2007

Biological removal of nutrients from mine waters
Biologinen ravinteiden poisto kaivosvedestä

Kaira-hankkeen loppuraportti

Katri Mattila, Gennadi Zaitsev, Jörg Langwaldt

METLA GTK TEKES

Biological removal of nutrients from mine waters

Biologinen ravinteiden poisto kaivosvedestä

Final report – Loppuraportti

Katri Mattila[1], Gennadi Zaitsev[1] and Jörg Langwaldt[2]

[1]Finnish Forest Research Institute, Rovaniemi Unit
[2]Geological Survey of Finland

METLA www.metla.fi

Mattila, K., Zaitsev, G. & Langwaldt, J. 2007. Biological removal of nutrients from mine waters. Biologinen ravinteiden poisto kaivosvedestä. Final report - loppuraportti. 99 p. ISBN 978-951-40-2060-5 (PDF).

http://www.metla.fi/hanke/7207/index-en.htm
http://www.ymparisto.fi/default.asp?node=8918&lan=fi

Publischer:
Finnish Forest Research Institute
Rovaniemi Unit
P.O. Box 16, FI-96101 Rovaniemi

Accepted by Martti Varmola, Director of Research Unit

Cover Jouni Hyvärinen

Contents

1 PREFACE

This Final Report on the research project entitled "KAIRA" summarizes the scientific results achieved during the duration of the project from 1.10.2005 to 31.8.2007. Initiation of the project was a result of the outcomes of the ARMI1 and ARMI2 research projects, which were carried out from 1.7.2001 to 30.8.2005 at the Finnish Forest Research Institute in Rovaniemi, Lapland. The research project KAIRA was established with support by the European Regional Development Fund provided by the Finnish National Technology Agency (TEKES) and industrial partners.

Project name:
Biological removal of nutrients from mine water (Biologinen typenpoisto kaivosvedestä)

Project no:
1141/3/05

Funding period and funding
Funding of the project was 415 050 EUR for the period 1.10.2005-30.8.2007. The funding was provided by the European Regional Development Fund of the European Commission, Tekes the Finnish Funding Agency for Technology and Innovations and the industrial partners.

1.1 Industrial partners

The industrial partners are listed below and mine (projects) are shown in Figure 1.

Outokumpu Chrome Oy, chromite mine in Kemi
ScanMining Oy, gold mine in Pahtavaara
Kemphos Oy, apatite mine in Siilinjärvi
Agnico-Eagle Finland, gold mine in Kittilä
Scandinavian Gold Prospecting Ab, Kevitsa mine project Ni, Cu, PGM and Au
Anglo American Exploration B.V. plc., Pulju Ni project
Oy Forcit Ab
Sarlin Hydor Oy
Tekno-Forest Oy

Figure 1. Location of participating mines.

1.2 The research group

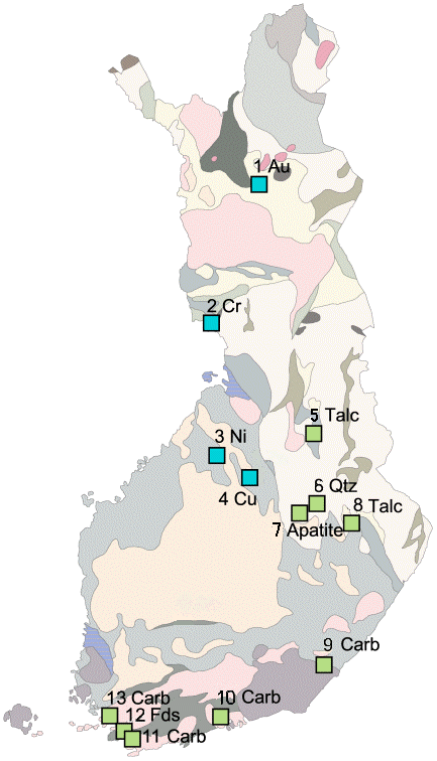
Finnish Forest Research Institute
Dr. Martti Varmola (Responsible project leader)
Dr. John Derome (project administrator)
Dr. Katri Mattila (Senior Researcher)
Dr. Gennadi Zaitsev (Senior Researcher)
LuK. Tarja Mettänen (Assistant Researcher)
yo Katri Häyrynen (Assistant Researcher 6.3.-10.9.2006, reverse osmosis)
yo Heidi Kontio (Assistant Researcher/half time 8.5.-31.8.2006, 18.6-31.8.2007 fate of N in ore and barren rock and TRFLP of clones)
yo Antti Karkman (Assistant Researcher 1.6.-30.11.2006, identification of biomass microbes)
Kimmo Kangas (Trainee 4.12.2006-30.4.2007, maintenance of pilot reactors at Kemi mine)

Geological Survey of Finland
Dr. Jörg Langwaldt (Researcher), Mineral Processing, Outokumpu

2 BACKGROUND

2.1 Mining activities in Finland

Finland has a long mining history and the number of new mining projects has increased since Finland joined the EU in 1995. The Finnish miming industry is an important sector for the economy and labor market. Today, 4 metal mines, about 40 industrial mineral mines and about 60 dimension-stone quarries are in operation (Mining Communications Ltd., 2005) (Figure 2).



Metal mines
1. Pahtavaara: Au 1996–
2. Kemi: Cr 1969–
3. Hitura: Ni, Cu 1970–
4. Pyhäsalmi: Cu, Zn, S 1962–

Industrial minerals mines
5. Lahnaslampi: Talc, Ni 1969–
6. Kinahmi: Quartz 1910–
7. Siilinjärvi: Apatite, limestone, mica 1979–
8. Horsmanaho: Talc, Ni 1980–
9. Ihalainen: Limestone, wollastonite 1910–
10. Sipoo: Limestone, dolomite 1939–
11. Förby: Limestone 1917–
12. Kemiö: Feldspar, quartz 1966–
13. Parainen: Limestone 1898–

Figure 2. Major mines in Finland (Geological Survey of Finland 2007).

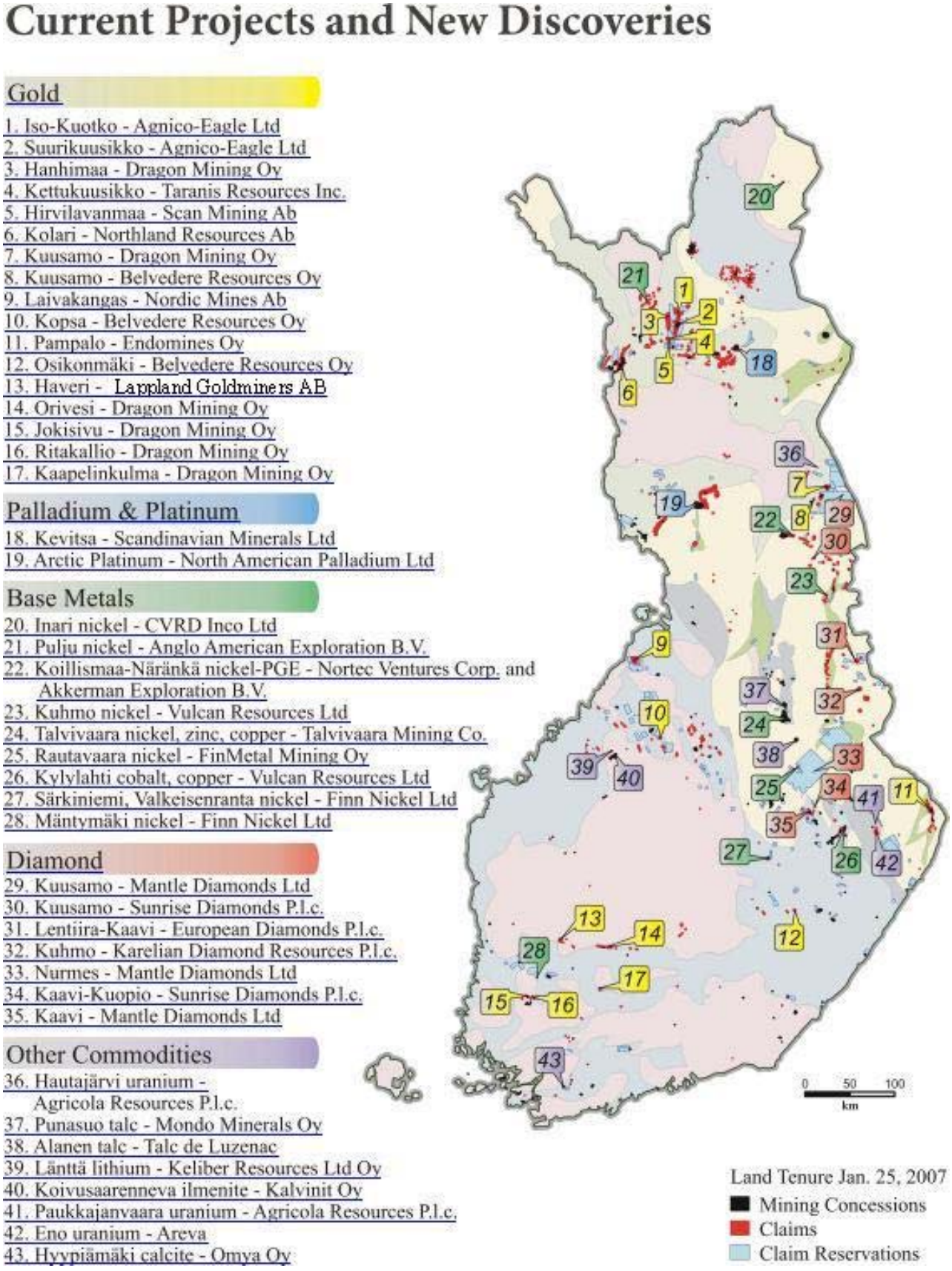


Figure 3. Current projects and new discoveries in Finland (Geological Survey of Finland 2007).

International interest in Finland's mineral resources has been increasing since 1995. Several large-scale valuable mineral deposits exist in Finnish Lapland (Mining Communications Ltd., 2005). The opening of new mines is envisaged in Lapland as described in a plan for Lapland until 2022 (Lapin Liitto, 2005). Current projects and new discoveries are shown in Figure 3. The ability to comply with new stricter requirements for environmental protection has been somewhat of an impediment in opening new mines and maintaining ongoing mines in Finland. The discharge of nutrients from mines into nearby water bodies is an emerging issue, which is expected to receive more attention in the future. This can be seen in manuals (EPA, 2003; Heikkinen et al., 2005) and BREF-documents (EU, 2004).

2.2 Origin of nitrogenous compounds in mine water

The presence of nitrogen compounds (ammonia, nitrite and nitrate) in surface water in the vicinity of mining areas can have detrimental effects to the environment. The main source of ammonium and nitrate in mine water originates from blasting agents such as ammonium nitrate fuel oil explosives (Forsberg and Åkerlund, 1999). Other sources of ammonium and nitrate in mine water are cyanide destruction, transformation of amines in flotation circuits, pH regulation agents, ammonium sulphate as eluent of uranium from ion exchange resins, ammonium hydroxide used in uranium precipitation, and ammonia used as lixiviant to recover copper and nickel in hydrometallurgical processes (EPA, 2003). Since the 1950s, one of the most used explosives is "Ammonium Nitrate Fuel Oil" (ANFO). ANFO explosives contain a mix of ammonium nitrate and fuel oil. More water-proof explosives like emulsion explosives have been developed by companies such as Oy Forcit Ab in Finland. These explosives are used for mass blasting, which are used outside populated areas and in tunnel blasting.

The total amount of extracted rock has been increasing significantly since the 1950s. In 2006, the total output of Finnish mining activities was 30.646.467 tons (Geological Survey of Finland, 2007). About 0.2-0.3 and 0.5-1.0 kg of explosives per ton of extracted rock are applied in open pit and underground mining, respectively (Oy Forcit Ab). In Finland, the total annual consumption of explosives might be about 12.000 tons (0.4 kg/ ton of rock). In a study by Forsberg and Åkerlund (1999) about 17% of applied explosives are transported with the ore to the refinement and barren rock piles. The study by Forsberg and Åkerlund (1999) and a survey at Kemi mine indicate that about 0.03% and 1% w/w of nitrogen in all loaded explosives, respectively, are discharged into the aquatic environment. The annual consumption of explosives in the Suhanko and Talvivaara mine project will be about 8000 to 12000 tons with a potential release of 80 to 120 tons of total nitrogen into the nearby water bodies if up to 1% of the total nitrogen in applied explosives are directed to the aquatic environment.

Furthermore, the extraction of groundwater to prevent flooding of mines, leachate discharge from barren rock piles and discharge of wastewater from the ore processing into tailing ponds results in large amounts of mine effluents in the order of several m^3/min or million m^3/year per mine. Discharge of nitrogen-loaded mine waters from the barren rock piles is largest during spring and after heavy rainfall events. The discharge of large volumes of nitrogen-loaded water in spring is critical since microbial activity is thought to be low after the cold winter. Barren rock plies are a long-term source of ammonia and nitrate release. Small streams and water bodies have only limited capacity to handle excessive N-loads and are at risk of eutrophication.

2.3 Reduction of nutrient load to the aquatic environment

The European Union Water Framework Directive (2000/60/EC, 23rd October 2000), which is the general legislation for the protection of Europe's aquatic environment demands a good state of all aquatic environments throughout the European Union by 2015. The assessment of the state of the aquatic environment is based on chemical as well as ecological conditions of streams and water bodies. Furthermore, in addition to the rules in Directive 80/68/EEC on the protection of groundwater against pollution caused by certain dangerous substances, also Article 17 of the WFD requires a Groundwater Daughter Directive on the protection of groundwater against pollution (2003/0210, COD). Nitrate is a key water pollutant and its minimization of discharge into the environment has been addressed by the EU for instance in directive 91/676/EC (protection of water from nitrate pollution from agricultural activities). Especially in Finland nitrate concentrations in rivers are increasing (EEA 2004).

The US Environmental Protection Agency addressed the issue of nutrient load from mines to the aquatic environment in its document "EPA and Hardrock Mining" by stating: "Specifically, residual chemicals may be present in mine drainage due to use of explosives. For example, blasting operations that use ANFO can produce elevated levels of ammonia (NH_4^+) and nitrate (NO_3^-) in mine effluent." (EPA, 2003).

However, possible NH_4^+ and NO_3^- removal processes were not discussed. The European Commission, Directorate General Joint Research Center, has prepared a reference document on "Best Available Techniques for Management of Tailings and Waste-Rock in Mining Activities" (EU, 2004). The best available techniques reference document (BREF) addresses only to a limited extent the discharge of inorganic nitrogen compounds from mining activities into the environment and doesn't provide a best available technique (BAT) to minimize the amount of nutrients released or removal of nitrogen from mine effluents. The BREF document (EU; 2004) states that "Ammonium and nitrate remaining from un-detonated explosives are easily leachate from waste-rock" (EU, 2004). In the recently published handbook for mine closure the environmental problem of nutrient discharge is addressed, but removal process is named (Heikkinen et al., 2005). Although different attempts have been made to develop a process for removal of dissolved nitrogen compounds from mine waters, currently no process for ammonia and nitrate removal is commercially available.

2.4 Removal of nutrients from mine water

In nature, a two-step process exists for the removal of nitrogen from water bodies. Ammonia NH_4^+ is first oxidized to nitrite NO_2^- (Eq. 1) and then to nitrate NO_3^- (Eq. 2) by bacteria. In Lapland, natural attenuation of ammonia and nitrate by nitrification and denitrification is limited by low temperature and lack of readily biodegradable carbon sources. The biological removal of total nitrogen by combined nitrification and denitrification in engineered processes is nowadays standard in municipal and industrial wastewater treatment. Nitrification is the initial step of total nitrogen removal which proceeds by ammonium oxidation to nitrite (Eq. 1) followed by nitrite oxidation to nitrate (Eq. 2). Ammonium is oxidized by bacteria of the genera *Nitrosomonas* and *Nitrospira* among others (Eq. 1).

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+ \qquad \text{decrease in pH} \qquad \text{Eq. 1}$$

Nitrite is oxidized by bacteria of the genera *Nitrobacter* and *Nitrospira* among others (Eq. 2).

$NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$ Eq. 2

Biological denitrification proceeds by reduction of nitrate to dinitrogen gas by facultative, anaerobic, heterotrophic bacteria (Eq. 3).

$2\ NO_3^- \rightarrow 2\ NO_2^- \rightarrow 2\ NO \rightarrow N_2O \rightarrow N_2$ Eq. 3

For the denitrification of inorganic water, the heterotrophic bacteria require external carbon sources such as methanol (Eq. 4). Methanol is often used, at a ration of 3 kg methanol/kg nitrate-nitrogen, due to its low cost and low excess biomass production (Tchobanoglous et al., 2003).

$6\ NO_3^- + 5\ CH_3OH \rightarrow 3\ N_2 + 5\ CO_2 + 7\ H_2O + 6\ OH^-$ Eq. 4

The nitrification is affected by temperature (Chen et al., 2006) and nitrification rates double with 10°C increase (Tchobanoglous et al., 2003). Denitrification is also strongly dependent on temperature, with denitrification rates doubling with every 4°C increase (Given and Meyer, 1998). In municipal wastewater treatment plants, the total nitrogen removal performance is usually about 1 kg $N/m^3/d$ (Tchobanoglous et al., 2003).

Recently a novel biological wastewater treatment process called "Anammox®" has been developed for warm wastewaters with ammonia concentrations above 200 mg/L and low organic carbon content (Kuenen and Jetten (2001). The temperature optimum of the process is 37 °C (Kuenen and Jetten, 2001). So far no microbial population capable of the "anammox" process at low temperature has been described. The biological process proceeds via a partial nitrification of ammonia (Eq. 5) and subsequent oxidation of ammonia with nitrite to nitrogen gas (Eq. 6).

Partial nitrification:
$2\ NH_4^+ + 1.5\ O_2 \rightarrow NH_4^+ + NO_2^- + H_2O + 2\ H^+$ decrease in pH Eq. 5

"Anammox" process:
$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O$ Eq. 6

For evaluating the presence of nitrifying and denitrifying bacterial species in nature molecular biological DNA based methods are needed. General understanding is that 1 % of all bacteria have been cultured or can be cultured from environmental samples. Amann et al (1995) reviewed that in water habitats 0.001-1 % of bacteria were culturable, in soil 0,3 % and in activated sludge about 1-15 %. The biomass samples of nitrifying and denitrifying bioreactors in this study present mixture of these three environments; water face where bacteria is growing as biofilm also on surface of fine stone particles.

The biological removal of ammonium and/or nitrate from mine water has been applied at a few mines in North America (Given and Meyer, 1998; Given et al., 1998; Reinsel and Plumb, 1999; Reinsel, 2001). During this process ammonium is converted to benign dinitrogen gas via nitrite/nitrate (Halling-Sørensen 1993). Nitrification as part of biological cyanide destruction has been extensively studied (e.g. Akcil, 2003; White and Schnabel, 1998). However, the combined removal of ammonium and nitrate from water of mining and mineral processing plants has only

been studied in suspended biomass systems as part of cyanide destruction (Given and Meyer, 1998; Given et al., 1998). Although plants treating water by suspended biomass processes perform successfully with organic wastewater, they are limited in treating dilute inorganic wastewaters. Reactors with biomass growing attached onto a carrier material, i.e a biofilm, have several advantages over suspended biomass water treatment systems. These advantages are a relatively short treatment time, low maintenance costs, slow excess biomass generation with generally no waste biomass, and good resistance against low temperature, toxification and changing feed quality (pH, salinity, metals, biological and chemical oxygen demand content) (Rowan et al., 2003). The attachment property of bacteria to the carrier material in the biofilm reactor is a major parameter and minimizes biomass washout of slow-growing microorganisms at high flowrates. Further, biofilm reactors yield higher removal rates in smaller units. Attached bacteria usually show higher specific activity than suspended bacteria (Singh et al., 2006). Dictor et al. (1997) applied fixed-bed reactors for biological cyanide conversion to ammonium. The fixed and moving bed biofilm processes are highly efficient in removing the soluble organic and nitrogennous compounds (Stowa, 2006a; b, c; Rusten et al., 1994; Odegaard et al., 2000). Processes based on biofilm growth on the surface of carrier elements have been applied in this study because of their superior behaviour at the low level of ammonium and BOD concentrations encountered in mine water. So far scientific laboratory studies have only applied synthetic mine effluents to study biological ammonium and nitrate removal at room temperature (e.g. Koren et al., 2000). The nitrification of ammonia in water of high salinity has not been studied in detail (Mizohuchi et al., 1998).

In general, the most common processes for removal of nitrogenous compounds beside biological treatment have been ion exchange and catalytic oxidation (Lee & Lueptow 2001). Reverse osmosis has been studied for the removal of total nitrogen from water (Awadalla and Kumar, 1994; Awadalla et al., 1993). Since the amount of formed mine effluents is extensive, a volume reduction step prior to biological treatment could be economically feasible. For this purpose, membrane filtration is a promising option, since it simultaneously can remove also other pollutants in addition to nitrogenous compounds (Shrimali and Singh, 2001) and requires less energy than many other concentrating processes (Chang, 1996). The molecular weight of ammonium and nitrate is below 70 g/mol and, therefore, viable membrane processes for their removal are reverse osmosis and electrodialysis (Shrimali and Singh, 2001). Membrane separation processes are quite simple, the equipments are compact and modular and capable for continuous operation. (Awadalla et al. 1994) In addition, the efficiency and performance of membrane treatment are stable, and predictable with proper feed treatment (Lee & Lueptow 2001). Although studies on removal of nitrogenous compounds from wastewater by RO have been published, very few studies have been conducted on separation of nutrients from mine water and none on RO as a pre-treatment for subsequent biological removal of nitrogen. Malaiyandi and Sastri (1981) as referred by Awadalla et al. (1994) reported less than 30 % retention of ammonium and nitrate by RO with a cellulose acetate membrane. The Du Pont company (1972) as referred by Awadalla et al. (1994) reported 80% rejection of ammonium and 85% rejection of nitrate ions from ammonium nitrate and sodium nitrate solutions, respectively, by a hollow fine fiber B-9, polyamide RO membrane. Awadalla et al. (1994) tested four different RO membranes for the removal of ammonium and nitrate from mining effluents with a 99% separation of ammonium and 97% separation of nitrates ions with a crosslinked thin-film composite RO membrane (Toray Ind. Japan). The operational costs for treatment of acid mine drainage by RO had been estimated to 0.60 –1.00 euro/m^3 (Robinson, 2003; Smalley and Reynolds, 2004). Although RO has been applied for the production of process water at mines (Allenby, 2004), the application of RO for neutral to alkaline mine effluents has not been studied in detail. For this reason, the use of reverse osmosis (RO) was tested to produce a permeate that

could be discharged directly to the nature and a concentrated brine including nitrogenous compounds for subsequent treatment in bioreactors.The aim of this study was thus to evaluate the applicability of RO in concentrating ammonium and nitrate from mine waters before the removal of nutrients from the concentrate in bioreactors.

3 PROJECT AIMS

The research project aimed to develop a biological high-rate treatment process for ammonia and nitrate containing mine effluents or RO concentrate at cold temperature. Within the project the enrichment was planned of cold-tolerant bacteria capable of nitrifying ammonia, oxidizing ammonia and nitrite, and denitrification of nitrate.

Since mine effluents contain only very small amounts of BOD and COD, an external organic carbon source, i.e. substrate, has to be added to allow denitrification of nitrate to nitrogen by heterotrophic bacteria. The most suitable external carbon source was to be established for the denitrification of nitrate. The effect of heavy metals on microbial activity was to be studied. The possibility of concentrating nutrients from mine water by RO was studied with the aim to minimize the bioreactor scale. The RO concentrate was biologically treated. The effect of high salinity and heavy metal content in the RO concentrate on nitrification and denitrification was studied. The different process combinations were investigated. The results of the pilot-scale tests were utilized to estimate operating costs for full-scale installations.

The original aims were as follows (progress towards aim)

A. Quantification of nitrogen release and fate of nitrogen at mine in Lapland. (completed)
B. Quantification of *in situ* nitrification of ammonia in ponds of mines. (completed)
C. Enriching and characterizing the bacteria found in nitrification or denitrification bioreactors. Monitoring of changes of the microbial population in the bioreactors. (completed)
D. Testing of biofilms for the nitrification and denitrification at low temperature with fresh mine effluents in the laboratory and at an operating mine. (completed)
E. Testing of biofilms for anaerobic oxidation of ammonia with nitrite (anammox) at low temperature with fresh mine effluents in the laboratory and at an operating mine. (Enrichment of anammox bacteria completed in batch assays at the bench scale)
F. Production of concentrated mine effluents, i.e. RO concentrate, by RO. Testing of biofilms for the nitrification and denitrification at low temperature of RO concentrate. (completed)
G. Studies on nitrification and denitrification of cold RO-concentrate with high salinity, heavy metal content and nitrogen compounds. (completed)
H. Quantification of biosorption of heavy metals in biofilms (completed)
I. Investigation and assessment of different process combinations (theoretically assessed)
J. Removal of total hydrocarbons in bioreactors (completed)
K. Characterization of biofilms in nitrifying and denitrifying bioreactors. (completed by molecular biological characterization based on TRFLP-analysis and not as proposed by microscopy using functional fluorescent stains)
L. Quantification of biosorption of heavy metals in biofilms (completed by water and biomass analyses and not as proposed by Energy Dispersive X-ray Spectrometre in combination with Scanning Electron Microscope for biofilm samples).

Following tasks were carried out additional to the proposed work plan:

1. Assessment of different biofilm reactor designs (fixed-bed versus moving-bed bioreactors)
2. Mass balance of C and N in high-rate denitrifying bioreactor (Pilot 5)
3. Characterization of suspended solids by particle size analysis at GTK and removal of suspended solids by hydrocyclones with support of GTK
4. Denitrification of nitrate-rich saline wastewater from Oy Forcit Ab (Pilot 10)
5. Nitrification and denitrification of water samples from Siilinjärvi mine, batch tests

4 MATERIALS AND METHODS

4.1 Fate of nitrogen at mines

The aim this work was to follow and quantify the amount of total nitrogen in water and rock at Kemi, Pahtavaara and Siilinjärvi mines. The fate of nitrogen originating from un-detonated explosives was quantified based on the amount of explosives used in a mine. The aim was also to define if in-situ nitrification and denitrification were occurring within the water treatment systems of operating mines in Finland.

Water, ore and barren rock samples were collected from Kemi, Pahtavaara and Siilinjärvi mines at least 5 times between 2005 and 2007. Water samples included all possible water treatment steps, barren rock influenced waters, natural water before and after mine area. The water samples were filtrated trough 0,45 µm filter at sampling site, stored frozen if not analyzed immediately. From each mine 4 different kinds of ore and barren rock were sampled. The samples were ore from the tunnel after explosion, ore after dry crushing and concentrate from storage. In Pahtavaara mine we sampled two different quality concentrates; a II concentrate from shaking table (tärypöytä) and a flotation concentrate. Also in Kemi mine, two concentrates were sampled (one from heavy media separation, palarikaste, and one from magnetic separation, hienorikaste). The concentrate from Siilinjärvi mine was apatite after flotation. The barren rocks in each mine were taken from the tunnel after blasting or freshly from the barren rock pile. Samples contained 5-10 kg of ore or barren rock and about half of the sample was dried and crushed and the other half stored. The crushing to -3 mm was performed by the Geological Survey of Finland in Rovaniemi. The crushed ore and barren rock samples were homogenized and inorganic nitrogenous compounds extracted to water by shaking in acidic conditions (Table 1).

Table 1. The method used in analysing the ore and the barren rock samples

Kemi (chromite)	Pahtavaara (gold)	Siilinjärvi (apatite)
dry crushing (particle size < 3 mm)		
25 g stone + 195 ml dH_2O add 5 ml 0.1 M H_2SO_4		50 g stone + 190 ml dH_2O add 10 ml 0.1 M H_2SO_4
shake horizontally 2 h 170 rpm		
filtration (pore size 0.45 µm)		
pH measurement		
analysis of nitrogen		

4.2 Separation of nutrients from mine water by reverse osmosis and nanofiltration

A DSS Labstak M20 laboratory equipment, manufactured by Danish Separation Systems AS was used. It can be equipped with flat sheet membranes. The effective membrane area is 0.036 m^2. It is possible to put a maximum of 20 membranes to the packing, thus increasing the total membrane area to 0.72 m^2. The feed was cooled to about 13-14 ºC, in order to simulate the conditions in the mine.Five different commercial membranes were tested. The characteristics of these membranes are presented in Table 2.

Table 2. Technical data for membranes used in the experiments.

Membrane identification	Supplier manufacturer	Membrane type and material	Retention %	Operating pH range
SW 30 HR	Filmtec Corp.	RO, thin film composite	99.6[1]	2-11
Espa 2	Hydranautics	RO, thin film composite	99.5[2]	3-10
TFC ULP	KOCH Membrane Systems	RO, thin film composite	98.0[3]	4-11
RO1	Separ	RO, thin film composite	98.0[4]	3-10
NF	Filmtec Corp.	NF, thin film composite	-	-

Figures from manufacturers' homepages:
[1] tested with 32 000 mg/l NaCl, 55 bar and at 25ºC
[2] tested with 1500 ppm NaCl feed water solution
[3] nominal rejection conductivity percent
[4] tested with 2000 ppm NaCl feedwater, with 8.6 bar pressure at 25ºC

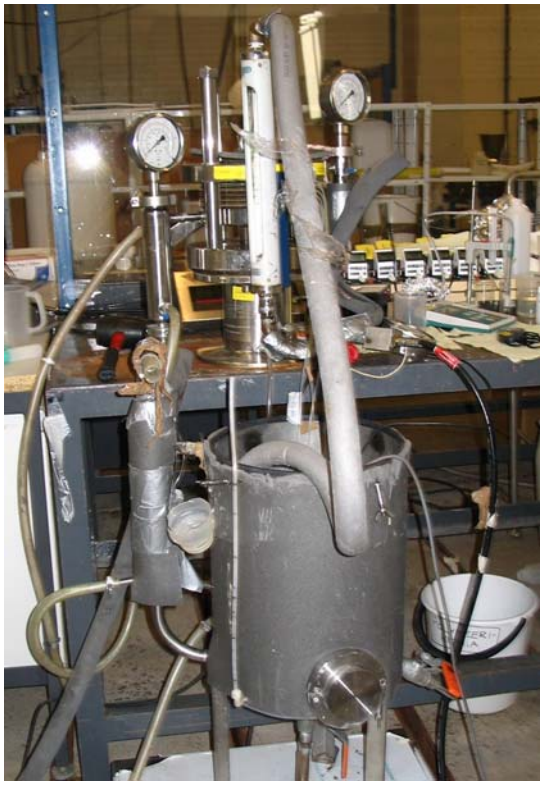


Figure 4. DSS-plate module

Figure 5. Packing of the membranes

The first stage of the experiment was carried out in a batch mode, both the permeate and concentrate were returned to the feed tank. The operating pressure was raised stepwise from 6 to 35 bar. The tested pressures were 6, 10, 15, 20 and 25 bar for Espa 2, TFC ULP, RO1 and NF membranes. Because of the small permeate flux from SW30 HR, the samples were taken only at 15, 20, 25, 30 and 35 bar. The optimum pressure was chosen based on the measured fluxes and calculated retentions of electric conductivities. The samples from the optimum pressure were further analysed, and based on the analysis results, the most suitable membrane for each mining effluent was selected. After the most suitable membrane and pressure value were selected, the effluent was concentrated using the selected membrane by using an effective membrane area of 0.144 m^2, by circulating the concentrate back to the feed vessel and by removing the permeate to a separate vessel. The pressure was maintained at 15 bar and the recirculation flow rate was kept at 80 % level. If the samples from the concentrate could not be returned, the amount of those samples was subtracted from the total feed water amount. A sample from every volumetric reduction factor concentrate was subjected to nitrification and denitrification tests (results not shown). Samples were collected from the feed and permeate vessels at five different volumetric reduction factors (VRF) which were calculated according to Eq. 7.

$$VRF = \frac{Total\ \ feedwater\ amount}{Total\ \ feedwater\ amount - The\ amount\ of\ \ permeate} \qquad \text{Eq. 7}$$

The retention of electric conductivity with different volumetric reduction factors was calculated according to Eq 8.

$$\mathrm{R(electric\,conductivity)} = 1 - \frac{C_{pa}}{C_c} \cdot 100\% \qquad \text{Eq. 8}$$

where C_{pa} is concentration in accumulated permeate at a specific VRF, mg/L
C_c is concentration in residual concentrate (feed) at a specific VRF, mg/L

The retention for ammonium, nitrate, chloride and metal ions was calculated according to Eq. 9.

$$\mathrm{R} = 1 - \frac{C_{pa}}{C_f} \cdot 100\% \qquad \text{Eq. 9}$$

where C_{pa} is concentration in accumulated permeate, mg/L
C_f is concentration in feed at the beginning, mg/L

When the feed is concentrated with high volumetric reduction factors, the osmotic pressure of the effluent becomes higher and, therefore, also higher operating pressures are required. Concentration also increases the concentration of elements in the formed permeate, as the feed solute concentration gets higher. Soluble salts, which concentrate to the feed, may to some extent precipitate to the membrane surface, causing fouling and resulting in a decrease of permeate flux (Awadalla and Kumar, 1994).

For the pilot scale concentrations, a spiral wound module equipment constructed by Lappeenranta University of Technology was used. The active membrane area depends on the type of membrane used. For the pilot scale concentration, a spiral wound RO1 membrane, with active membrane area of 1.2 m^2 was used to produce about 0.2 m^3 of RO-concentrate from the mine waters 1 and 2.

Figure 6. Spiral wound module used in large scale concentration

Volumetric reduction factors were applied based on the biological experiments done on the samples from initial concentration tests. The RO-filtration was operated at nearly constant concentrate flow rate of 800 l/h and permeate flux of 25-33 l/(m^2h). Membrane fouling was estimated by comparing the water fluxes before and after the filtration to be low when flux difference was 10% or less (Lee and Lueptow, 2001). The membrane fouling was calculated by comparing the pure water permeability before and after the filtration (Eq. 10).

$$\text{Fouling} = (1 - \frac{PWP_a}{PWP_b}) \cdot 100\% \qquad \text{Eq. 10}$$

where PWP_a is pure water permeability after effluent filtration, [L/(m^2h bar)]
PWP_b is pure water permeability before effluent filtration, [L/(m^2h bar)]

Table 3. The pH, electrical conductivity (EC) and elemental compositions of the three mine waters.

Mine	pH	EC	NO_3-N	NH_4-N	Cl	Li	Na	Mg	Al	S	K	Ca
		µS/cm	[mg/l]									
Pahtavaara	7.9	464	15.5	9.53	14.03	<0.01	28.1	15.9	<0.01	14.6	15.0	32.2
Kemi	7.5	6840	20.3	5.5	2400	0.04	834	120	0.59	75.4	76.8	544
Siilinjärvi	7.9	988	44.0	11.8	57.6	<0.01	94.1	17.8	0.13	44.9	89.9	52.4
Mine	**Mn**	**B**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Cd**	**Ba**	**Pb**	**Cr**
	[mg/l]											
Pahtavaara	0.04	0.10	0.01	0.01	<0.01	0.02	0.08	<0.01	<0.01	0.12	<0.01	<0.01
Kemi	0.09	0.40	<0.01	0.04	<0.01	0.03	0.16	<0.01	<0.01	1.62	<0.01	0.02
Siilinjärvi	<0.01	0.09	0.02	<0.01	<0.01	<0.01	0.021	<0.01	<0.01	1.68	0.01	0.01

The amount of ammonium, nitrate and chloride was measured with Hach Lange DR2800 spectrophotometer. The analysis method of nitrate is equivalent to the ISO 7150-1 method and the analysis of nitrate to ISO 7890-1-2-1986 method. The elemental composition of feed and concentrate was analysed by ICP-AES. The pH and electric conductivity were measured with conventional instruments.

4.3 Biological removal of nitrogenous compounds from mine water at laboratory-scale

The biological removal of nutrients in mine water was studied by laboratory- and pilot-scale biofilm reactors (Table 4). Two biofilm reactor designs, i.e. fixed-bed and suspended-bed, were tested at bench-scale. The fixed-bed bioreactors were constructed as trickling and submerged filter, whereas the suspended-bed was a moving bed (Figure 7 and 8). Different carrier media with a total surface area was applied to support attachment and growth of microorganisms (Fig 9). The nitrification and denitrification rates are the key parameters in the design of a biological wastewater treatment plants for nitrogen removal. For this reason, it is essential to experimentally determine the maximum nitrification and denitrification rates in similar conditions, to those of proposed full-scale treatment plant. In the laboratory experiments water was used from the dewatering system of two operating underground mines (ScanMining Oy Pahtavaara mine and Outokumpu Chrome Oy Kemi mine), one operating open pit mine (Kemphos Oy Siilinjärvi apatite mine and process wastewater from Oy Forcit Ab. Pilot-scale bioreactor systems were operated at Kemi mine. The mine water was sampled into 30 l or 220 l barrels and stored in the cold. The concentrations of ammonia and nitrate in Pahtavaara mine water were in the range from 5 to 32 and 11 to 42 and in Kemi mine water from 0.5 to 26.8 and 7.2 to 52.5, respectively. (Figures 21 and 25). The pH of the feed was about 8, which is favourable for the treatment of weakly buffered waters in nitrifying bioreactors (see Eq. 1). Water contained mainly following chemical elements Na (838±43), K (159±39), Mg (117±10), Ca (502±44), S (84±7) from Kemi mine and Na (24±1), K (24±7), Mg (18±1.3), Ca (33±1), S (15±2) from Pahtavaara mine. Concentrations of these elements were approximately 10 times higher in Kemi mine water than in water from Pahtavaara mine. From heavy metals only strontium was found in significant amounts in Kemi mine water (22±0.9 mg/l). The content of suspended solids in water from Kemi mine was usually 402±102 g/l dry weight and from Pahtavaara 1080 g/l dry weight. The effluent of the nitrifying reactor was treated in the denitrifying reactor, using methanol as external carbon sources. Methanol was diluted with tap water to concentration of 1.5 %. Phosphoric acid (0.5 to 5 mg P total /L) was dosed to the mine water to avoid deficiency of phosphorus in the mine waters (Figures 22 and 26). A mixture of methanol and phosphoric acid was added to bioreactors as separate solution in excess amount in order to ensure that neither of phosphorus or carbon sources was limiting denitrification rate.

Table 4. Operational parameters and capacity of the laboratory bench-scale systems

Pilot number and installation place	Scientific aim Test duration	Description of pilot installation
Pilot 1 Laboratory at 5°C	Evaluation of the low temperature effect on removal of total nitrogen from mine waters by nitrification and denitrification process. 5.3.2005-21.9.2005 Nitrification 3.11.2005-6.10.2006 Denitrification	System consists of nitrifying bioreactor, clarifier, anoxic unit and denitrifying bioreactor. - The nitrifying bioreactor is combining trickling filter and submerged fixed bed aerobic bioreactor. It is a column in height of 1.2 m and diameter 0.14 m with total volume of 18.5 liters aerated from the bottom with air. -- The trickling filter has volume of 12.8 liters filled by Hufo 200 plastic carrier with total surface of 1.52 m^2 and 50 g of lignite coke. -- The submerged fixed bed bioreactor has volume of 5.7 liters filled by Hufo 200 with total surface of 0.33 m^2. - The anoxic unit is column in height of 0.6 m and diameter 0.085 m with submerged fixed bed filled by Hufo 200 with total surface of 0.47 m^2. Filter has water volume of 2.7 liters with downstream flow. - The denitrifying bioreactor is submerged fixed bed anaerobic column in height of 0.6 m and diameter 0.14 m filled by F2 with total surface of 1.11 $m^2$and 6.82 liters of water. Reactor has working volume of 7.4 liters with upstream flow.
Pilot 2 Laboratory at 5°C	Evaluation of the low temperature effect on nitrification using bioreactor with moving carrier and Kemi mine water. 31.10.2005-13.8.2006	System consists of submerged moving bed aerobic nitrifying bioreactor and clarifier. - The bioreactor has working volume of 29.2 liters filled by F2 with total surface of 5.867 m^2 and 26.15 liters of water.
Pilot 3 Laboratory at 5°C	Enrichment of nitrifying bacteria on hydrophobic glass carriers 24.1.2006-31.5.2006	Type of the nitrifying bioreactor is combining trickling filter and submerged fixed bed aerobic bioreactor. It is a column in height of 1.2 m and diameter 0.14 m with total volume of 18.5 liters aerated from the bottom by air. - The trickling filter with volume of 11.1 liters filled by F2 with total surface of 0.53 $m^2$and 1.75 dm^3 of glass fiber. - The submerged fixed bed bioreactor with volume of 7.4 liters filled by Hufo 200 with total surface of 0.47 m^2.
Pilot 4 Laboratory at 5°C	Evaluation of the low temperature and high salinity effects on nitrification process. 23.1.2006-31.8.2006	Type of the nitrifying bioreactor is combining trickling filter and submerged fixed bed aerobic bioreactor. It is a column in height of 1.2 m and diameter 0.14 m with total volume of 18.5 liters aerated from the bottom by air. - The trickling filter has volume of 10.5 liters filled by Hufo 200 with total surface of 1.52 m^2 and 400 g of lignite coke. - The submerged fixed bed bioreactor has volume of 7.7 liters filled by Hufo 200 with total surface of 0.39 m^2 and 250 g of lignite coke.

Pilot 5 Kemi mine at 10°C	Evaluation of the low temperature effect on removal of total nitrogen by nitrification and denitrification process from Kemi mine water with high hydraulic retention time. 2.2.2006-4.5.2007	System consists of nitrifying bioreactor, anoxic filter and denitrifying bioreactor. - The nitrifying bioreactor is combining trickling filter and submerged fixed bed aerobic bioreactor. It is a column in height of 1.2 m and diameter 0.14 m with total volume of 18.5 liters aerated from the bottom by air. -- The trickling filter has volume of 10 liters filled by Hufo 200 with total surface of 1.74 m^2 and 120 g of lignite coke. -- The submerged fixed bed bioreactor has volume of 8.5 liters filled by Hufo 200 with total surface of 0.5 m^2. - The anoxic filter is column in height of 0.93 m and diameter 0.085 m with water volume of 4.9 liters and downstream flow. - Denitrifying bioreactor is submerged fixed bed anaerobic column in height of 1.2 m and diameter 0.14 m filled by F2 with total surface of 3.2 m^2. Reactor has water volume of 16.9 liters with upstream flow.
Pilot 6 Laboratory at 12°C	Evaluation of the low temperature and high salinity effects on denitrification applying methanol. 8.11.2006-16.1.2007	The denitrifying bioreactor is submerged fixed bed anaerobic column in height of 0.6 m and diameter 0.14 m filled by F2 with total surface of 1.17 m^2. Reactor has working volume of 8.3 liters with upstream flow.
Pilot 7 Laboratory at room temperature	Enrichment of anaerobic ammonium oxidizing (ANNAMOX) bacteria. RO-concentrate is used. 1.2.2006-15.4.2006	System consists of anoxic filter and denitrifying bioreactor. - The anoxic filter is column in height of 0.53 m and diameter 0.085 m with submerged fixed bed filled by 11 pieces of Hufo 200. Filter is blown from the bottom by nitrogen and has water volume of 1.7 liters with downstream flow. - The denitrifying bioreactor is submerged fixed bed anaerobic column in height of 0.6 m and diameter 0.14 m filled by 104 pieces of F2 and 7 liters of water. Reactor has working volume of 7.58 liters with upstream flow.
Pilot 8 Laboratory at 5° and Pilot 9 Laboratory at 12° 11.4.2006-20.7.2006	Evaluation of the low temperature effect on nitrification applying Pahtavaara mine water using bioreactor with moving carrier.	System consists of submerged moving bed aerobic nitrifying bioreactor and clarifier. - The bioreactor has working volume of 32.2 liters filled by F2 with total surface of 7.467 m^2.

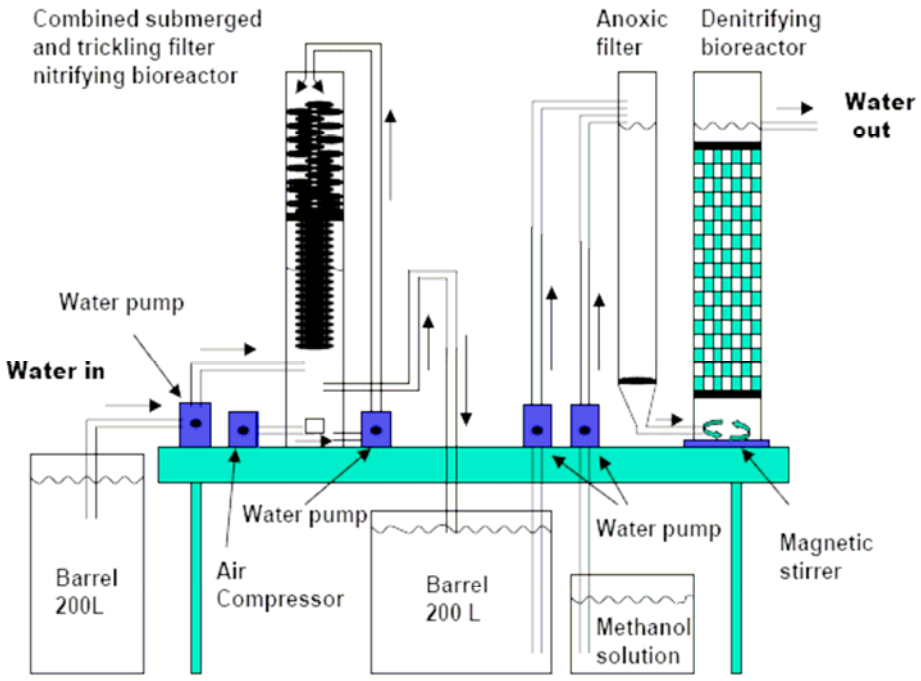


Figure 7. A schematic presentation of the continuous flow laboratory-scale system of nitrifying and denitrifying bioreactors (Pilot 5).

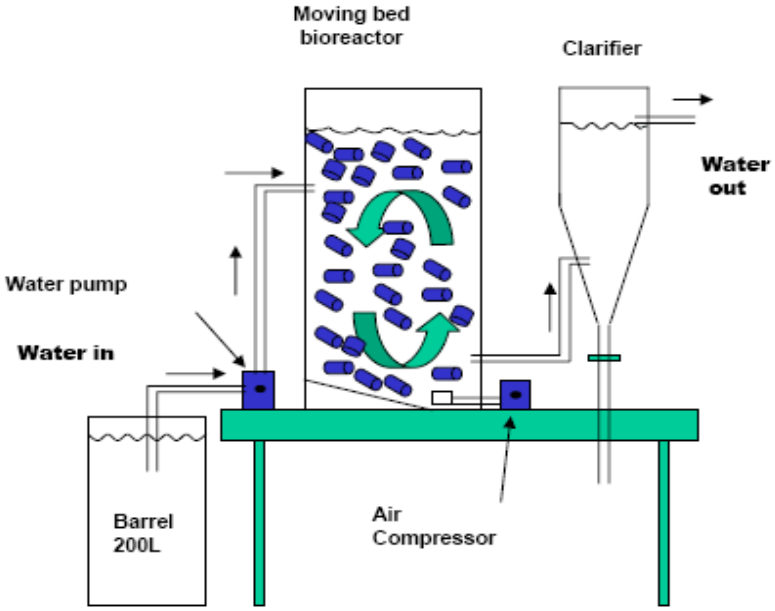


Figure 8. A schematic presentation of the nitrifying continuous flow suspended carrier biofilm reactor (pilot 2).

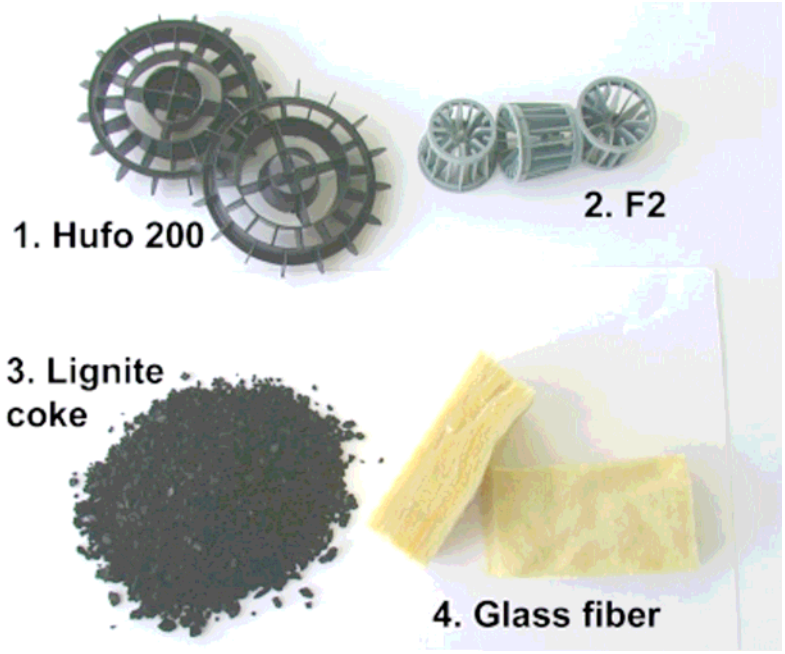


Figure 9. Carrier materials for biofilm in the studied reactors.

1. Hufo 200, polypropylene (PP), total surface of 200 m^2/m^3 (Nordiska Plast AB, Sweden).
2. F2, polypropylene (PP) with density of 0.96 $/cm^2$ and total surface of 320 m^2/m^3 (3F GmbH & Go. KG, Germany).
3. The lignite coke from AQUA-bioCarbon Ltd, specific surface of 300 m^2/g.
4. Glass fiber.

4.3.1 Inocula

The nitrifying reactor was inoculated with cultures from four nitrifying bioreactors:1) Laboratory-scale nitrifying bioreactor treating synthetic wastewater of low BOD and high ammonia for one year at room temperature (BN-1A); 2) Full-scale bioreactor treating cold municipal landfill in arctic climate (BN-1B); 3) Laboratory-scale nitrifying bioreactor inoculated with sludge from a fishpond maintained with ammonium rich water, 50 to 100 mg /l, for one year at 8°C (BN-1C); 4) Laboratory-scale nitrifying bioreactor treating landfill leachate, 1500 to 2000 mg/l NH4+-N for three months at 15 to 20°C (BN-1D). The denitrifying bioreactor was inoculated with a mixed population of cold-tolerant methylotrophic denitrifying bacteria (BDN-1A and BDN-1B) developed from activated sludge (Helsinki wastewater treatment plant) (Zaitsev et al., 2007). The mixed culture had earlier shown excellent performance in full-scale denitrification of cold landfill leachate (unpublished results) and biodegradation of methyl *tert*-butyl ether at low temperature (Zaitsev et al., 2007). The mixed culture had been adapted to methanol as external carbon source over a period of two months in the laboratory.

4.3.2 Biological nitrification and denitrification in RO concentrate and saline water

As feed in the experiments with saline effluents, reverse osmosis (RO) concentrate of water samples from Pahtavaara, Kemi and Siilijärvi mines were used. The characteristics of the RO concentrates used in the experiments are shown in Table 5.

Table 5. Basic composition of reverse osmosis concentrates used in experiments

Feed	Type of experiment	$N\text{-}NH_4^+$ (mg/l)	$N\text{-}NO_3^-$ (mg/l)	$N\text{-}NO_2^-$ (mg/l)	COD_{Cr} (mg/l)	Cl^- (g/l)	Total salinity (g/l)	pH
RO concentrate, Pahtavaara	Batch	112	203	7	<500	0,37	4	7,8
	Continuous	103	155	2	527	0.16	3	7,9
RO concentrate, Kemi	Batch	44	184	7	627	31	48	8,1
	Continuous	45	111	1,5	<500	11.2	17	8,7
RO concentrate, Siilijärvi	Batch	105	585	39	1,14	<500	10	8,1

4.3.3 Nitrification in RO concentrates in batch experiments

Batch tests were performed on ability of nitrifying biofilm for oxidation of ammonium at low temperature. The biomass was obtained from Pilot 4 and was adapted to high salt concentrations. These tests were carried out prior to continuous flow experiments. Aerobic batch experiments were performed in 3 liter bottles with 0.5 liter of RO concentrate and 20 ml of culture from bioreactor (Pilot 4). The bottles were incubated on a gyratory shaker (152 rpm) at 12 ± 1°C.

4.3.4 Nitrification in RO concentrates using a carrier biofilm process with continuous operation

Nitrification in RO concentrates was studied in bioreactor with continuous operation (Pilot 4) containing a biofilm adapted to high concentration of salts at 5± 1°C.

4.3.5 Denitrification in RO concentrates using a carrier biofilm process with continuous operation

The effluents of the nitrifying reactor from Pilot 4 were further treated in the denitrifying bioreactor (Pilot 6) at 12±1°C, using methanol as external carbon sources. A mixture of methanol and phosphoric acid was added to bioreactor in excess amount in order to ensure that neither of phosphorus or carbon sources was limiting denitrification rate (see above, page 13). The concentration of nitrate in effluents after oxidation of ammonium in Pilot 4 was 157 mg/l for RO concentrate from Kemi mine water and 262 mg/l for RO concentrate from Pahtavaara mine water. The concentrations of nitrite were low in both RO concentrates and not exceed 2 mg/l. The denitrifying reactor had biofilm, which was enriched from mixed population of methylotrophic denitrifying bacteria from Pilot 5 during two months adaptation to high concentration of salts (Figure 42).

4.3.6 Denitrification in saline water using a carrier biofilm process with continuous operation

Explosives production wastewater from Oy Forcit Ab was used in this experiment. The main characteristics of wastewater were as follows:

COD_{Cr}	1300 mg/l
Total salinity	67000 mg/l
Ammonium	3 mg/l
Nitrate	13700 mg/l
Nitrite	15 mg/l
Sulphate	3500 mg/l
Chloride	2600 mg/l
Total phosphorus	0,1 mg/l
Sodium	7700 mg/l
Magnesium	4000 mg/l
Sulphur	1200 mg/l
Calcium	3800 mg/l
Heavy metals less than	0,02 mg/l
pH	8

Denitrification was studied in a bioreactor with suspended carrier (Pilot 10). The reactor, which had a working volume of 30,5 liter was filled up to 59% with Kaldnes (K1) carrier, placed in a temperature controlled room and maintained at 12±1°C. The carrier was kept in suspension with the aid of a cycling water pump. The bioreactor was inoculated with mixed population of methylotrophic denitrifying bacteria from Pilot 5. A mixture of phosphoric acid (0,01% v/v) and methanol (3,3% v/v) was added to bioreactor as a separate solution. The flow rate was approximately 10 % of that in the wastewater.

4.3.7 Biological removal of nitrogenous compounds from mine water at pilot-scale

The denitrification test work with a pilot-scale system took place at Kemi mine at the level of –115 m and was carry out 238 days between October 26th 2006 and June 20th 2007. Three denitrifying reactors, 0.8 m^3 working volume each have been used (Figure 10). The reactors contain F2 carrier with total volume of 0.7 m^3, so that surface area was 93 m^2/m^3 reactor working volume. Water in denitrifying bioreactors is mixing by the stirrers with adjustable from 10 to 150 rpm (Figure 11 and 13). The reactors were fed with mine water containing 10.8±0.7 mg/l NO_3^--N, 0.2±0.05 mg/l NH_4^+-N, less than 0,05 mg/l total phosphorus and 44±4 mg/l COD. From 4 to 10% of methanol and from 0.01 to 0.03% of phosphoric acid solution was used as sources of carbon and phosphorus, respectively. The reactors were operated under carbon and phosphorus limiting conditions to be sure that these elements would not present in effluent water in significant amounts. Additionally, aerobic reactor was installed after denitrifying reactors, to remove the rest of methanol and products of its incomplete oxidation, which can be present in the effluent water after denitrification (Figure 12 and 14).

4.3.8 Analyses

Ammonium, nitrite, nitrate, total-phosphorus, chloride and CODCr concentrations were colorimetrically determined based on the method indophenol blue, sulfanilic acid, 2,6.dimethylphenol, phosphormolybdenum blue, iron(III)thiocyanate and chromosulphuric acid oxidation, respectively (LCK standard test kits, HACH Dr. Lange). The pH was measured with a SCHOTT model CG 842 pH meter. Composition of gases accumulated in the denitrifying bioreactor was determined by gas chromatography with an ECD detector. The geochemical analysis of water samples was measured by inductively coupled plasma atomic emission spectroscopy (Thermo Jarrell Ash model ICAP 1100) at GTK Outokumpu. The geochemical analysis of solid samples, i.e. dry biomass, was performed by atomic absorption spectroscopy (Perkin Elmer AAS 4100) at GTK Outokumpu. The Total Hydrocarbon (THC) content was determined by GC/MS-instrument (scan mode), where C10-C40 hydrocarbon fraction is measured, based on C10 decane and C40 tetracontane retention times. Method is based on ISO 16703 method.The particle size distribution analysis of suspended solids in a Kemi mine water sample (-550 m pumping station) was carried out by a Coulter LS Particle Size Analyzer at GTK, Outokumpu. Sedimenation tests on suspended solids in Pahtavaara and Kemi mine water were performed. The minerals and the mineral composition of the Siilinjärvi barren rock and ore samples were determined at GTK Outokumpu by using a scanning electron microscope and an image analysis system - MLA equipment, that is a Scanning Electron Microscopy (SEM) and energy dispersive X-ray analysis (EDX) based image analysis system. The MLA is state-of-the-art software designed to quantitatively analyse inorganic samples. The MLA is an off-the-shelf FEI Quanta 600 scanning electron microscope and an EDAX energy dispersive X-ray analysis system with two detectors combined with a software written at the JKTech Pty Ltd.

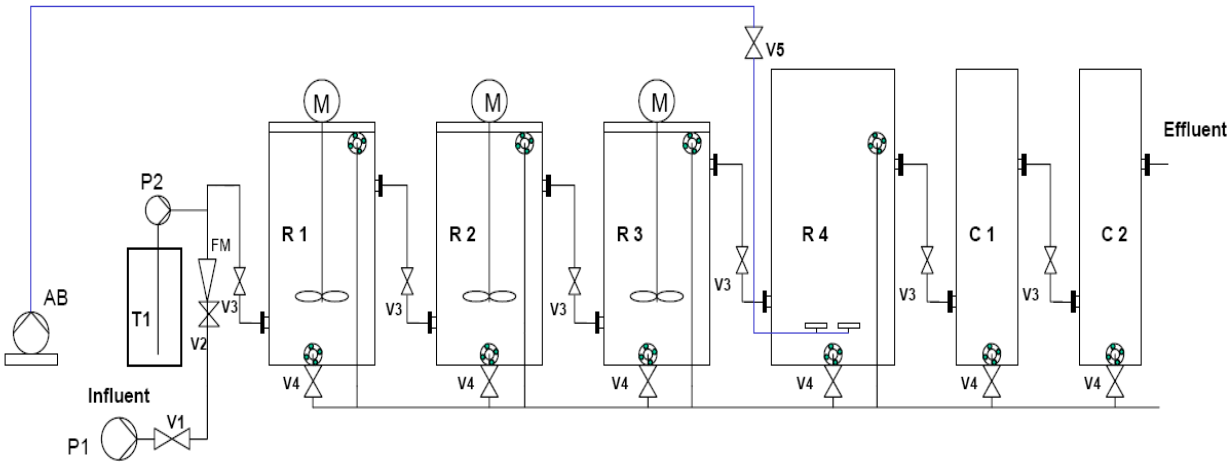


Figure 10. A schematic presentation of the continuous flow pilot-scale bioreactor system for denitrification test at Kemi mine at level of –115 m (Phase 2).

T1	Barrel for methanol solution (0.2 m^3)
R1, R2 and R3	Denitrifying bioreactors (total volume of each reactor is 0.9 m^3, working volume is 0.8 m^3)
R4	Aerobic bioreactor (total volume of reactor is 1 m^3, working volume is 0.74 m^3)
C1 and C2	Clarifier (total volume of each clarifier is 0.36 m^3, working volume is 0.26 m^3)
M	Stirrer
AB	Air blower
P1	Water pump
P2	Pump for methanol solution
V1 and V2	Valves for regulation of water flow (Water flow regulation valves)
V3	Valve between bioreactors
V4	Channel valve
V5	Valve for regulation of air flow (Air flow regulation valve)
FM	Water flow meter

Fig. 11. Denitrifying bioreactor with suspended carrier (R1)

Fig. 12. Moving bed aerobic bioreactor (R4) and clarifier (C1)

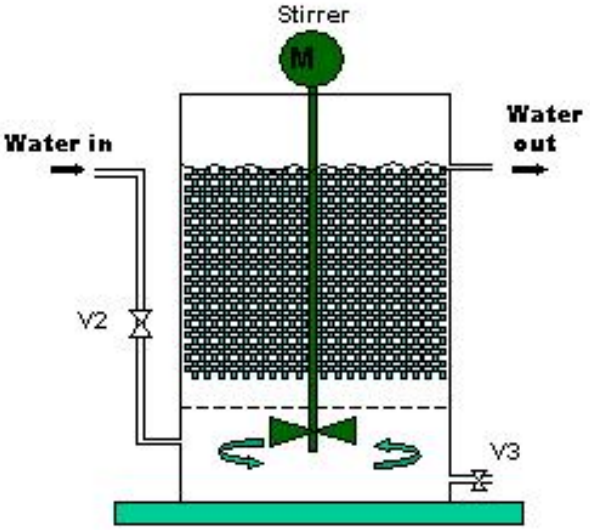


Fig. 13. A schematic presentation of denitrifying bioreactor with suspended carrier

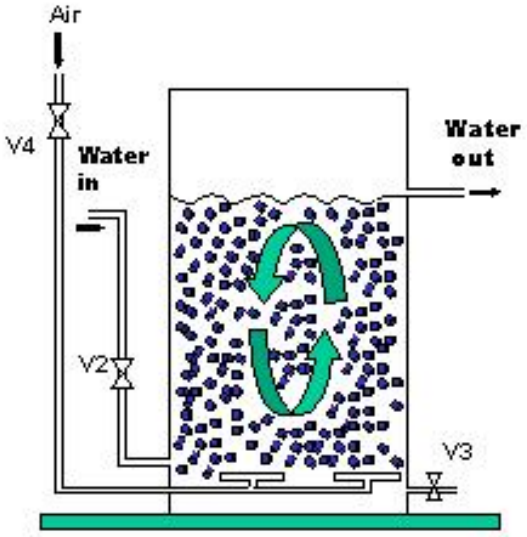


Fig. 14. A schematic presentation of aerobic moving bed bioreactor

4.4 Molecular biological population profiling and characterization of bacteria from bio-reactor biomass

Bacterial biomass from the bioreactors was collected at different time points and characterized using molecular biological and cultivation independent methods. Whole genomic DNA was extracted from the biomass samples and 16S rRNA genes were amplified with PCR. For generating clone library the PCR 16S rDNA products of 6 representative (nitrifying, denitrifying and high salinity nitrifying bioreactors) biomass samples were cloned in to E. coli and sequenced. Clones and all biomass samples (total 63) were processed to form TRFLP data for population profiling. Profiles will give option to see changes in bacterial diversity and identify major groups of bacteria.

4.4.1 Sampling

The biomass samples were taken from the bioreactors during maintenance and stored in -20°C until DNA extraction. The samples were kept in 50 ml Falcon tubes that contained biomass and water from the bioreactors. The amount of the biomass varied among the samples. For identification study three samples from nitrifying (BN), two from denitrifying (BDN) and one from high-salinity (HSN) nitrifying bioreactor were chosen (Table 6).

Table 6. Description of sequenced samples. N means nitrifying, DN denitrifying and HSN high-salinity nitrifying bioreactor.

sample	bioreactor	Time of sampling
BN-3	Pilot 1 (N)	1.2.2006
BN-11	Pilot 5 (N)	14.8.2006
BN-13	Pilot 2 (N)	14.9.2006
BDN-4	Pilot 5 (DN)	17.7.2006
BDN-5	Pilot 1 (DN)	3.8.2006
BHSN-1	Pilot 4 (HSN)	4.4.2006

4.4.2 Evaluation of DNA extraction methods

Three different DNA extraction methods were evaluated. Extractions were made with basic miniprep protocol (Ausubell et al, 1987), an enzymatic method (Purkhold et al, 2000) and commercial kit (Fast DNA spin kit, Qbiogene) according to the instructions given.

Extracted DNA was amplified with Taq polymerase (Fermentas, Canada) and with universal primers pA and pH´ (Table 7)(Oligomer, Finland) (Edwards et al, 1989). The reaction mixture (50 µl) contained 1 x Taq Buffer with KCl, 0,2 mM each dNTP, 1 µM both primers, 2 mM $MgCl_2$, 1 µl extracted DNA as template and 1 unit of polymerase. The PCR program contained an initial denaturating step at 94°C for 3 min and 25 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min followed by a final extension at 72°C for 10 min.

RFLP was done using restriction enzymes Hae III (NEB, USA) and Hinf I (Fermentas, Canada). The restrictions were done in separate tubes and the mixtures (20 µl) contained 18 µl of PCR product, 1 x appropriate buffer (see manufacturer´s catalog) and 5 units of enzyme. The reactions were held at 37°C overnight and stopped with 6 x gel loading dye before loading to 3 % agarose gel.

The three extraction methods were first evaluated with restriction fragment lenght polymorphism (RFLP) and two of them also with sequencing and comparing the bacterial diversity observed with Libcompare in the Ribosomal Database Project (RDP) website.

4.4.3 DNA extraction

DNA extractions for identification and profiling were made with FastDNA spin kit for soil (Qbiogene, USA) and a FastPrep bead-beating machine. Sample tubes were vortexed and 0,5 ml of sample was used in the extraction, which was made according to the manufacturer´s instructions. DNA was eluted in 50 µl of distilled water. No replicas were made in the extraction step due to limited sample amount.

4.4.4 Molecular biological identification of the samples

16S rDNA PCR was done using Phusion DNA polymerase (Finnzymes, Finland) and the universal primers pA and pH´. The reaction mixtures (20 µl) for PCR contained 1 x HF buffer, 0,2 mM of each dNTP, 10 pmol both primers, 1 µl extracted DNA as template and 0,4 units of Phusion polymerase. The PCR program contained an initial denaturating step at 98°C for 30 s and 15 cycles of 98°C for 10 s, 61°C for 10 s and 72°C for 45 s followed by a final extension at 72°C for 10 min. Four replicas from each sample were amplified and a negative control with distilled water as template was used.

Before cloning the four replicas were mixed in one tube and purified on filter membrane with 0,1 x TE (10 mM Tris-HCl, 1 mM EDTA) and Milli-Q water and eluted in 0,1 x TE. A overhang addition was made before TA-cloning with DynaZyme polymerase. The reaction mixture (25 µl) contained 1 x DynaBuffer, 0,2 mM of each dNTP, 22 µl of mixed and purified PCR-product and 0,6 units of polymerase. The mixture was incubated 1 h at 72°C. The A-addition mixture was purified on filter membrane with 0,1 x TE and Milli-Q water and eluted in 0,1 x TE.

Cloning was made with PCR cloning *plus* kit (Qiagen, USA) which included also the competent cells. The ligation to Qiagen pDrive-vector was made according to the manufacturer´s instructions with 4 µl of the purified A-addition mixture and incubated at 16°C overnight. Transformation was made to competent *E. coli* cells with heat-shock. 25 µl cells and 0,5 µl 1:0 and 1 µl 1:50 diluted ligation mixture was used. Transformation reactions were plated on LB-agar + ampicilin and clone libraries grown at 37°C overnight. Colonies from the transformation plates were picked with Qpix robot, 96 from each clone library, and growth on LB-medium at 37°C overnight. Glycerolstocks were made from each clone library (150 µl of 50 % glycerol and 200 µl of overnight cultivation in LB-medium) and stored at -80°C. Plasmid were purified with Qiagen 3000 robot and eluted in 0,1 x TE. The cloned insert was reamplified from the plasmids with primers for the vector and the products were checked on agarose gel.High-troughput sequencing was done with ABI 3730 -sequencer (Applied Biosystems, USA) in the core facility of University of Helsinki.

The sequencing reactions were done with BigDye v.3.1. chemistry (Applied Biosystems, USA) and the reaction mixture were made according to the manufacturer´s instructions. Sequencing primer was pD´ (Table 7). Sequencing reactions were purified after amplification and run on the sequencer.

Sequences from sequencer were processed with Staden Package (http://staden.sourceforge.net/), which is a free, open source program for sequence assembly and analysis. With Trev, the vector and primer sequences were cut and then sequences were complemented and same sequences (<1,5 % mismatch) grouped as one with Gap4.

The sequences were imported as a fasta file to Ribosome Database Project II (RDP) (http://rdp.cme.msu.edu/) and the identification and phylogeny was made with programs in the RDP. The phylogenetic tree was made with ARB software (www.arb-home.de).

Table 7. Primers used in PCR and sequencing reactions

Primer	Sequence	Lenght	Location (E.coli - 16S)
pA	AGA GTT TGA TCC TGG CTC AG	20	8-28
pD´	GTA TTA CCG CGG CTG CTG	18	536-518
pH´	AAG GAG GTG ATC CAG CCG CA	20	1542-1522
27f	AGA GTT TGA TCC TGG CTC AG	20	27-47
907r	CCG TCA ATT CCT TTG AGT TT	20	887-907

4.4.5 Biomass population profiling with terminal restriction fragment length polymorphism (TRFLP) method

PCR amplification of the 16S rRNA gene was performed using forward primer 27f and reverse primer 907r (Table 7) (Johnson, 1994). PCR was carried out in a final volume of 50 µl using 0.5 µl template DNA, 200 µM of each dNTP, 0.3 µM of each primer and 1 U DyNAzyme II polymerase with 1x concentration of the supplied buffer (Finnzymes, Espoo, Finland). PCR amplification was performed in a PTC-100 thermal cycler (MJ Research, Waltham, Mass.) as follows: initial denaturation at 94°C for 2 min, 30 cycles at 94°C for 45 sec, 55°C for 1 min and 72°C for 1 min, and the final extension step at 72°C for 10 min. For T-RFLP analysis the forward primer was labeled with FAM (6-carboxyfluorescein) or HEX (6-carboxy-2′,4,4′,5′,7,7′-hexachlorofluorescein). Three PCR amplification products from each biomass DNA preparation were separately purified using E.Z.N.A™ Cycle-Pure Kit purification kit (Omega Bio-Tek). Two differently labeled PCR amplification products of clones were pooled and purified using E.Z.N.A™ Cycle-Pure Kit. Approximately 100 ng of the purified PCR product from biomass and clone samples was digested with restriction enzymes *Msp*I (Fermentas). T-RFLP profiles were analyzed by the Fragment analysis service of University of Turku using an ABI PRISM 377 automatic sequencer and GeneScan -500 TAMRA (Applied Biosystems) DNA fragment length standard.

5 RESULTS AND DISCUSSIONS

5.1 Fate of nitrogenous compounds at mines

5.1.1 In-situ nitrification/denitrification

The concentration of total nitrogen in water samples collected at the three mines were combined with total volumes of water used to yield the total nitrogen load. The information concerning the use of explosives were provided by the mines and used as input value of total nitrogen to the system.

Ammonium in water samples from Kemi and Siilinjärvi mine was disappearing before the second clarification pond. Thus indicating occurrence of in-situ nitrification of ammonium in aerated cold mine water (Table 8). Whereas at Pahtavaara mine ammonium was detected at low concentrations after pond treatment. The lack of sufficient in-situ nitrification may be overcome by longer retention time of water in ponds or aeration by circulative pumping. After pond treatment Pahtavaara water is directed to wetland system. At all mines the total nitrogen concentration decreased significantly during the water treatment in ponds. However, the treatment process is not sufficient to remove all nitrogen. The water treatment efficiency was independent of seasons (Figures 15 and 16) (Outokumpu Chrome Oy, velvotetarkkailu 2005/2006). The calculated nitrogen load was calculated based on volume and concentration data of each mine. The internal N input was different at each mine. At Kemi mine, 30 000 kg N is annually directed to the refinery with ore and only 1 500 kg N is directed outwards with final products (Figure 17). Major part of N is directed to pond system, tailings pond 6 and clarification ponds 4 and 5. The total nitrogen input to clarification ponds have been estimated to beat least 31 000 kg N where *in situ* removal is 18 000 kg N (58%) annually. The key elements for nitrogen removal at the ponds of Kemi are long retention time and the rich vegetation that provides carbon source for denitrifying process in otherwise oligotrophic mine water. In Siilinjärvi mine, 69 000 kg N is received at the refinery with water and ore while only 45 000 kg N has been pumped to Musti tailings pond (Figure 18). It seems that removal of 24 000 kg N occurs in the refining process. In apatite flotation, the pH of flotation pulp is about 11 and sufficiently high to strip ammonia gas with flotation aeration of the pulp. In Pahtavaara mine, the N input to the refinery is 6 500 kg N but the outflow is 30 000 kg N which indicates another source of nitrogen in the refinery (Figure 19). The chemicals used in the flotation circuit do not contain such amounts of nitrogen so that the more probable explanation is un-representative sampling. The annual discharge to receiving water bodies is at all mines about 10 000 kg total nitrogen. Some un-quantified barren rock pile originating waters not directed to pond systems may increase the total discharge.

5.1.2 Differences of explosives used

All three studied mines used different type of explosive. The oil emulsion explosive Kemiitti 810 used in Kemi mine have been considered to be most environmental friendly due to its stable structure in wet conditions (Oy Forcit Ab, Vuolio 2001) while the water gel explosive Kemiitti 110 used in Siilinjärvi is more easy to solve in water. The ANFO explosive used at Pahtavaara is known for its even better water solubility. At Pahtavaara mine, the relation between nitrogen determined from ore and water indicates that most of un-detonated nitrogen is in water due to good solubility of ANFO (Table 9). At Kemi mine, the higher percentage of nitrogen is found from ore and barren rock after thorough washing (Figure 20) and only little from water. In Siilinjärvi both percentages (1,8-3,4) are lower than in Kemi mine.

Table 8. Content of total nitrogen and ammonia in water samples collected from Kemi, Siilinjärvi and Pahtavaara mines.

Kemi mine		N-tot		NH4-N	
code	type	mg/l	stdev	mg/l	stdev
KK0	mine water	25,31	11,90	5,1	4,2
KK1	natural water	1,6	0,8	0,1	0,02
KK2	natural water	1,6	0,5	0,1	0,02
KK3	barren rock water	2,8	1,1	0,1	0,1
KK4	Viianmaa open pit	9,5	2,8	0,1	0,04
KK5	barren rock water	32	4	0,03	0
KK6	barren rock water	7,8	2,4	0,1	0,1
KK7	clarification pond 5	3,5	2	0,5	0,7
KK8	clarification pond 4	5,9	3,3	0,7	0,9
KK9	Iso-Ruonaoja	1,6	0,6	0,3	0,2

Siilinjärvi mine		N-tot		NH4-N	
code	type	mg/l	stdev	mg/l	stdev
SK0, SK00	mine water, Itälampi	68,8	34	16,9	13
SK1	Jaakonlampi	7,5	3,4	0,7	0,7
SK2	Sikopuro	4,5	1,3	1,7	1,6
SK3	Raasio	1,5	0,7	0,05	0,03
SK4	Musti tailings pond	8,8	5,4	0,7	0,7
SK5	Pirttioja	4,6	4,9	0,04	0,01
SK6	Raasio dam flow trough	0,6	0,3	0,03	0
SK7	barren rock water	10,3	10,2	0,04	0,02
SK8	barren rock water	117,2	49,3	0,1	0,1
SK9	barren rock water	2	0,2	0,1	0,1
SK10	barren rock water	3	1,5	0,1	0,1
SK11	Sulkavanjärvi	1,1	0,4	0,04	0,02

Pahtavaara mine		N-tot		NH4-N	
code	type	mg/l	stdev	mg/l	stdev
PK0	mine water	32,0	32,2	17,2	13,3
PK1	refinery pond	26,2	9,4	8,7	5,4
PK2	tailings pond	28,4	10,4	11,2	5,2
PK3	clarification pond	17,2	7,1	3,9	3,6
PK4	after ponds	5,6	3,8	1,9	3,6
PK5	barren rock water	8,2	4	0,1	0,01
PK6	Soasjoki	0,6	0,4	0,03	0,01
PK7	Pitkäkoskenoja	1,3	0,8	0,1	0,03

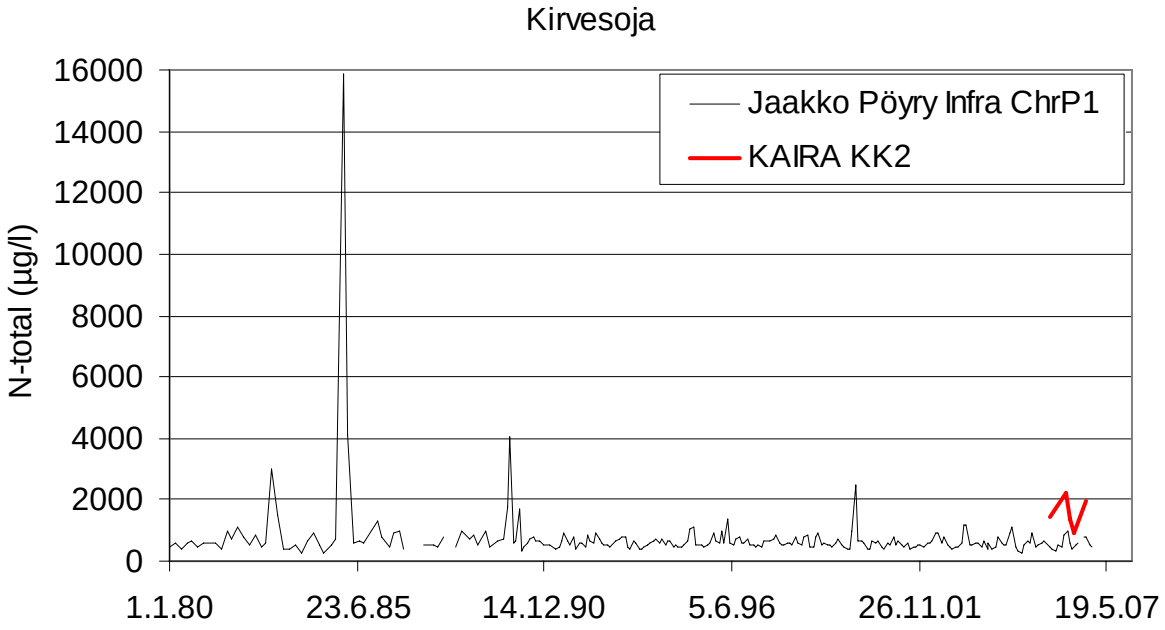


Figure 15. Comparison between long term monitoring (Pekkala and Rantala, 2006; Pekkala et al. 2007) and KAIRA results of total nitrogen content in Kirvesoja at Kemi mine.

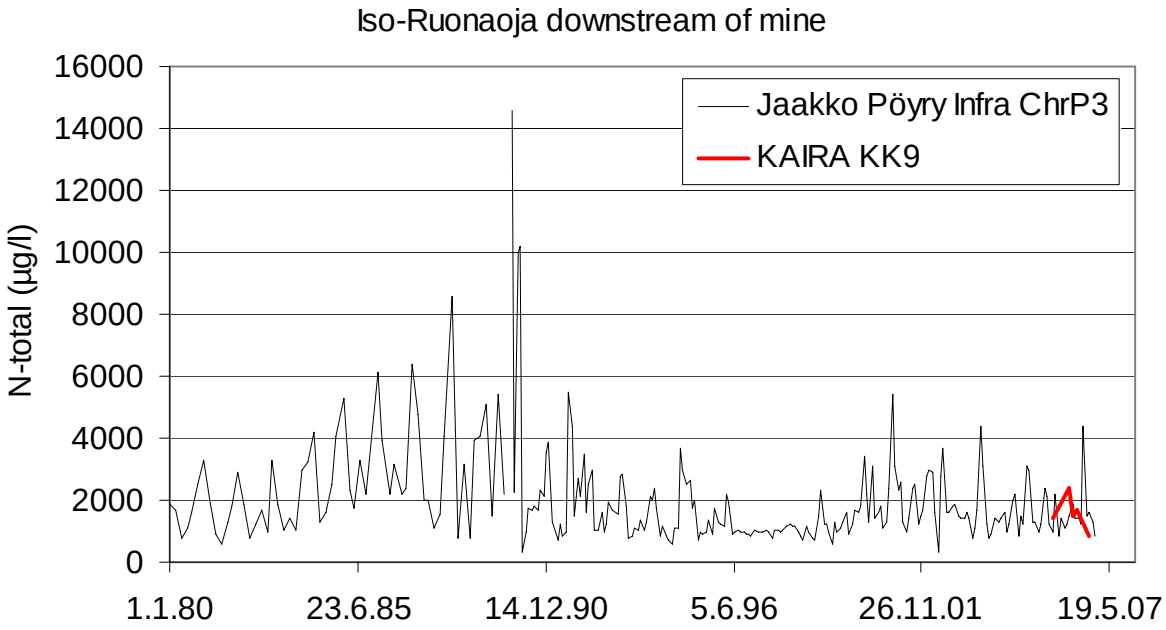


Figure 16. Comparison between long term monitoring (Pekkala and Rantala, 2006; Pekkala et al. 2007) and KAIRA results of total nitrogen content in Iso-Runoaoja downstream of Kemi mine.

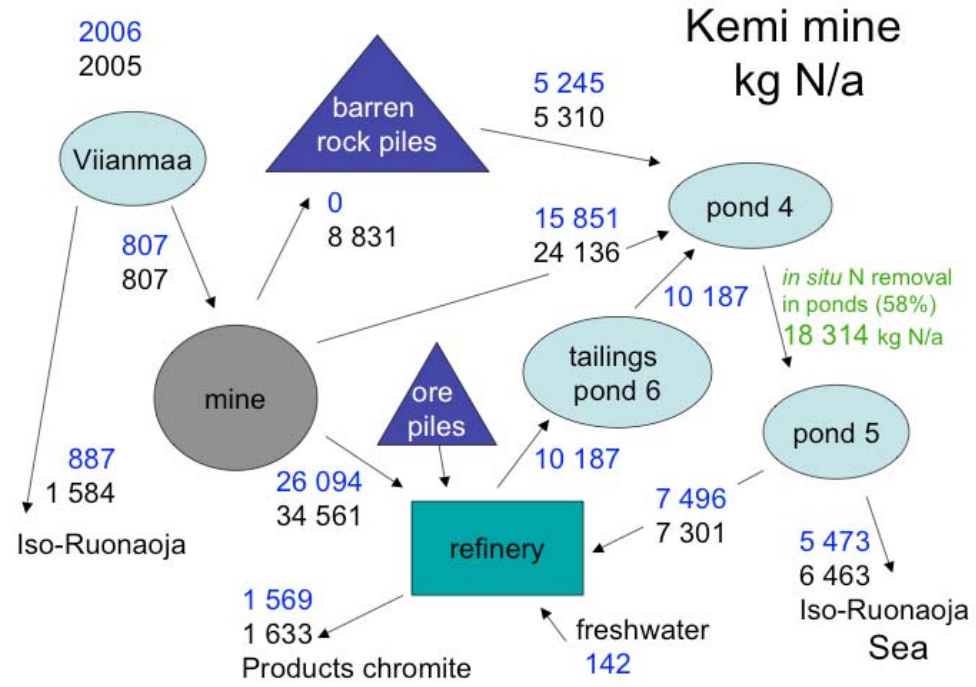


Figure 17. Flow chart of nitrogen in Kemi mine. Values given are kg N/a.

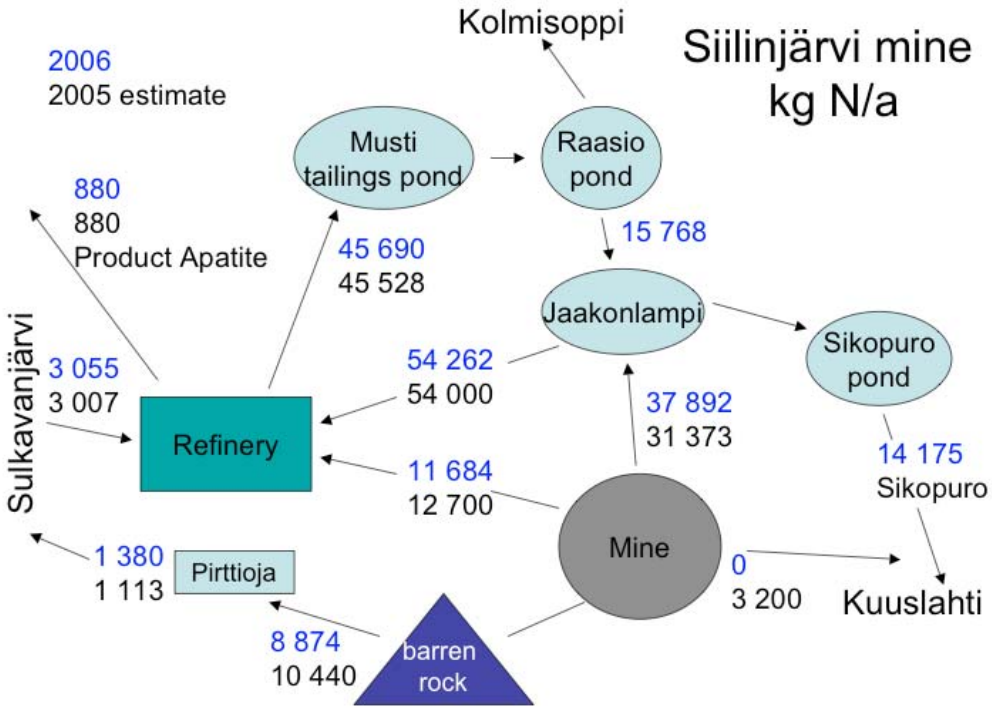


Figure 18. Flow chart of nitrogen in Siilinjärvi mine. Values given are kg N/a.

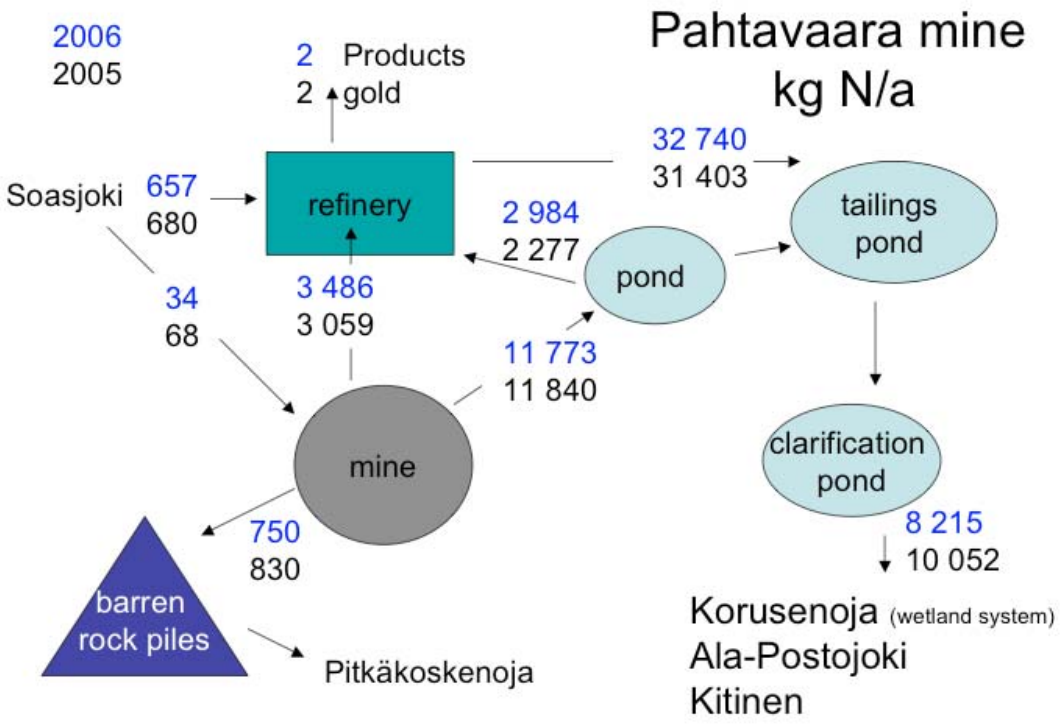


Figure 19. Flow chart of nitrogen in Pahtavaara mine. Values given are kg N/a.

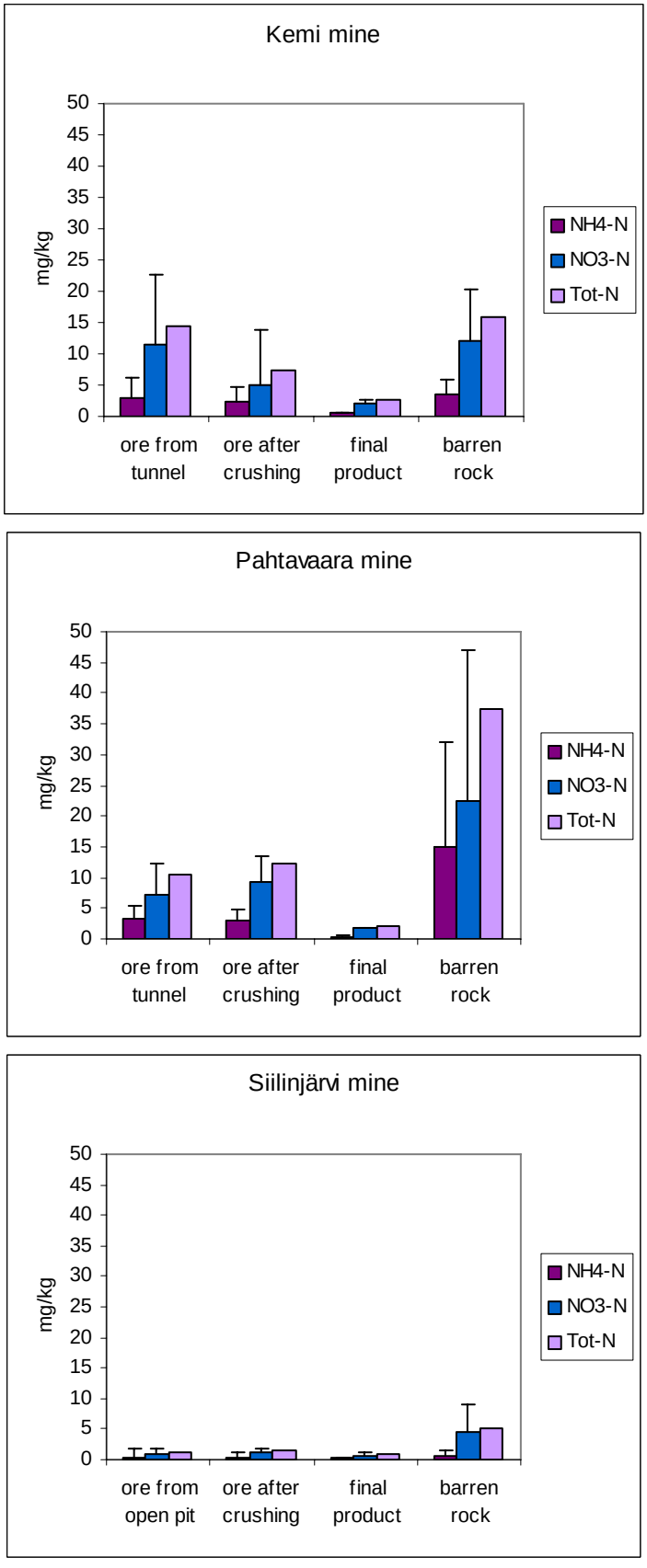


Figure 20. Content of nitrate, ammonia and total nitrogen in ore, barren rock extracted to water.

5.1.3 Explosive nitrogen input and output comparison

Based on the result presented in Table 9 the relation of used explosive per ton of extracted ore is in same range (0,3-0,4) in Siilinjärvi and Kemi mines while the values of Pahtavaara are higher (0,6) and also closer to estimates of Oy Forcit Ab (0,2-0,3 in open pit, 0,5-1 in underground). When the content of total nitrogen originating from un-detonated explosives was measured from ores and barren rocks of all three mine the results were surprisingly different. The analysis method used was adapted from a similar study done in Kiiruna LKAB mine (Forsberg & Åkerlund 1999), where 15-17% of total nitrogen content of explosive could be analyzed from ore and mine air after blasting. The values of 2% of total nitrogen analyzed from rock at Siilinjärvi and 6% at Pahtavaara were lower than expected when compare to 18% of Kemi mine. The concentration of total nitrogen measured from mine waters was highest at Siilinjärvi mine (69 mg N/l) but presented in only 3% of total nitrogen used in explosives. The comparison of total nitrogen contents analyzed from water and rock samples showed 30% lost of explosive input at Kemi, 15-19% at Pahtavaara while the lost at Siilinjärvi was only5%.

Table 9. Comparison of the explosive efficiency in Siilinjärvi, Kemi and Pahtavaara mines.

	Siilinjärvi mine			Kemi mine			Pahtavaran mine		
years	2005	2006		2005	2006		2005	2006	
explosive kg	4508 457	4800 000	kemiitti 110	804 523	408 342	kemiitti810 + anfo	291 961	370 865	anfo+kemix
contains totN kg/a	1059 487	1128 000		225 266	114 335		91 206	117 493	
explosive kg /t extracted	0,38	0,44		0,4	0,35		0,63	0,61	
Total extraction Mt	12	10,9		2,21	1,15		0,462	0,61	
ore Mt	10	9,2	cont. 1,3 mg N/kg	1,46	0,88	14,5 mg N/kg	0,437	0,498	10,6 mg N/kg
barren rock out Mt	2	1,7	cont. 5,2 mg N/kg	0,561	0	15,8 mg N/kg	0,025	0,0226	37,6 mg N/kg
barren rock stay Mt				0,23	0,27				
N out with rock kg N/a	23 140	20 558		30 034	12 760		5 572	6 129	
% totN of explosive in rock	**2,2**	**1,8**		**15**	**15**		**6,1**	**5,2**	
Dewatering m3	456 000	550 892	cont. 68,8 mg N/l	1 035 156	691 200	25,3 mg N/l	370 000	367 920	32,0 mg N/l
Out with water kg N/a	31 373	37 892		26 200	17 494		11 840	11 773	
% totN of explosive in water	**3**	**3,4**		**12**	**15**		**13**	**10**	
total kg N/a	**54 513**	**58 450**		**59 868**	**34 520**		**17 412**	**17 902**	
% totN of explosive	**5,1**	**5,2**		**27**	**30**		**19**	**15**	

5.1.4 Extraction methods and mineral types

At Kemi and Pahtavaara mine ore is extracted from underground while in Siilinjärvi ore is extracted from open pit. The drill hole diameters and distance between drill holes were differing greatly also in underground mining depending on whether the operation was drifting (perän ajo) or stoping (louhinta) (Table 10). Also the differences in hardness of ore and barren rock will influence on the extraction method chosen. Differences in mining methods were originally not considered to be a major parameter and therefore ore samples were collected by mine personnel only with the objective to get samples right after blasting. At Pahtavaara and Kemi mine the difference between samples from drifting or stoping operation were not obtained. Based on calculations only the use of explosives in drifting would be 10 times higher compared to other types of mining referred to possible explosives contact surface (Table 10). All other combinations of drill hole diameter and drill hole distance would give close to similar values.

The mineral composition of ore/barren rock however may give some explanations to the observed nitrogen concentration. The content of nitrogen detected from barren rock was in all mines higher compared to ore samples. This may indicate that the minerals of barren rock have been harder or that a major part of drifting has been done in it. The ore of Siilinjärvi mine has also an especially high content (60%) of biotite/phlogopite, which is used commercially as nutri-

ent trap for land filtration fields in municipal wastewater treatment (Vilpas et al 2005, Kemira GrowHow 2006). This mineral may remove un-detonated nitrogen, too, by sorption and thus hinder release even during extraction with agitated water (Table 1). The biotite may get regenerated in mineral processing of apatite (high temperature, high pH) and evaporate from process in gaseous form.

Table 10. Difference of mining methods in numbers

Type	Drill diameter mm	Distance m	Surface contact M^2/m^3	Source
Open pit	98	3	0,031	Oy Forcit Ab
Open pit	200	6	0,036	Pekka Särkkä, TKK
Underground	51	0,7	0,328 (drifting)	Oy Forcit Ab
Underground	51	2	0,041	Oy Forcit Ab

The three studied mines differed from each other in such a way that most of the conclusions can only be made for each individual case. Some common conclusions can be made on the influence of barren rock on the water quality. The relatively high total nitrogen values were found from each mine when compared to natural back ground values. Seepage from barren rock piles should be directed to water treatment processes in order to avoid nutrient discharge to the environment. The results here are based on some uncertainty connected to limited numbers and size (rock weight) of samples. However, the same methods have been used for each mine. The total input of N is considered here to come only from explosive used. It is anyhow not studied weather the air in underground mine (containing 78% on nitrogen) give some surplus nitrogen in explosion event. Therefore, the calculated loss values would be different if nitrogen compounds in mine air would be considered. In this study the focus was in mine water and its treatment.The nitrogen content of barren rock and ore samples were measured from rock samples of 10 cm size prior to crushing. This relatively small rock size may have been generated only with short distance from detonated drill holes and therefore containing more nitrogen than larger rock pieces extracted. The other rock samples (ore after crushing and concentrate) have been taken after several homogenization steps and therefore would represent all mining activity.

5.1.5 Monitoring of water at Pahtavaara mine

The analytical results of water samples from the dewatering system of Pahtavaara mine showed that the ratio between ammonium and nitrate remained nearly constant during one year of monitoring and did not depend on seasons (Figure 21). Further the often growth limiting element phosphorous was usually below 0.2 mg/l (Figure 22). The chemical oxygen demand (COD) is a sum parameter for natural organic matter as well as man-made contaminations of the water. Natural organic matter and other organic compounds can serve as carbon source in denitrifying bioreactors. The COD value was at anytime below 80 mg O_2/l (Figure 22). The content of organic compounds in Pahtavaara mine water was usually below detection limit (<50 µg THC/l, results not shown. The pH was on average 8.2 and the inorganic chloride concentrations from 4 to 27 mg/l (Figure 23).

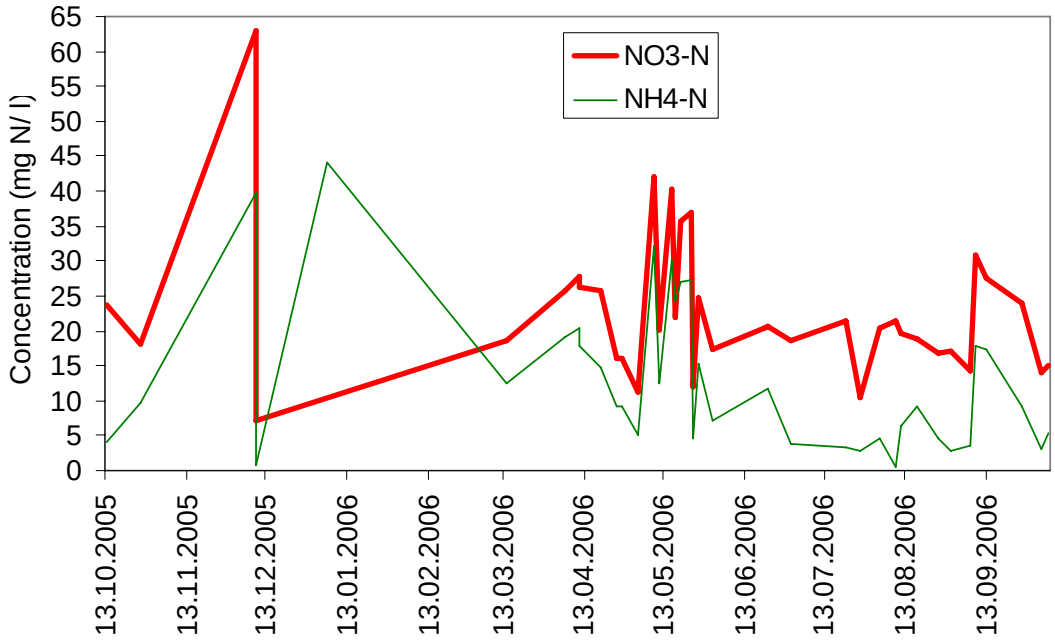


Figure 21. Ammonium and nitrate concentrations in water sampled from the dewatering system of the Pahtavaara underground mine.

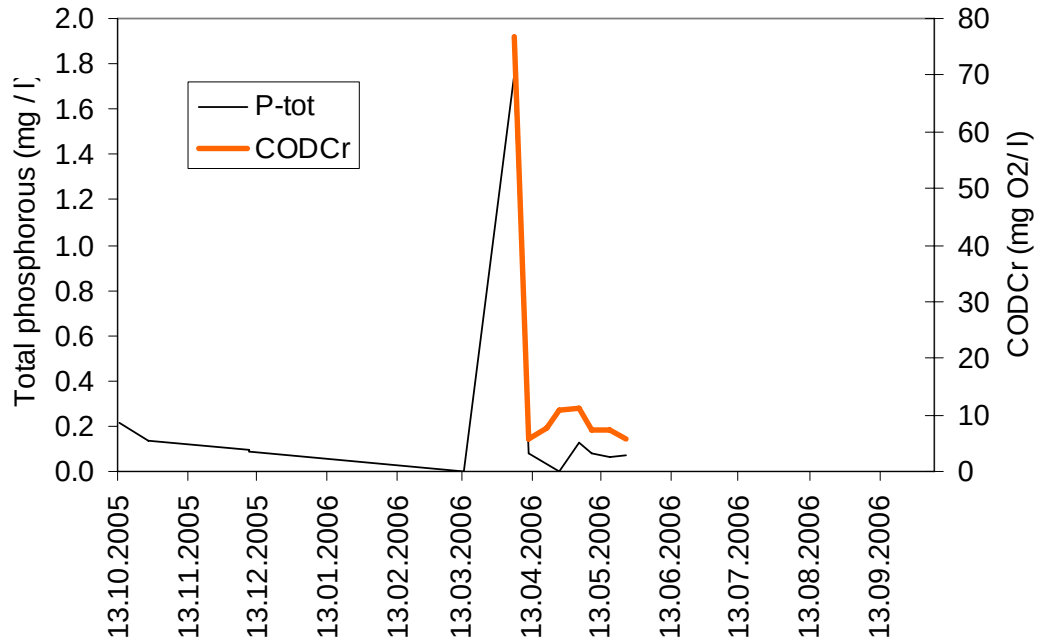


Figure 22. Total phosphorous content and chemical oxygen demand (COD) in water sampled from the dewatering system of the Pahtavaara underground mine.

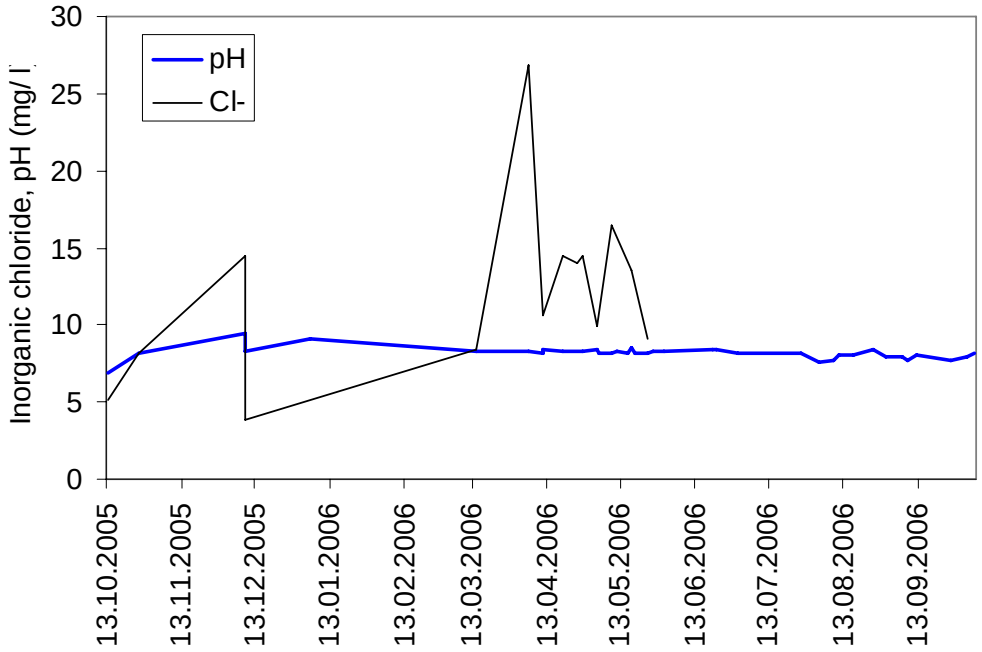


Figure 23. Inorganic chloride concentration and pH of water sampled from the dewatering system of the Pahtavaara underground mine.

The elemental composition of water in the dewatering system at Pahtavaara mine was as shown in Figure 24. In Pilot 8 the removal of elements from the mine water only in the case of Na, Fe and Sr at best 11, 23 and 10%, respectively (Figure 24).

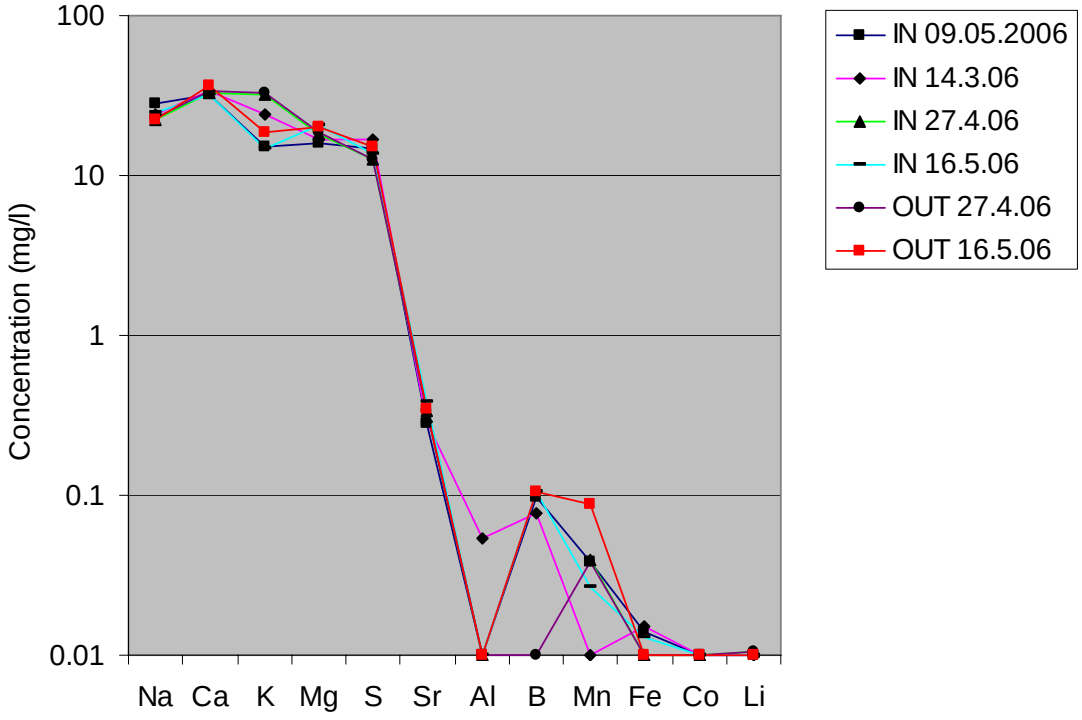


Figure 24. Reduced elemental composition of influent and effluent of Pilot 8 treating water of the dewatering system of the Pahtavaara underground mine.

5.1.6 Monitoring of water at Kemi mine

The analyses of water samples from the dewatering system of Kemi mine showed that concentration of total nitrogen changed significantly during two years and also the ratio between ammonium and nitrate varied (Figure 25). Ammonia concentration changed from 1 up to 12 mg/l, nitrate concentration from 7 up to 40 mg/l (Fig. 25). The total phosphorous content and COD showed sharp peaks (Figure 26). The COD was similar to Pahtavaara mine water while the total phosphorous content was less. The pH was in the range of 7.3 to 8.6 (Figure 27). The inorganic chloride content in water at Kemi mine was about 100 times higher than at Pahtavaara, thus likely to affect microbial activity (Figure 27). The chloride concentration showed a downward trend from May to December 2006.

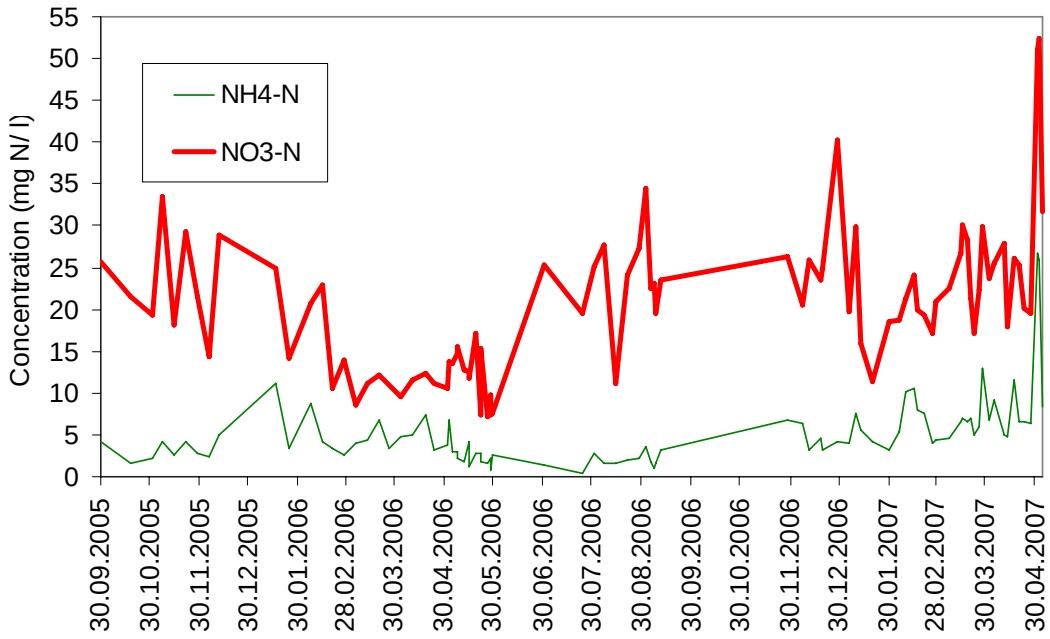


Figure 25. Ammonium and nitrate concentrations in water sampled from the dewatering system of the Kemi underground mine.

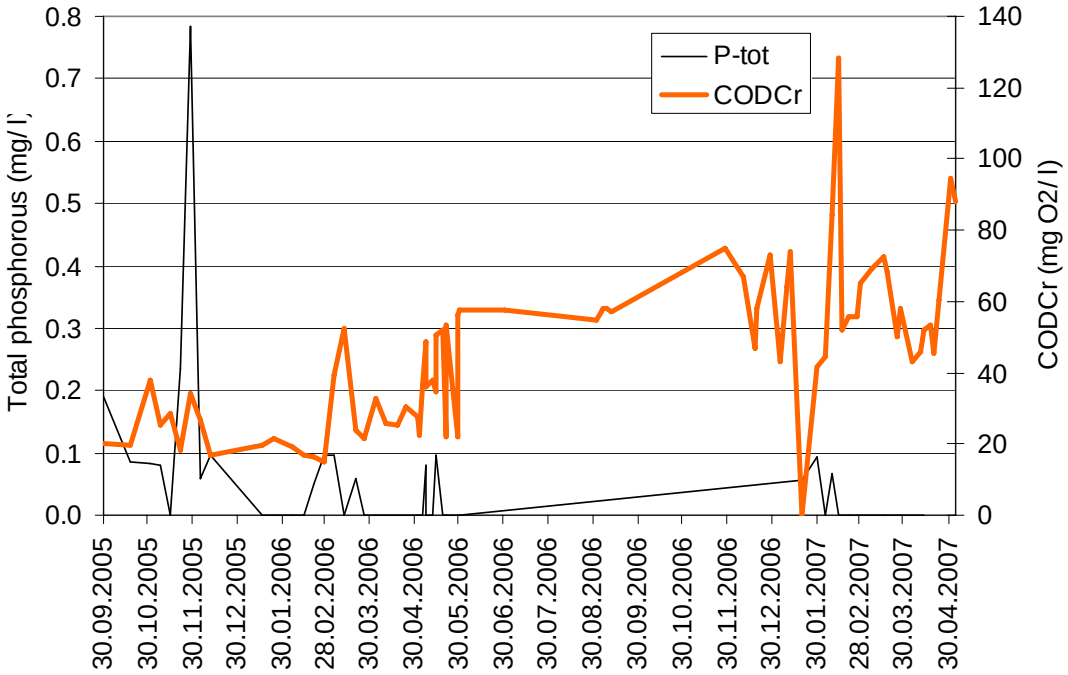


Figure 26. Total phosphorous content and chemical oxygen demand (COD) in water sampled from the dewatering system of the Kemi underground mine.

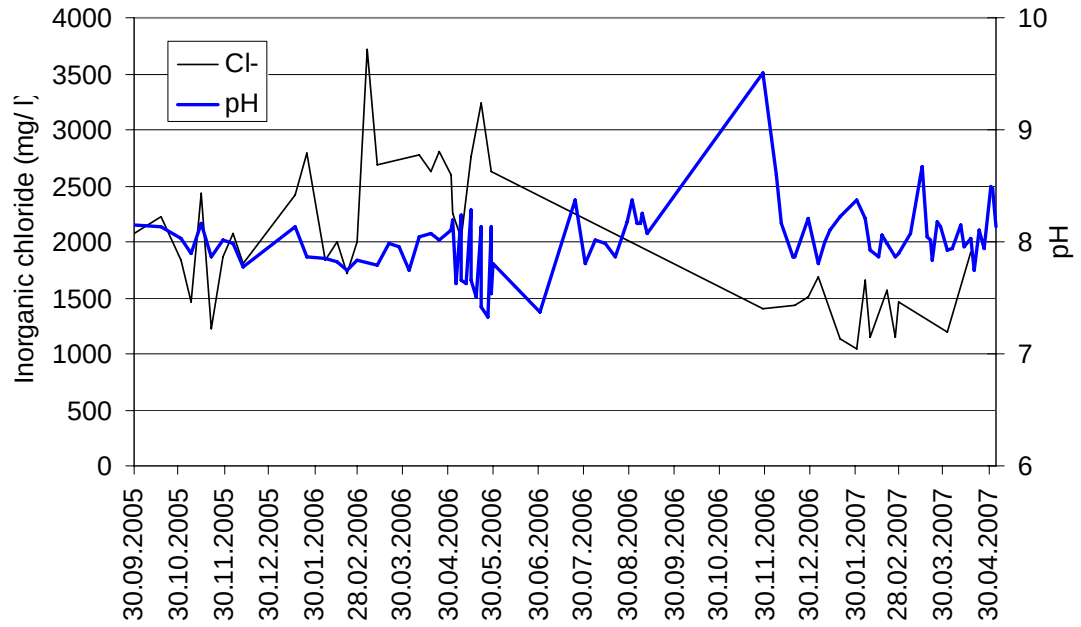


Figure 27. Inorganic chloride concentration and pH of water sampled from the dewatering system of the Kemi underground mine.

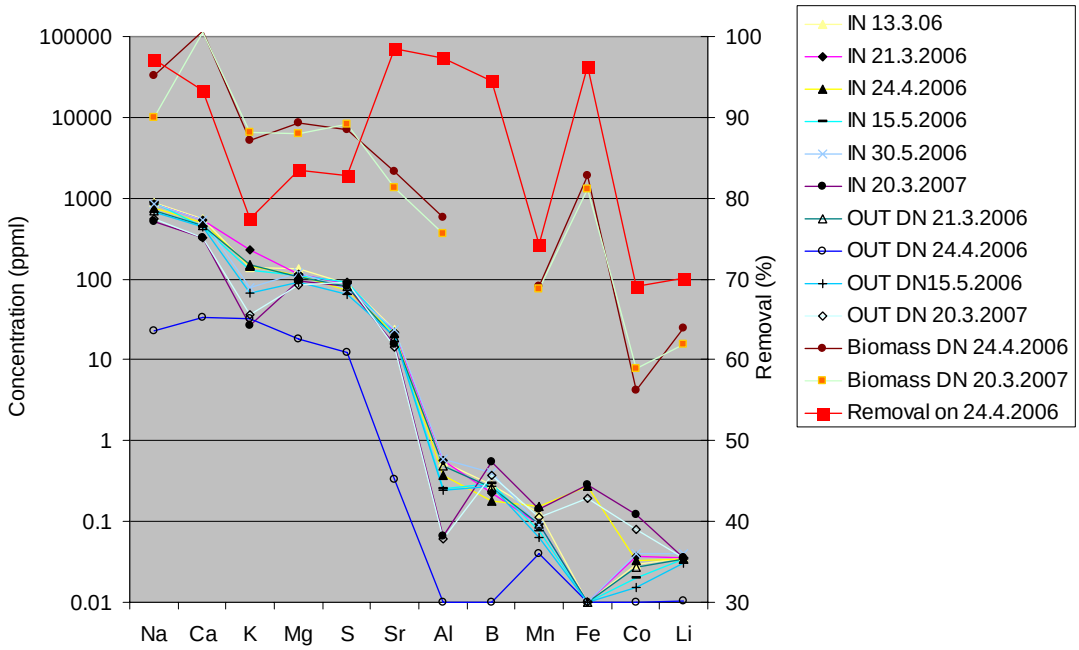


Figure 28. Reduced elemental composition of influent, effluent and biomass in Pilot 5 at Kemi mine. The calculated removal of elements is based on influent and effluent concentration.

The results show that the elemental composition of the Kemi mine water was relatively constant throughout one year. The heavy metal removal was best about two months after start-up of Pilot 5 (removal on 24.4.2006). Before and after this date heavy metal removal was greatly reduced. The biomass was mainly enriched in comparison to the mine water on Cr, Cu, Zn, Fe, Ni and Al. The sorption of Sr onto the biomass was minor if seen in relation to influent concentration. However, the Sr concentration in the biomass was high at about 2000 ppm and might have caused inhibition of the biofilm. The poor heavy metal removal after the first two months can be also seen in comparison of the very similar metal concentration in biomass from the denitrifying bioreactor in 2006 and 2007. This result is contrary to the expectation since the biomass was already exposed to Kemi mine water prior to inoculation. The heavy metal concentration in the biomass can be seen as a steady-state concentration due to the biomass specific sorption capacity. New grown biomass is expected to only take up as much metals as the older biomass before.

5.2 Concentration of nitrogenous compounds by filtration

The selection of most suitable membranes (nanofiltration and reverse osmosis) for concentration of nitrogenous compounds from mine water was performed with a DSS-plate module. Since NF failed to fulfill the filtration requirements, i.e. concentration of nitrogenous compounds, a comparison between results of RO and NF tests are only given for Pahtavaara mine water (Figure 29, Table 11). The results of bench-scale membrane selection tests with all three studied mine waters are given in Table 12. As can be seen from Figure 29, the reverse osmosis membranes had quite similar retentions based on measured electric conductivity, whereas the nanofiltration membrane had much poorer retention. In the case of SW30 HR, it can be seen that the permeate fluxes were quite small even with the highest pressure due to the tight structure of the membrane. The other three reverse osmosis membranes had more similar fluxes, Espa2 had the smallest flux and RO1 the highest of these three. The retention of electric conductivity for all the tested RO membranes was more than 90 % in all pressures. The retention improved as the pressure was raised, how-

ever, at higher pressure values it started to decrease slightly. The fluxes with the NF membrane were much higher than the four RO membranes had. When the pressure was increased to 20 bar the retention of the NF membrane started to decrease because the flux became too high. With high fluxes also the concentration polarization is larger and, therefore, also the diffusion through the membrane becomes higher. Another reason for the poor retention with the NF membrane is that it tends to let monovalent ions to penetrate the membrane. None of the membranes had a substantial influence on the permeate pH. In all cases the permeate pH was smaller than the pH of the feed water and, therefore, all the tested membranes retained more OH^- ions than H^+ ions due to negative charge of the membranes at the applied pH. No direct relationship between the fluxes and pH was observed. Based on the fluxes and retentions of the conductivity, samples from 15 bar were chosen for further analysis with Espa2, TFC ULP, RO1 and NF. The pressure chosen for the SW30 HR was 25 bar, because of the smaller permeate flux.

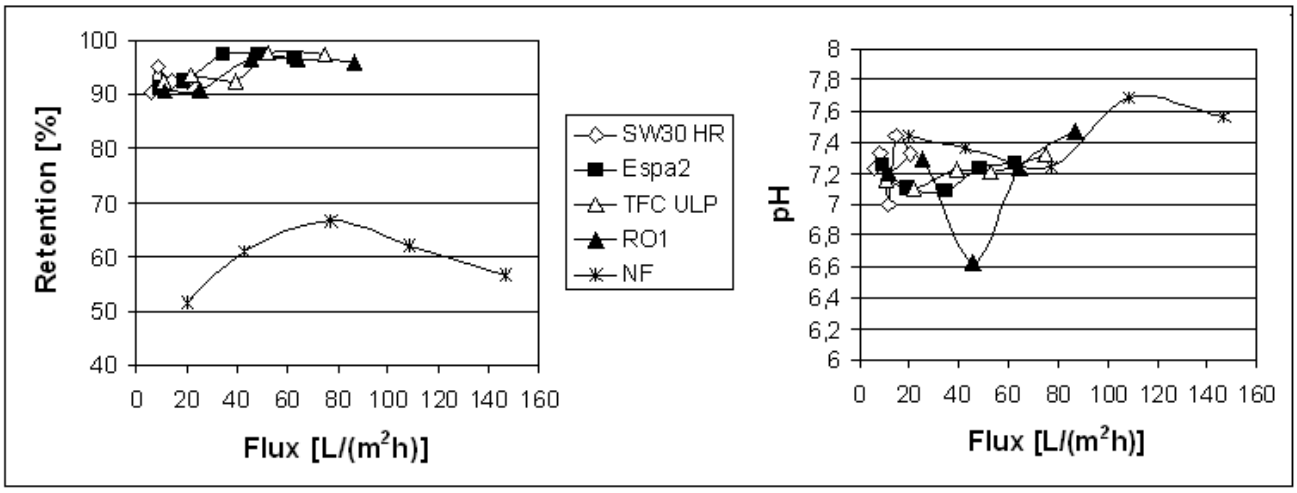


Figure 29. Permeate flux versus retention based on electric conductivity and pH for the five studied membranes (pressures 6, 10, 15, 20 and 25 bar except SW30 HR 15, 20, 25, 30, 35 bar) in test with Pahtavaara mine water.

The results of nitrate, ammonium and chloride analyses for the Pahtavaara case are presented in Table 11. The TFC ULP membrane had the best retention to NO_3-N and Espa2 to NH_4-N. Out of these two membranes, TFC ULP had a slightly better permeate flux. The results of ICP-AES -analyses from the "Pahtavaara" permeate are presented in Table 11. There were no substantial differences in the ion retentions of the membranes. The NF membrane removed some of the ions similarly as the RO membranes did, however, the ions separated were multivalent. One exception was zinc (Zn^{2+}) which had a deficient retention. Poor retention can be partly due to the small size of the ion. The only considerable difference between the four reverse osmosis membranes was in their ability to remove boron, which was retained best by SW 30 HR and TFC ULP. The amount of arsenic was higher in permeate than it was in the feed water with all the other membranes except for TFC ULP. The amount of lead was also higher in the Espa 2 permeate than it was in the feedwater. The membrane permeation rate for these elements was higher than that of water in these cases. The fouling percentages to Espa2 membrane was 0.4%, TFC ULP 5.3%, RO1 4.6% and to NF 11.7 %. With SW30 HR no fouling had occurred. All of these percentages are acceptable. The selected membrane for the concentration was TFC ULP due to the membrane's rejection of NO_3-N and NH_4-N and the high permeate flux.

Table 11. The results from the permeate analyses of batch tests with five different membranes and Pahtavaara mine water.

		Feed	SW 30 HR	Espa 2	TFC ULP	RO1	NF
NO_3-N	**mg/l**	15.6	0.94	0.40	0.36	0.61	11.5
	retention [%]		93.9	97.4	97.7	96.0	24.9
Cl	**mg/l**	14.0	2.86	<1.30	< 1.02	<1.16	5.07
	retention [%]		79.6	> 90.8	> 92.7	> 91.8	63.9
NH_4-N	**mg/l**	9.53	1.64	0.54	0.81	0.80	4.17
	retention [%]		82.8	94.3	91.5	91.6	56.2
S	**mg/l**	14.6	0.83	0.07	0.07	0.24	0.17
	retention [%]		94.3	99.5	99.5	98.4	98.8
Na	**mg/l**	28.1	1.96	1.60	0.67	1.85	13.7
	retention [%]		92.0	93.3	96.6	92.4	50.7
Mg	**mg/l**	15.9	0.85	0.09	0.06	0.15	2.89
	retention [%]		94.7	99.4	99.6	99.1	81.8
K	**mg/l**	15.0	2.50	2.13	1.77	2.21	7.04
	retention [%]		83.3	85.8	88.2	85.3	53.1
Ca	**mg/l**	32.2	1.37	0.11	< 0.01	0.17	7.24
	retention [%]		95.8	99.7	99.9	99.5	77.5
Sr	**mg/l**	0.28	0.01	< 0.01	< 0.01	< 0.01	0.06
	retention [%]		95.4	> 96.4	> 96.4	> 96.4	79.1
B	**mg/l**	0.10	0.02	0.04	0.03	0.04	0.09
	retention [%]		81.4	60.8	71.1	55.7	4.1
Zn	**mg/l**	0.08	0.03	< 0.01	< 0.01	< 0.01	0.07
	retention [%]		67.5	> 87.5	> 87.5	> 87.5	7.8
Ba	**mg/l**	0.12	< 0.01	< 0.01	< 0.01	< 0.01	0.02
	retention [%]		> 91.4	> 91.4	> 91.4	> 91.4	80.8
Mn	**mg/l**	0.04	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	retention [%]		> 75.0	> 75.0	> 75.0	> 75.0	> 75.0
Cu	**mg/l**	0.02	< 0.01	< 0.01	0.06	< 0.01	0.07
	retention [%]		> 50.0	> 50.0	0	> 50.0	0
As	**mg/l**	< 0.01	0.02	0.02	< 0.01	0.13	0.04
Pb	**mg/l**	< 0.01	< 0.01	0.07	< 0.01	0.01	< 0.01

Table 12. Separation of nitrate, ammonium, chloride and sodium chloride of the formed permeates during the membrane selection tests for all three tested mine waters.

Pahtavaara		**Feed**	**SW 30 HR**	**Espa 2**	**TFC ULP**	**RO1**
NO_3-N	**mg/l**	15.6	0.94	0.40	0.36	0.61
	retention %		93.9	97.4	97.7	96.0
Cl	**mg/l**	14.0	2.86	<1.30	< 1.02	<1.16
	retention %		79.6	> 90.8	> 92.7	> 91.8
NH_4-N	**mg/l**	9.53	1.64	0.54	0.81	0.80
	retention %		82.8	94.3	91.5	91.6
NaCl	**mg/l**	28.10	1.96	1.60	0.67	1.85
	retention %		92.0	93.3	96.6	92.4
Kemi						
NO_3-N	**mg/l**	20.8	1.66	1.33	1.94	0.89
	retention %		92.0	93.6	90.7	95.7
Cl	**mg/l**	2400	240	152	195	183
	retention %		90.0	93.7	91.9	92.4
NH_4-N	**mg/l**	5.50	0.86	0.33	0.60	0.60
	retention %		84.4	94.0	89.1	89.1
NaCl	**mg/l**	834	62.2	34.4	65.4	24.7
	retention %		92.5	95.9	92.2	97.0
Siilinjärvi						
NO_3-N	**mg/l**	44.0	1.17	1.76	2.68	3.05
	retention %		97.3	96.0	93.9	93.1
Cl	**mg/l**	57.6	1.11	<1.28	1.39	<1.05
	retention %		98.1	>97,8	97.6	>98.2
NH_4-N	**mg/l**	11.8	1.07	0.65	0.82	1.64
	retention %		90.9	94.5	93.0	86.1
NaCl	**mg/l**	94.1	1.96	4.65	5.63	2.63
	retention %		97.9	95.0	94.0	97.2

Bench-scale concentration tests were performed at operational pressures of 15 bar for Pahtavaara mine water (Table 13, 14) and for 20 bar for Siilinjärvi mine water. For Kemi mine water initially 20 bar pressure was used, however, it had to be raised to 40 to increase the flow. Sampling of permeate was planned at volumetric reduction factors (VRF) of 9, 10, 12, 15 and 20. The actual sampling occurred at VRF close to the planned VRFs. The retentions of electric conductivity were at least 99.0, 98.3 and 98.8 % for the Pahtavaara, Kemi and Siilinjärvi mine water. These percentages are comparable to the technical data provided by the manufacturers. For Kemi and Siilinjärvi mine water, the pH values were lower in the concentrates than in the feed. The concentrate of Pahtavaara mine water had higher pH values than the feed. The highest achieved VRF for Pahtavaara, Kemi and Siilinjärvi mine water were 20.4, 19.4 and 23.5. With these VRFs, the retentions for ammonium were 82.2, 71.0 and 68.3% for Pahtavaara, Kemi and Siilinjärvi mine water, respectively. While retentions were 89.9, 70.0 and 83.4% for nitrate and 84.7, 89.6 and 94.1% for chloride with Pahtavaara, Kemi and Siilinjärvi mine water. Since the RO-feed became more concentrated during the filtration, the retention percentages are less than reported by Awadalla et al. (1994).Since nitrifying and denitrifying bacteria are sensitive to salinity, it was monitored during the bench-scale concentration tests. The total salinity increased with VRF and was 4.0, 75.4 and 10.1 g/l at the highest VRF for Pahtavaara, Kemi and Siilinjärvi mine water. With Pahtavaara and Siilinjärvi mine water the VRF could be higher than 20 based on

biological tests. The obtained total salinity with Kemi mine water is relatively high and might decrease the microbial activity in bioreactors. Thus, the VRF should be less than 5 for Kemi mine water in the pilot-scale concentration tests. In tests with Pahtavaara mine water, the heavy metals Cu and Al were enriched to the concentrate. These metals might hinder the microbial activity of the bacteria in bioreactors. Membrane fouling was 0.4-4.2 %, 15.4-20.7% and 30.4-57.2 % with the Pahtavaara, Kemi and Siilinjärvi mine water, respectively. In the case of Siilinjärvi mine water, the increased fouling was probably due to higher permeate flux than with the two other mine waters. The results of the bench-scale tests were utilized to select the process parameters for pilot-scale concentration of Pahtavaara and Kemi mine water. Siilinjärvi mine water was not included in these tests.

The spiral wound module concentration test with Pahtavaara mine water was carried out with an initial pressure of 4.5 bar and the final VRF of 17.2 was reached. Since no pre-treatment was applied prior to reverse osmosis, suspended solids affected the filtration. Therefore, the operational pressure was raised during the test up to 35 bar to maintain a sufficient permeate flux (Figure 30). During the run, the mean flux was about 23.6 l/(m^2h). Additionally, the membrane had to be cleaned chemically, which temporarily improved the situation. The retention of the electric conductivity and permeate pH during the concentration test were as shown in Figure 31. Initial and final pH values were similar. In the beginning of the filtration of Pahtavaara mine water retentions were above 94%. Retention remained approximately constant up to a VRF value of 7 but, after that, it started to decrease notably, mainly due to the decrease in permeate fluxes caused by fouling. At the VRF value of 8.9, the retention improved temporarily, due to chemical cleaning of the membrane. The reverse osmosis filtration with Kemi mine water was started at 12.5 bar. The operational pressure was stepwise increased up to 28.5 bar and the final VRF was 4.7 (Figure 32). The retention of electric conductivity was stable and over 95%, but the pH decreased during the concentration test (Figure 33). Comparable to Kemi mine water, the retention percentages were lower than given by the manufacturers due to membrane fouling. During the run, the mean flux was approximately 25.2 l/(m^2h). Ammonium and nitrate were analysed in the feed water of both mine waters and in the final concentrates (Table 13). The membrane retention and the permeate quality was not as favourable as it had been in the small-scale filtrations (Figure 33). Ammonium and nitrate were analysed in the feed water of both mine waters and in the final concentrates (Table 15). The membrane retention and the permeate quality was not as favourable as it had been in the small-scale filtrations (Figure 33).

Table 13. The results from the permeate analyses and electric conductivity measurements at different volumetric reduction factors after the concentration stage with the TFC ULP membrane and Pahtavaara mine water.

			Volumetric reduction factor				
		Feed	**8.9**	**10.1**	**12.7**	**15.5**	**20.4**
EC	**permeate [µs/cm]**	460	30.2	29.7	31.2	31.7	33.2
	concentrate [µs/cm]		3080	3260	3680	3940	4380
NO_3-N	**permeate [mg/l]**	15.3	1.33	1.38	1.48	1.47	1.54
	separation [%]		91.3	91.0	90.3	90.4	89.9
	concentrate [mg/l]		135	173	139	163	203
Cl	**permeate [mg/l]**	15.7	2.00	< 1.18	< 1.03	< 1.25	2.4
	separation [%]		87.3	92.5	93.4	92.0	84.7
	concentrate [mg/l]		105	119	138	150	161
NH_4-N	**permeate [mg/l]**	9.48	1.53	1.54	1.6	1.63	1.69
	separation [%]		83.9	83.8	83.1	82.8	82.2
	concentrate [mg/l]		74.3	86.3	83.6	98.2	104

Table 14. Elemental composition of permeate and concentrate at different volumetric reduction fac-tors after the concentration stage with the TFC ULP membrane and Pahtavaara mine water.

	Feed		**Volumetric reduction factor**				
	[mg/l]		**8.9**	**10.1**	**12.7**	**15.5**	**20.4**
S	14.6	**permeate [mg/l]**	0.32	0.29	0.19	0.15	0.28
		retention [%]	97.8	98.0	98.7	99.0	98.1
		concentrate [mg/l]	107	111	127	133	151
Na	28.1	**permeate [mg/l]**	3.04	2.90	3.02	3.04	3.17
		retention [%]	89.2	89.7	89.3	89.2	88.7
		concentrate [mg/l]	218	228	261	278	312
Mg	15.9	**permeate [mg/l]**	0.25	0.21	0.21	0.21	0.21
		retention [%]	98.4	98.7	98.7	98.7	98.7
		concentrate [mg/l]	122	126	143	151	169
K	15.0	**permeate [mg/l]**	1.29	2.04	1.93	2.00	2.32
		retention [%]	91.4	86.4	87.1	86.7	84.5
		concentrate [mg/l]	104	108	123	130	146
Ca	32.2	**permeate [mg/l]**	0.74	0.54	0.49	0.50	0.51
		retention [%]	97.7	98.3	98.5	98.5	98.4
		concentrate [mg/l]	253	259	282	283	316
Sr	0.28	**permeate [mg/l]**	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
		retention [%]	> 96.4	> 96.4	> 96.4	> 96.4	> 96.4
		concentrate [mg/l]	2.76	2.94	3.43	3.59	4.21
B	0.10	**permeate [mg/l]**	0.07	0.06	0.06	0.07	0.08
		retention [%]	27.8	33.4	33.3	23.0	17.9
		concentrate [mg/l]	0.37	0.38	0.40	0.44	0.48
Ba	0.12	**permeate [mg/l]**	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
		retention [%]	> 91.7	> 91.7	> 91.7	> 91.7	> 91.7
		concentrate [mg/l]	0.92	0.95	1.05	1.06	1.15
Mn	0.04	**permeate [mg/l]**	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
		retention [%]	> 75.0	> 75.0	> 75.0	> 75.0	> 75.0
		concentrate [mg/l]	0.21	0.20	0.21	0.20	0.28
Zn	0.08	**permeate [mg/l]**	0.02	0.01	< 0.01	< 0.01	< 0.01
		retention [%]	76.5	82.5	> 82.5	> 82.5	> 82.5
		concentrate [mg/l]	0.34	0.29	0.28	0.19	0.47
Cu	0.02	**permeate [mg/l]**	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
		retention [%]	> 50.0	> 50.0	> 50.0	> 50.0	> 50.0
		concentrate [mg/l]	0.32	0.36	0.40	0.36	0.69
P	< 0.01	**permeate [mg/l]**	< 0.01	< 0.01	0.04	< 0.01	< 0.01
Li	< 0.01	**concentrate [mg/l]**	0.03	0.03	0.03	0.03	0.04
Al	< 0.01	**concentrate [mg/l]**	0.47	0.33	0.35	0.34	0.39
Co	0.01	**concentrate [mg/l]**	0.02	0.02	0.03	0.02	0.03
Ni	< 0.01	**concentrate [mg/l]**	0.08	0.08	0.09	0.09	0.11
Cr	< 0.01	**concentrate [mg/l]**	< 0.01	< 0.01	< 0.01	0.01	0.02

For Pahtavaara mine water, membrane fouling caused smaller concentration increases for ammonium and nitrate as expected from the bench-scale concentration tests. The aim was to keep the feed temperature below 15 ºC, however, due to increased temperature of the municipal water used for cooling, the feed temperature of Pahtavaara mine water could not be kept below 19.0 ºC. The ammonium increase in the concentrate was lower than expected and might indicate biological nitrification of ammonium to nitrate during filtrations. For Kemi mine water, the feed temperature was 19-21 ºC promoting biological nitrification. However, the ammonium content in permeate could not be analysed to quantify the nitrification. The results from the ICP-AES - analyses of the concentrates are presented in Table 16. With Pahtavaara mine water, the concentration of arsenic was higher in the feed than in the formed concentrate. This decrease occurred also in Pahtavaara mine water membrane selection stage, therefore, penetration of arsenic to the permeate can be considered as a characteristic of the RO1 membrane. None of the elements had enriched to a great extent into the concentrate of Pahtavaara mine water. With Kemi mine water, the amount of phosphorous was smaller in the concentrate than it had been in the feed and, hence, it has penetrated through the membrane to the permeate. As it can be observed in Table 16, iron, copper, zinc, cadmium and lead were enriched in Kemi mine water concentrate. Dalzell et al. (2002) reported 50% inhibition of nitrification by cadmium, copper and zinc at each 15, 30 and 10 mg/l. These IC50 concentrations are well below the metal content in the RO-concentrate of Kemi mine water, thus unlikely to affect bacteria. Depending on the origin of the nitrifying bacteria, they tolerate different salinities. Koops et al. (1991) described new *Nitrosomonas* species, which show optimal growth at NaCl concentration of 17.5 to 23.4 g/l. Dupla et al. (2006) studied extensively the denitrification of seawater, 28 to 30 g NaCl/l, and achieved good denitrification at 10°C. Thus, the salt content in the RO-concentrates is not expected to significantly inhibit the biological nitrification or denitrification of the RO-brine. When concentrating Pahtavaara mine water, fouling percentage for the RO1 spiral membrane was 98 % because suspended solids were not removed prior to RO. Therefore, it has been concluded that a prefiltration step would be needed before treating Pahtavaara mine water with the RO-module. With Kemi mine water, the fouling percentages to the membrane were from 6.4% to 18.9 % depending on the pressure. The 10 % limit of low fouling is exceeded, however, the fouling percentages are still tolerable. Notwithstanding, a prefiltration step is recommended also in this case.

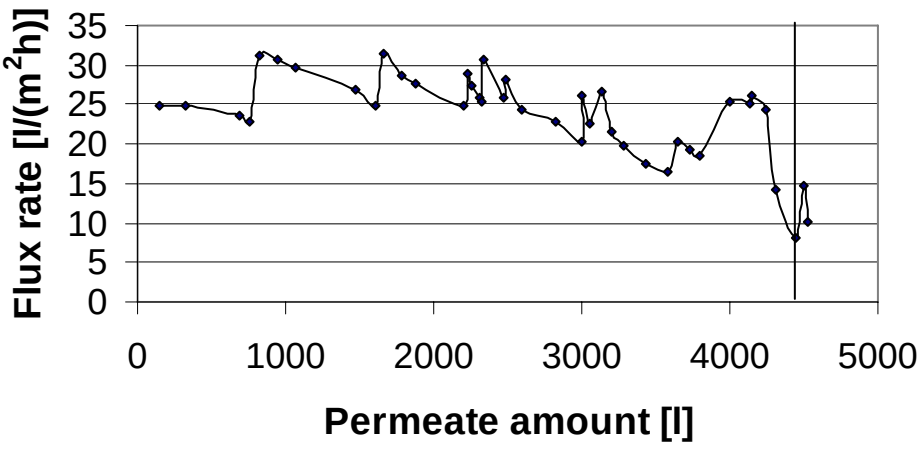


Figure 30. Development of permeate flux during concentration of Pahtavaara mine water. Increases in pressure are shown as rapid flux improvements. The chemical cleaning of membrane is marked with a vertical line.

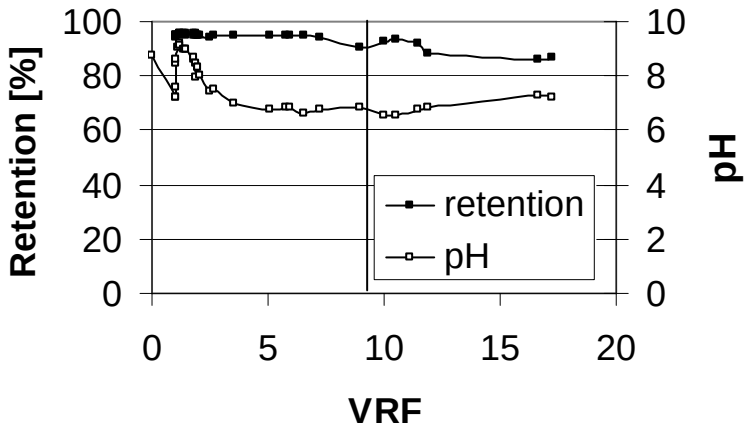


Figure 31. Development of retention of electric conductivity and permeate pH with increased volumetric reduction factor (VRF) during filtration of Pahtavaara mine water. The chemical cleaning of membrane is marked with a vertical line.

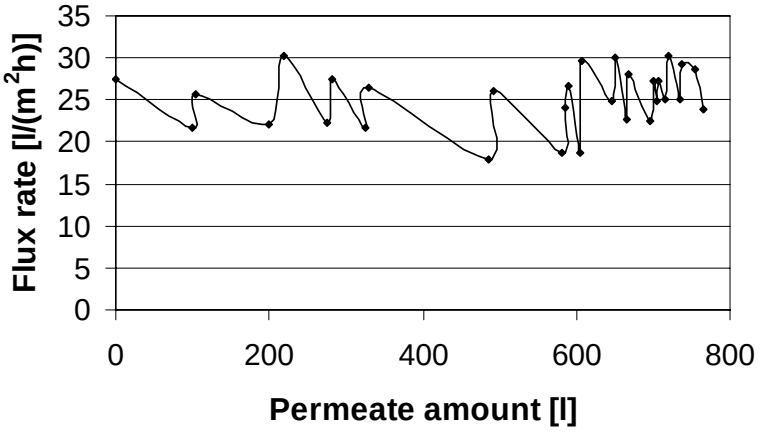


Figure 32. Development of permeate flux during concentration of Kemi mine water. Increases in pressure are shown as rapid flux improvements.

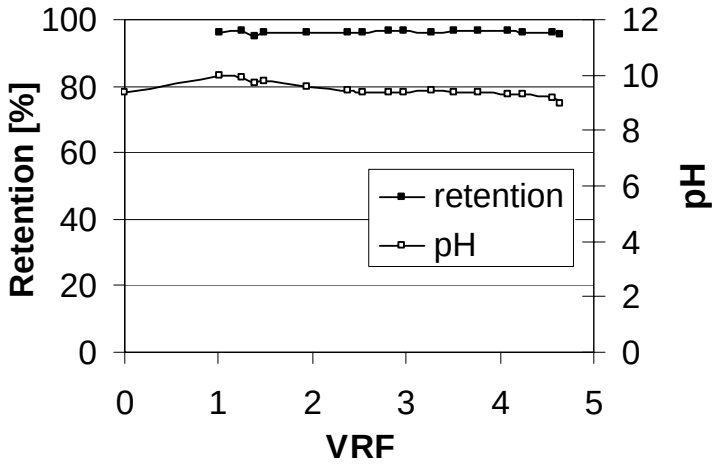


Figure 33. Development of retention of electric conductivity and permeate pH with increased volumetric reduction factor (VRF) during filtration of Kemi mine water.

Table 15. Ammonium and nitrate content in the reverse osmosis concentrate from the pilot scale concentration tests.

		Pahtavaara mine Feed	Pahtavaara mine VRF 17.2 concentrate	Kemi mine Feed	Kemi mine VRF 4.7 concentrate
NO3-N	mg/l	20.7	118	28.1	98.7
	increase		5.71		3.51
NH4-N	mg/l	8.56	31.0	3.12	6.03
	increase		3.63		1.93

Table 16. Elemental composition of the feed and reverse osmosis concentrate obtained from the pilot scale concentration tests.

		Li	Na	Mg	Al	P	S	K	Ca	Mn	B	Fe
Pahtavaara	mg/l	<0.01	24.5	21.6	0.15	0.07	17.0	17.6	35.6	0.02	0.09	0.03
VRF 17.2	mg/l	0.02	164	169.	0.29	0.19	177	82.7	146	0.04	0.15	0.06
	increase	>2.0	6.69	7.82	1.95	2.58	10.40	4.70	4.10	1.80	1.63	1.78
Kemi	mg/l	0.03	617	90.4	0.53	0.09	111	94.3	395	0.06	0.27	<0.01
VRF 4.7	mg/l	0.14	2820	386.0	2.03	<0.01	351	425	1440	0.25	0.43	0.41
	increase	5.19	4.57	4.27	3.83	-	3.16	4.51	3.65	4.44	1.60	>41
		Co	Ni	Cu		Ba	As	Zn	Cd	Pb	Cr	
Pahtavaara	mg/l	0.02	0.02	0.01		0.16	0.10	<0.01	<0.01	<0.01	<0.01	
VRF 17.2	mg/l	0.05	0.04	<0.01		0.61	<0.01	<0.01	<0.01	<0.01	<0.01	
	increase	2.10	1.73	-		3.77	-	-	-			
Kemi	mg/l	0.04	0.01	<0.01		0.19	<0.01	<0.01	<0.01	<0.01	<0.01	
VRF 4.7	mg/l	0.08	0.02	0.39		0.84	<0.01	0.24	0.18	0.10	0.04	
	increase	2.27	1.94	>39		4.31	-	>24	18	10	>4	

5.2.1 Process economics

An estimate of the process economics can be calculated. The calculations were done for a plant capacity of 250 000 m^3/a at VRF 20 and for a plant capacity of 1 000 000 m^3/a at VRF 5. Estimated capital and operational cost are listed in Table 17. The operational costs are formed from membrane replacement, energy consumption, cleaning chemicals purchase and labour costs. The calculations were done for a spiral wound module with a membrane area of 1.2 m^2 in one module. The incoming flux rate was assumed to be 700 L/(m^2h) and the permeate flux from one unit 33.3 l/(m^2h). The pretreatment unit before the reverse osmosis unit is considered to be cartridge filters. By using larger RO modules, the costs for the membrane changes would be much lower, since the price for membrane square meter is lower. The energy consumption depends mainly on the operational pressure and, therefore, the energy costs are higher with a larger plant capacity and higher operational pressures. The calculations were done considering two chemical cleanings per year. The estimated labour hours would form from turning the plant on and off, recording the flow and pressure, maintaining the log (Taylor et al., 1989) and performing the chemical cleaning. According to Awadalla et al. (1994), the membrane costs typically represent about 20 to 30 % of system costs for an aqueous separation system. In the present calculation, membrane costs were assumed to be 30 % of system costs. With amortizing time of 30 years and with an interest rate of 5% the capital costs would be 0.13 €/m^3 of processed feed with the

lower plant capacity and 0.11 €/m^3 with the larger plant capacity. Based on these considerations the total estimated total costs was calculated to be 0.34 €/m^3 of processed feed with the plant capacity of 250 000 m^3/a and with VRF 20. For a plant capacity of 1 000 000 m^3/a and VRF 5, the total estimated costs are 0.31 € /m^3. This is considerably less than the $0.95 to $1.06 /$m^3$ total costs of ammonium removal by RO from surface water estimated by Koyuncu et al (2001).

Table 17. Estimated total costs for processing 250 000 m3/a and 1 000 000 m3/a of mine water.

	Used parameters		250 000 m^3/a, VRF 20	1 000 000 m^3/a VRF 5
	Plant capacity 250 000 m3	Plant capacity 1 000 000 m3		
Energy	energy price 7.20 c/kWh		0.03 €/ m^3	0.07 €/ m^3
	pump efficiency 70 %			
	average operating pressure 9 bar	average operating pressure 20 bar		
Chemicals	The prices 3.73 €/kg (used as 1% solution) and 2.86 €/kg (used as 0.5 % solution)		0.002 €/ m^3	0.002 €/ m^3
	two chemical cleanings per year			
Membrane and pre-filtration cartridge changes	678 modules, 218.24 € a piece.	2283 modules, 218.24 € a piece.	0.17 €/ m^3	0.15 €/ m^3
	Membrane life expectancy 4 years			
	Pre-filtration cartridges changed once a week			
	7 cartridges, 18 € a piece.	22 cartridges, 18 € a piece.		
Labour	75 hours in a year		0.006 €/ m^3	0.002 €/m^3
Capital costs	30 years amortizing time, 5% interest rate		0.13 €/ m^3	0.11 €/ m^3
Total			0.34 €/ m^3	0.31 €/ m^3

5.3 Biological removal of nitrogenous compounds from mine water at laboratory-scale

The present study shows the near complete removal of total nitrogen (ammonium, nitrite, nitrate) from suspended solid-free mine water by using a biological two-step system consisting of nitrifying and denitrifying biofilm reactors (Table 4, Figure 7 and 8). It was shown that stable nitrification and low effluent ammonium concentration (below 0.3 mg/l) were achieved during 224 days of operation of bioreactor at 10±2°C (Pilot 5). Close to complete nitrification (90 – 95%) was obtained when the loading rate was in the range from 3 to 150 g (NH_4^+-N)/m^3/d and hydraulic retention time from 5.5 to 0.25 hours (Figure 34). Nitrification rate remained high during the following 148 days of operation with high load of ammonium. Ammonium removal percentage of 70 – 90% was obtained at a loading rate from 100 to 435 g (NH_4^+-N)/m^3/d and hydraulic retention time from 0.7 to 0.17 hours (Fig. 22). The process was stable, but an increase of the ammonium loading rate up to 595 - 750 g (NH_4^+-N)/m^3/d resulting in a decrease of the ammonium removal percentage down to 45% (Figure 34). Maximum removal rate obtained was 383 g NH_4^+-N/m^3/d or 3.2 g NH_4^+-N/m^2/d. The similar ammonium removal rate (3.17 g NH_4^+-N/m^2/d) was obtainer by Salvetti et al. (2006) in pure-oxygen moving-bed biofilm reactor, which was fed with the secondary effluent of a waste water treatment plant.

The effluent of the nitrifying reactor was treated in the fixed bed denitrifying reactor, using methanol as an external organic carbon source. The removal rate obtained at 10°C was 522 g $N/m^3/d$ or 3.6 g N/m^2 carrier/d (Pilot 5). Stable denitrification (90 to 96 %) was achieved at a loading rate from 47 to 480 g $(NO_3^-\text{-}N)/m^3/d$ and hydraulic retention time from 7 to 1.4 hours during 210 days of operation of bioreactor at 10±2°C. Stable denitrification and low effluent nitrate concentration (below 1 mg/l) were achieved during the following 168 days of operation (Fig. 23 and Fig. 24). The bioreactor was able to reduce 544 g of $NO_3^-\text{-}N/m^3/d$ to nitrogen gas with an efficiency of 96% at hydraulic retention time of 1.4 hours. The maximum removal rate obtained was 522 g $NO_3^-\text{-}N/m^3/d$ or 3.6 g $NO_3^-\text{-}N/m^2$ carrier/d. These results can be compared with those obtained in studies performed with Kaldnes K1 carrier. Welander and Mattiasson (2003) obtained the maximum denitrification rate of 2.6 g $NO_3^-\text{-}N/m^2$ carrier/d at 11°C with hydraulic retention time of 0.86 hours. A mixture of sodium acetate, yeast extract and peptone was used as carbon sources. A study perfomed on municipal waste water, using the same carrier gave a maximum denitrification rate of 2.2 g $NO_3^-\text{-}N/m^2$ carrier/d (Rusten et al., 1995) and 3.2 g $NO_x^-\text{-}N/m^2$ carrier/d (Rusten and Ødegaard, 2007).

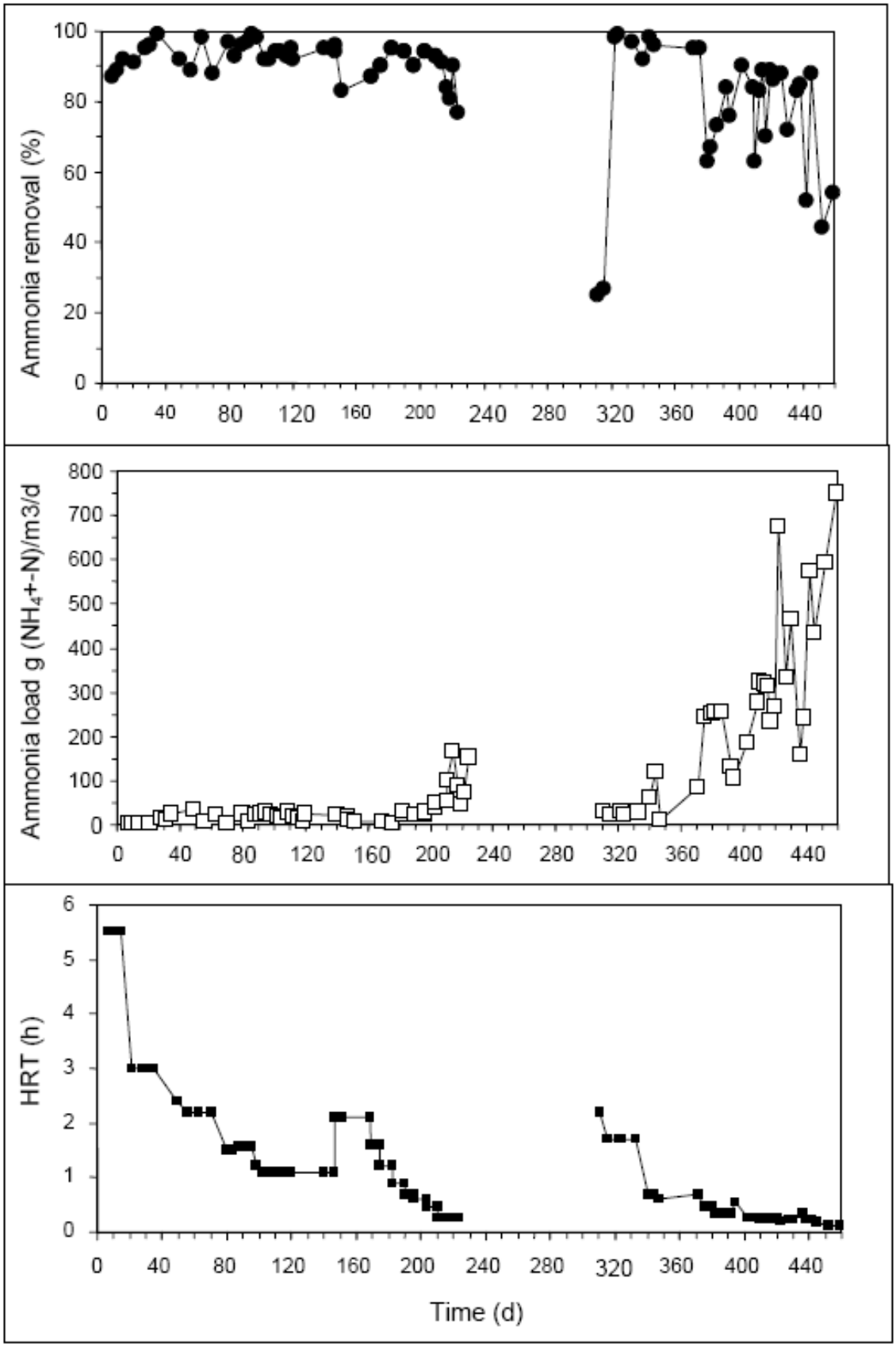


Figure 34. Ammonia removal (measured as difference between the inlet and the outlet of the bioreactor) in the bioreactor at 10°C with different flow rates and different load of ammonia (Pilot 5 at Kemi mine).

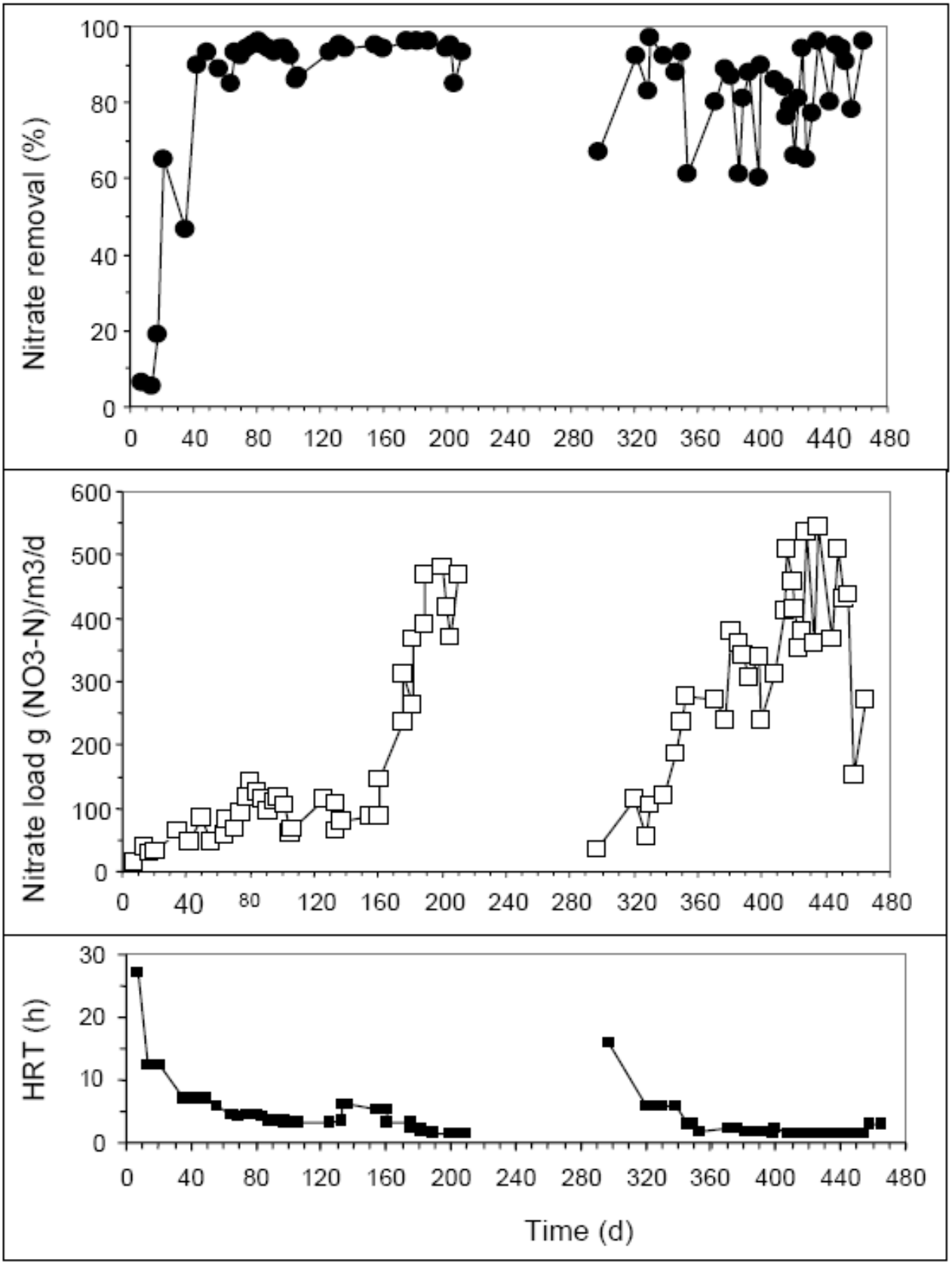


Figure 35. Nitrate removal (measured as difference between the inlet to the anoxic unit and the outlet of the bioreactor) with different load of nitrate and different flow rates (Pilot 5 at Kemi mine).

Compositions of the gas samples collected from bioreactor (Pilot 5) are shown in Table 18. The gases CO_2 and N_2 were founded to be the major components in the gas produced in the bioreactor. Only traces of N_2O, an important greenhouse gas, were detected. From 84 to 89% of assimilated NO_3^--N was reduced to nitrogen (Table 18) and rest was converted to biomass.

Table 18. The percentage and volume of off-gasses collected from the denitrifying bioreactor of Pilot 5 under different concentration and load of nitrate.

Number of samples	Concentration of NO_3^--N (mg/l)		Volume of treated water	Volume of collected gases	Collected gas	Amount of NO_3^--N converted to N_2
	Influent	Effluent	(liter)	(liter)	(%)	(%)
1	49,2	<1	157	6	N_2 - 92 CO_2 - 6 CH_4 - 2 N_2O – 0.004	89
2	34,2	<1	337	7,7	N_2 - 91 CO_2 – 7.4 CH_4 – 1.6 N_2O – 0.002	84

The denitrifying bioreactor utilized organic compounds measured as Total Hydrocarbon (THC) content in water (Figure 36). Thus, the denitrifying bioreactor also acts as a polishing step for organic compounds in mine water.

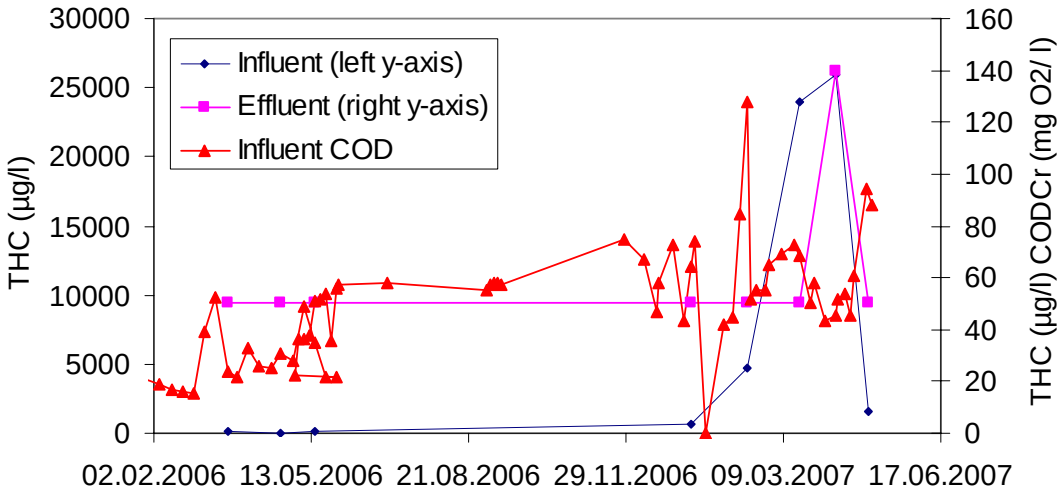


Figure 36. Total Hydrocarbon Content (THC) and COD_{Cr} in influent to nitrifying bioreactor and effluent of denitrifying bioreactor of Pilot 5 at Kemi.

5.4 Biological removal of nitrogenous compounds from high salinity water at laboratory-scale

5.4.1 Reverse osmosis concentrate

The characteristics of the reverse osmosis (RO) concentrates used in the experiments are shown in Table 19.

Table 19. Basic composition of reverse osmosis concentrates used in experiments

Feed	Type of experiment	$N\text{-}NH_4^+$ (mg/l)	$N\text{-}NO_3^-$ (mg/l)	$N\text{-}NO_2^-$ (mg/l)	COD_{Cr} (mg/l)	Cl^- (g/l)	Total salinity (g/l)	pH
RO concentrate, Pahtavaara	Batch	112	203	7	<500	0,37	4	7,8
	Continuous	103	155	2	527	0.16	3	7,89
RO concentrate, Kemi	Batch	44	184	7	627	31	48	8,1
	Continuous	45	111	1,5	<500	11.2	17	8,67
RO concentrate, Siilijärvi	Batch	105	585	39	1,14	<500	10	8,1

The Figures 37, 38 and 39 present the nitrification activity of bacterial culture from pilot 4 in different RO concentrates. Nearly 95 % of ammonium was oxidized for 20 – 24 days of incubation in all RO concentrates. During ammonium oxidation, there is release of hydrogen ions that decrease the pH to an extent related to the buffering capacity of the RO concentrate but at the further pH increased approximately to an initial level. Accumulation of nitrite was also observed during ammonium oxidation in all RO concentrates. Nitrite was oxidized further to nitrate in ten days in RO concentrate from Siilijärvi and Pahtavaara mine waters (Figure 37 and 38). On the other hand, oxidation of nitrite was completely inhibited in RO concentrate from Kemi mine water with total salinity of 48 g/l (Fig. 39, Table 19). These results agree with those of other authors, which found that nitrite oxidation was more sensitive to salt concentration than ammonium oxidation (Catalan et al. (1997), Schenk and Hegemann (1995). Vredenberg et al., (1997) found that nitrite accumulations were produced at chloride concentrations higher than 20 g Cl^-/l, while Hunik et al. (1992; 1993) used batch assays to study the effect of different salts on ammonium and nitrite oxidation, finding ammonium oxidation more sensitive to saline effect. In our experiments nitrifying biofilm from Pilot 4 adapted to high concentration of salts was capable to oxidize ammonium in RO concentrate from Kemi mine water with total salinity of 48 g/l.

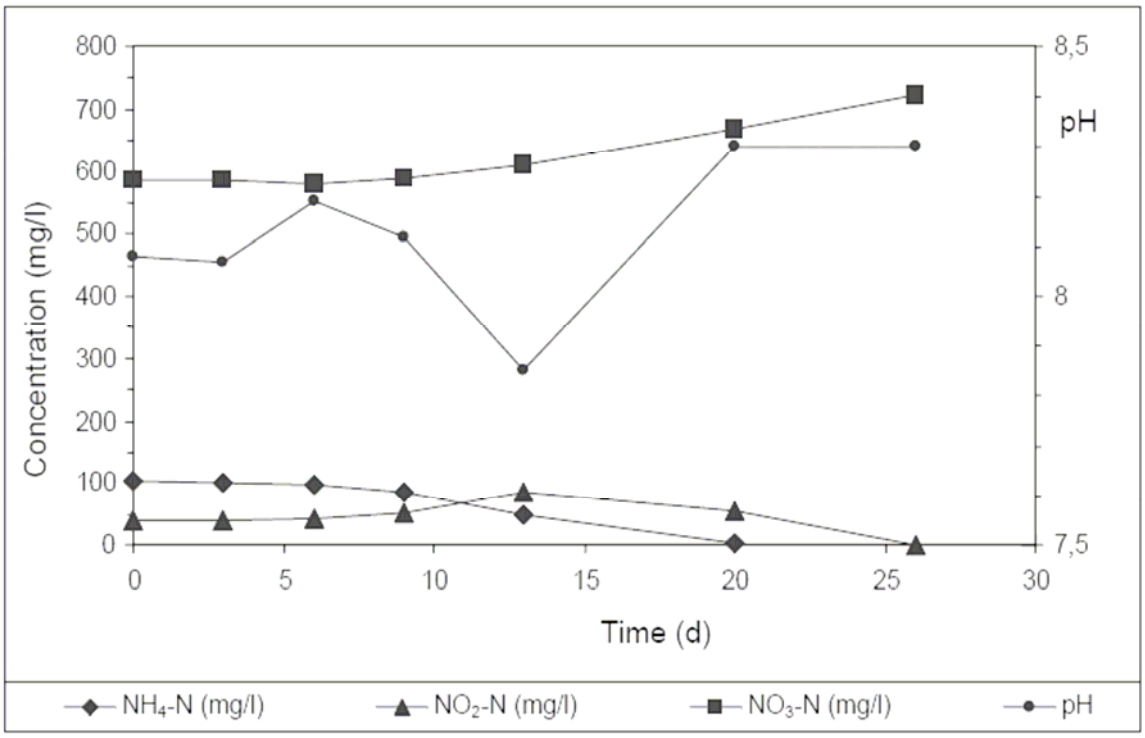


Figure 37. Concentration of ammonium, nitrite, nitrate and pH value during the batch experiment on nitrification of reverse osmosis concentrate from Siilinjärvi mine water.

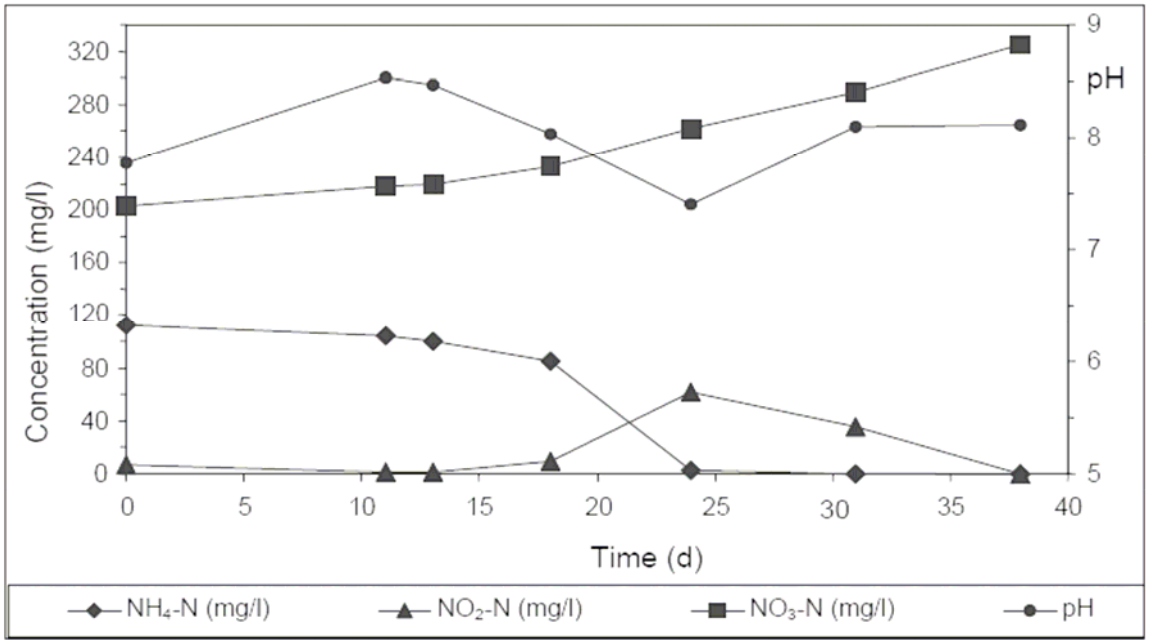


Figure 38. Concentration of ammonium, nitrite, nitrate and pH value during the batch experiment on nitrification of reverse osmosis concentrate from Pahtavaara mine water.

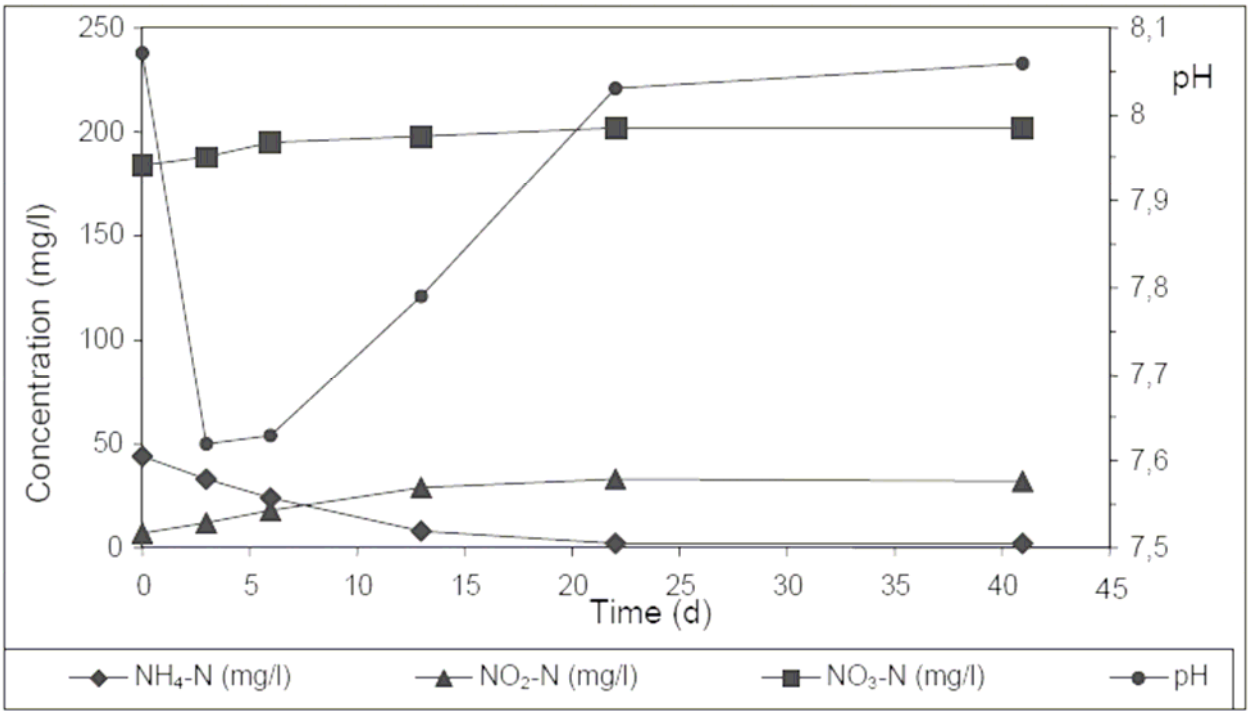


Figure 39. Concentration of ammonium, nitrite, nitrate and pH value during the batch experiment on nitrification of reverse osmosis concentrate from Kemi mine water.

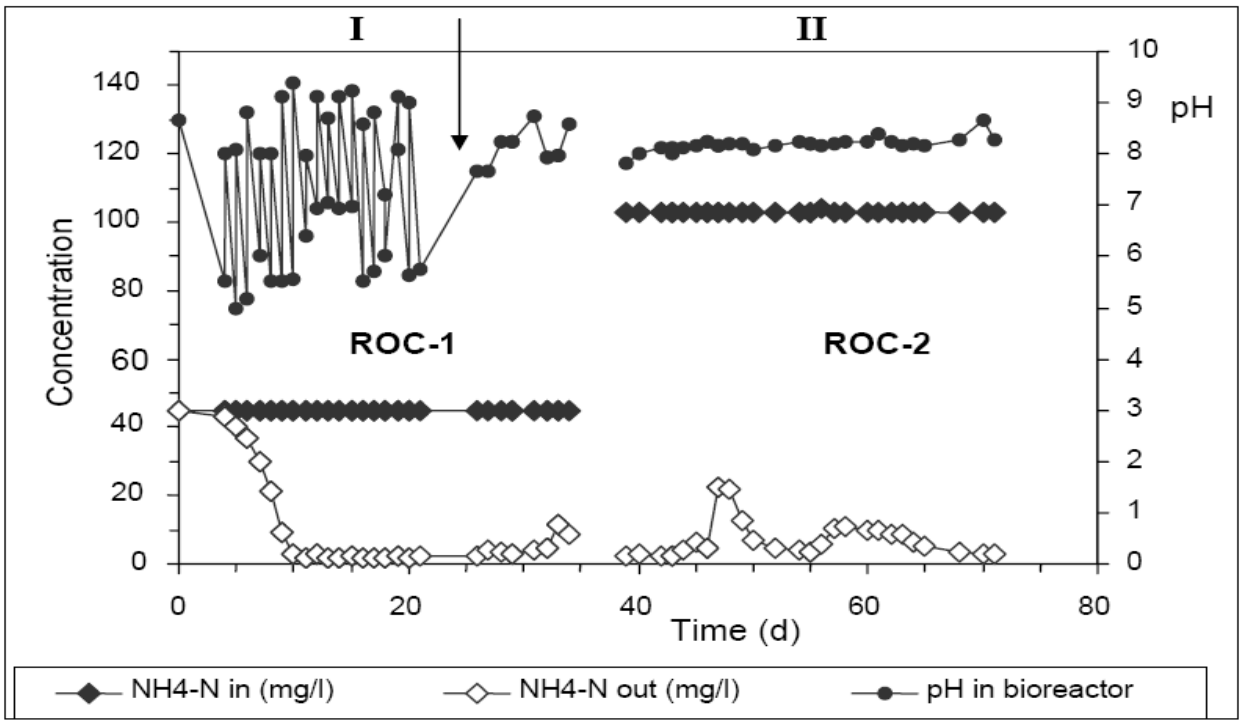


Figure 40. Nitrification in bioreactor with reverse osmosis concentrates from Kemi mine (ROC-1) and Pahtavaara mine (ROC-2) at 5°C (Pilot 4). I – manual pH regulation; II – automatic pH regulation (arrow indicates time of installation of automatic pH regulation).

The reactor performance with RO concentrates at 5± 1°C is depicted in Figures 40 and 41. At the beginning reactor was operated with RO concentrate from Kemi mine water with manually pH regulation. From day 28 onwards pH in the bioreactor was regulated automatically in the range of 7.9 to 8.3. The experiment was started with hydraulic retention time of 91 hours and ammonium load of 5 g of NH_4^+-N/m^3/d (Figure 41). After 10 days of operation bioreactor reached almost complete nitrification (94 – 96 %). From day 19 onwards the ammonium loading rate was increased in several steps to 102 g of NH_4^+-N/m^3/d by increasing the inflow rate. The bioreactor was able to oxidise 48 g of NH_4^+-N/m^3/d to nitrate with an efficiency of 90% at initial concentration of 45 mg NH_4^+-N/l. From day 39 the bioreactor was operated with RO concentrate from Pahtavaara mine water. In this case, bioreactor oxidised 45 g of NH_4^+-N/m^3/d with an efficiency of 95 – 98 % at initial concentration of 103 mg NH_4^+-N/l (Figures 40and Fig. 41). Maximum removal rate obtained was 0.4 g NH_4^+-N/m^2 carrier/d for both RO concentrates. These results similar with those of Isid (2003) who while studying the nitrification in high salinity waters using Kaldnes moving bed bioreactor obtained removal rate of 0.28 g NH_4^+-N/m^2 carrier/d at 33 – 34 °C and concentration of NaCl 29 g/l.

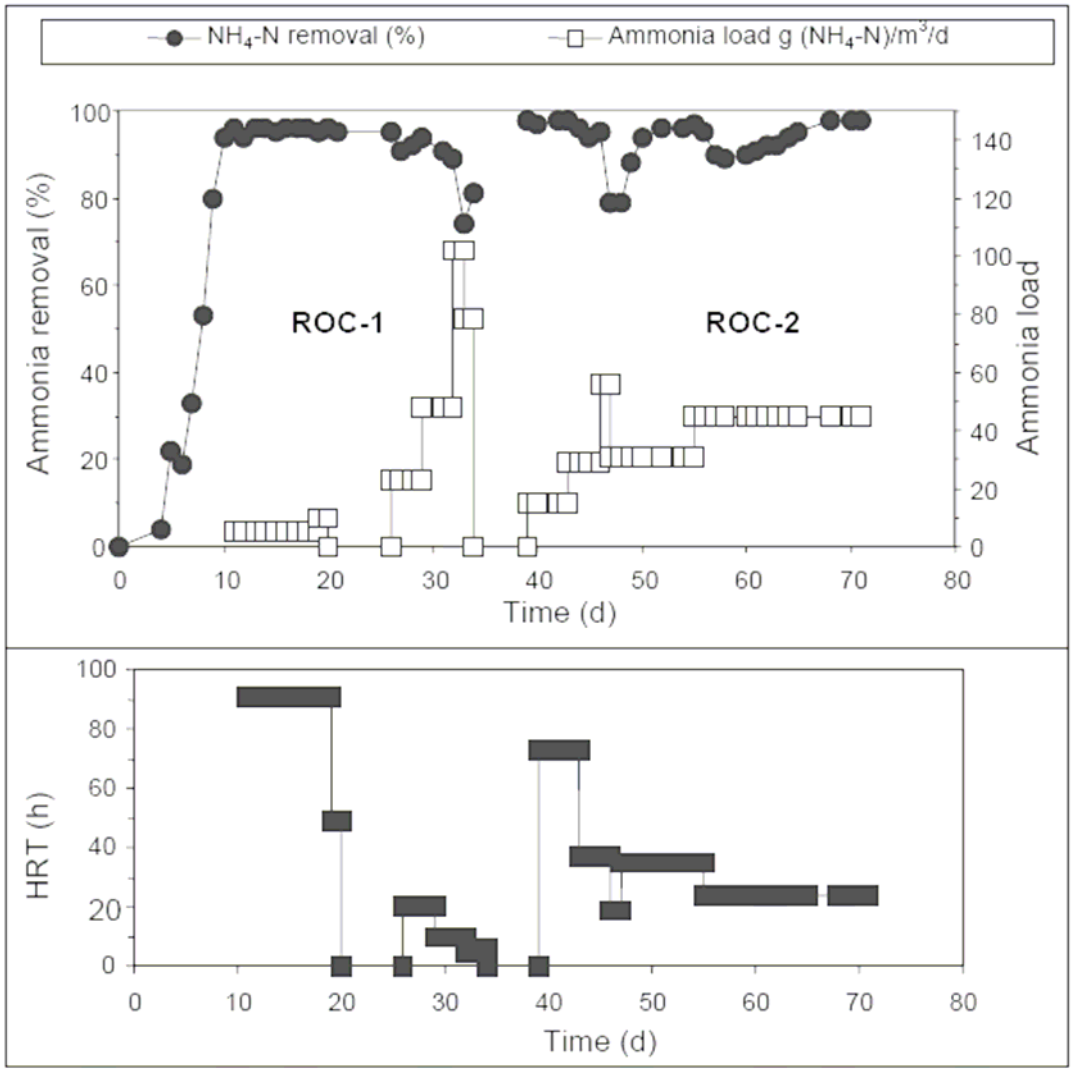


Figure 41. Ammonium removal (measured as difference between the inlet and the outlet of the bioreactor) from reverse osmosis concentrates in bioreactor with different ammonium loads and flow rates at 5°C. (Pilot 4). ROC-1 reverse osmosis concentrate from Kemi mine and ROC-2 reverse osmosis concentrate from Pahtavaara mine.

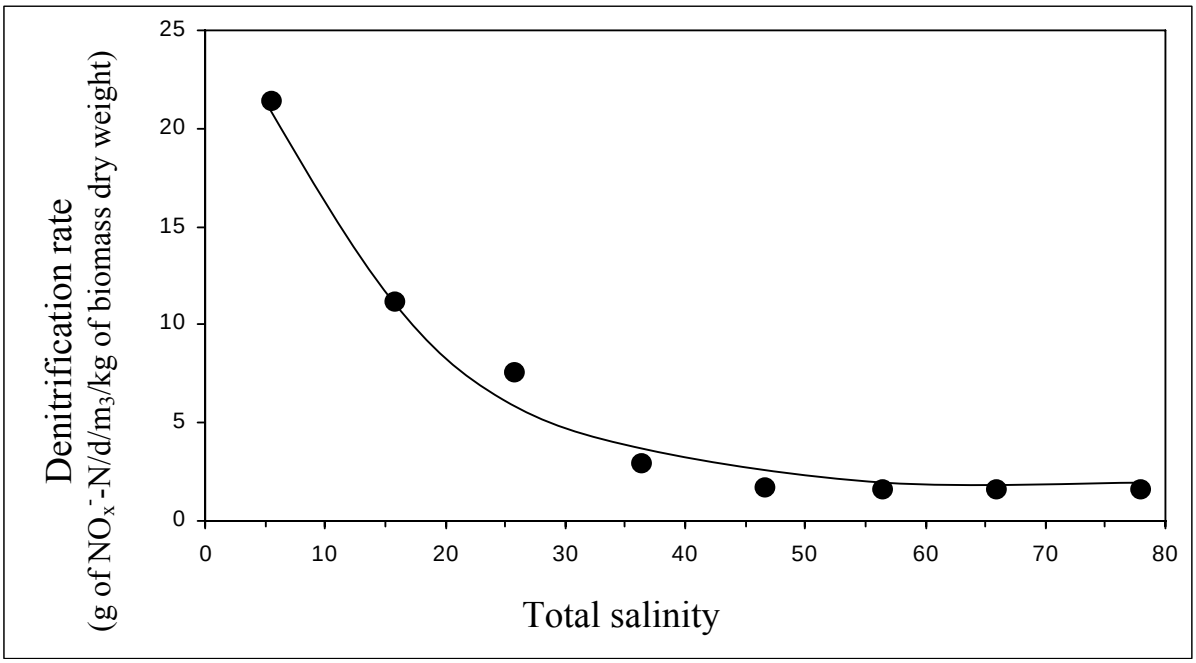


Figure 42. Denitrification rate versus total salinity in tests with a population of methylotrophic denitrifying bacteria adapted to high concentration of salts.

The effluent of the nitrifying reactor (Pilot 4) was treated in the fixed bed denitrifying reactor (Pilot 6), using methanol as an external organic carbon source. From 84 to 92 % nitrate removal was obtained with loading rate of 91 g (NO_3^--N)/m^3/d for RO concentrate from Kemi mine water and from 91 to 97 % nitrate removal was obtained with loading rate of 174 g (NO_3^--N)/m^3/d for RO concentrate from Pahtavaara mine water (Figures 43 and 44). Hydraulic retention time in both experiments was 40 hours.

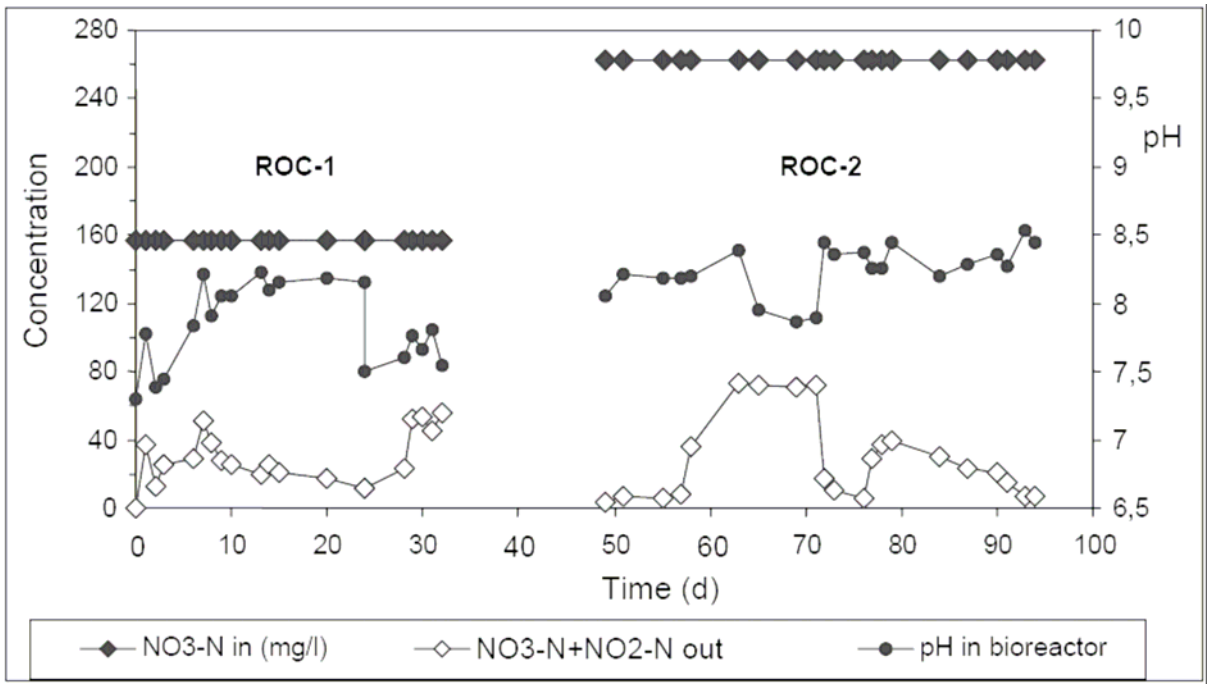


Figure 43. Denitrification in the bioreactor with reverse osmosis concentrates from Kemi mine (ROC-1) and Pahtavaara mine (ROC-2) at 12°C (Pilot 6).

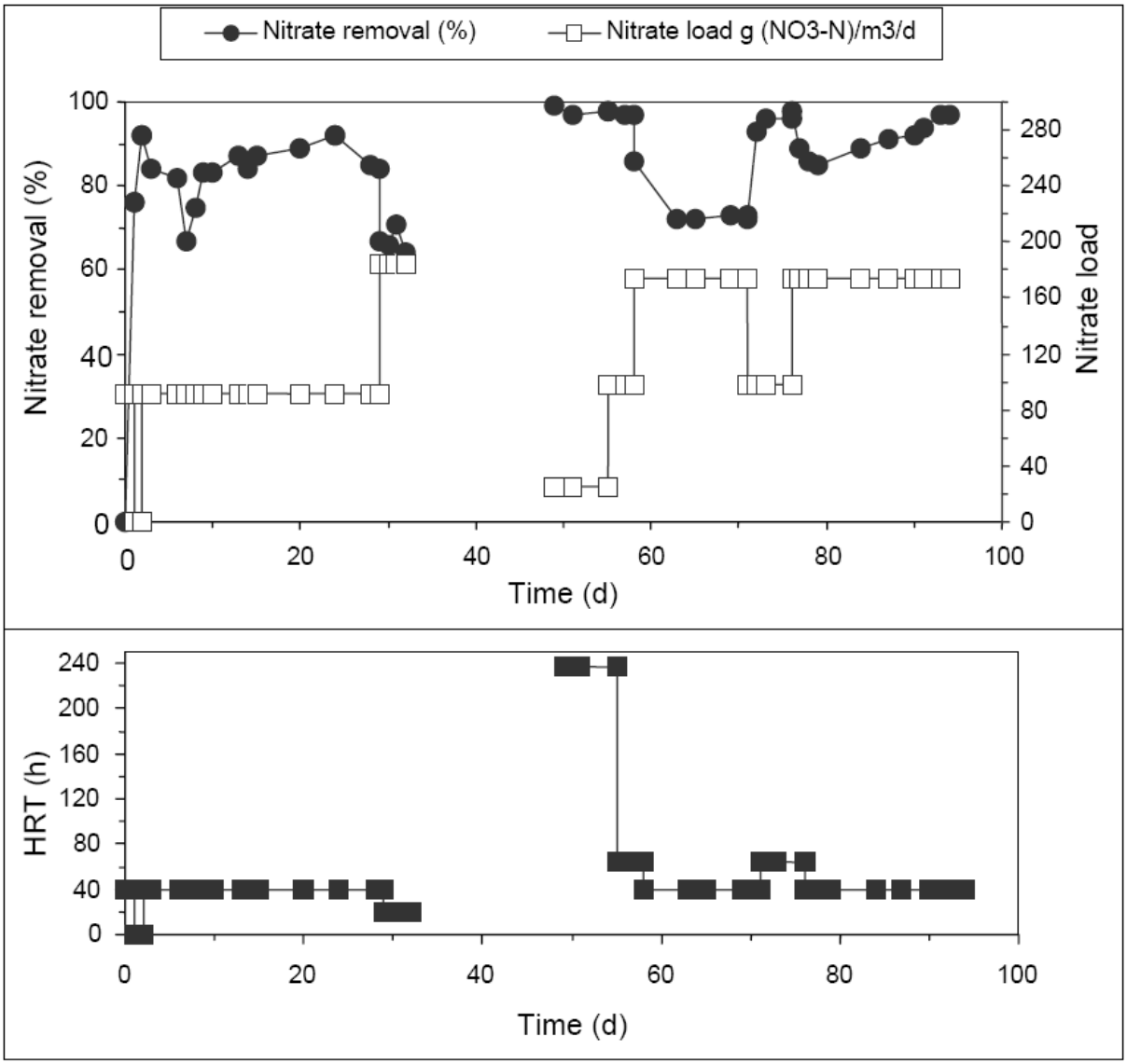


Figure 44. Nitrate removal (measured as difference between the inlet and the outlet of the bioreactor) from reverse osmosis concentrates in bioreactor with different nitrate loads and flow rates at 12°C (Pilot 6). ROC-1 reverse osmosis concentrate from Kemi mine and ROC-2 reverse osmosis concentrate from Pahtavaara mine.

5.4.2. Denitrification in saline wastewater with high nitrate concentration in a continuous-flow biofilm reactor

Explosives production wastewater from Oy Forcit Ab was used in this experiment. The experiment was started with wastewater diluted in 10 times by tap water. At the beginning reactor was run with hydraulic retention time of 22 hours and nitrate load of 52 g of NO_3^--N/m^3/d (Fig. 45 and 46). After 40 days of operation bioreactor reached almost complete denitrification (94 %). From day 40 the nitrate loading rate was increased in several steps to 199 g of NO_3^--N/m^3/d by decreasing waste water dilution and increasing the inflow rate. The bioreactor was able to oxidise 180 g of NO_3^--N/m^3/d to nitrogen gas with an efficiency of 80% at hydraulic retention time of 12 hours and initial concentration of 2,23 g NO_3^--N/l.

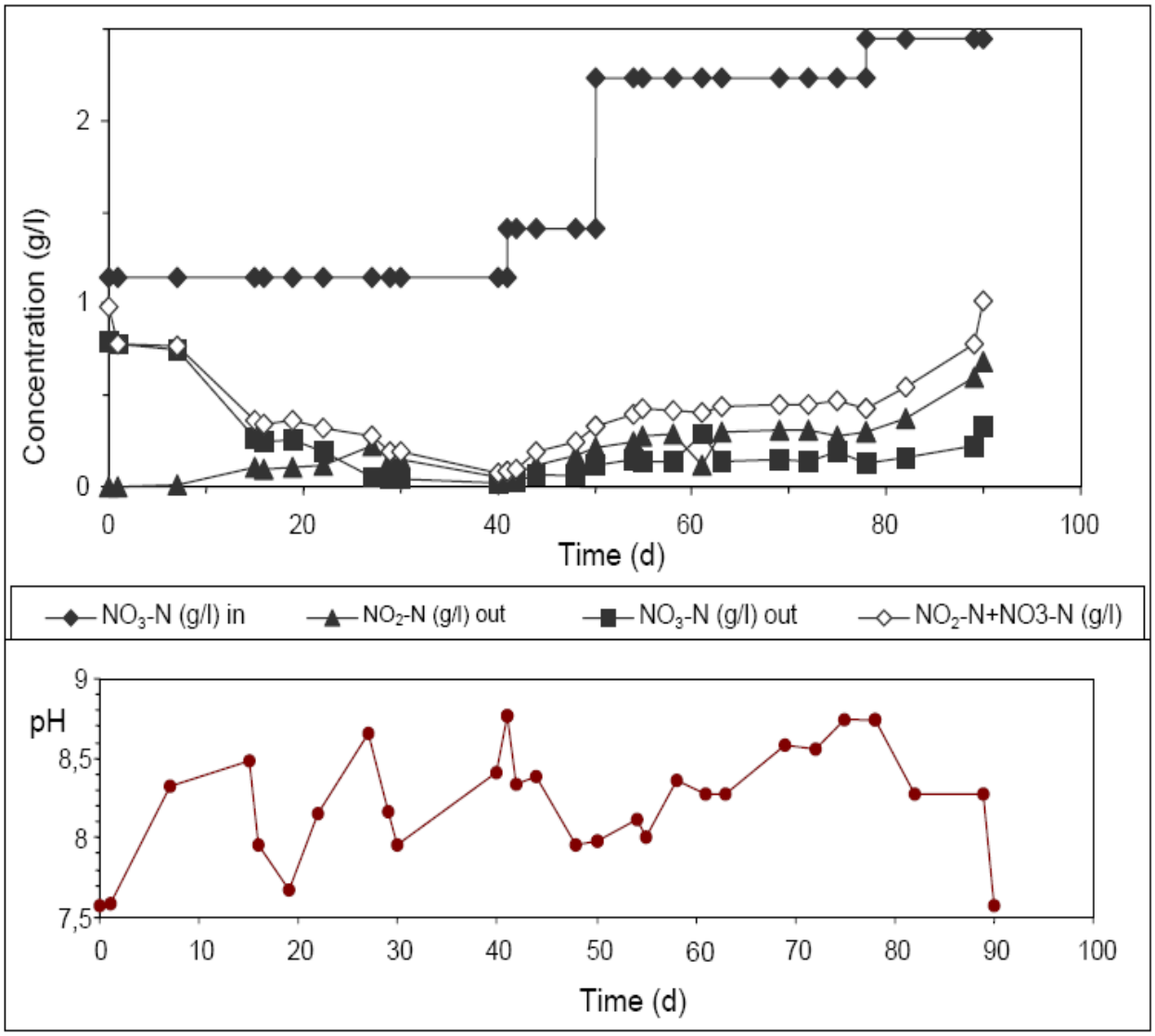


Figure 45. Denitrification in the bioreactor with high nitrate concentration waste water from Oy Forcit Ab at 12°C.

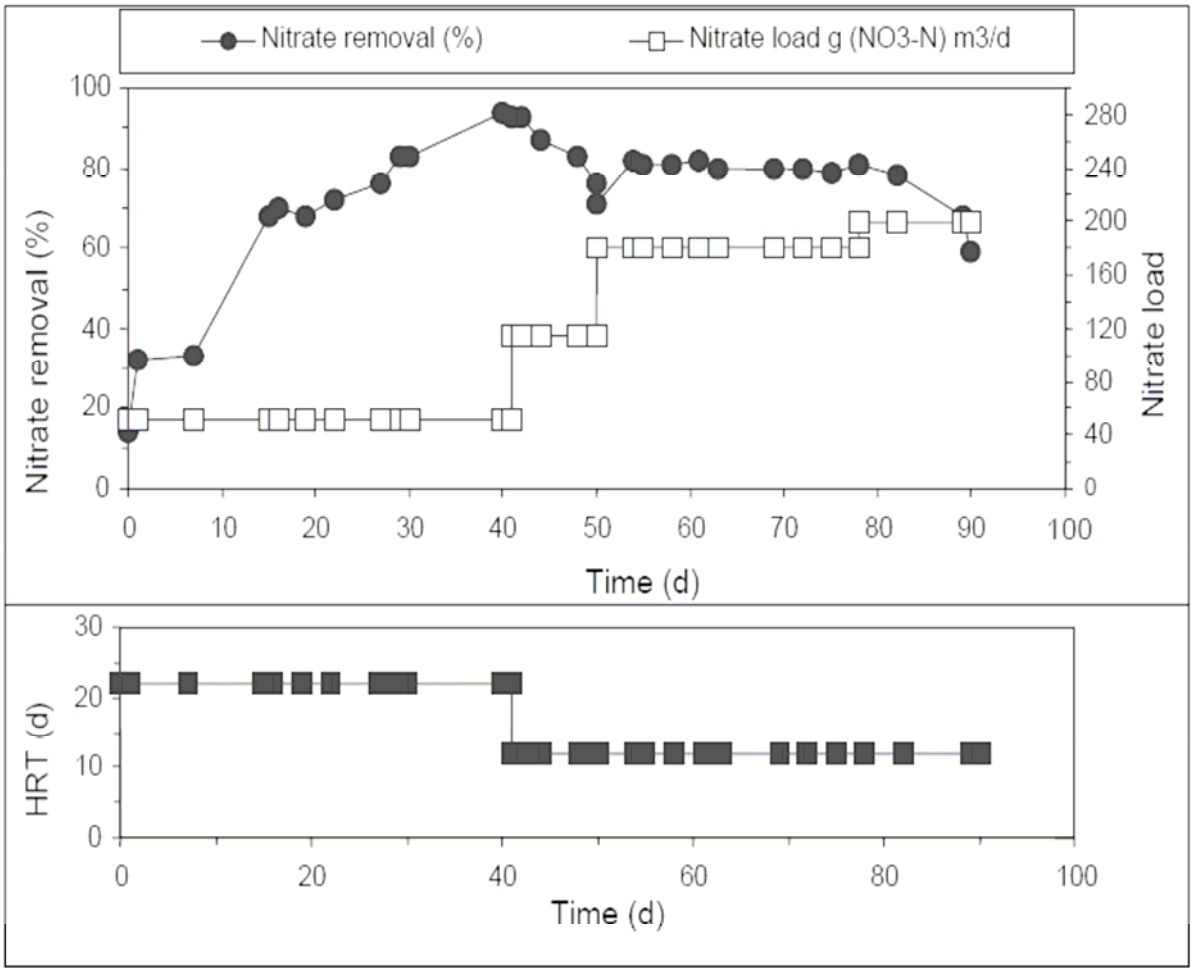


Figure 46. Nitrate removal (measured as difference between the inlet and the outlet of the bioreactor) from high nitrate concentration wastewater (Oy Forcit Ab) with different nitrate loads and flow rates at 12°C.

Denitrification of nitrate-rich brine has also been investigated at the Mineral Hill Mine in Montana in 1996 (Hiebert, 1998). The study demonstrated that denitrification of nitrate in raw mine water (about 25 mg/L NO_2/NO_3-N) as well as ion exchange brine (300-400 mg/L NO_2/NO_3-N) was possible. The bioreactor's treatment capacity was 26 L/min and nitrate removal was 31-65% with methanol as organic substrate at temperature of 6 °C (Hiebert, 1998).

5.5 Pilot phase 2

The first 28 days after inoculation by methanol-degrading bacteria from Pilot 5 bioreactors were operated in batch mode to enrich biomass. After day of 28 the system was operated in continuous mode with water flow from 1.7 to 64 m^3/d. Hydraulic retention time in denitrifying reactors varied from 32 to 0,9 hours and nitrate load from 8.2 to 284 g NO_3^--N /m3/d. The temperature of influent water was from 3.2 to 15.9°C. The performance of pilot-scale system is shown in Figures 47 and 48. At C/N ratio from 2.5 to 3 nitrate removal efficiency of 80 –98% was obtained (Figure 49). COD of the effluent water exceed of COD of influent water insignificantly and was 65±14 mg/l. If the C/N ration was higher than these values, quantities of the organic matter

measured as COD in the treated effluent water increased. At C/N ratio lower than 2.5 efficiency of denitrification decreased. Thus, optimal C/N ratio for initial nitrate concentration of 10.8±0.7 mg/l NO_3^--N was 2.5 – 3. This result generally agrees with those published by other researchers as listed in Table 20.

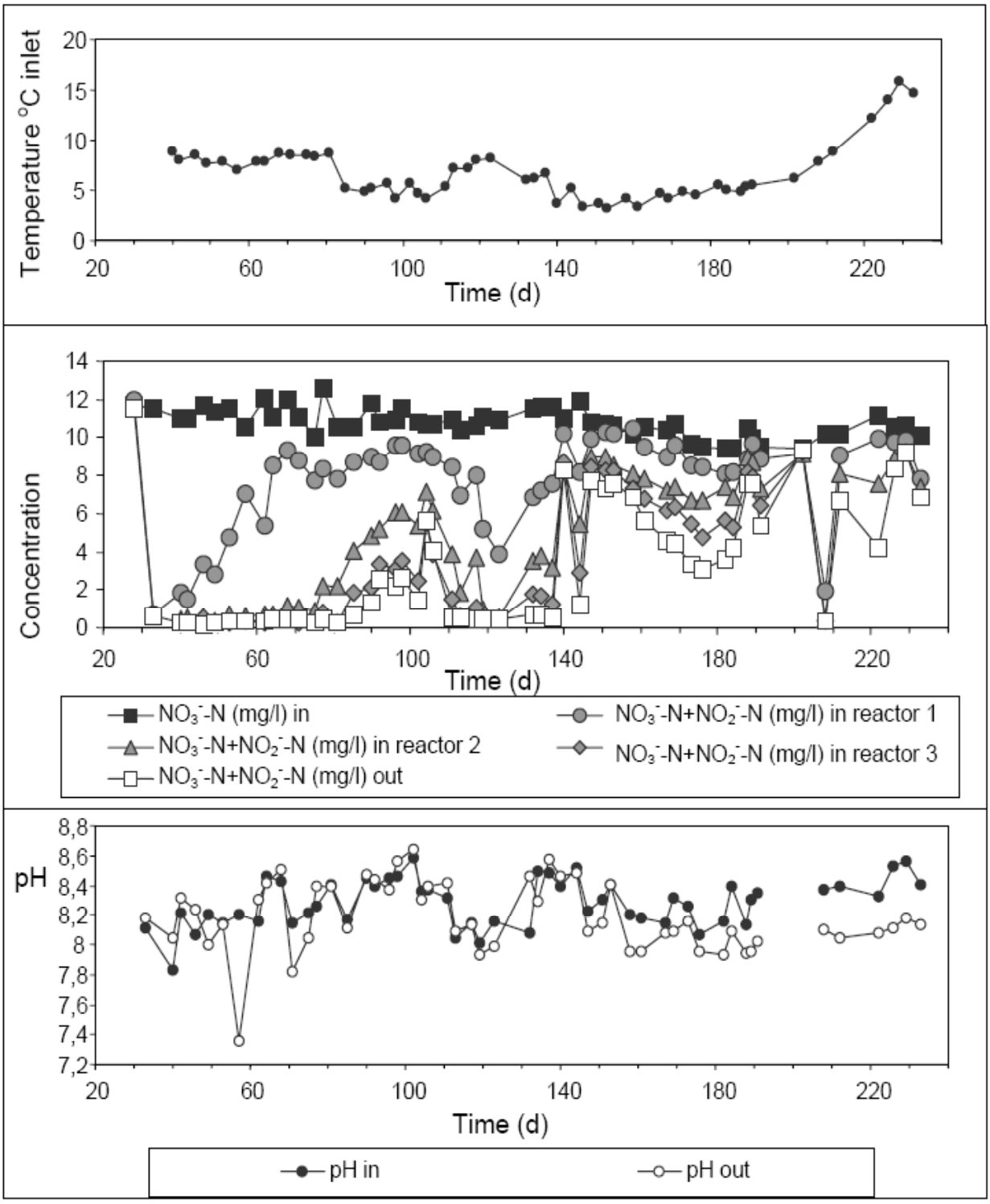


Figure 47. Denitrification in the bioreactors at Kemi mine (2nd phase pilot scale system).

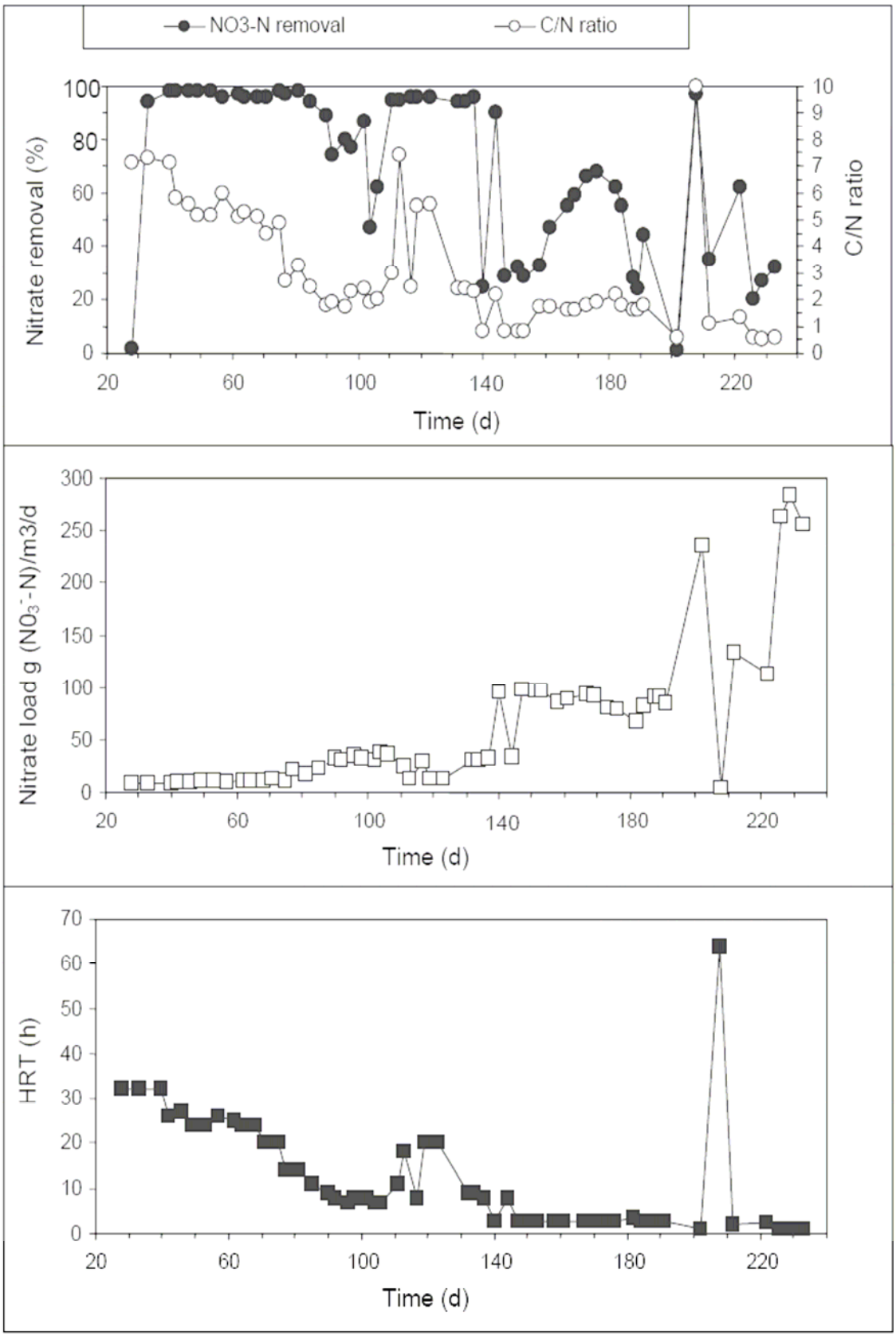


Figure 48. Nitrate removal with different loads of nitrate, flow rates and C/N ratio (2nd phase pilot scale system).

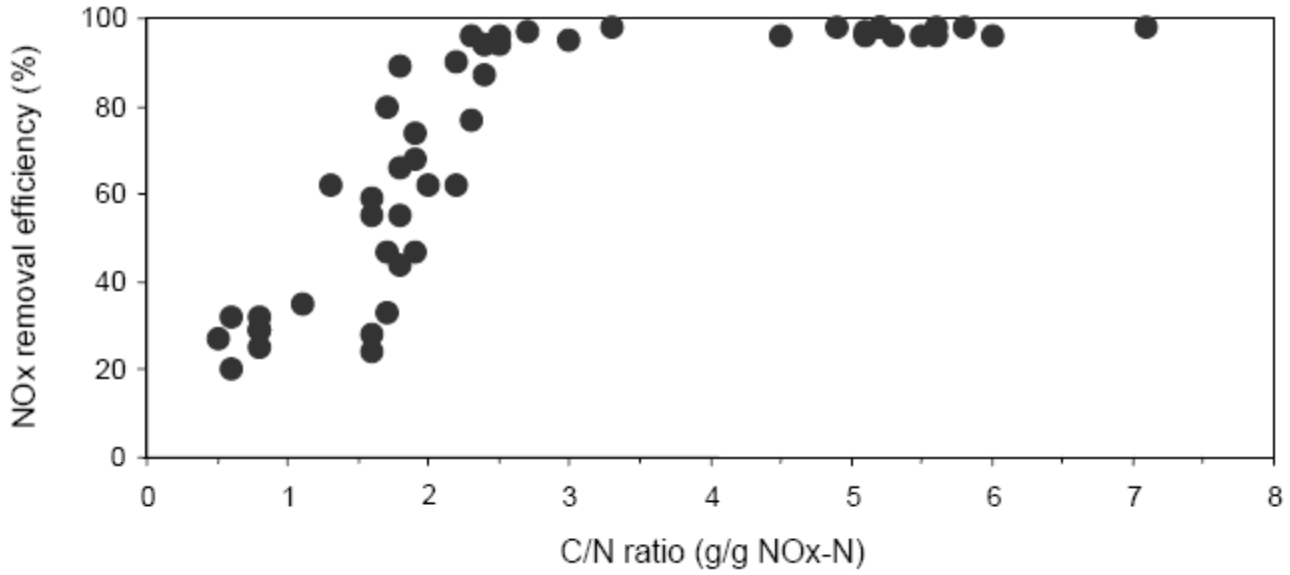


Figure 49. NOx^--N removal efficiencies versus C/N-ratios for denitrification reactors of 2nd phase pilot scale system.

Table 20. Comparison of reported optimal organic carbon/nitrogen ratios

Reference	Water	C source	C/N
Kuba et al., (1993)	Waste water	Acetate	3.4
Chiu and Chung, (2003)	Synthetic media	Acetate	5.5
Christensson et al., (1994)	Synthetic media	Ethanol	3.85
Rusten and Odegaard, (2007)	Waste water	Ethanol	>3.8 (COD/N)
Christensson et al., (1994)	Synthetic media	Methanol	4.45
Timmermans and Van Haute, (1983)	Waste water	Methanol	2.55
Cifford and Liu, (1993)	Ion-exchange brine	Methanol	2.2 – 3.2
Labelle et al., (2005)	Seawater	Methanol	4.2 – 4.3 (COD/N)
This study	**Mine water**	**Methanol**	**2.5 - 3**

In summary, the results show that total nitrogen can be almost completely removed from suspended solid-free oligotrophic mine water by using a biological two-step system consisting of nitrifying and denitrifying bioreactors (Table 21). High nitrification rates were achieved in fixed bed nitrifying bioreactors without pH regulation. The removal rate obtained at 10°C was 383 g $N/m^3/d$ or 3.2 g N/m^2 carrier/d (Pilot 5). The ammonium removal percentage was also high (90%), regardless of concentration of nutrients in the influent and hydraulic retention times. The effluent of the nitrifying reactor was treated in the fixed bed denitrifying reactor, using methanol as organic carbon source. The removal rate obtained at 10°C was 522 g $N/m^3/d$ or 3.6 g N/m^2 carrier/d (Pilot 5, Table 21). The nitrate removal percentage was high (90–96%), regardless of concentration of nutrients in the influent and hydraulic retention times. The nitrifying and denitrifying reactors operated stable during 459 days and rapidly attained the steady state. Results obtained from Pilot 5 are comparable with municipal waste water treatment plants using high-rate biofilm system such as Kaldnes Moving Bed technology (Table 22, 23). The ammonium and nitrate removal capacities in reverse osmosis concentrates were much lower (Table 21). Compared to the rate in Pilot 5, the nitrification rate in Pilot 4 was estimated to be a factor of nine lower and denitrification rates in Pilot 6 were estimated to be a factor of six and three lower for RO concentrates from Kemi and Pahtavaara main waters, respectively.

Table 21. Operational parameters for nitrifying and denitrifying fixed- and moving-bed biofilm reactors throughout the study.

Pilot number, installation place and reactor type	Water	Temperature (°C)	Hydraulic retention time (h)	Removal rate	
				(g $N/m^3/d$)	(g $N/m^2/d$)
Nitrification					
Pilot 1, Laboratory, Fixed bed	Kemi mine	5	0.95	29	0.3
Pilot 5, Kemi mine, Fixed bed	Kemi mine	10	0.17	383	3.2
Pilot 2, Laboratory, Moving bed	Kemi mine	5	3.5	33	0.16
Pilot 8, Laboratory , Moving bed	With suspended solids, Pahtavaara mine	5	28	3-6	0.01-0.03
Pilot 9, Laboratory, Moving bed	With suspended solids, Pahtavaara mine	12	21	16-20	0.07-0.09
Pilot 4, Laboratory, Fixed bed	RO concentrate, Kemi mine	5	10	43	0.4
Pilot 4, Laboratory, Fixed bed	RO concentrate, Pahtavaara mine	5	24	44	0.4
Denitrification					
Pilot 1, Laboratory, Fixed bed	Kemi mine	5	3	605	3.9
Pilot 5, Kemi mine, Fixed bed	Kemi mine	10	1.4	522	3.6
Pilot 6, Laboratory, Fixed bed	RO concentrate, Kemi mine	12	40	84	0.6
Pilot 6, Laboratory, Fixed bed	RO concentrate, Pahtavaara mine	12	40	169	1.14

Table 22. Comparison of reported operational parameters for nitrifying and denitrifying fixed- and moving-bed biofilm reactors.

Reactor type	Water	Temperature (°C)	Removal rate (g $N/m^2/d$)	Reference
Nitrification				
Kaldnes moving bed	Municipal waster water	11	1.4	Rusten and Ødegaard, 2007
Moving bed with pure oxygen	Secondary effluent of a waster water treatment plant	21	3.17	Salvetti et al., 2006
Fixed bed	**Kemi mine**	**10**	**3.2**	**This study**
Moving bed	**Kemi mine**	**5**	**0.16**	**This study**
Moving bed	**Pahtavaara mine water with suspended solids**	**10**	**0.07-0.09**	**This study**
Nitrification in saline water				
Kaldnes moving bed	Synthetic ammonium-buffer solution with 29 g/l NaCl	33-34	0.28	Isid, 2003
Fixed bed	**RO concentrate, Kemi mine and Pahtavaara mine**	**5**	**0.4**	**This study**
Denitrification				
Kaldnes moving bed	Synthetic medium with acetate	11	2.6	Welander and Mattiasson, 2003
Kaldnes moving bed	Municipal waster water	7 - 18	2.2	Rusten et al., 1995
Kaldnes moving bed	Municipal waster water with ethanol	11	3.2	Rusten and Ødegaard, 2007
Fixed bed	**Kemi mine with methanol**	**5**	**3.9**	**This study**
Denitrification in saline water				
Moving bed	Municipal landfill leachate with methanol	17	15.7	Welander et al., 1998
Moving bed	Seawater	-	17.7	Labelle et al., 2005
Fixed bed	**RO concentrate with methanol, Kemi mine**	**12**	**0.6**	**This study**
Fixed bed	**RO concentrate with methanol, Pahtavaara mine**	**12**	**1.14**	**This study**

Nitrifying biofilms can be established in reactors treating inorganic mine water at low temperature without external carbon or phosphorus sources. The activity of defined nitrifying and methylotrophic denitrifying biofilms can be maintained during long-term operation for more than one year. Denitrifying biofilms require long incubation times at low temperature to reach stable op-

eration. However, excellent denitrification is achievable with methanol as an external carbon source and supplementation of phosphate. Nitrifying biofilms are strongly affected by temperature and feed salinity. Nitrification of ≤99% at load of ≤0.77 kg NH_4^+-N/m^3/d is possible at 12°C. Nitrification >99% can be achieved at temperature as low as 5°C, as can denitrification of ≤95% at load of ≤0.91 kg NO_3^--N/m^3/d and 5°C. Thus, nitrification and denitrifiation rates are similar to operating biofilm process for organic wastewater treatment. Thus, fixed-bed biofilm reactors have potential to remove ammonium and nitrate also from mine water.

Table 23. Performance of Kaldnes Moving Bed Natrix process for removal of total nitrogen from organic-rich municipal waster water. Adapted from (http://www.stowa-selectedtechnologies.nl/Sheets/Sheets/Kaldnes.Moving.Bed..KMB..Natrix.Process.html)

Design capacity	350.000 p.e.
Total empty bed volume	570 m^3
Tank depth	4,8 m
Reactors	aerobic – 6, anoxic – 2
Spec. active biofilm surface area	~300 m^2/m^3
Average biomass concentration	4 kg DS/m^3
Average specific sludge production	0,36 kg DS/kg $COD_{removed}$
Temperature wastewater	**6,9 - 15,9°C**
Nitrification started at volumemetric loads	1,5 - 2,2 kg BOD_7/m^3.d
Nitrification rates at low organic loads	**300 - 400 g NH_4-N/m^3.d**
Denitrification rates	**700 - 750 g NO_x-N/m^3.d**
C/N – ratio	3,5 g COD_{added}/NO_3-N
NO_x-N removal (at C/N = 3,5)	85%

5.5.1 Process economics

At Homestake's Nickel Plate mine in British Columbia, Canada, a process was developed for treatment of cyanide containing water from tailing ponds. The process sufficiently removed cyanide via biological destruction of cyanide to ammonia. Ammonia was converted to nitrogen gas via nitrification and denitrification at flow rates of 0.7-1.1 m3/min.

Since 1996, the Homestake's Nickel Plate mine in British Columbia, Canada, successfully applied a full-scale suspended sludge process for cyanide and thiocyanate removal from effluents of tailing facilities (Given and Meyer, 1998; Given et al., 1998). The biological destruction of cyanide and thiocyanate generates ammonium, which is biologically converted to dinitrogen gas via nitrification and denitrification. The bacteria involved in the nitrification and denitrification have long generation times and low cell mass yields. Therefore, in the applied suspended sludge process a high sludge age has to be maintained to achieve good performance results. The activated sludge process was designed for total nitrogen removal of about 20 mg N/l at flow rates of 0.7-1.1 m^3/min. The ammonium concentration is from 20 to 50 mg N/l and the nitrate content is from 100 to 130 mg N/l (Given and Meyer, 1998; Given et al., 1998). At Nickel Plate mine, nitrification requires temperature above 11°C and the heating costs are about 5% of the total operational costs of about 0.01 euro/m^3. Methanol is used as a carbon source in the denitrification at a ratio of 3 kg methanol/kg nitrate-nitrogen and accounts for another 5% of treatment costs. However reagents costs are only about 10% of the treatment costs with labor as the main costs of 40% (Given and Meyer, 1998; Given et al., 1998).

Full-scale denitrification of nitrate in mine effluents has been carried out at two mines in the Montana nearby Yellowstone National Park in the USA (East Boulder Mine and Stillwater Mine) (Reinsel and Plumb, 1999; Reinsel, 2001). Denitrification of slightly alkaline mine effluents has been successfully demostrated (Reinsel and Plumb, 1999) at temperature as low as 2 °C. A full-scale low-maintenance bioreactor had been installed at the Stillwater Mine in Montana. The bioreactor has a treatment capacity of 4.2 m^3/min and operating costs have been stated to be in the range of 0.08 to 0.20 euro/m^3 (Reinsel, 2001).

5.6 Molecular biological population profiling and characterization of bacteria from bioreactor biomass

5.6.1 DNA extraction evaluation

The FastDNA spin kit (Qbiogene,USA) and the enzymatic DNA extraction (Purkhold et al, 2000) methods give broader diversity than miniprep protocol and therefore it was not taken in to sequencing analysis. The results comparing (RDP Libcompare) enzymatic method and FastDNA kit showed no significantly difference (Table 24) and therefore FastDNA kit was chosen to be used for the remaining samples.

Table 24. Comparison of the two extraction methods, FastDNA kit (total 41 sequences) and enzymatic extraction of Purkhold (total 55 sequences) with the RDP Libcompare.

FastDNA kit, %	Enzymatic method, %	Phylum ID
0,0	3,7	*Actinobacter*
2,4	1,9	*Nitrospira*
2,4	1,9	*Bacteroidetes*
7,3	5,6	*Planctomycetes*
85,4	81,5	Proteobacteria
2,4	5,6	unclassified Bacteria

5.6.2 Molecular biological identification

Nitrifying bioreactor. Most of the sequences obtained in the nitrifying bioreactors were proteobacteria (Table 26), mostly β-,α- and a few γ-proteobacteria. Important β-proteobacteria family was *Nitrosomonadacae,* which includes the genera *Nitrosomonas* and *Nitrosospira.* And a α-proteobacterial genera *Nitrobacter.* Other big proteobacterial groups were *Sphingomonadaceae, Rhodobacteraceae* and *Rhizobiales,* but their significance is not clear in the bioreactor. Other bacteria identified, involved in the nitrogen removal were of genera *Nitrospira* and order *Planctomycetales.* Especially *Nitrospira* were very abundant. Few sequences representing *Firmicutes, Bacteroides, Actinobacteria* and *Verrumicrobia* were found, but their role is also unclear. Also many unknown or uncultured species sequences were found and something about them was predicted from phylogenetic threes.

Denitrifying bioreactors. The proteobacteria group was the most prevalent in the denitrifying bioreactors (Table 25). Most common sequence referred to an uncultured γ-proteobacteria and there were much more γ- and δ-proteobacterial sequences than in nitrifying bioreactors. Species found from DN-bioreactor included *Methylophilus* and *Methylobacillus, Acidovorax, Aquaspirillum, Aminomonas* and mostly same α- and β-proteobacterial species as N-biofilms. From DN-bioreactors was also found few sequences representing *Firmicutes, Bacteroides, Actinobacteria*

and *Verrumicrobia.* Species diversity was not as broad in the DN-bioreactors as in the N-bioreactors.

High-salinity bioreactor. From high-salinity bioreactor was found mostly the same species as from normal N-bioreactors, a bit more *Actinobacteria, Bacteroides, Firmicutes and Verrumicrobia,* but the sequences were unique, exactly same sequences were not found from other samples. No representatives of *Nitrospira* or *Planctomycetales* were found from HSN-bioreactor biomass.

Table 25. Percentage values of different bacterial groups in the cloned samples (RDP Classifier).

Phylum	BN-3	BN-11	BN-13	HSN-1	BDN-4	BDN-5
Bacteroidetes	3,2	2	2,5	10	3	25
Proteobacteria	77,4	71,4	77,5	63,3	69,7	70
Planctomyces	8,1	2	7,5	0	0	0
Verrumicrobia	0	6,1	2,5	3,3	3	0
Nitrospira	1,6	4,1	2,5	0	0	0
Actinobacteria	3,2	0	0	10	6,1	0
Firmicutes	0	0	0	3,3	3	5
Unclassified Bacteria	6,5	14,3	7,5	10	15,2	0

Proteobacteria were the most predominant group in all biomass samples (Table26) and that *Nitrospira* and *Planctomycetes* were only found in the nitrifying bioreactors (not in the high-salinity nitrifying bioreactor). Where as the *Firmicutes* were only found from the denitrifying bioreactors and in the high-salinity bioreactor. The identification data based on cloning were used on identifying also the groups of bacteria separated in TRFL analysis of other nitrifying and denitrifying bioreactor biomasses.

5.6.3 Population profiling of biomass samples with TPFLP method

Nitrifying bioreactors. The inoculas used for starting the nitrifying bioreactors differ greatly on their TRFLP profiles. The inoculate BN1A originating from room temperature laboratory bioreactor was dominated by *Erythromonas, Rhodobacter* (genus level identification) and unidentified group of bacteria (not included in clone library). Their coverage of total population was together close to 80%. The inocula BN1B originating from full-scale landfill leachate bioreactor was dominated with one unidentified bacterial group with 42% coverage of total population. Minor findings were also for *Caulobacter, Roseovarius* and *Nitrosomonas* (an Ammonium oxidizing bacterium, AOB) (together 10%). The inocula BN1C originating from fishpond sludge was dominated by again unknown group of bacteria (35%) with minor findings of *Rhodopseudomonas* (14%), unclassified *Proteobacteria* (4+10%), *Hydrogenophaga* (7%) and *Rhodobacter* (6%). The last inocula BN1C originating from bioreactor treating high ammonia landfill leachate was dominated by *Aquabacterium* (19%) and with minor findings of *Sterolibacterium* (9%) and *Erythrobacter* (5%). The three unidentified bacterial groups (total 18%) were also seen. Generally none of the major bacterial groups found in TRFLP profiles were present in all of the inocula biomasses.

The Pilot 5 nitrifying bioreactor is used as example here for following the changes of bacterial population in time. After 11 months on inoculating first mine water fed bioreactor the Piloit 5

was started with biomass BN3. The biomass contained *Erythrobacter* (13%) as one of the major groups identified already from inoculas. While all the other bacterial groups found were different to any of groups found from inoculas. Dominating groups were *Roseovarius* (19%), *Methylophilus* (7%) and *Nitrosomonas* AOB (6%). After 17 months the *Nitrosomonas* group AOB (48%) dominated biomass population with only minor findings of *Nitrospira* (a nitrite-oxidizing bacterium, NOB) (4%), *Erythrobacter* (3%), *Roseovarius* (6%) and *Methylophilus* (7%). The biomass samples taken after 25 and 26 months were similar to each other. Dominating groups in both were *Nitrospira, Erythrobacter, Hydrogenophaga* and *Nitrosomonas*. The group of unclassified *Alpha Proteobacteria* (uncultured) has grown to about 15% of total population and was one of the major groups in both samples.

Denitrifying bioreactors. The inoculas used for starting the denitrifying bioreactor (Pilot 1) were originating from the same barrel (top and bottom) and showed great similarity (Figure 50). The dominating groups were same but their amount was differing due to circumstances (oxic / anoxic). *Methylobacillus* (49%) was dominating the BDN1B bottom sample while the *Methylophilus* (32%) was dominant in BDN1A top sample. Both of them indicate also that methanol has been used as carbon source for biomass. Other groups found were *Methylophaga*, *Xanthomonas*, *Acidovorax* and *Aquaspirillum*. Only one major group of the bottom inocula remained unidentified.

The Pilot 5 denitrifying bioreactor was inoculated with BDN6 that was sampled after 6 months from pilot 1. From the dominating groups of original inocula only *Methylophilus* (18%) group was found. Other dominating groups *Acidovorax* (16%), *Hydrogenophaga* (unidentified 9%), *Acidaminobacter* (6%) and *Rhodanobacter* (18%) were not detected from inocula. After 13 and 14 months dominance of *Methylophilus* group (24-29%) was strong and the other bacterial groups (unidentified *Fulvimonas, Hydrogenophaga, Rhodobacter* and *Flavobacterium*) were smaller than 10% of total population.

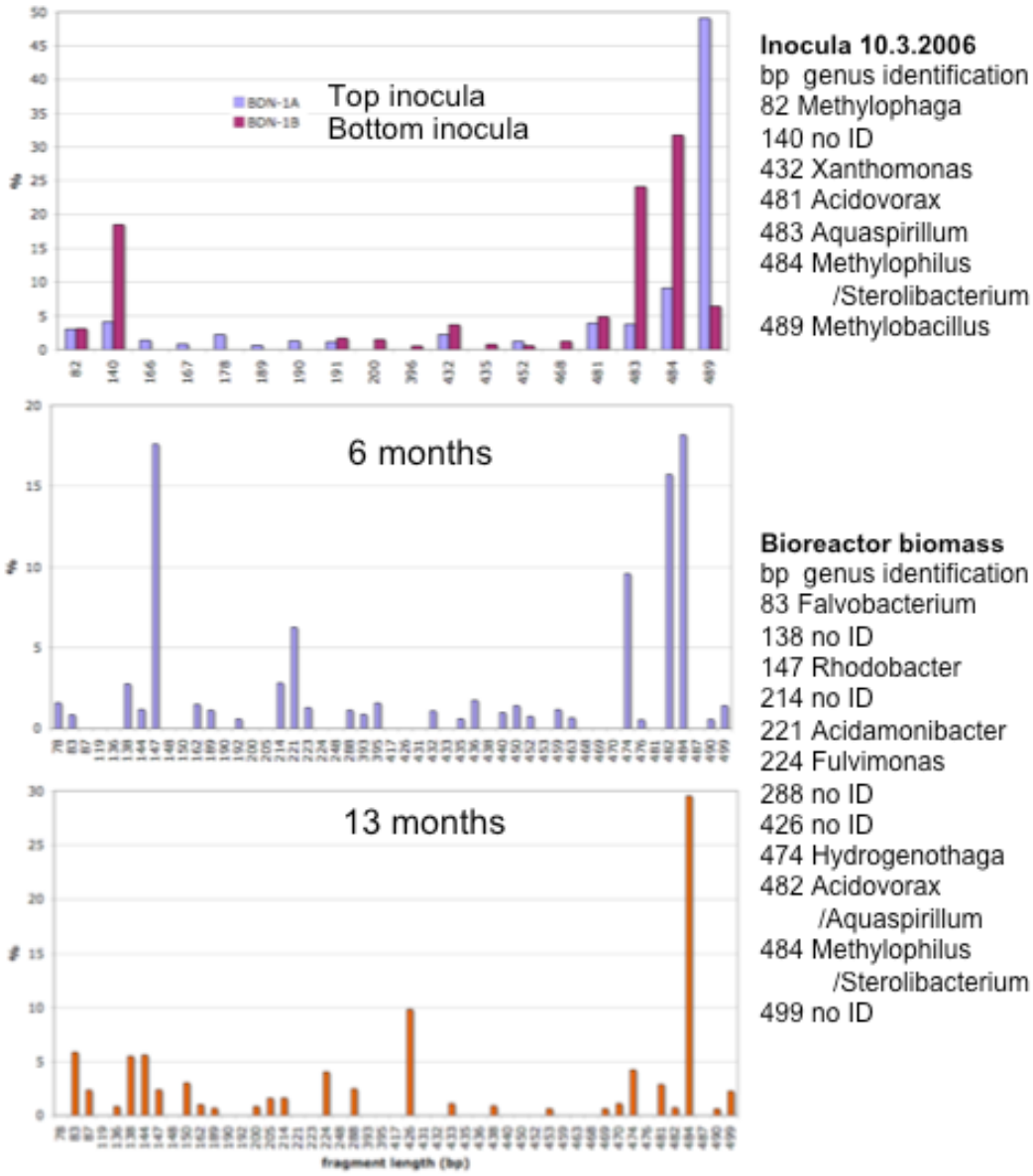


Figure 50. Population profiles of bacteria from denitrifying bioreactors starting from original inocula (no mine water contact) to 13 months (fed with mine water).

High salinity nitrifying bioreactor. Kemi mine water was spiked with different salts (total salinity 15.5g/l) and treated in bioreactor 4. The high salinity biomass BHSN1 of bioreactor 4 showed wide diversity bacteria. No dominance of one group was seen. The major groups of bacteria identified were Defluvibacter (14%), Nitrobacter NOB(12%), Nitrosomonas AOB (8%), Hyphomicrobium (6%) and Leifsonia (5%). Most of the minor bacterial groups remained unidentified (did not belong to 30 identified clones from BHSN1)

The genus level identification have been given here when the similarity if sequenced fragment has shown more than 93% similarity to it. Remarkably many of the clone identification connected to major groups in TRFL profiles belong to uncultured species. Total number of clones generated from 6 biomass samples (Table 6) was 227and still the population profiles contained remarkable amount of unidentified groups.

6 SUMMARY

The two year KAIRA research project yielded following international novel key results.

- Extensive comparison study on fate of nitrogenous compounds from different explosives at two metallic and one non metallic ore mine(s).
- In detail comparison study on filtration as concentration process of nutrients from three different mine waters by five different membranes and two different scales. Production of RO-concentrate for subsequent tests on biological removal of total nitrogen.
- High-rate biological removal of total nitrogen from cold inorganic (oligotrophic) water of three different mines by combination of nitrifying and denitrifying biofilm reactors of different types and scales. The achieved rates are higher than any published rates in similar systems and conditions.
- Biological removal of nitrogenous compounds from cold inorganic RO-concentrate and saline water by nitrifying and denitrifying biofilm reactors in batch and continuous tests.
- Estimates of the process economics have been calculated for mine water pre-treatment by RO and total nitrogen removal in biofilm reactors.

Following recommendations are given for the biological removal of nutrients from mine water.

- Explosives should be applied in the most water insoluble form (e.g. emulsion, gel) to minimize immediate transfer of nutrients to mine water.
- Water management at a mine should aim to collect seepage water from barren rock piles, ensure sufficient aeration of tailings and discharge of all mine water at a single location.
- In cases of significant in-situ nitrification, biological denitrification systems for removal of residual nitrate should be placed at the point of discharge to the aquatic environment to treat high water volumes with low suspended solids content.

The main results of the project aims are briefly summarized. The fate of nitrogenous compounds has been studied at one non-metallic ore open pit and two metallic ore underground mines using different explosives. In summary, 20-30 % of total nitrogen in the explosives, i.e. 20 000 to 50 000 kg N, will remain after detonation and can be detected from mine water, ore or barren rock. Only at Siilinjärvi mine, where phlogopite and biotite make up 60% of the mineral composition of the ore, nitrogenous compounds remain sorbed to these minerals. Barren rock piles are a reservoir of nitrogen, thus seepage water of these piles have higher nitrogen content. Ammonium of explosive will be in-situ nitrified in well aerated mine water. Furthermore, the in-situ removal of nitrite/nitrate in mine, barren rock and tailings water requires sufficient retention time in ponds and suitable organic carbon source (vegetation). In Kemi mine 58% of the annual total nitrogen input, i.e. 18 000 kg N, is removed by denitrifying bacteria and pond vegetation.

Membrane technology was tested for the concentration of nitrogenous compounds in mine water prior to treatment in a bioreactor. Four reverse osmosis and one nanofiltration membranes were tested. The nanofiltration membrane had a poor retention to ammonium, nitrate and chloride, therefore, it is not applicable as a pretreatment process prior to a nitrification-denitrification process in a bioreactor. Whereas, all the tested RO membranes removed ammonium and nitrate adequately from the tested mine water. The best membranes were clearly Espa2, TFC ULP and RO1 for all tested mine waters, with the retention value of above 90.7 % for NO_3-N and above 86.1 % for NH_4-N. The concentration of the mine waters deteriorated the permeate quality compared to the permeate formed in membrane selection stage, however, the retention for ammo-

nium and nitrate were satisfying. For the part of the reverse osmosis process, the mine effluent could have been concentrated by higher volumetric reduction factor than 20 but, because the processability of the concentrate in a bioreactor defines the highest possible VRF, it can not be very high, especially for Kemi mine water when the salt content in the feed water is high. For pilot scale concentration, the results were poorer than expected due to higher feed temperature and membrane fouling. However, concentration on a large scale is possible and, using adequate pretreatment it should give similar results as the smaller scale concentration. Membrane age is an important factor in operational costs, and chemical cleaning is a major limitation. To determine the best cleaning intervals and chemicals, further tests with longer filtration periods would be necessary. According to the economical analysis, the estimated cost for the treatment of the tested mine effluents with reverse osmosis would be from 0.31 to 0.34 €/m^3. This is a reasonable price for a water treatment plant, however, for a mine, the overall costs are too high.

The microbial consortium in the bioreactors were characterized by molecular biology tools. In nitrifying bioreactors *Nitrosomonas / Nitrosospira* (AOB)*, Nitrospira / Nitrobacter* (NOB) and *Planctomycetales* have the main responsibility on the nitrification, these bacteria have also been found from different municipal and industrial wastewater treatment plants and from marine sediments when identified ammonia oxidizing bacteria (Purkhold et al., 2000,Urakawa et al., 2006, Bothe et al., 2000). Denitrification capability is much more widespread in bacteria, so it is difficult to point out the denitrifying organisms (Bothe et al., 2000) We found many sequences related to sequences previously found from activated sludge denitrification reactors (*Firmicutes, Bacteroides, Acidovoraz, Azoarcus, Aquqspirillum)*(Heylen et al., 2006, Yoshie et al., 2001). The appearance of genus *Methylophilus* and *Methylobacillus* are probably due the methanol used as fed in there. The TRFLP profiles showed that the bacterial population used as inocula was replaced within a few months with bacteria originating from water used as feed. After the adaptation changes in bacterial population were minor. The bacterial diversity in nitrifying bioreactor remained large, whereas in denitrification reactor one dominating group (30% total population) was present. Results presented here are not statistically tested. Statistical analysis of the data will be carried out at a later stage. The evaluation of bioreactor biomass bacterial composition and quality has been presented here only with examples, since the bioreactors were operated as long as possible. The last samples for population profiling were obtained after termination of the bioreactors at May 2007. The remaining results and statistical tests will be published later on. The further steps for identification and profiling study would have been sequencing the whole 16S rRNA genes for describing new species. The cold and oligotrophic environment of the nitrifying bioreactors supports reservoir for novel species.

The bioreactors tests show that total nitrogen (ammonium, nitrite and nitrate) can be almost completely removed from suspended solid-free mine water by using a biological two-step system consisting of nitrifying and denitrifying bioreactors (Table 22). The achieved nitrification and denitrification rates, including nitrification in saline water, were higher than rates published in the scientific literature. High nitrification rates were achieved in fixed bed nitrifying bioreactors without pH regulation. The removal rate obtained at 10°C was 383 g N/m^3/d or 3.2 g N/m^2 carrier/d (Pilot 5). The ammonium removal percentage was also high (90%), regardless of concentration of nutrients in the influent and hydraulic retention times. The effluent of the nitrifying reactor was treated in the fixed bed denitrifying reactor, using methanol as organic carbon source. The removal rate obtained at 10°C was 522 g N/m^3/d or 3.6 g N/m^2 carrier/d (Pilot 5). The nitrate removal percentage was high (90–96%), regardless of concentration of nutrients in the influent and hydraulic retention times. Generally behaviour of the system consists of nitrifying and denitrifying reactors was stable during 459 days of operations, rapidly attaining the steady state. At the same time ammonium and nitrate removal capacities in reverse osmosis con-

centrates were much lower (Table 22). Compared to the rate in Pilot 5, the nitrification rate in Pilot 4 was estimated to be a factor of nine lower and denitrification rates in Pilot 6 were estimated to be a factor of six and three lower for RO concentrates from Kemi and Pahtavaara main waters, respectively. Based on this study the optimal C/N ratio for initial nitrate concentration of 10.8±0.7 mg/l NO_3^--N was determined to be 2.5 – 3 that corresponding 6.7 - 8 kg or 8.4 - 10 liters of methanol for 1 kg of NO_3^--N reduced.

Results obtained from Pilot 5 are comparable with municipal waste water treatment plants using high-rate biofilm system such as Kaldnes Moving Bed technology (Table 23). Nitrifying biofilms can be established in reactors treating inorganic mine water at low temperature without external carbon or phosphorus sources. The activity of defined nitrifying and methylotrophic denitrifying biofilms can be maintained during long-term operation for more than one year. Denitrifying biofilms require long incubation times at low temperature to reach stable operation. However, excellent denitrification is achievable with methanol as an external carbon source and supplementation of phosphate. Nitrifying biofilms are strongly affected by temperature and feed salinity. Nitrification of ≤99% at load of ≤0.77 kg NH_4^+-N/m^3/d is possible at 12°C. Nitrification >99% can be achieved at temperature as low as 5°C, as can denitrification of ≤95% at load of ≤0.91 kg NO_3^--N/m^3/d and 5°C. Thus, nitrification and denitrifiation rates are similar to operating biofilm process for organic wastewater treatment. Thus, fixed-bed biofilm reactors have potential to remove nutrients also from mine water.

The project has been made public with two web pages (Appendix 1). The scientific results have been disseminated on several international conferences and research publications.

At the end of the KAIRA project two mine projects of the industrial partners have developed rapidly. The mining project at Suurkuusikko has developed in the Kittilä gold mine of Agnico-Eagle Finland, which is scheduled to start operation in 2008. The mine project at Kevitsa by Scandinavian Gold Prospecting Ab has also strongly developed and the mine will start production in 2010. The expected increase in mining activities in Finland as well in other countries requires further steps in protection of the aquatic environment.

7 ACKNOWLEDGEMENT

The funding provided by the European Regional Development Fund, the Finnish Funding Agency for Technology and Innovation TEKES and industrial partners is greatly acknowledged. We kindly acknowledge the technical support generously provided by numerous mine personnel. All the individuals, we discussed with aspects of this research work, we thank for their constructive support. Riitta Keiski, Eva Pongrácz, Nora Papa, and Virpi Väisänen of the University of Oulu we thank for supporting the work of Katri Häyrynen. We are grateful for advices on filtration tests by Mika Mänttäri of the Lappeenranta University of Technology. We also express our gratitude to Marko Virta and Manu Tammi, University of Helsinki, for supporting Antti Karkman in his identification work. We would also like to acknowledge the support of Pekka Särkkä, Helsinki University of Technology, on blasting techniques aspects. We also thank Mauri Heikkinen and Rainer Peltola from Finnish Forest Research Institute in Rovaniemi for cooperation in analysis of gas samples.

8 REFERENCES

Akcil, A. 2003. Destruction of cyanide in gold mill effluents: biological versus chemical treatment. Biotechnology Advances 21(6): 501–511.

Alleny, M. 2004. Aluminum Compounds Cause Operational Problems in a Reverse Osmosis Plant. Materials Performance 44–46.

Amann, R.I, Ludwig, W. and Schleifer, K.H. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev. 59(1): 143–69.

Ausubell, F., eds. (1987) Current Protocols in Molecular Biology, Wiley, New York.

Awadalla, F.T. and Kumar, A. 1994. Opportunities for Membrane Technologies in the Treatment of Mining and Mineral Process Streams and Effluents. Separation Science and Technology 29(10): 1231–1249.

Awadalla, F.T., Striez, C. and Lamb, K. 1994. Removal of Ammonium and Nitrate Ions from Mine Effluents by Membrane Technology. Separation Science and Technology 29(4): 483–495.

Bothe, H., Jost, G., Scholter, M., Ward, B.B. and Witzel, K.-P. 2000. Molecular analysis of ammonia oxidation and denitrification in natural environments. FEMS Microbiology Reviews. 24: 673–690.

Campos, J. L., Mosquera-Corral, A., Sánchez, M., Méndez, R. and Lema, J. M. 2002. Nitrification in saline wastewater with high ammonia concentration in an activated sludge unit. Water Research 36(10): 2555–2560.

Catalan, M.A.B., Wang, P.C. and Matsumura, M. 1997. Nitrification performance of marine nitrifies immobilized in polyester and macro-porous cellulose carriers. J. Fermentation Bioeng. 84(6): 563–571.

Chang, L., An industrial wastewater pollution prevention study: Evaluation of precipitation and separation processes. Environmental Progress 1996 15(1): 28–36.

Chen, S., Ling, J. and Blancheton, J.-P. 2006. Nitrification kinetics of biofilm as affected by water quality factors. Aquacultural Engineering 34(3): 179–197.

Chiu, Y.-C. and Chung, M.-S. 2003. Determination of optimal COD/nitrate ratio for biological denitrification. International Biodeterioration and Biodegradation 51: 43–49.

Christensson, M., Lie, E. and Welander, T. 1994. A comparison between ethanol and methanol as carbon sources. Water Sci. Technol. 30(6): 83–90.

Clifford, D. and Liu, X. 1993. Biological denitrification of spent regenerant brine a sequencing batch reactor. Water Research. 27(9): 1477–1484.

Dalzell, D.J.B., Alte, S., Aspichueta, E., de la Sota, A., Etxebarria, J., Gutierrez, M., Hoffmann, C.C., Sales, D., Obst, U. and Christofi, N. 2002. A comparison of five rapid direct toxicity as-

sessment methods to determine toxicity of pollutants to activated sludge. Chemosphere. 47 (5): 535–545.

Dictor, M.C., Battaglia-Brunet, F., Morin, D., Bories, A. and Clarens, M. 1997. Biological treatment of gold ore cyanidation wastewater in fixed-bed reactors. Environmental Pollution. 97(3): 287–294.

Dupla, M., Comeau, Y., Parent, S., Villemur, R. and Jolicoeur, M. 2006. Design optimization of a self-cleaning moving-bed bioreactor for seawater denitrification. Water Research 40(2): 249–58.

Edwards, U., Rogall, T., Blockerl, Emde, M. Bottger, E.C. 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Research 17(19): 7843–7853.

EEA 2004. EEA Signals 2004. European Environment Agency, Copenhagen, Denmark. 36 p.

EPA 2003. EPA and Hardrock Mining: A Source Book for Industry in the Northwest and Alaska, U.S. Environmental Protection Agency (EPA), Seattle, USA. 64 p.

EU 2004. Reference Document on Best Available Techniques for Management of Tailings and Waste-Rock in Mining Activities. EUROPEAN COMMISSION, Sustainability in Industry, Energy and Transport, European IPPC Bureau. 557 p.

European Council Directive 80/68/EEC of 17 December 1979 on the protection of groundwater against pollution caused by certain dangerous substances as amended by Council Directive 91/692/EEC (further amended by Council Regulation 1882/2003/EC). Official Journal L 020, 26/01/1980 P. 0043–0048.

European Union Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Communities. 22.12.2000 [referred 31.5.2006]. 72 p. Accessible as a PDF-document: http://europa.eu.int/eurlex/pri/en/oj/dat/2000/l_327/l_32720001222en00010072.pdf.

Forsberg, H. and Åkerlund, H. 1999. Nitrogen and the residues of explosives in ore-, waste rock - and production flows of LKAB. (Kväve och sprängämnesrester I LKAB:s malm-, gråbergs- och produktflöden) MSc-thesis, Luleå University of Technology. 122 p. (in Swedish)

Geological Survey of Finland 2007. http://en.gtk.fi/ExplorationFinland/Posteri/index.html.

Ginige, M.P., Hugenholtz, P., Daims, H., Wagner, M., Keller, J. and Blackall, L.L. 2004. Use of stable-isotope probing, full-cycle rRNA analysis and fluorescence *in situ* hybridization-microautoradiography to study a methanol-fed denitrifying microbial community. Appl. and Environ. Microbiol. 70: 588–596.

Given, B., Dixon, B., Douglas, G., Mihoc, R. and Mudder, T. 1998. Combined aerobic and anaerobic biological treatment of tailings solution at the Nickel Plate Mine. In: Mudder, T.I., Botz, M. (Eds.) The Cyanide Monograph, 2nd ed. The Cyanide Compendium on CD by Mining Journal Books Limited, London, UK, ISBN 0-9537-33602, 1998. p. 391–421.

Given, B. and Meyer, S. 1998. Biological Treatment of Tailings Solution at the Nickel Plate Mine. In: Proceedings of the 22nd Annual British Columbia Mine Reclamation Symposium, Penticton, British Columbia, 1998. p. 157–171.

Halling-Sørensen, B. 1993. Biological nitrification and denitrification. In: B. Halling-Sørensen and S. E. Jørgensen (eds.). The removal of nitrogen compounds from wastewater. Elsevier, Amsterdam, The Netherlands. p. 41–53.

Heikkinen, P. et al. 2005. Kaivosten sulkemisesta. TEKES-projekti "Kaivostoiminnan ympäristötekniikka" (käsikirja).

Heylen, K., Vanparys, B., Wittebolle, L., Verstraete, W., Boon, N. and De Vos, P. 2006. Cultivation of Denitrifying Bacteria: Optimization of Isolation Conditionsand Diversity Study. Applied and Environmental Microbiology 72(4): 2637–2643.

Hieber, R. 1998. Final Report—Nitrate Removal Demonstration Mine Waste Technology Program Activity III, Project 4. MSE Technology Applications, Inc., Montana, USA. 60 p.

Hunik, J.H., Meijer, H.J.G. and Tramper, J. 1992. Kinetics of *Nitrosomonas europaea* at extreme substrate, product and salt concentrations. Appl. Microbiol. Biotechnol. 37: 802–807.

Hunik,. J.H., Meijer, H.J.G. and Tramper, J. 1993. Kinetics of *Nitrobacter agilis* at extreme substrate, product and salt concentrations. Appl. Microbiol. Biotechnol. 40: 442–448.

Häyrynen, K., Langwaldt, J., Pongrácz, E., Väisänen, V., Mänttäri, M. and Keiski, R.L. 2007. Separation of nutrients from mine water by reverse osmosis for subsequent biological treatment. Mineral Engineering (Special Edition Bio and Hydromet ‘07) (in press).

Isid, D. 2003. Moving bed biological nitrification in high salinity waters. Masters’s thesis. University of Oulu, Department of Process and Environmental Engineering.

Jetter, M.S.M., Wagner, M., Fuerst, J., van Loosdrech, M., Kuenen, G. and Strous, M. 2001. Microbiology and application of the anaerobic ammonium oxidation (“anammox”) process. Current Opinion in Biotechnology 12: 283–288.

Johnson,J.L. 1994. Similarity analysis of rRNAs in Methods of General and Molecular Biotechnology. American Society of Microbiology. Washington, USA.

Kemira GrowHow. 2006. http://www.kemira-growhow.com/NR/rdonlyres/D7CBEEFC-2FA3-4C7B-87A9-6D0BEA2BD436/0/BIOTIITTIesitepainom.pdf

Khin, T. and Annachhatre, A.P. 2004. Novel microbial nitrogen removal processes. Biotechnology Advances 22: 519–532.

Koops, H.-P., Böttcher, B., Möller, U.C., Pommerening-Röser, A. and Stehr G. 1991. Classification of eight new species of ammonia-oxidizing bacteria : *Nitrosomas communis* sp. nov., *Nitrosomonas urea* sp. nov., *Nitrosomas aestuarii* sp. nov., *Nitrosomonas marina* sp. nov., *Nitrosomas nitrosa* sp. nov., *Nitrosomonas eutropha* sp. nov., *Nitrosomas oligotropha* sp. nov., *Nitrosomonas halophila* sp. nov. Journal of General Microbiology. 137(7): 1689–1699.

Koren, D.W., Gould, W.D. and Be´dard, P. 2000. Biological removal of ammonia and nitrate from simulated mine and mill effluents. Hydrometallurgy 56(2): 127–144.

Koyuncu, I., Topacik, D., Turan, M., Celik, M.S. and Sarikaya, H.Z. 2001. Application of the membrane technology to control ammonia in surface water. Water Science and Technology: Water Supply 1(1): 117–124

Kuba, T., Smolderrs, G., van Loosdrecht, M.C.M. and Heijnen J.J. 1993. Biological phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor. Water Sci. Technol. 27(5-6): 241–252.

Kuenen, J.G. and Jetten, M.S. 2001. Extraordinary anaerobic ammonium-oxidizing bacteria. ASM News 67: 456–463.

Labelle, M.-A., Juteau, P., Jolicoeur, M., Villemur, R., Parent, S. and Comeau, Y. 2005. Seawater denitrification in a closed mesocosm by a submerged moving bed biofilm reactor. Water Res. 39(14): 3409–3417.

Lapin Liitto 2005. Eläköön Lappi. Lapin maakuntasuunnitelma 2022. Report. 19 p.

Lee, S. and Lueptow, R.M. 2001. Membrane rejection of nitrogen compounds. Environmental Science and Technology 35(14): 3008–3018

Lipponen, M.T.T., Suutari, M.H. and Martikainen, P.J. 2002. Occurrence of nitrifying bacteria and nitrification in Finnish drinking water distribution system. Water Research 36: 4319–4329.

Malaiyandi, M. and Sastri, V.S. 1981. Reverse Osmosis Separation of Sulfate, Nitrate and Ammonia from Mining Effluents. Separation Science and Technology: 16(4): 371–376.

Mining Communications Ltd., 2005. Finland. R. Ellis (Eds), Mining Journal Special Publication. 24 p.

Mizohuchi, M., Omotani, J., Mizuno, Y., Takahashi, R., Kanehira, T., Shinohara, M. and Tokutama, T,. 1998. Newly isolated marine ammonia-oxidizing bacterium. J. Fermen. Bioeng. 86: 406–409.

Odegaard, H., Gisvold, B. and Strickland, J. 2000. The influence of carrier size and shape in the moving bed biofilm process. Water Science and Technology. 41(4-5): 383–391.

Oy Forcit Ab; Anfo-, Kemiitti 510-, Kemiitti 810 -käyttöturvallisuus tiedotteet (in Finnish).

Painter, H.A. 1986. Nitrification in the treatment of sewage and waste waters. Spec. Publ. Soc. Gen. Microbiol. 20: 185–213.

Patterson, B.M., Grassi, M.E., Robertson, B.S., Davis, G.B., Smith, A.J. and McKinley, A.J. 2004. Use of Polymer Mats in Series for Sequential Reactive Barrier Remediation of Ammonium-Contaminated Groundwater: Field Evaluation. Environ. Sci. Technol. 38(24): 6846–6854.

Pekkala, M. and Rantala, L. 2006. Outokumpu Chrome Oy, Kemin kaivos. Velvoitetarkkailu vuosiyhteenveto v. 2005. Jaakko Pöyry Infra, PSV-Maa ja Vesi, Oulu 2006. p. 17+10 appendices (in Finnish)

Pekkala, M., Rantala, L and Tuomela, P. 2007. Outokumpu Chrome Oy, Kemin kaivos. Velvoitetarkkailu vuosiyhteenveto v. 2006. Pöyry Environment Oy, Oulu 2007. p.16+9 appendices (in Finnish)

Pienimäkki, M. and Rantala, L. 2004. Outokumpu Oy Kemin kaivoksen ympäristölupaan liittyvät lisäselvitykset. PSV-MAA JA VESI. 20 s.

Prosser, J.I. 1986. Nitrification. Society for general Microbiology. Oxford.

Purkhold, U., Pommerening-Röser, A., Juretschko, S., Schmid, M.C., Koops, H-P. and Wagner, M. 2000. Phylofeny of all recognized species of amminia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: Implications for molecular diversity surveys. Applied and Enviromental Microbiology 66(12): 5368–5382.

Reinsel, M.A. 2001. Analyzing a new biological treatment process for wastewater. Water Online News. http://www.wateronline.com/content/news/article.asp?docid={CCA99FC1-2783-11D5-A770-00D0B7694F32}

Reinsel, M.A.2001. Analyzing a new biological treatment process for wastewater. Water Online News, 4.4.2001: (http://www.wateronline.com/content/news/article.asp?DocID=%7BCCA99FC1-2783-11D5-A770-00D0B7694F32%7D&Bucket=Case+Studies)

Reinsel, M.A. and Plumb, P.D. Anoxic biotreatment cell (ABC) for removal of nitrate and selenium from mining effluent waters. In: D. Kosich and G. Miller. (Eds.) Closure, Remediation & Management of Precious Metals Heap Leach Facilities. January 14 -15, 1999. p. 99–104.

Robinson. 2003. The Amanzi concept for acid mine drainage. http://www.scienceinafrica.co.za/2003/may/amd.htm

Rowan, A.K., Snape, J.R., Fearniside, D., Barer, M.R., Curtis, T.P. and Heard, I.M. 2003. Composition and diversity of ammonia-oxidising bacterial communities in wastewater treatment reactors of different design treating identical wastewater. FEMS Microbiology Ecology 43(2): 195–206.

Rusten, B., Hem, L.J. and Ødegaard, H. 1995. Nitroge removal from dilute wastewater in cold climate using moving-bed biofilm reactors. Water Environ. Res. 67(1): 65–74.

Rusten, B., Siljudalen, J.G., Nordeidet, B. 1994. Upgrading to nitrogen removal with the KMT moving bed biofilm process. Water Science and Technology. 29(12): 185–195.

Rusten, B., Wien, A, and Skjefstad, J. 1996. Spent aircraft deicing fluid as external carbon source for denitrification of municipal wastewater: From waste problem to beneficial use. 51. Purdue Industrial Waste Conference Proc., Ann Arbor Press Inc., Chelsea, Michigan, 1996. 481–518.

Rusten, B. and Ødegaard, H. 2007. Design and operation of nutrient removal plans for very low effluent concentrations. Water Environment Federation. Nutrient Removal 2007. 1307–1331.

Salvetti, R., Azzellino, A., Canziani, R. and Bonomo, L. 2006. Effect of temperature on tertiary nitrification in moving-bed biofilm reactors. Waret Research 40: 2981–2993.

Schenk, H. and Hegemann W. 1995. Nitrification inhibition by high salt concentrations in the aerobic biological treatment of tannery wastewater. Wasser/Abwasser 136(9): 465–470.

Sharon, B. and Ahlert, R.C. 1977. Nitrification and nitrogen removal. Water Research. 11: 897–925.

Shop, V. 2004. Membranes in Water Treatment and Recycling: Operational and Performance Issues S. Khan (Eds.), May 20, 2004, Wollongong, Australia.

Shrimali, S. and Singh, K.P. 2001. New methods of nitrate removal from water. Environmental Pollution 112(3): 351–359.

Singh, R., Paul, D. and Jain, J.K. 2006. Biofilms, implications in bioremediation. Trends in Microbiology 14(9): 389–397.

Smalley, J. and Reynolds, S. 2004. Treatment of mine drainage water using reverse osmosis and other membrane processes. Proceedings of Aquarec Workshop V: Membranes in Water Treatment and Recycling: Operational and Performance Issues S. Khan (Eds.), May 20, 2004, Wollongong, Australia.

Smith, R.L., Miller, D.N., Brooks, M.H., Widdowson, M.A. and Killingstad, M.W. 2001. In Situ Stimulation of Groundwater Denitrification with Formate To Remediate Nitrate Contamination. Environ. Sci. Technol. 35: 196–203

Stowa, 2006a. http://www.stowa-selectedtechnologies.nl/Sheets/Sheets/Kaldnes.Moving.Bed..KMB..Natrix..Process.html

Stowa, 2006b. http://www.stowa-selectedtechnologies.nl/Sheets/Sheets/Submerged.Fixed.Beds.html#nitrogen%20removal

Stowa, 2006c. http://www.stowa-selectedtechnolgies.nl/Sheets/Sheets/Sbbr...Bfsbr...Upflow.Fixed.Bed.Biofilter.html#nitrogen%20removal

Taylor, J.S., Mulford, L.A., Durenceau, S.J. and Barrett, W.M. 1989. Cost and performance of a membrane pilot plant. Journal AWWA 81(11): 52–60

Tchobanoglous, G., Burton, F. and Stensel, H. D. 2003. Wastewater Engineering: Treatment and Reuse. Metcalf & Eddy, Inc., 4th edition, McGraw-Hill College, New York, 2003. 1819 p.

Timmermans, P. and Van Haute, A. 1983. Fundamental study of the growth and denitrification capacity of *Hyphomicrobium* sp. Water Research 17(10): 1249–1255.

Urakawa, H., Kurata, S., Fujiwara, T., Kuroiwa, D., Maki, H., Kawabata, S., Hiwatari, T., Ando, H., Kawai, T., Watanabe, M. and Kohata, K. 2006. Characterization and quantification of am monia oxidizing bacteria in eutrophic coastal marine sediments using polyphasic molecular approaches and imunofluorescence staining. Environmental microbiology 8(5): 787–803

Vilpas R., Kujala-Räty K., Laaksonen T. ja Santala E. 2005. Haja-asutuksen ravinnekuormituksen vähentäminen – Ravinnesampo. Osa 1: asumisjätevesien käsittely. Helsinki. Suomen ympäristökeskuksen julkaisu nro 762. 111 s.
[http://www.ymparisto.fi/download.asp?contentid=37356&lan=fi]

Vredenbregt, L.H.J., Nielsen, K., Potma, A.A., Kristensen, G.H. and Sund C. 1997. Fluid bed biological nitrification and denitrification in high salinity wastewater. Water Sci. Technol. 36(1): 93–100.

Vuolio, R. 2001: Räjäytysopas 2001 (Explosive guide 2001). SML:n Maarakentajapalvelu Oy. p. 207. (in Finnish)

Welander, U. and Mattiasson, B. 2003. Denitrification at low temperatures using a suspended carrier biofilm process. Water Research 37: 2394–2398.

White, D.M. and Schnabel, W. 1998. Treatment of cyanide waste in a sequencing batch biofilm reactor. Water Research 32(1): 254–257.

Wilderer, P.A., Bungartz, H.-J., Lemmer, H., Wagner, M., Keller, J. and Wuertz, S. 2002. Modern scientific methods and their potential in wastewater science and technology. Water Research 36: 370–393.

Witzig, R., Manz, W., Rosenberger, S., Krüger, U., Kraume, M. and Szewzyk, U. 2002. Microbiological aspects of a bioreactor with submerged membranes for aerobic treatment of municipal wastewater. Water Research 36: 394–402.

Wolkersdorfer, C. and Göbel, G.J. 2002. Environmental Regulation of Mine Waters in the European Union. D2. Overview of the EU and Eastern Europe. 11 Finland. April 2002. EU-project report.

Yoshie, S., Noda, N., Miyano, T., Tsuneda, S., Hirata, A., Inamori, Y. 2001. Microbial Community Analysis in the Denitrification Process of Saline-Wastewater by Denaturing Gradient Gel Electrophoresis of PCR-Amplified 16s rDNA and the Cultivation Method**.** Journal of Bioscience and Bioengineering 92(4): 346–353.

Zaitsev, G.M., Uotila, J.S., Häggblom, M.M. 2007. Biodegradation of methyl tert-butyl ether by cold-adapted mixed and pure bacterial cultures. Appl. Microbiol. Biotechnol. 74: 1092–1102.

Zhu, S. and Chen, S. 2002. The impact of temperature on nitrification rate in fixed film biofilters. Aquaculture Engineering 26(4): 221–237.

Kaira-project 2004-2007

Kaivosveden biologinen **ra**vinteidenpoisto - KAIRA - Biological removal of nutrient from mine water

The mining sector in important for the economy and labor market in Northern Finland. Nitrogen-based explosives used during mining are the major source of ammonia and nitrate in mine waters. In Lapland, mine water receiving streams and water bodies are small and vulnerable to eutrophication. The aim of KAIRA-project is to develop different treatment processes for the effective removal of total nitrogen in mine waters at low temperature.

The studied treatment processes for mine waters combine physical concentration of nutrients by reverse osmosis and biological removal of nitrogen via nitrification and denitrification. The biological removal of nitrogen from saline, heavy metal rich effluents at low temperature is cutting-edge technology. The parameters affecting the processes, e.g. temperature, heavy metal content and salinity, will be determined. Further, potential retardation (biosorption) of heavy metals in biofilms of the nitrifying and denitrifying bioreactors will be studied. Within the project, pilot-scale experiments are carried out at operating mine. Furthermore, the fate of ammonium and nitrate during mining activities will be monitored in respect to different explosives and working methods used at a mine.

Finally, the KAIRA-project aims to recommend an economical feasible BAT option for treatment of nutrient-containing mine waters in cold climate.

Project run by Finnish Forest Research Institute
METLA, Rovaniemi Research unit

METLA

Metla Project 7207

Biological removal of nutrients from mine waters

[Suomeksi | Objectives | Project leader | Researchers | Media releases | Metla Research]

Duration: 2005-2007 **Keywords:** bioreactor, denitrification, explosives, microbes, mining, nitrification, nutrients, water

Research Program: Distinct projects 1 - Structure and function of forest ecosystems

Objectives

The mining sector in important for the economy and labor market in Northern Finland. Nitrogen-based explosives used during mining are the major source of ammonia and nitrate in mine waters. In Lapland, mine water receiving streams and water bodies are small and vulnerable to eutrophication. The aim of KAIRA-project is to develop different treatment processes for the effective removal of total nitrogen in mine waters at low temperature.

The studied treatment processes for mine waters combine physical concentration of nutrients by reverse osmosis and biological removal of nitrogen via nitrification and denitrification. The biological removal of nitrogen from saline, heavy metal rich effluents at low temperature is cutting-edge technology. The parameters affecting the processes, e.g. temperature, heavy metal content and salinity, will be determined. Further, potential retardation (biosorption) of heavy metals in biofilms of the nitrifying and denitrifying bioreactors will be studied. Within the project, pilot-scale experiments are carried out at operating mine. Furthermore, the fate of ammonium and nitrate during mining activities will be monitored in respect to different explosives and working methods used at a mine.

Finally, the KAIRA-project aims to recommend an economical feasible BAT option for treatment of nutrient-containing mine waters in cold climate.

More about the KAIRA-project:

- Partners
- Contacts
- Process
- Posters and press releases

Project leader: Varmola, Martti
The Finnish Forest Research Institute, Rovanniemi Research Unit, PL 16, FI-96301 ROVANIEMI, FINLAND
Phone: +358 10 211 4410 *Telefax:* +358 10 211 4401
E-mail: martti.varmola@metla.fi

Other researchers: Häyrynen, Katri (2006), Karkman, Antti (2006), Kontio, Heidi (2006-07), Mattila, Katri (2005-07), Mettänen, Tarja (2005-07), Zaitsev, Guennadi (2005-07)

Top of page

Kaldnes Moving Bed (KMB) Natrix Process
Sheets

13-jun-2006, STOWA

Country		Norway
Stage of development		Full scale
Process -	Line	Water
	Function	COD removal – Nitrogen removal
	Input	Effluent from primary treatment – Reject water
	Concept	Moving bed biofilm reactor - Attached growth
Keywords: decrease area demand; water line; COD removal; nitrogen removal		

Background

The moving bed biofilm process combines the technologies of activated sludge processes and biofilm processes. The moving bed biofilm process is frequently used for the upgrading of an existing plant, especially when space is an issue. High-rate biofilm systems such as the Kaldnes Moving Bed Reactor (KMB) or the NATRIX technology are highly efficient in removing the soluble organic and nitrogen load.

The first KMB reactor was built in the beginning of the 1990s. Nowadays there are about 20 full-scale plants in Norway using the KMB process for municipal wastewater treatment, mainly for BOD removal but it can also be applied for removal of nitrogen. A number of similar process designs have been developed and patented. The Kaldnes Moving Bed Reactor (KMB) and the NATRIX process are presented together, since there is a close cooperation between the suppliers of these technologies.

Description and working principle

The KMB and the Natrix process are suspended carrier biofilm processes. They are based on the use of plastic carrier media, which are kept in suspension and continuous movement in the treatment reactor. Excess biomass sloughs off from the media and is washed out of the process with the treated effluent.

Several process combinations have been realised in upgrading activated sludge treatment plants. The existing tanks can be either retrofitted to MBBR tanks or they are preceded by a so-called roughing MBBR reactor. However, it is also possible to realise hybrid solutions using the biofilm carriers in the existing tanks without any retrofitting.

When a pure KMB process is employed, a typical overall treatment design consists of pre-treatment (screening) followed by one or more MBBR tanks, where the degradation processes of soluble matter take place. The final stage of the treatment is normally the particle separation. See also heading Graphics for a schematic visualisation.

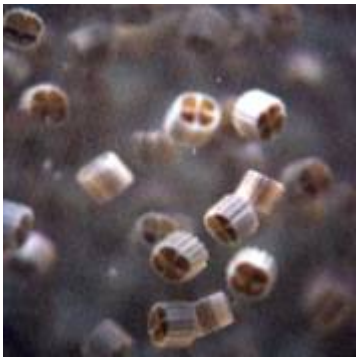
Design Guidelines / Technical Data

Carrier elements

The core of the process are the biofilm carrier elements on which attached growth of microorganisms takes place. The elements are made from polyethylene with a density slightly lower than water. The biofilm carrier elements are kept suspended in the water by air from the diffusors in aerobic reactors and by means of propeller mixers in anaerobic and anoxic reactors. The carrier elements are retained by means of suitably sized sieves or plates.

The original KMB carrier elements are about 7 mm long and 10 mm in diameter and designed to provide a large protected surface for the biofilm and optimal conditions for the bacteria culture when the elements are circulated in the water. The carrier elements are wheel shaped with longitudinal fins at the outside. They are made of polyethylene having a density of 0.96 g/cm^3, which allow easy movement of the carrier material in the completely mixed tanks. The carrier material is retained by perforated plates (usually 5x25 mm).

The reactors are normally filled up to 67% of their volume with biofilm carrier elements corresponding to an effective biofilm area of 250 to $350 m^2/m^3$ water volume, depending on the carrier shape and size used. Due to the shape of the carrier elements, only 12% of the water is displaced.



KMB carrier [1]

The NATRIX technology is different in the carrier it uses. The patented NATRIX carrier is normally from 31 to 50 mm in length and from 31 to 60 mm in diameter. The carriers are injection moulded high density polyethylene/calcium carbonate. The filling volume is typically 30 to 50% of the reactor volume. The NATRIX process can treat wastewater with total suspended solids contents of 1000 to 2000 mg/l, up to this moment the process has been nearly exclusively used in industrial applications.

Process design

Retention times in the MBBR tanks are in the order of 15 to 30 minutes. Since the effect of hydrolysis will not take place at retention times less than 1 hour the particulate COD and slowly biodegradable fraction will pass through an MBBR process largely unaltered. Thus, the biological treatment step has to be followed by an efficient particle separation to remove particulate organic matter.

This has to be taken into account in, for example, the design of secondary settling tanks. The settling of particulate organic matter has to be enhanced by coagulation, with typical retention times 10 to 15 minutes, which is then followed by particle separation such as flotation, cloth or membrane filtration.

In aeration tanks the concentration of dissolved oxygen (DO) has to be relatively high because of the DO concentration being the limiting factor in biofilm processes. A high driving force in terms of DO concentration across the biofilm is therefore required. Typically MBBR's are operated at a DO concentration of 5 - 7 mg/l. The oxygen transfer rate in a MBBR is similar to the one achieved in conventional aeration tanks by fine bubble aeration even though coarse bubble aeration is used in the MBBR's. The aeration devices are tapered pipes with a converging diameter at both ends, through which the air flows. Common aeration tank depths are given as 4 to 5 meters. Unlike trickling filters and rotating biological contactors, the biofilm in the Kaldnes process becomes thinner at higher loadings rates because of the mixing intensity in the aerated or stirred tanks.

More technical information can be found in patent WO9111396 and related patents.

Performance

General performance

Research, carried out by research institutes in Norway, indicates that shape and size of the carrier material can be varied without an decrease in removal efficiency, as long as the effective surface area stays the same [4]. Based on these findings typical design parameters are given for different treatment ambitions in the table below.

Purpose	Removal in %	Design load rate [g/m^2.d]	Design loading at 67% fill [kg/m^3.d]	
BOD removal				
high-rate	75-80	25	8	
normal	85-90	15	5	
low	90-95	7,5	2,5	
Nitrification (O_2>5 mg/L)				
NH_4-N >3 mg/L	90	1	0,35	
NH_4-N <3 mg/L	90	0,45	0,15	
Denitrification				
Pre-DN (C/N >4)	70	0,9	0,3	
Post-DN (C/N >3)	90	2	0,7	[3]

Performance at specific installtions

WWTP Bekkelaget (350.000 p.e.) (Nitrification)

An activated sludge plant was upgraded to remove nitrogen. A post-denitrification treatment was set up with methanol as a carbon source. With a well-functioning pre-precipitation

process 80 to 90% N-removal with a total hydraulic retention time of 2,6 hours was possible. Using online DO, NH_4-N and NO_3-N analyzers for controlling the aeration/impeller mixing and methanol dosage resulted in significant savings in methanol dosing.

Design capacity	350.000 p.e.
Total empty bed volume	570 m^3
Tank depth	4,8 m
Reactors	aerobic – 6, anoxic – 2
Spec. active biofilm surface area	~300 m^2/m^3
Average biomass concentration	4 kg DS/m^3
Average specific sludge production	0,36 kg DS/kg $COD_{removed}$
Temperature wastewater	6,9 - 15,9°C
Nitrification started at volumetr. loads	1,5 - 2,2 kg BOD_7/m^3.d
Nitrification rates at low organic loads	300 - 400 g NH_4-N/m^3.d
Denitrification rates	700 - 750 g NO_x-N/m^3.d
C/N – ratio	3,5 g COD_{added}/NO_3-N
NO_x-N removal (at C/N = 3,5)	85%

Capital and operating cost

Sufficient information has not been available.

Operational stability and maintenance

The Kaldnes Moving Bed process requires no sludge recirculation and has a robust operational design regarding factors such as temporary limitation of nutrients, toxicity, as well as pH- and temperatures shocks.

These factors may temporarily reduce the biological capacity of the biofilm system, but will not significantly affect the biomass in the reactor. Due to the continuous movement and pre-treatment by means of screening the KMB process is generally not prone to clogging. However, in upgrading existing treatment plants that operate without primary settling and rather large screen sizes, the carrier material should be chosen correspondingly to prevent clogging.

Reference installations

The first KMB installation was realised in 1990. At the moment there are in total about 90 municipal reference installations for different process combinations, capacities ranging from 500 to 375.000 p.e. Countries, where KMB is realised, are Norway, Sweden, Germany, United Kingdom, Switzerland, Austria, Spain, New Zealand, Japan, and the United States. Another 90 references installations for industrial applications of the KMB process are reported.

WWTP Sjölunda (SWE) (375.000 p.e.) (1998) (N-removal, denitrification)

For the NATRIX technology about 25 industrial reference installations are reported mainly in the paper mill industry.

Suppliers / Patents

Kaldnes Miljøteknologi AS

P.O. Box 2011 - 3103 Tønsberg – Norway

Tel: 0047 - 3337 6700 - Fax: 3331 5518 - E-mail: kaldnes@kmt.no - Web: www.kmt.no

The process was invented and developed in close cooperation with the SINTEF/NTNU in Trondheim. Kaldnes Miljøteknologi AS is owned by Anglian Water PLC with 85% and by ANOX, a swedish company with 15%. Kaldnes holds the patent on process design and carrier material - patent number WO9111396 and related patents.

At the turn of the year 2001 Kaldnes Miljøteknologi AS agreed on a close cooperation with ANOX, which is marketing the NATRIX process. License agreement from KMT will therefore also include biofilm applications developed by ANOX.

There are licensees in Germany, Switzerland, United Kingdom, Sweden, Spain, Italy, Korea, Japan, Taiwan, and Brazil.

Literature references

[1] Website owned by Kaldnes Miljøteknologi AS - www.kmt.no

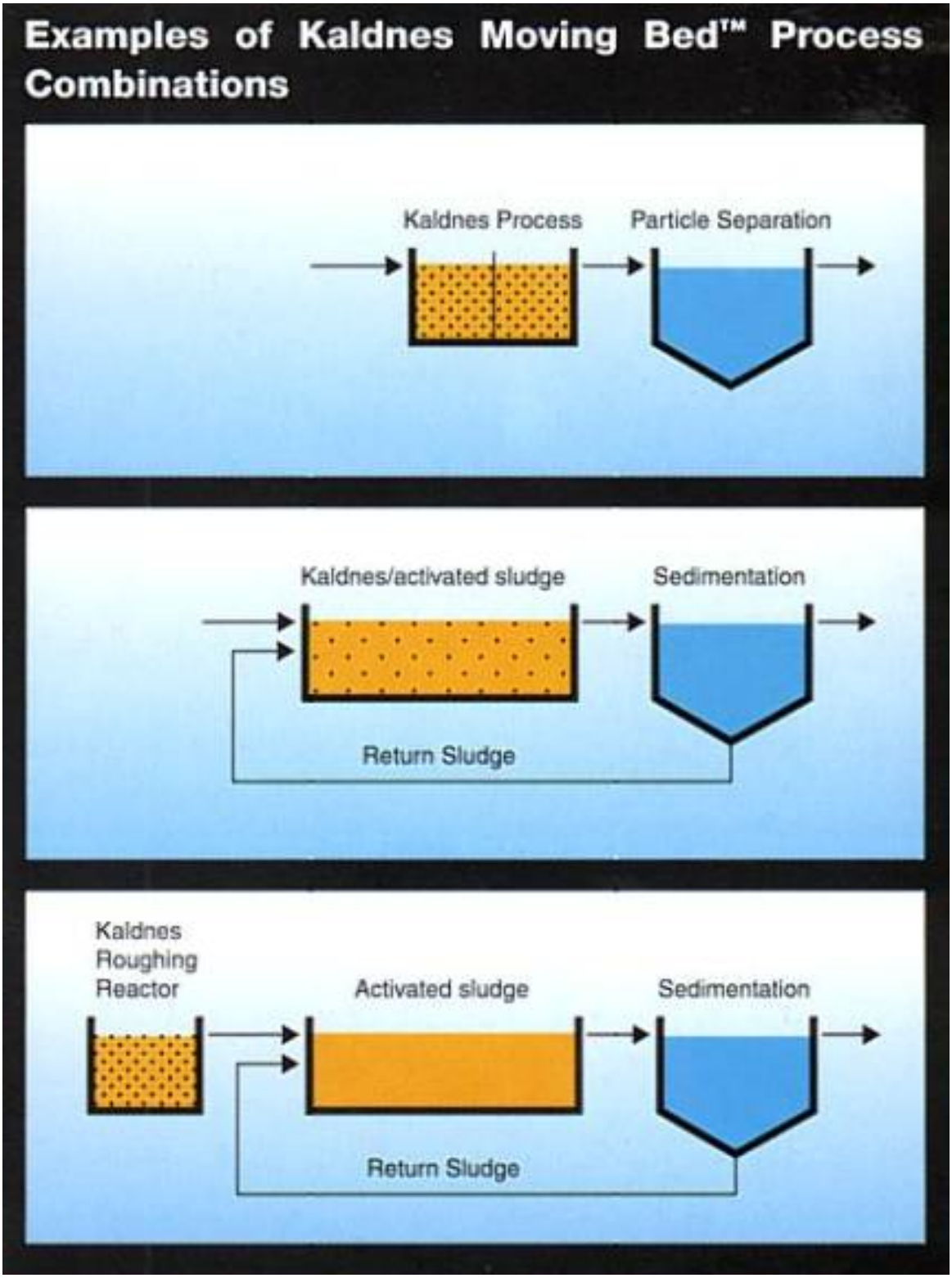
[2] Rusten, B.; Siljudalen, J.G.; Nordeidet, B.: Upgrading to nitrogen removal with the KMT moving bed biofilm reactor. Wat. Sci. Tech. Vol. 29, No. 12, pp. 185-195

[3] Odegaard, H.; Rusten, B.; Siljudalen, J.: The development of the moving bed biofilm process - from idea to commercial product

[4] Odegaard, H.; Gisvold, B.; Strickland, J.: The influence of carrier size and shape in the moving bed biofilm process. Water Science and Technology Vol. 41 No 4-5 pp 383-391. 2000.

[5] Telephone conversation with F. Greulich, Purac GmbH - German Kaldnes licensee

Graphics



Process configurations KMB [1]

Submerged Fixed Beds
Sheets

13-jun-2006, STOWA

Country		Germany
Stage of development		Full scale
Process	Line	Water
	Function	BOD removal – nitrogen removal
	Input	Effluent from primary and secondary treatment
	Concept	Fixed bed biofilm reactor – Submerged carrier material
Keywords: decrease area demand; water line; BOD removal; nitrogen removal		

Background

Biofilm processes have been known to the wastewater industry since the early days. One of the most common application was the trickling filter, which showed reliable operation. However, in the light of more stringent effluent standards and requirements for nutrient removal trickling filters could not always give the desired performance when compared to modern biological treatment processes such as activated sludge. The principle of providing large surface areas for the growth of micro-organisms has been taken up again in the new generation of submerged fixed beds. The technology is applied on a full-scale basis now and several process designs have been developed and patented.

Submerged fixed beds are particularly suited for pre-treatment of industrial effluents and small WWTP's, especially if the inflow is subject to high seasonal fluctuations.

Description and working principle

The submerged fixed bed is a version of the biofilm process, where structured packing material is submerged in one or more basins to provide a surface area for micro-organisms to grow on. The specific design of the packing material enables a high concentration of active micro-organisms on the one hand and relatively large cross sectional areas and free volumes on the other hand. This decreases the head loss across the packing and reduces the risk of clogging. Moreover a good mixing and an intensive mass transfer are realised.

Submerged fixed beds can be used for the following applications:

- Biological pre-treatment, e.g. preceding an activated sludge system.
- Full biological (secondary) treatment with or without nitrogen removal. Denitrification can be realised as pre-, post- or simultaneous denitrification.
- Nitrification as part of a multi-stage process.
- Post (tertiary)-treatment, e.g. residual nitrification.

In general the submerged fixed bed processes can be classified in systems with and without back-wash. The latter have typically a low continuous excess sludge production. The production rate of excess sludge can be considerably lower than in process designs with a periodic backwash.

Design guidelines / Technical data

General guidelines

The submerged fixed bed is a relatively new technology and no general design guidelines are available yet. Results from a few full-scale plants indicate that similar sizing methods as for biological contactors can be used provisionally.

The following recommendations regarding the design are given:

- Biological treatment with nitrification: Design load < 4 g BOD/(m3.d).
- Separate nitrification (after removal of C-compounds): Design load < 1,0 - 1,5 g TKN/ (m2.d).
- Sizing of denitrification volume according to volume ratio used for activated sludge systems by considering the specific surface area of the packing material.
- Pre-denitrification: Internal recycle ratio < 4.
- Aeration performs two functions: Oxygen supply and 'flushing' of the fixed bed (packing material) to prevent clogging.
- Oxygen requirements: Use similar design guidelines as for activated sludge systems.
- Concentration of dissolved oxygen in basin: Design concentration ~4 mg/l.
- Even though the oxygen utilisation in the submerged fixed bed is better than in an activated sludge system, it is recommended to use an alpha <0,8 until more results from full-scale plants are available.
- Recommended specific surface area for reactors without backwash:
- 150 m2/m3 for organic carbon removal, nitrification and simultaneous nitrification.
- 200 m2/m3 for separate nitrification.
- 100 m2/m3 for denitrification.
- Reactors with backwash: Backwash water requirement amounts to 5 to 10% of the inflow. Backwash water requirement: ~7 $m^3/m^2_{Basin\ area}$.h.
- Good pre-treatment and sufficiently sized primary clarification required. The design of the primary clarification should be similar to the design for trickling filter plants.
- Design of secondary clarification similar to the design for trickling filter plants, but lamella separators or filtration are also possible.
- Packing should have grid type structure to ensure good mixing.
- Basins in series (cascades) improve the biological treatment efficiency.

Technical data for specific installations

- Plastic packing manufactured mainly with a specific, theoretical surface area in the range of 100 to 400 m^2/m^3.The free volume of the packing ranges theoretically between 85% - 90%.
- Fixed bed heights of up to 6 m have been realised so far.
- Materials like polyethylene are generally more suitable than for example PVC, due to their chemical inertness (no inhibition in start-up phase).

Examples of operating parameters of small WWTP's with submerged fixed bed systems:

	Hamburg	Garching	Kiemerts-hofen	Lauterbach-Siedlung	Hamburger Stahlwerke	Heiligen-roth	Flöha
P.E.	4	8	75	80	500	1.000	8.000
BOD loading*	1,2	3,4	2,4	4	3	11,1	17,6
NH4-N loading*	0,28	0,6	0,45	0,8	0,4	-	-
Aeration time [%]	40	50	100	50	100	100	100
Recirculation [%]	300	0	0	0	100	0	0

* Loading in [g/m^2.d] relates to effective surface area.

Performance

General performance

Experiences with the submerged fixed bed systems so far can be summarised as:

- Easy and stable operation. Not sensitive to fluctuating loads and flows. Even after prolonged standstill full capacity can be achieved within a relatively short period.
- Low control requirements.
- Fixed bed enables growth of micro-organisms with long generation times, thus enhancing the removal of substances, which are difficult to degrade. Example WWTP Kiemertshofen: Even at 3°C, almost complete nitrification and 60% simultaneous denitrification occurs.
- Smaller basin volumes required as compared to activated sludge systems.
- Fixed bed reduces the rising velocity of air bubbles, thus increasing the oxygen utilisation by 50% as compared to activated sludge systems.
- No sludge recycle (return activated sludge) required.
- Lower sludge production as compared to activated sludge systems. Can be as low as 0,1 kg DS/kg BOD.
- Higher loading of secondary clarification possible.
- Removal efficiency of submerged fixed bed systems similar to advanced wastewater treatment systems if loading and operating conditions are chosen properly.

Performance of small WWTP's with submerged fixed bed systems

	Hamburg	Garching	Kiemerts-hofen	Lauterbach-Siedlung	Hamburger Stahlwerke	Heiligen-roth	Flöha
P.E.	4	8	75	80	500	1.000	8.000
BOD influent	250	173	262	93	160	136	60-170
BOD effluent	3	12	4	6	1,4	12	16
COD influent	500	343	462	444	445	250	150-350
COD effluent	26	50	39	51	19	35	70
NH_4-N influent	60	55,6	40	-	38	56	-
NH_4-N effluent	1,4	11	0,7	17	0,2	-	-
NO_3-N effluent	7,5	0,4	20	-	9,5	-	-
N-removal [%]	85	> 80	~65	-	>73	-	-

(concentrations are given in [mg/l])

Operational stability and maintenance

Please refer to section Performance.

Capital and operating cost

Sufficient information has not become available.

Suppliers / Patents

Particular suppliers are not mentioned in the ATV-report. However, there are various suppliers on the European market which provide proprietary technological solutions.

Reference installations

There are 8 reference installations for the treatment of municipal wastewater using submerged fixed beds. Capacities are ranging from 4 to 12.000 p.e.. Another 3 plants are treating industrial effluents, capacities ranging from 30.000 to 120.000 p.e. (COD). All of these reference installations are situated in Germany.

Literature references

[1] Arbeitsbericht der ATV-Fachgruppe 2.6.3: ‘Anlagen mit getauchten Festbetten’, Korrespondenz Abwasser, Vol. 43, No. 11, 1996, pp.2013-2023.

[2] Brochure of Emscher Gesellschaft für Wassertechnik (EW) and Lippe Gesellschaft für Wassertechnik (LW): ‘Das getauchte Festbett - ein flexibles und wirtschaftliches Verfahren’.

Sbbr / Bfsbr - Upflow-Fixed Bed Biofilter

Sheets

13-jun-2006, STOWA

Country		Germany
Stage of development		Pilot scale
Process -	Line	Water
	Function	Nitrification
	Input	Effluent from primary treatment
	Concept	Fixed bed biofilm - Filtration

Keywords: decrease area demand; water line; nitrogen removal

Background

The design of fixed bed reactors is mainly based on empirical data and due to the relatively short hydraulic retention times the reactors are generally designed for peak loads. Under normal operating conditions this leads to an 'inactive' volume of up to 25% and it is not certain whether this volume will be fully available under peak conditions. It was therefore investigated to what extent the operating parameters influence the performance of the biofilter and how the operation could be made more flexible by varying these operating parameters [1].

Description and working principle

In the upflow-fixed bed process design the reactor is filled with expanded clay pellets (‘Blähtonkugeln’), which serve as carrier material for the biofilm. Wastewater and air enter the reactor at the bottom and flow upwards through the packed bed. The reactor can be operated either in continuous flow - through or batch (recirculation) mode. The latter one is also called SBBR (Sequencing Batch Biofilm Reactor). Depending on the operation mode plug-flow or fully mixed conditions will be prevalent.

In the biofilm filter SBR design (BFSBR) the reactor is also filled with expanded clay pellets (‘Blähtonkugeln’), serving as carrier material. The reactor is operated in batch mode and one cycle consists of three phases. In the first phase the reactor is filled with wastewater from a holding tank until the packed bed is submerged. Subsequently wastewater from the holding tank and air enter the reactor at the bottom at loading rates, which will expand the packed bed by 20%. The treated wastewater leaves the reactor at the top and flows back into the holding tank. In this phase the reactor content is fully mixed. In the third phase the wastewater from the holding tank flows downwards (or upwards) through the packed bed. Since expansion and recirculation do not take place a plug flow of wastewater is achieved. The packed bed now acts as a biofilter to eliminate pollutants of low concentration.

Design guidelines / Technical data

Generic BFSBR plant

Example for the design of a BFSBR for nitrification in a wastewater treatment plant with a capacity of 9.500 population equivalents:

Nitrification rate	0,75 g NH_4-N/(m^2.d)
Packing material	Expanded clay pellets
Average diameter	3,2 mm
Specific surface area	1.125 m^2/m^3
Porosity of the packed volume	0.4
Upflow velocity	20 m/h
Temperature	10°C
Reaction rate	0,84 kg NH_4-N/(m^3.d)
Reactor	2 reactors in parallel
Volume	46 m^3
Packing depth	6,5 m
Diameter	3 m
Filtration velocity	10 m/h
Filtration efficiency	3,0 kg suspended solids/m^2 surface area
Aeration requirements	4,6 g O_2/g NH_4-N

Parameters for calculating the required air flow are: The free volume in the reactor, ammonia load to be eliminated, concentration of dissolved oxygen in the reactor, oxygen saturation concentration in water at a given temperature, and the alpha-factor.

Fixed bed (pilot plant)

Reactor height	5 m
Diameter	0,48 m
Packing depth	4,35 m
Packing material (diameter)	Liapor (4-8 mm)

BFSBR (pilot plant)

Reactor height	5 m
Diameter	0,192 m
Packing depth	3,0 m
Packing material (diameter)	Liapor (4-8 mm)
Aeration	Coarse or fine bubble

Performance

Upflow-Fixed bed

The experimental results showed that a short-term peaks of up to 50% in the inflow concentration can be handled by the fixed bed reactor by varying the air flow. If pure oxygen is used for aeration, the reaction rate can even be increased by four times. Higher hydraulic loadings result in an increased reaction rate of up to 70%. These results show that fixed bed reactors can be designed for average loads instead of peak loads as is common practise and that they will still be able to handle short-term peaks just by varying the operational parameters.

- Nitrification rates of up to 1,57 kg NH_4-N/(m_3.d) at an upflow velocity of 15 m/h and an air flow rate of 20 m/h.
- By aeration with pure oxygen, nitrification rates of up to 3,5 kg NH_4-N/(m_3.d) were achieved.

BFSBR

The BFSBR represents a process for handling high inflow concentrations and at the same time achieving very low effluent concentrations due to the combination of completely mixed (re-circulation mode) and plug-flow (filter mode) conditions.

- In the fully mixed (recirculation mode) conversion rates of about 1,3 kg NH_4-N/(m_3.d) at an upflow velocity between 20 to 30 m/h and an air flow rate of 20 m/h are reported.
- In the plug flow (filter mode) a suspended solids removal efficiency of up to 90% at a filtration rate of 10 m/h is reported.

Capital and operating cost

Not reported.

Reference installations

Pilot plants. No full-scale installations of the BFSBR are known.

Supplier / Patents

The process design has been developed at the Technische Universität Hamburg.

Literature references

[1] Brinke-Seiferth, S.: ”Beitrag zur Erhöhung des Reinigungsvermögens und der Flexibilität von Biofilmreaktoren (Festbett, Schwebebett, Filter)”, Hamburger Berichte zur Siedlungswasserwirtschaft Nr. 27, Technische Universität Hamburg-Harburg, 1998.