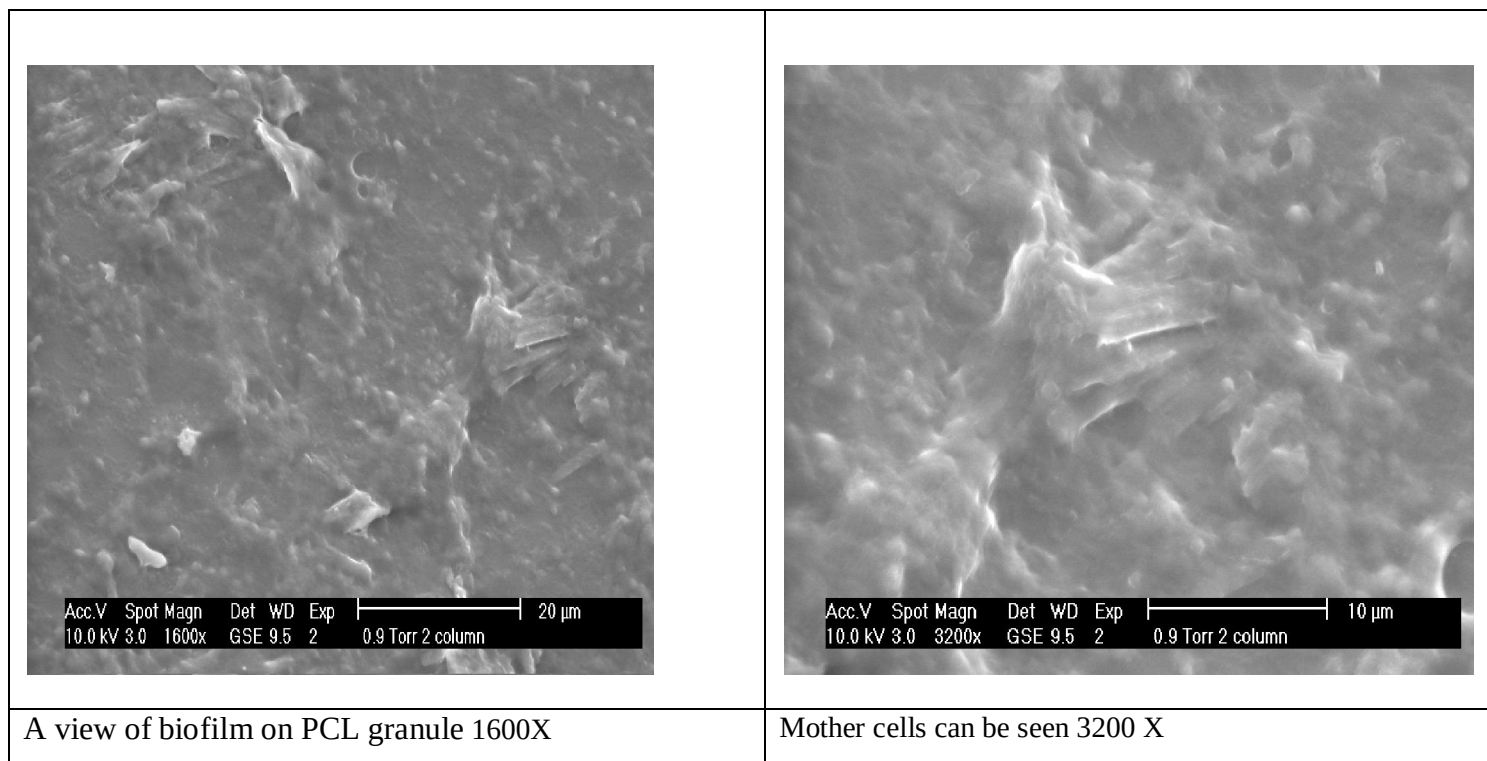


Figure 4.17 Biofilm on PCL taken from the denitrification column with 2,4-D contamination.

4.7 Mathematical Modeling of the Continuous Biological Denitrification

It has been generally accepted that design of a commercial-scale reactor, which is the heart of a plant, cannot be accomplished by a purely theoretical approach alone. To start with, at least laboratory and/or pilot plant data on the reactions involved must be available. A satisfactory scale-up procedure is the method of mathematical modeling. This method requires basic kinetic data from bench-scale or pilot-plant scale studies as well as practical experience (Perry, 1973).

Yani et al. investigated biological removal characteristics of ammonia by a peat biofilter on a laboratory scale experiment. Kinetic analysis was conducted by assuming a plug gas flow in the biofilter column and using Michaelis-Menten kinetics (Yani et al., 1998). Kiranmai et al. investigated purification of wastewater in a fixed-bed biofilm reactor. An alternative methodology was proposed in this work, which makes use of a novel optimization method to evaluate the bio-kinetic parameters, in the course of the solution of an inverse problem with the aid of measured data of fractional conversion of the substrate. (Kiranmai et al., 2005). Ueda et al. used an upflow fixed-bed biofilm reactor for biological denitrification of potassium-nitrate and dilute molasses solutions. In order to examine the effect of influent TOC on nitrate removal rates, observed nitrate removal rates were simulated by a Monod model (Ueda et al., 2006). Gabaldón et al. investigated biological nitrate removal in a continuous fixed bed reactor for 5

months. A simple model based on Monod kinetics for substrate consumption, and constant biomass concentration was applied to model the industrial wastewater treatment, and a reasonably good fitting was obtained (Gabaldón et al. , 2007). In the thesis modeling studies were conducted according to Monod and Michaelis Menten kinetics.

4.7.1 Modeling According to Monod Kinetics

The data belong to the first 39 days of the first continuous denitrification column were used calculating kinetic constants (Fig.4.5). A simple model development was used to simulate the experimental results. Assuming plug-flow regime, Monod kinetic for substrate consumption, and constant biomass concentration, the nitrate balance for the reactor is as follows (Gabaldon et al., 2007).

$$- \frac{dS}{dt} = (k - \frac{kS}{K_s + S}) \tau \quad (4.7)$$

where S is the nitrate concentration (mg NO_3^- -N/L); τ the empty bed residence time ($\tau = V_{bed}/\text{flow rate}$) (h); k the maximum denitrification rate; K_s is the Monod half saturation constant (mg NO_3^- -N/L).

Notice that the units of k are in reference to the limiting substance; in this case the units of k are mg NO_3^- -N removed/(L reactor*h). If Eq. 4.7 is rearranged it takes the following form:

$$\frac{S_o - S}{a + S} = -\theta \quad (4.8)$$

Eq. (4.8) integrated and turns to Eq. (4.9)

$$-\theta + \ln(S_o - S) = -\theta S_o + \ln S_o \quad (4.9)$$

Denitrification rate (DR) is defined according to inlet and outlet NO_3^- -N concentrations and empty bed residence time as follows:

$$DR = \frac{S_o - S}{\theta} \quad (4.10)$$

where S_o and S are inlet and outlet nitrate concentration (mg NO_3^- -N/L); respectively. Eq. (4.9) turns to Eq. (4.11) if denitrification rate is placed in it.

$$-\theta + \ln(S_o - S) = -\theta S_o + \ln S_o \quad (4.11)$$

Eq (4.11) was simplified making a definition of “ a ”

$$a = \ln \frac{S_o - S}{S_o - S_o} \quad (4.12)$$

If “ a ” term is placed in Eq. (4.11) it turns to Eq. (4.13):

$$+ = \quad (4.13)$$

Eq. (4.13) was divided by τ and the model equation of Eq.(4.14) obtained. This is the equation of a straight line defined as $y= ax + b$. If a straight line plotted using a/τ values as y-axis and DR as x-axis, slope and intercept of the line were $-1/K_s$ and k/K_s , respectively.

$$- = - - - \quad (4.14)$$

Values of a/τ and DR in Eq. 4.14 were calculated using data belong to the first 39 days of the continuous denitrification experiment (Fig. 4.5). Fig. 4.18 shows a plot of a/τ values as y-axis and DR values as x-axis. Slope and intercept of the line were found as -0,904 and 0,363 from the line equation as can be seen in the graph. k and K_s constants were calculated as shown in Table 4.9.

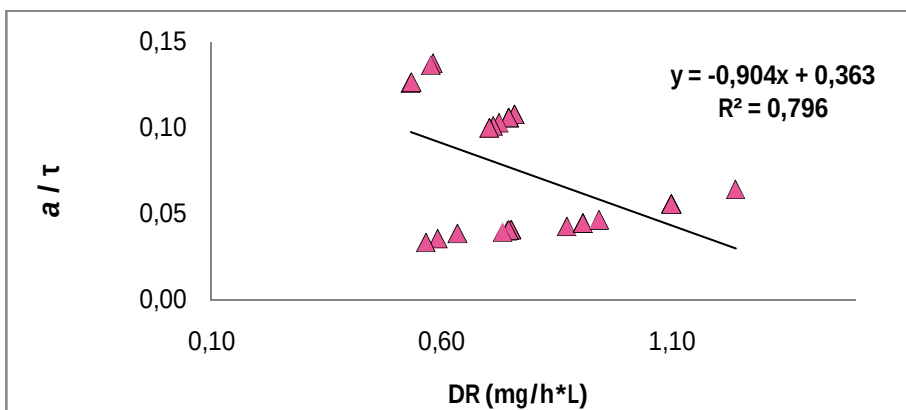


Figure 4.18 A graph of a/τ versus denitrification rate for Monod model.

Table 4.9 Monod constants for biological denitrification with PCL

$y = - 0,904 x + 0,363$	
k/K_s	0,363
$-(1/ K_s)$	-0,904
$K \text{ (mg NO}_3^-\text{-N /L*h)}$	0,40
$\text{(mg NO}_3^-\text{-N/L)}$	1,11

Comparison of experimental and model denitrification rates must be examined to see whether the obtained constants really represent the system. For this purpose the value of $\frac{r}{K} = \frac{r}{K_s} - \frac{r}{K_s} \cdot \frac{1}{K}$ from Eq. 4.19 was placed in Eq.4.10 and the following equation obtained:

$$= - \frac{1}{K_s} \ln \frac{r}{r - K} \quad (4.15)$$

Eq. 4.15 was used in obtaining model *DR* values. Fig. 4.19 was plotted using experimental and model denitrification rates as x- and y- values, respectively. As can be seen in Fig. 4.19 this model approach didn't coincide with the experimental results.

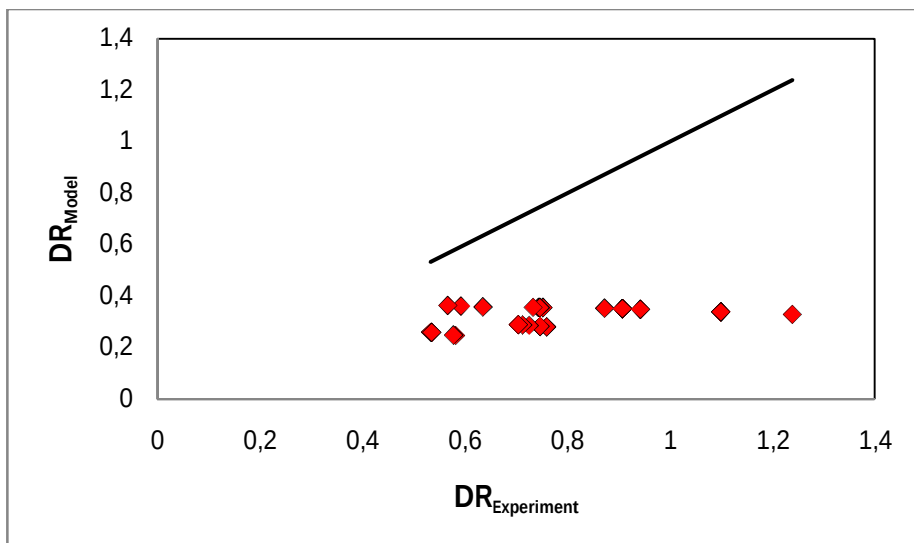


Figure 4.19 Comparison of experimental and model denitrification rate results for Monod model.

4.7.2 Modeling According to Michaelis-Menten Kinetics

Again the data belong to the first 39 days of the first continuous denitrification column (Fig. 4.5) were used obtaining the kinetic parameters of biological removal of nitrate. Assuming a plug-flow regime in the biofilm column and based on Michaelis-Menten-type equation the following derivations were made (Yani et al., 1998). Michaelis Menten equation was written in Eq. 4.16.

$$= \frac{V_{max} \cdot S}{K_m + S} \quad (4.16)$$

Since empty bed residence time defines as in Eq. 4.17

$$= \frac{V}{Q} \quad (4.17)$$

Diferentiation of eq.4.17 leads to Eq. 4.18

$$= \frac{dV}{dQ} + \frac{V}{Q^2} \frac{dQ}{d\tau} \quad (4.18)$$

Since

$$= 0 \left(\frac{dV}{dQ} \right)$$

$$= 0 \left(\frac{dQ}{d\tau} \right)$$

$d\tau$ takes the following form:

$$= \frac{d\tau}{dC} \quad (4.19)$$

If $\frac{dC}{d\tau} = \left(\frac{dC}{d\tau} \right) /$ placed in Eq.4.18 and proceeded in following way

$$= \frac{dV}{dQ} + \frac{V}{Q^2} \frac{dQ}{d\tau} = \frac{dV}{dQ} + \frac{V}{Q^2} \frac{dQ}{dC} \frac{dC}{d\tau} \quad (4.20)$$

If $d\tau$ value in Eq. 4.20 placed in $dC/d\tau$ Eq. 4.21 is obtained .

$$\frac{dC}{d\tau} = \frac{dC}{dQ} \frac{dQ}{d\tau} = \frac{dC}{dQ} \frac{dQ}{dC} \frac{dC}{d\tau} \quad (4.21)$$

If the value of $dC/d\tau$ in Eq. 4.21 placed in Eq. 4.16 the following equation is obtained

$$- \frac{dC}{d\tau} = \frac{K_s C}{K_s + C} \quad (4.22)$$

If (F/S_a) term placed in the right hand side of the Eq. 4.22 then an equation indicating changing of concentration according to column length is obtained (Eq. 4.23)

$$- \frac{dC}{dL} = \frac{K_s C}{K_s + C} \frac{F}{S_a} \quad (4.23)$$

Placing space velocity value ($SV = F/S_a * L$) in Eq.4.23 leads to Eq.4.24

$$- \frac{dC}{dL} = \frac{K_s C}{K_s + C} \frac{1}{L} \quad (4.24)$$

where C is NO_3^- -N concentration (mg/L), L length of column (m), V_m maximum removal rate (mg NO_3^- -N/L*d), K_s saturation constant (mg NO_3^- -N/L), S_a cross section of column (m^2), F flow rate (m^3/d), L height of packed bed (m), SV space velocity (h^{-1}) = $F/(S_a * L)$. Equation 4.25 was obtained by integrating Eq. 4.24 under the condition of $C=C_o$ at $L=0$, and $C=C$ at $L=L$.

$$\frac{1}{\left(\frac{C_o - C_e}{C_o} \right)} = \frac{1}{\frac{\left(\frac{C_o - C_e}{C_o} \right)}{\ln \left(\frac{C_o}{C_e} \right)}} + \frac{1}{\ln \left(\frac{C_o}{C_e} \right)} \quad (4.25)$$

Eq.4.25 was simplified by setting $R = SV^* (C_o - C_e)$ and $C_{ln} = (C_o/C_e) / \ln (C_o/C_e)$ and the model equation Eq.4.26 was obtained

$$\frac{C_{ln}}{R} = \frac{1}{V_m} + \frac{K_s}{V_m} \cdot \frac{1}{C_{ln}} \quad (4.26)$$

Eq. 4.26 is the equation a straight line. A plot of C_{ln}/R according to C_{ln} was presented in Fig. 4.20. The slope ($1/V_m$) and intercept (K_s/V_m) of the line were obtained from the line equation as 0,045 and 0,1607. Using these values the maximum removal rate (V_m) and the saturation constant (K_s) were calculated as 22.06 mg/L*day and 3,54 mg/L, respectively (Table 4.10).

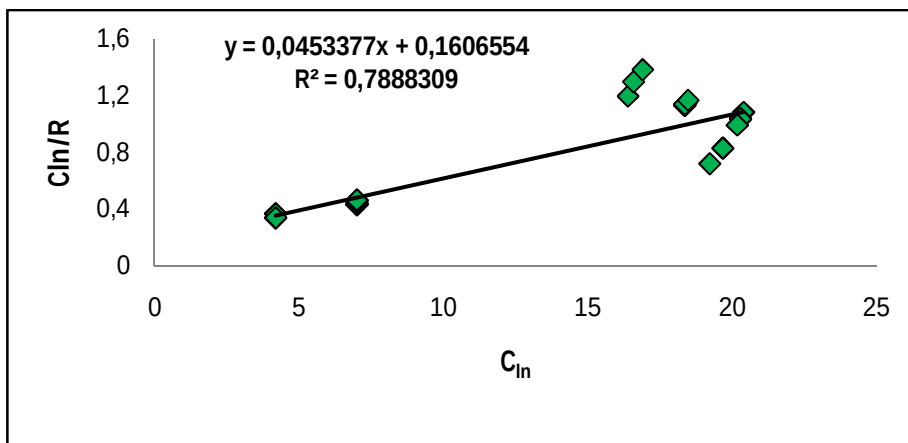


Figure 4.20 Kinetic analysis of nitrate removal by packed-bed of PCL for Michaelis-Menten model.

Table 4.10 Michaelis-Menten costants for continuous biological denitrification

$= -0,0453377 + 0,1606554$	
$1/V_m$	0,160
K_s/V_m	-0,045
V_m (mg NO_3^- -N /L*d)	22,06
K_s (mg NO_3^- -N /L)	3,54

Experimental and model denitrification rates must be compared to see whether the obtained constants really represent the system. For this purpose obtained V_m and K_s and experimental C_{ln} values were placed in the model equation and Eq.4.27 formed.

$$\frac{1}{R} = \frac{1}{V_m} + \frac{K_s}{V_m R} = 0,1607 + \frac{3,54}{22,06 R} \quad (4.27)$$

Fig. 4.21 was a plot of $(C_{ln}/R)_{Model}$ (from Eq.4.27) versus $(C_{ln}/R)_{Experimental}$. As can be seen in the graph model and experimental results were fitted, that is V_m and K_s represent the system truly, so they can be used in design of biological denitrification system.

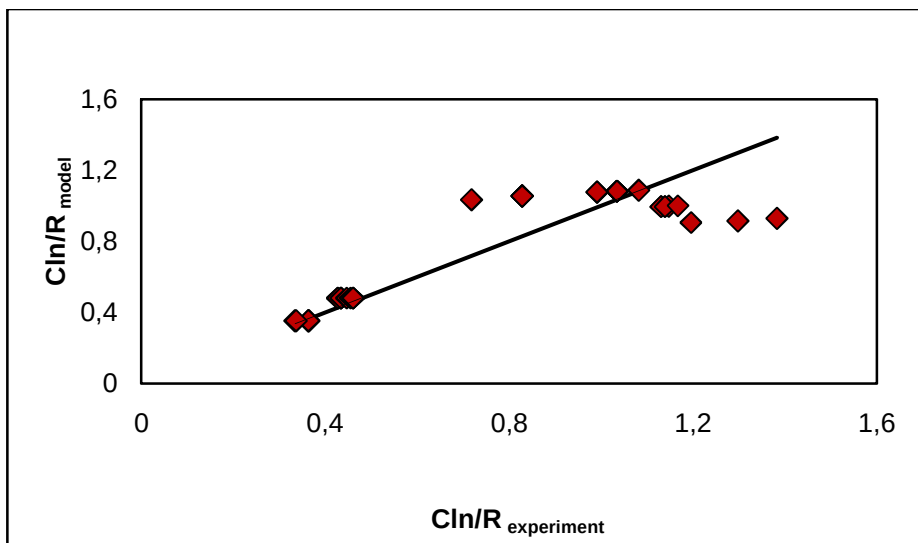


Figure 4.21 Comparison of experimental and model denitrification rate results for Michaelis-Menten model.

Modeling constants obtained in different biological denitrification reactors were listed together with results from the thesis in Table 4.11. As shown in Table 4.11 Gabaldon et al. observed very high nitrate removal from wastewater using methanol as carbon source.

Table 4.11 Modeling constants in different biological denitrification reactor systems

Type of process	Carbon source	To be purified	Model	Maximum removal capacity	V_m	K_s	Reference
Ammonia adsorption in a peat biofilter seeded with night soil sludge	-	ammonia	Michaelis-Menten	3,24 g N/(kg adsorbent*d) at a nitrogen load of 3,5 g N/(kg adsorbent*d)	11,40 g N/kg adsorbent*d	226 mg/L	Yani et al., 1998
bagasse charcoal pellets	sugar-industry waste (molass)	KNO ₃ and dilute molass	Monod	85 % nitrogen removal	195 g NO ₃ -N/m ³ *d	13,80 mg/L	Ueda et al., 2006
Nylon rings	Methanol	waste water	Monod	135 g NO ₃ ⁻ -N /m ³ *h at a nitrate load of 250 g NO ₃ -N/m ³ *h	217 g NO ₃ ⁻ -N/m ³ *h	112 g /m ³	Gabaldon et al., 2007
PCL pellets	PCL	drinking water	Michaelis-Menten	12 mg NO ₃ ⁻ -N/L*d	22,06 mg NO ₃ ⁻ -N/L*d	3,54 mg/L	PhD Thesis

4.7.3 Design of the Packed-Bed Biological Denitrification

In the design operation inlet and outlet nitrate concentrations assumed to be 45 and 8 mg NO_3^- -N/L. Inlet concentration of 45 NO_3^- -N /L was chosen being a high level of nitrate contamination in drinking water. Outlet nitrate concentration was chosen as 8 mg NO_3^- -N/L because this number is under the maximum allowable limit of 10 mg NO_3^- -N/L. V_m and K_s values were obtained during modeling. Operational characteristics for nitrate removal in biological denitrification can be seen in Table 4.12.

Table 4.12 Operational characteristics for nitrate removal by PCL packed bed in biological denitrification

Operational data	Symbol	Unit	Value
Inlet nitrate concentration	C_o	mg NO_3^- -N /L	45
Outlet nitrate concentration	C_e	mg NO_3^- -N /L	8
Maximum denitrification rate	V_m	mg/L*d	22,06
Half saturation constant	K_s	mg/L	3,54

Model equation Eq.4.27 was used in the design of fixed bed biological denitrification column. Left hand side of the model equation (C_{ln}/R) was calculated using assumed inlet and outlet nitrate concentrations, column

diameter, bed height, and any flow rate. Right hand side of the model equation $((K_s/V_m) + C_{ln}/V_m))$ was calculated using K_s , V_m , and C_{ln} values. Left hand side minus right hand side of the model equation was equaled to zero by applying Excel Solver.

Design operation was firstly applied to the denitrification column worked in the laboratory ($D=0,038$ m, height of packed bed= $0,38$ m, optimum flow rate= 350 mL/d). In the first row of Table 4.13, keeping column diameter constant, height of packed bed was changed from $0,38$ m to $1,00$ m and flow rate found as $0,60$ L/d. In the second row keeping height of packed bed constant and changing the column diameter from $0,038$ m to $0,1$ m led to a flow rate of $1,50$ L/day. In the third row keeping flow rate constant height of packed bed was changed and column diameter found as $0,027$ m. As shown in Table 4.13 (rows 1 and 2) increased column dimensions, that is increased surface area of reaction, led to larger flow rates. When working at constant flow rate (row 3) a smaller diameter enough if bed height increased to $1,00$ m.

Table 4.13 Design alternatives for the denitrification column worked in the laboratory

Column diameter (m)	Height of packed bed (m)	Flow rate (L/d)
0,038	1,000	0,60
0,100	0,380	1,50
0,027	1,000	0,35

In Table 4.14 assuming a constant column diameter of 0,30 m and changing height of packed bed in the range of 1 to 3 m flow rates were calculated. Increase in bed height led to increase in flow rates as shown in Table 4.14.

Table 4.14 Design alternatives for nitrate removal with constant column diameter of 0,30 m and changing bed height

Height of packed bed (m)	Flow rate (L/d)
1	36
2	72
3	108

Assuming a constant height of packed bed of 3 m and changing column diameter from 0,20 m to 0,50 m flow rates were calculated. As shown in Table 4.15 flow rates proportionally increase with increasing column diameters.

Table 4.15 Design alternatives for nitrate removal with constant height of packed bed of 3 m and changing column diameters

Column diameter (m)	Flow rate (L/d)
0,20	48
0,40	193
0,50	301

Assuming a constant flow rate of 200 L/d and changing bed height from 1 to 3 m column diameters were calculated in Table 4.16. Column diameters gradually decreased with increasing bed heights as shown in Table 4.16.

Table 4.16 Design alternatives for nitrate removal with constant flow rate of 200 L/d and changing height of packed bed

Column diameter (m)	Height of packed bed (m)
0,65	1
0,46	2
0,38	3

5 CONCLUSION

In this thesis the biodegradable synthetic polymer poly(ϵ -caprolactone) (PCL) was investigated as a substrate for denitrification. Experiments were started with mixed bacteria culture from soil suspension and waste water treatment. Afterwards the production of a pure culture '*Acidovorax avenae subsp. Avenae* LMG 17238 from University of Gent, bacteria collection in the sterilized laboratory condition was investigated as a more reliable basis of comparison.

Batch denitrification experiments were carried out in order to investigate the denitrification process with PCL and bacteria cultures (mixed and pure culture). Different carbon sources were also investigated to provide a baseline for comparison. However the experiments showed PCL is not highly responsive as a carbon source within the studied parameters.

Continuous experiments were conducted to further understand the process. Highly efficient denitrification was achieved in continuous experiments using PCL with the pure bacteria culture LMG 17238. The removal ratio varied between 80 and 85%, depending on the flow rate. The maximum denitrification rate was observed as $12 \text{ mg NO}_3^- \text{-N/m}^2 \cdot \text{d}$ at a nitrate load of $15 \text{ mg NO}_3^- \text{-N/m}^2 \cdot \text{d}$ in the continuous denitrification reactor.

The study of modeling of denitrification column was based on Michaelis-Menten and Monod kinetics. The experimental data of denitrification column fitted to Michaelis-Menten kinetics and model constants were found as maximum denitrification rate and saturation constant respectively; $V_m = 22,06 \text{ mg NO}_3^- \text{-N}/(\text{L} \cdot \text{d})$ and $K_s = 3,54 \text{ mg NO}_3^- \text{-N}/\text{L}$. The design of new denitrification columns were made according to model constants and assuming experimental inlet and outlet nitrate concentrations.

The purpose of this study included the simultaneous removal of pesticides and nitrates. To this end the ability of PCL to adsorb the phenoxyacid herbicide 2,4-D from water was investigated. Adsorption behavior of 2,4-D on PCL fitted both Langmuir and Freundlich isotherm models. The linear regression correlation coefficients for Langmuir and Freundlich were 0,995 and 0,998. The total capacity of the adsorbent were defined from experimental data for 2,4-D (10,68 mg/g).

The comparative denitrification experiments were conducted because it was necessary to investigate the effect of pesticide contamination. These experiments were carried out using phenoxyalkanoic acid herbicide; 2,4-D in water. The liquid chromatography-mass spectrometry (LC-MS-MS) analysis of effluent water showed pesticide adsorption in the fixed bed. So it was shown that pesticide and nitrate can be removed together in heterotrophic denitrification. Pesticide adsorption had no major effect of column outlet nitrate concentrations. At the end of 2,4 D contaminated 50 days, denitrification column needed to recover. The

film of LMG 17238 pure bacteria culture creates a brownish color on the PCL particles. The intensity of this brownish color increased especially at the bottom part of column. At the end of this recovery time the brownish color on the PCL particles waned considerably.

To sum up the combination of PCL with pure bacteria culture LMG 17238 is a feasible denitrification process which can remove both pesticides and nitrates simultaneously from water. Further research is required to understand the ability of this process to effectively adsorb other pesticides and to learn the interactions of and between different bacteria cultures in this denitrification process.

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RESUME

Nuran Böke graduated from Ege University Chemical Engineering Department in 1977. She had a MSc degree from the same Department in 1982.