KTH Land and Water
Resources Engineering

STUDY ON ONE-STAGE PARTIAL NITRITATION-ANAMMOX PROCESS IN MOVING BED BIOFILM REACTORS: A SUSTAINABLE NITROGEN REMOVAL

Andrea Bertino

December 2010

TRITA-LWR Degree Project
ISSN 1651-064X
LWR-EX-11-05

© Andrea Bertino 2010

Degree Project in the Master's program Water Systems Technology

Water System Technology

Department of Land and Water Resources Engineering

Royal Institute of Technology (KTH)

SE-100 44 STOCKHOLM, Sweden

Reference should be written as: Bertino, A. (2010) "Study on one-stage Partial Nitritation-Anammox process in Moving Bed Biofilm Reactors: a sustainable nitrogen removal" TRITA LWR Degree Project

Dedicated to my Family and Federica

"We forget that the water cycle and the life cycle are one." Jacques Cousteau

SUMMARY IN SWEDISH

Under det senaste decenniet har flera nya och kostnadseffektiva biologiska kvävereningstekniker utvecklats. Upptäckten av anaerob ammoniumoxidation (Anammox), för ca 15 år sedan, har resulterat i nya möjligheter för forskning och utveckling av hållbara kvävereningssystem. Jämfört med konventionell nitrifikation/denitrifikation, eliminerar Anammox behovet av organisk kolkälla, har en mindre produktion av överskottsslam, minskar efterfrågan på energi för luftning (upp till 60-90%) och CO_2-utsläpp (upp till 90%). System baserade på Anammox kan vara till stor hjälp för att uppfylla strängare utsläppskrav för avloppsvatten och minska miljöproblem som orsakas av utsläpp av näringsämnen (t.ex. eutrofiering).

Denna avhandling undersöker partiell nitritation/Anammox i ett enstegssystem under syrebegränsande villkor (även kallad CANON eller Deammonifikation) och med Moving Bed Biofilm Reactor (MBBR™) teknik. Anammoxprocessen kopplad till partiell nitritation kan vara särskilt lämpad för att behandla ammoniumrikt avloppsvatten med lågt innehåll av biologiskt nedbrytbart organiskt material, som rejektvatten från avvattning av rötslam, som vanligen recirkuleras tillbaka till huvudströmmen i avloppsreningsverk och står för 15-20% av den totala kvävebelastningen.

Partiell nitritation/Anammoxprocessen testades framgångsrikt på en anläggning i pilotskala i fyra månader vid 25 ° C, i en 200 L Continuous Stirred Tank Reactor (CSTR), fylld till 40% av Kaldnes bärarmedia (modell K1). Vid en ammoniumytbelastning (ASL) på 3,.45 gN m^{-2} d^{-1}, var kvävereningsgraden 2,85 gN m^{-2} d^{-1}. Avlägsningseffektiviteter på 95%, 85% och 83% uppnåddes för respektive NH_4^+-N, oorganiskt kväve och Total kväve (TN). Bakterieaktiviteten bestämmdes med batchtester såsom S Specific Anammoxaktivitet (SAA), syreupptagshastighet (OUR) och nitratupptagshastighet (NUR), som avslöjade en ökning i aktiviteten för Nitrosomonas- och Anammoxbakterier i biofilmen. Koncentrationen löst syrgas i vattenfasen var en avgörande parameter, medan pH och konduktivitet visade sig vara två användbara verktyg för övervakning.

Två reaktorer i laboratorieskala drevs tidigare i två månader vardera, för att utvärdera en enstegs partiell nitritation/Anammoxprocess med lägre ASL. En reaktor tillfördes utspätt rejektvatten, medan den andra behandlade utflödet från UASB-reaktorn (Up-flow Anaerobic Sludge Blanket) efter sandfiltrering. Ganska bra verkningsgrad (> 75%) uppnåddes, men i det sista fallet kan låg ammoniumkvävebelastningen innebära ett problem för en stabil fullskaleinstallation och långsiktig tillväxt av Anammoxbakterier.

Några förslag för en fullskalig implementering och fortsatt forskning föreslås i det sista kapitlet i detta examensarbete.

Nyckelord: Anammox Biofilm; Deammonifikation; CANON; Moving Bed Biofilm reaktor; Rejektvatten.

ACKNOWLEDGMENTS

It is a pleasure to thank my supervisor Professor Elżbieta Plaza who made this interesting thesis possible. It allowed me to investigate a subject of current importance and to learn a lot, from both theoretical and practical points of view. I am indeed grateful to you and I wish you good luck in the hope of achieving rewarding results from this excellent research on the deammonification process.

The research on deammonification process was funded by Swedish Institute and Swedish Water Development (SVU).

I am heartily grateful to my teachers, Professors Giuseppe Genon and Maurizio Onofrio, who, despite the problem of distance, have been always promptly available for me in order to help me and solve my doubts during my study.

Jingjing Yang, PhD student at KTH (Department of Land and Water Resources Engineering) and friend, deserves an enormous thank for her patience and helpful support provided during the whole study. I have really appreciated your effort and your guidance that, especially at the beginning of the study, allowed me to develop the necessary understanding of the subject. You have always been available to discuss issues of experimentation with me and share your knowledge and views throughout the whole study. I do hope that all your efforts will be rewarded in your professional carrier in the best way.

I would like to acknowledge Dr. Eng. Jozef Trela (KTH), the leader of the "deammonification project" for his help and his knowledge.

Special thanks go to Dr. Eng. Christian Baresel and Dr. Eng. Lars Bengtsson from Swedish Environmental Research Institute (IVL), for their kindness, assistance and help when needed and for creating a friendly and pleasant atmosphere at the Hammarby Sjöstadsverk research facility.

I am also thankful Dr. Eng. Monika Żubrowska for sharing her experience about experimental work and Rune Bergström for his contribution regarding the pilot plant reactor.

I would like to extend my gratitude to the excellent KTH Library service that allowed me to have access to scientific literature necessary to carry out this Master Thesis.

Further thanks should also go to Politecnico of Turin (Servizio Gestione Didattica) for granting a scholarship for development of thesis abroad and EDISU (Ente regionale per il DIritto allo Studio Universitario), Politecnico of Turin and European Union for the financial support within the ERASMUS programme.

Many thanks go also to my family for all their love, support and encouragement during my studies.

I am heartily thankful to my girlfriend Federica, who, even though I have been away for several months, has always been present with her love, kindness and understanding. You are unique and I cannot thank you enough.

A special thank you also to all my friends who have made my time in Stockholm enjoyable, with countless memories. I am grateful for having shared with you this amazing international experience.

Finally, I want to thank everyone, both within and outside of the University, who has sparked my interest in environmental issues including wastewater treatment and protection of water resources.

Thank you!

Stockholm, December 2010.

TABLE OF CONTENTS

ACRONYMS AND SYMBOLS

AOB = Ammonium Oxidizing Bacteria

ANAMMOX = ANaerobic AMMonium OXidation

ASL = Ammonium Surface Load

ATP = Adenosine-5'-triphosphate

BABE = Bio Augmentation Batch Enhanced

BOD = Biochemical Oxygen Demand (mg O_2/l)

CANON = Completely Autotrophic Nitrogen Removal Over Nitrite

CBOD =Carbonaceous Biochemical Oxygen Demand (mg O_2/l)

COD = Chemical Oxygen Demand (mg O_2/l)

CSTR = Continuous Stirred-Tank Reactor

DEAMOX = DEnitrifying AMmonium Oxidation

DEMON = DEamMONification

DENAMMOX – DENitrification-anAMMOX

DO = Dissolved Oxygen

DNRA = Dissimilatory Nitrate Reduction to Ammonia

FA = Free ammonia

FISH = Fluorescent In Situ Hybridization

FNA = Free Nitrous Acid

HRT = Hydraulic Retention Time

HT = Heterotrophs

MAP = Magnesium Ammonium Phosphate

MBBR = Moving Bed Biofilm Reactor

MBR = Membrane Biological Reactor

MLSS = Mixed Liquor Suspended Solids

MLSS = Mixed Liquor Volatile Suspended Solids

NOB = Nitrite Oxidizing Bacteria

NUR = Nitrate Uptake Rate

ORP = Oxidation Reduction Potential

OUR = Oxygen Uptake Rate

p. e. = Population Equivalent

R = molar gas constant = 8.314 J mol^{-1} K^{-1}

Rpm = Revolutions per minute

RBC = Rotating Biological Contactor

SAA = Specific Anammox Activity

SBR = Sequencing Batch Reactor

SHARON = Single Reactor for High Activity Ammonium Removal Over Nitrite

S.D. = Standard Deviation

SRT = Sludge Retention Time

SS = Suspended Solids

T = Temperature

TKN= Total Kjeldahl-Nitrogen

TN = Total Nitrogen

TP = Total Phosphorus

TSS = Total Suspended Solids

UASB – Upflow Anaerobic Sludge Bed

VSS = Volatile Suspended Solids

WWTP = Wastewater Treatment Plant

μ_{max} = maximum growth rate (day^{-1})

ABSTRACT

In the last decade, several novel and cost-effective biological nitrogen removal technologies have been developed. The discovery of anaerobic ammonium oxidation (Anammox), about 15 years ago, has resulted in new opportunities for research and development of sustainable nitrogen removal systems. Compared to conventional nitrification/denitrification, Anammox eliminates necessity of external organic carbon source, has a smaller production of excess sludge, reduces energy demand for aeration (up to 60-90%) and CO_2 emissions (up to 90%). Systems based on Anammox can be of great help to comply with stricter wastewater discharge regulations and reduce environmental problems caused by nutrients discharges (e.g. eutrophication).

This thesis investigates the partial nitritation/Anammox in one stage system under oxygen limited conditions (also called CANON or Deammonification) and with the Moving Bed Biofilm Reactor (MBBR™) technology. Anammox process coupled with partial nitritation can be particularly suitable to treat ammonium-rich wastewater with low content of biodegradable organic matter, such as the reject water from dewatering of digested sludge, which is usually recirculated back to the main stream of wastewater treatment plants, accounting for the 15-20% of the total nitrogen load.

Partial nitritation/Anammox process was successfully tested on a pilot plant scale for four months at 25°C, in a 200 L Continuous Stirred Tank Reactor (CSTR), filled with 40% of Kaldnes media (model K1). At an Ammonium Surface Load (ASL) of 3.45 gN m^{-2} d^{-1}, the removal rate was about 2.85 gN m^{-2} d^{-1}. Removal efficiencies of 95%, 85% and 83% were respectively achieved for NH_4^+-N, inorganic nitrogen, and Total Nitrogen (TN). Bacteria activity was followed by batch tests such as Specific Anammox Activity (SAA), Oxygen Uptake Rate (OUR) and Nitrate Uptake Rate (NUR), which revealed an increase in activity for Nitrosomonas and Anammox bacteria within the biofilm. Dissolved oxygen concentration in the bulk liquid was a crucial parameter, whereas pH and conductivity turned out to be two useful monitoring tools.

Two laboratory-scale reactors were previously run for two months each, in order to evaluate the one-stage partial nitritation/Anammox process with a lower ASL. One reactor was fed with diluted reject water, whereas the other one treated the effluent from UASB (Up-flow Anaerobic Sludge Blanket) reactor after sand filtration. Fairly good efficiency (>75%) were reached but, however, in the last case the low ammonium nitrogen load could represent a problem for a stable full-scale installation and long-term growth of Anammox bacteria.

Some suggestions for full-scale implementation and further research are proposed in the last chapter of this master thesis.

Key words: Anammox Biofilm; Deammonification; CANON; Moving Bed Biofilm Reactor; Reject water.

1 INTRODUCTION

Nowadays the world is facing a steady increase in world population and drink-ing water demand as well as an increment of industrial sites. Therefore a higher and higher pressure is applied on the surrounding environment and ecosystem, which has been affected by innumerable cases of pollution. In order to prevent further degradation there is a strong need for sustainable technologies, cleaner production and wastewater treatment. These important concepts should also be applied to effluent streams with unacceptable levels of nitrogen.

Nitrogen is the most abundant element in the atmosphere and the fourth most common element found in cells as a building block of proteins and nucleic acids.

Nitrogen can be found in the environment under several forms as shown in Table 1.

The nitrogen cycle is a complex biogeochemical cycle in which nitrogen is converted from its inert atmospheric molecular form (N_2) into a form that can be used in biological processes. The classical nitrogen cycle includes:

- *Nitrogen fixation*: conversion of the inert form N_2 to an organic (or fixed) form which organism can use. Nitrogen fixation is mostly carried out by biological processes (e.g. nitrogen-fixing bacteria such as Rhizobium or Azotobacter and cyanobacteria). A small amount of nitrogen is 'fixed' through high-energy natural events such as lightning and forest fires.

Table 1. Forms of nitrogen in the environment

Unoxidized form	Oxidized form
Nitrogen Gas (N_2)	Nitrite NO_2^-
Ammonia (NH_4^+, NH_3)	Nitrate NO_3^-
Organic Nitrogen (urea, amino acids, peptides, proteins, etc...)	Nitrous Oxide (N_2O)
	Nitric Oxide (NO)
	Nitrogen Dioxide (NO_2)

Nitrogen can also be fixed through man-made processes (e.g. ammonia and nitrogen-rich fertilizers, explosives or combustion of fossil fuels which release NOx).

- *Nitrification*: conversion of ammonia into nitrite (NO_2^-), and then into nitrate (NO_3^-), which is the form that plants take up mostly. It is carried out by nitrifying bacteria under aerobic conditions.
- *Assimilation*: uptake of nitrogen compounds (i.e. nitrate, nitrite, ammonia, and ammonium) from soils by plants which used them for the formation of proteins.
- *Ammonification* (or mineralization): is the conversion of organic nitrogen to ammonia-nitrogen. It is carried out by microorganism (decomposers) which produce ammonium (NH_4^+) from dead organic matter (plants and animal tissues) and animal fecal matter.
- *Denitrification*: conversion of nitrate (NO_3^-) back to gaseous nitrogen (N_2) and, to a lesser extent, nitrous oxide gas, which is a strong greenhouse gas. It is carried out anaerobically by denitrifying bacteria. Through denitrification nitrogen is removed from ecosystems and it is a way to contrast the increased nitrogen fixation.
- *Dissimilatory Nitrate Reduction to Ammonia* (DNRA): it is a form of anaerobic respiration process where nitrate (NO_3^-) is used as electron acceptor instead of oxygen and it is recycled to ammonia (NH_4^+). In contrast to denitrification, this process does not remove the nitrogen from the habitat, but it remains available to primary producers. An example of dissimilatory nitrate reducer is Escherichia coli.

In this traditional version of the N-cycle, the ammonium oxidation was assumed to take place only under aerobic conditions and the possibility of an anaerobic ammonium oxidation was not contemplated. Recently it was discovered that ammonium can also be oxidized under anaerobic conditions. This new discover created a "short-cut" in the traditional nitrogen cycle (Fig. 1) and was called (ANaerobic AMMonium Oxidation - ANAMMOX), which is described in paragraph 1.2.5.

Over the last century, anthropogenic processes (e.g. fertilizers production, fossil fuel combustion, industrial production, livestock ranching and cultivation of crops such as legumes and rice) have substantially altered the global nitrogen cycle by increasing both the availability and mobility of nitrogenous compounds in the environment including water systems (Kumar & Lin, 2010).

In this first section the environmental concerns and risks connected to the discharge of nutrients (e.g. nitrogen) in water bodies will be discussed.

In order to shed light upon the current situation and give a general background of the nowadays available technologies for nitrogen reduction from wastewater, a second section will focus on different strategies and treatment methods.

A particular attention will be devoted to biological processes with special regard to the new promising sustainable technologies based on the recently discovered ANAMMOX® (ANaerobic AMMonium Oxidation), such as the one-stage Partial Nitritation-Anammox process (also called "Deammonification" or "CANON"), using the

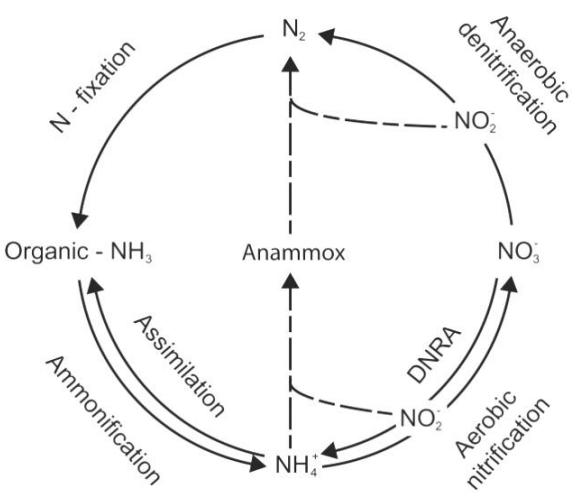

Fig. 1. The updated nitrogen cycle following the discovery of ANAMMOX (source: http://aem.asm.org/cgi/content/full/69/11/6447).

moving bed biofilm technology. These technologies have proved to be particularly suitable to treat wastewaters with high content of ammonium nitrogen and low content of biodegradable organic compounds.

1.1 Environmental problems related to nitrogen discharges

The discharge of nitrogen compounds can cause environmental impacts on the surrounding ecosystem and receiving water bodies or watersheds.

The commonly nitrogen compounds present in a wastewater treatment plant which may adversely impact the receiving waters are:

- ammonium ions (NH_4^+);
- nitrite ions (NO_2^-);
- nitrate ions (NO_3^-).

The main risks related to the presence of these compounds in concentrations above the water quality standard or guidance values may cause:

- dissolved oxygen (O_2) depletion;
- toxicity;
- eutrophication ;
- methemoglobinemia;
- deterioration of water aesthetic quality and odors from decomposing algae.

Ammonium ions are oxidized to nitrite ions by bacteria and nitrite ions are then oxidized to nitrates ions. Both these two reactions (nitrification) require *dissolved oxygen* which is depleted and reduced within the water.

Besides this, these three ions represent forms of nitrogen nutrients which aquatic plants (i.e. algae) can use for their growth. With their death, the dead plants will induce an increment of organic matter to be decomposed by bacteria, which will lead to a further reduction of dissolved oxygen. Moreover the water bodies might face the accumulation of parts of plants that do not decompose.

In the last decades several lakes, estuaries and coastal zones have faced problems due to the high nutrients contents, mainly deriving from different sources and human activities such as sewage discharges and the extensive use of fertilizer in agriculture.

This nutrient enrichment can lead to localized eutrophication, which in turn is associated with more frequent or severe algal blooms (WHO, 2000) with losses in ecological, commercial, recreational and aesthetic value of these water and changes in species composition and diversity of plant and animal communities. Prolonged and excessive eutrophication has also been responsible for algal blooms on a regional basis, such as those in the Adriatic and Baltic seas in recent years (WHO, 2000).

Eutrophication is defined by the European Commission – Environment as "the enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned".

Eutrophication is recognized as a pollution problem in European, North American and Asian lakes and reservoirs since the mid-20th century (Rodhe, 1969).

Although nitrogen is an essential nutrient for biological health and aquatic ecosystem integrity, it becomes a pollutant if its amount is beyond the natural capacity of the system to assimilate or flush the excess. This is particularly true for water bodies characterized by a low turnover rate (i.e. lagoons, lakes, coastal areas). Human activities are responsible for increasing and accelerating the natural process of eutrophication in the surrounding watersheds (Bricker et al, 1999).

Table 2 - Pollution concerns related to excess of NH_4^+,NO_2^- and NO_3^- (Gerardi, 2002)

Nitrogenous ion	Pollution concerns
NH_4^+	Overabundant growth of aquatic plants Dissolved Oxygen depletion Toxicity as NH_3
NO_2^-	Overabundant growth of aquatic plants Dissolved Oxygen depletion Toxicity Methemoglobinemia
NO_3^-	Overabundant growth of aquatic plants Toxicity Methemoglobinemia

Direct consequences of this enhanced growth in a lake are a limited amount of light reaching the lower regions (leading to a loss of submerged aquatic vegetation), color, odor (associated with the growth and death of aquatic plants) and, above all, low levels of dissolved oxygen at the bottom (i.e.hypolimnion), which, in very eutrophic lakes with high concentration of organic matter could lead to the reduction of sulphate to hydrogen sulphide, before the end of summer stagnation. HS^- is very toxic for aquatic organisms. .Recently it has been found that some cyanobacteria have the capacity to produce toxins dangerous to human beings and cyanobacterial toxins have become widely recognized as a human health problem arising as a consequence of eutrophication (WHO, 1999). Besides this, cyanobacteria ("blue-green algae") are nitrogen-fixing bacteria which increase ammonium concentration in the aquatic ecosystem.

According to the Swedish Environmental Protection Agency (2000) total phosphorus, total nitrogen and the nitrogen/phosphorus ratio are parameters used to assess lakes". The ratio TN/TP shows the availability of nitrogen in relation to phosphorus in lakes. When the ratio is higher than 30, the production of algae is governed by availability of phosphorus.

The Total Nitrogen (TN) is the sum of dissolved inorganic nitrogen (i.e. nitrate-nitrogen (NO_3^--N), nitrite-nitrogen (NO_2^--N), ammonia-nitrogen (NH_4^+-N)) and organic nitrogen (e.g. urea, peptides, proteins). Total Kjeldahl-Nitrogen (TKN) is used to indicate the sum of organic N and NH_3, which are the two typical forms of nitrogen in the sewage treatment plant inflow.

The nitrogenous ions can be *toxic* to aquatic life, especially to fish. (Gerardi, 2002). Ammonium ions and nitrite are extremely toxic, and nitrite ions are the most toxic of the three nitrogenous ions form. Ammonium ions, actually, are one of the most preferred nitrogen nutrient for most organisms, but they can be converted to ammonia with increasing pH of water (above 8-9), which can toxic for aquatic life at concentration as low as 0.025 mg/l NH_3. The reference value for water suitable for fish life is equal to 0.005 mg/l (Decreto Legislativo 152/2006 - allegato alla parte terza). High temperature and low salinity (freshwater) are other parameters that can contribute to a higher unionized-ammonia concentration in the water.

The toxic effects of nitrate exposure result from the conversion of nitrate to nitrite. *Methemoglobinemia* is a well-recognized hazard of ingestion of nitrates and nitrites (Comly H.H., 1945); nitrates are reduced to nitrites in the digestive system and, combining with the hemoglobin of the blood, stop the transport mechanism of oxygen. Infants younger than 4 months of age who are fed with water from rural domestic wells are at highest risks to developing health effects from nitrate exposure (American Academy of Pediatrics Committee on Nutrition, 1970).

The current legislations in Europe provide the following requirements for discharges of nitrogen from urban wastewater treatment plants (Table 3).

The current environmental legislation in Italy is *Decreto legislativo 3 aprile 2006, n. 152 -Norme in materia ambientale* and it contains also the limits for nitrate-nitrogen (NO_3^--N), nitrite-nitrogen (NO_2^-N) and ammonium (NH_4^+) (Table 4).

There are several typology of wastewater from industrial production which can be sources of high concentration of ammonium, nitrite and nitrate ions. The main are listed in Table 5 (Gerardi, 2002).

Table 3 - Requirements for discharges from urban waste water treatment plants. (Directive 91/271/EEC)

	Population equivalents (p.e.) (1)			
	10.000-100.000		>100.000	
Nitrogenous specie (annual average)	Concentration [mg/l]	% of reduction (2)	Concentration [mg/l]	% of reduction (2)
Total Nitrogen	≤ 15	70-80	≤ 10	70-80

(1) 1 unti PE = 0.2 m^3/d (Henze et al, 2002)

(2) Reduction in relation to the load of the influent.

Table 4 - Discharge limit values in surface water and sewer. (D.Lgs 3 aprile 2006, n. 152 - Norme in materia ambientale)

	Concentration [mg/l]	
Nitrogenous specie (annual average) (2)	Discharge into surface waters	Discharge into public sewers (1)
NH_4^+	< 15	< 30
NO_2^--N	< 0.6	< 0.6
NO_3^--N	< 20	< 30

(1) The limits for discharge into the public sewer are required in the absence of limits established by the competent authority or in the absence of a final treatment plant can meet the emission limits final discharge.

(2) As regards discharges of urban waste waters, the limits indicated in Table 4 for sensitive areas are applied. As regards discharges of industrial waste water into the sensitive areas total nitrogen concentrations must be less than 10 mg/l.

Table 5 - Industrial streams containing relatively high concentrations of Ammonium, Nitrite and Nitrate ions (modified after Gerardi, 2002)

Pollutant	Industry	Concentrations [mg/l]	Reference
NH_4^+	Reject water from a sludge digester	600 – 1600 mg/l TN	-
	Landfill leachate	400-2500 mg/l TN	Chung et al, 2003
	Molasses-based distillery wastewaters	1660–4200 mg/l TN	Mahimairaja & Bolan, 2004
	Pectin industry	1600 mg/l TN	Deng Peterson et al, 2003
	Starch production	800-1100 mg/l TN	Abeling and Seyfried, 1993
	Crude palm oil wastewater	770 mg/l TN 35 mg/l NH_4^+-N	Nemerow and Dasgupta, 1991
	Livestock manure (e.g. piggery manure)	1000-5000 mg/l TN	Ahn et al, 2004
	Oil Refinery	5-80 mg/l NH_4^+-N 9-90mg/l TKN	Jørgensen, 1979
	Slaughterhouse and packinghouse wastes	150-400 mg/l TN 113-324 mg/l org N	Zhan et al, 2008 Nemerow & Dasgupta, 1991
	Tannery	128 – 185 mg/l TN	Murat et al, 2003
	Wood-preservation	89 mg/l TN 32 mg/l NH_4^+-N	Middlebrooks, 1968
	Automotive		-
	Chemical		-
	Coal		-
	Fertilizer		-
	Petrochemical		-
	Ordnance		-
	Metallurgical		-
	Mining industries (blasting residuals)		-
	Pharmaceutical		-
	Primary metal		-
NO_2^-	Corrosion inhibitor	1100 mg/l NO_2^-	http://www.environet.ene.gov.on.ca/instruments/9430-725LUQ-14.pdf
	Meat (pre-treated)		-
	Steel		-
NO_3^-	Fertilizer Industry	600-950 mg/l NO_3^--N	Zala et al, 2004
	Mining industries (blasting residuals)		-
	Meat (flavoring)		-
	Meat (pre-treated)		-
	Steel		-
	Electroplating plants	10-120 mg/l NO_3^--N	Jørgensen, 1979

Table 6 – Centrifugated anaerobically digested sludge (reject water)

Parameter	Value / Range	References
pH	7.18 - 8.42	Marsalek et al, 2004
BOD_5 (mg/l)	230	Helliga et al, 1999
	109 ± 44	Vandaele et al, 2000
	1400 - 2000	Galì Serra A., 2006
COD (mg/l)	650	Köz Utku, 2007
	700 - 1000	Wett et al, 1998
BOD_5/ COD	0.14 - 0.2	Vymazal, 2010
COD_{sol}/ NH_4^+-N	0.29 - 1.19	Marsalek et al, 2004
NH_4^+-N (mg/l)	943 - 1513	Marsalek et al, 2004
	1180 ± 140	Van Dongen et al, 2001
	800 - 900	Dosta et al, 2007
	450 - 750	Vymazal, 2010
TKN (mg/l)	1053	Helliga et al,1999
	859	Vymazal, 2010
NO_2^--N, NO_3^--N (mg/l)	0-3	Vymazal, 2010

The effluent from the sludge line and the landfill leachate are the two most important and common wastewater sources of high nitrogen load. Anaerobic digestion of sludge and the sanitary landfill under acid or methanogenic phases are characterized by anaerobic conditions.

Regarding the sludge treatment, nitrogen is initially present as organic nitrogen bound in proteins of the biomass. During anaerobic digestion proteins are broken down into amino acids (hydrolysis) which are further broken down releasing ammonium (acidogenesis). The liquor effluent from the digester can have concentrations around 1000 mg N/l. A similar process occurs within a sanitary landfill, where, under acidogenic phase ammonia nitrogen concentration may gradually raise up to over 1000 mg/l.

The characterizations of these two kinds of wastewater are shown with regard to nitrogen forms and organic material in Table 6 and 7.

1.2 Nitrogen removal in WWTPs

Nowadays there are several ways to reduce nitrogen content from wastewater. This chapter deals with treatments for nitrogen removal, considering both the well-established techniques and the innovative ones with greater chances of success, and examines the advantages and drawbacks.

Most of the wastewater treatment plants carry out nitrogen removal by biologi-cal methods rather than physical-chemical ones. The main reasons are, generally, the lower operational costs, the lower complexity of the plant and management and less use of chemicals.

In general, a municipal wastewater treatment plant which removes organic matter and nutrients can achieve concentrations in the effluent as the ones shown in Table 8.

Regarding wastewater with high concentration of nitrogen (i.e. leachate, reject water from dewatering of sludge, slurry from farms, etc.) a more

Table 7 – Landfill leachate (Ehrig, 1989; Nuovo Colombo - Manuale dell'Ingegnere 84° ed. 2003; Renou et al, 2008)

Parameter	Acidogenic phase		Methanogenic phase	
	mean	range	mean	range
pH	6.1	4.5 - 7.5	8	7.5 - 9
BOD_5 (mg/l)	13000	4000 - 40000	180	20 - 550
COD (mg/l)	22000	6000 - 60000	3000	500 - 4500
BOD_5/ COD	0.58	0.20 - 0.70	0.06	0.03 - 0.20
Organic N (mg/l)	600	10 - 4250	600	10 - 4250
NH_4^+ (mg/l)	750	30 - 3000	750	30 - 3000
TKN (mg/l)	1350	40 - 3425	1350	40 - 3425
NO_2^--N (mg/l)	0.5	0 - 25	0.5	0 - 25
NO_3^--N (mg/l)	3	0.1 - 50	3	0.1 - 50

Table 8 - Range of concentration in urban sewage water before and after treatment to remove organic matter and nutrient. (Nuovo Colombo - Manuale dell'Ingegnere 84ª ed. 2003)

Parameter	Concentration [mg/l]	
	Raw sewage water	Effluent from biological treatment for removal of organic matter and nitrogen
BOD_5	150-300	5-20
COD	300-600	40-120
NH_4^+-N	20-40	0-3
NO_2^--N	0-1	0-0.5
NO_3^--N	0-5	5-10
N_{tot}	25-60	6-15

stringent treatment for nitrogen removal is necessary in order to reduce the high concentrations of am-monia in the wastewater and prevent the high potential impacts from discharge. These special kinds of wastewater should be treated through an appropriate treat-ment which can allow reducing the high content of nitrogen and the presence of any toxic compounds, before these effluents are discharged to the sewage system and sent back to the head of the municipal WWTP, for further purification and nutrient reduction.

1.2.1 Physical - chemical methods

Here below some physical-chemical methods are briefly discussed. Among them there are:

- Mechanical separation
- Membrane filtration
- Ammonia stripping
- Ion exchange
- Breakpoint chlorination
- Electrodialysis
- Struvite precipitation

Mechanical separation is mainly used for cattle slurry treatments and is a physical separation, with the goal to obtain an easier handling of the liquid by concentrating it. The separation efficiency of the process is rather low and removal rates for nutrients are less than 30% (Barker, 1993). In order to obtain a higher separation a prior coagulation/flocculation step is required. Some examples of mechanical separation are flotation separator and horizontal centrifugal.

Membrane filtration includes Ultrafiltration (UF), Nanofiltration (NF) and Reverse Osmosis (RO). Ultrafiltration method uses membranes with a pore size of 0.1-0.01 μm, whereas Nanofiltration membranes have a pore size of 0.01-0.001 μm. The removal mechanism of these two methods is based on physical separation (i.e. size exclusion). Reverse Osmosis removal mechanism is instead based on a diffusive mechanism. A pressure (high enough to exceed the osmotic pressure) is applied to the side with high concentration to force water to flow through the semi-permeable membrane in the opposite direction of the natural osmotic flow and the separation efficiency is dependent on influent solute concentration, pressure and water flux rate. Reverse Osmosis can achieve very high level of purity. Nitrogen separation trials by RO were performed on domestic wastewater and combined domestic-industrial wastewater achieving a separation efficiency of 95% for total nitrogen (Bilstad T., 1995). The main disadvantages are that it is expensive and can be subject to membrane fouling (caused by deposits of inorganic, organic and colloidal and suspended substances on the membrane surface), which requires maintenance and increases significantly operational costs. Some possible solutions to this problem are pH adjustment, pre-filtration and coagulation. Membrane technology is usually used as polishing step. Another drawback is that the concentrates obtained by means of membrane technology (UF, NF, RO) - although they may theoretically be used as fertilizer - have a high salt concentrations and it is unclear the type of market that will have, and it should not be excluded that they have to be "disposed" with associated costs.

The use of a combination of membrane separation technology (micro-and ultrafiltration) and bioreactors is steadily increasing and can contribute to very compact systems working with a high biomass concentration and achieving a low sludge production with a good effluent quality (Van Dijk & Roncken, 1997). For instance, a Membrane bioreactor (MBR), which combines activated sludge process with micro- or ultrafiltration membranes, does not need for a secondary clarifier and can provide several advantages such as a high efficiency of selectivity, an im-

proved retention of the biomass and compact dimension of the whole system. Moreover, in a membrane assisted bioreactor excess sludge production is lower than in conventional activated sludge systems (Ghyoot et al, 2000; Rols & Goma, 1997).

Ammonia Stripping consist of removing ammonia present as solute in wastewater and transfer it to gaseous form by means of air flow. Nitrogen is simply transferred from one form to another with characteristics more suitable for further processing. Higher pH (10.5-11.5), temperature and air flow, as well as greater packed bed depth, increase the efficiency and the removal of ammonia from solution. Temperature can be increased by using steam instead of regular air. Once the ammonia is removed from the wastewater, it can be concentrated as ammonium sulfate or equal or can undergo thermal destruction. The first option is carried out through the combination of a stripping tower and a scrubber where the flow of air loaded with ammonia is brought into contact with an acidic solution, usually acid-based sulfuric acid, to obtain a salt, ammonium sulphate. The ammonium salt thus formed can be treated as spent solution or, less frequently, crystallized, precipitated and handled as solid form. Some of the main drawbacks are the need to increase the pH (with lime) and then the need to decrease it before discharge, the need of large quantities of sulfuric acid, fouling (calcification) of the packed stripping tower, low efficiency of the process in cold weather, potential for odors generation and release of ammonia with potential environmental and health effects. Carbonates precipitation due to high pH can be prevented by acidifying the water and stripping CO_2 as pre-treatment, but this will increase the use of chemicals (Henze, 2008). Ammonia stripping is a method widely established in industrial applications and in the treatment of landfill leachate (Piccinini et al, 2007). Ammonia stripping may be preceded by an anaerobic digestion step, in order to reduce costs, where it is feasible.

Ion Exchange is a process in which ions on the surface of a solid are exchanged for ammonium ions in the wastewater. It can be carried out through the use of materials with high affinity for ammonium ion such as clinoptilolite, a naturally occurring zeolite (Jorgensen & Weatherley, 2003; Thornton et al, 2007). Ammonium ions are usually exchanged for ions with the same charge, typically sodium. When all the exchange sites have been replaced, the resin must be regenerated. Ion exchange is typically used for small flows. The optimum ammonium exchange by clinoptilolite occurs at pH between 4 and 8. If the ammonium concentration is high or large volumes need to be treated, frequent regeneration may be required, with an increase in operational costs. A combined ion-exchange and nitrification column can be an attractive solution.

Breakpoint chlorination is a process in which chlorine is added to the wastewater in an amount sufficient to oxidize ammonia-nitrogen into nitrogen gas. The ratio Cl_2/NH_3-N needed for the oxidation is 10:1, which makes this technique expensive. Another disadvantage is the addition of chloride to the water, which might give chlorination by-products. On the other side, one advantage is represented by the low spatial requirement.

Electrodialysis is a process in which ions are transported through a semipermeable membrane under the action of an electric field. Membranes can be cation or anion-selective (i.e. positive ions or negative ions can pass through them) and may be arranged in series. The total nitrogen removal efficiency is low compared to other treatment methods (about 40-50%) (Halling-Sørensen & Jørgensen, 1993). Some disadvantages are chemical precipitation of salts with low solubility on the membrane surface, clogging of the membrane by residual colloidal organic matter.

Struvite precipitation. Precipitation of nitrogen (in the form of ammonia) as struvite. The efficiency of nitrogen recovered as struvite can be beyond 70% (Shin & Lee, 1997) and time required for reaction is very short (i.e. 10-15 minutes). Struvite is an inorganic magnesium ammonium phosphate (MAP) mineral with the chemical formula $Mg(NH_4)PO_4 \cdot 6H_2O$. It is a valuable by product, which can be used as slow-release fertilizer, as a raw material for the phosphate industry, for use in making fire-resistant panels and as binding material in cements (Schuiling & Andrade, 1999; Sarkar, 1990). One limiting factor to the application of this technology to wastewaters with high content of ammonium nitrogen is the stoichiometry for the precipitation; for a molar ratio of magnesium, ammonium and phosphate of 1:1:1, ammonium is in large excess in the influent wastewater and therefore additional magnesium phosphate has to be added. One way to solve this problem is to remove ammonium in precipitated magnesium ammonium phosphate and then recycle magnesium and phosphate ions to the influent. Recycling can be based on chemical dissolution and ammonia removal or dissolution by bacteria as performed in introductory studies at KTH (Hultman and Plaza, 2009). Another

alternative is to recover the ammonium from the MAP sludge by heat treatment which makes ammonium volatilize, allowing to a recover of ammonium and a reuse of magnesium phosphate in the treatment (Henze, 2008).

1.2.2 Conventional Nitrification/Denitrification

The combined process of Nitrification/Denitrification is the most common method used for wastewater treatment at municipal wastewater treatment plants nowadays. It is a treatment process known and well established and with high stability of operation.

This biological treatment consists of two steps called Nitrification and Denitrification.

Nitrification is a biological process whereby free and saline ammonia is oxidized to nitrite and then nitrate. It is mediated by autotrophic organism (nitrifying bacteria) which obtain their energy requirement (catabolism) for biomass synthesis from inorganic nitrogen compounds, oxidizing ammonia to nitrite and nitrite to nitrate, and their carbon requirement (anabolism) from dissolved CO_2 (Gerardi, 2002).

Nitrification is therefore made up of two sequential steps:

1) Ammonia is oxidized to nitrite (NO_2^-) by *Nitrosomonas spp.* bacteria:

   $NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+$

   These bacteria are also called Ammonium Oxidizing Bacteria (AOB).

2) Nitrite is converted to nitrate by *Nitrobacter spp.* bacteria:

   $NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$

   These bacteria are also called Nitrite Oxidizing Bacteria (NOB).

The stoichiometric oxygen required for these reactions is: $1.5 \cdot 32/14 = 3.43$ mg O_2/mg N for ammonia oxidation and $0.5 \cdot 32/14 = 1.14$ mg O_2/mg N for nitrite oxidation. The first reaction consumes alkalinity (about 7.1 g of alkalinity as $CaCO_3$ for each gram of N-NH_4^+ oxidized).

The most commonly recognized genus of bacteria that carries out ammonia oxidation is *Nitrosomonas*; however, *Nitrosococcus*, *Nitrosopira*, *Nitrosovibrio* and *Nitrosolobus* are also able to oxidize ammonium to nitrite (Ahn, 2006).

The main responsible for nitrite oxidation is *Nitrobacter* genus but several other genera such as *Nitrospira*, *Nitrospina*, *Nitrococcus*, and *Nitrocystis* are known to be involved (Ahn, 2006).

As mentioned by Gerardi (2002), recent molecular techniques have discovered that there are several genera of nitrifying organisms (i.e. Protozoa, Actinomycetes, Algae, Fungi, and other bacteria such as Pseudomonas, Bacillus, Vibrio, Proteus and Arthrobacter), but however, most of nitrification is carried out by Nitrosomonas spp. and Nitrobacter spp., whose rate of nitrification is often 1000 to 10000 times greater than the nitrification achieved by other organisms (Gerardi, 2002).

The main factors which might influence the kinetics of nitrification are:

- pH; the two reactions mentioned above produce H^+ and therefore lower the pH. The optimum pH for Nitrosomonas and Nitrobacter is between 7.2 and 8.5. At pH of 6.0 normally the nitrification stops. The pH also controls the concentration of free ammonia (NH_3) and nitrous acid (HNO_2) which are strong inhibitor of bacterial activity. Free ammonia can inhibit Nitrosomonas at concentration as low as 10 mg/l and Nitrobacter at concentration as low as 0.1 mg/l. Free nitrous acid inhibits them at concentrations as low as 1 mg/l (Gerardi, 2002). The pH can control these two equilibria: $NH_4^+ \leftrightarrow H^+ + NH_3$ and $NO_2^- + H^+ \leftrightarrow HNO_2$.
- DO; dissolved oxygen is an important parameter for nitrifiers growth. The DO concentration should be kept above 2-3 mg/l (Nuovo Colombo - Manuale dell'Ingegnere, 2003) in order to not unduly depress the rate of removal. A DO between 0.5 and 2.5 mg/l may limit the nitrification (NSF International and US EPA, 2003) in suspended or attached growth system under steady state conditions, depending on the degree of diffusional resistance, especially in attached biomass growth systems.
- Temperature; a too low temperature (below 10-15 °C) as well as sudden changes in temperature can decrease the removal rate. Nitrification reaches a maximum rate at temperatures between 30 and 35 °C.
- Heavy metals and organic compounds; some heavy metals (Zn^{2+}, Cd^{2+}, Cr^{3+}, Pb^{2+}, Ni^{2+}) may exert their inhibitory action from concentration of 1 mg/l. Active carbon or acclimatization of biomass can reduce the inhibitory action of many compounds.

Denitrification is a biological process whereby nitrate is reduced to nitrite and the produced nitrite to nitrogen gas. It is mediated by heterotrophic microorganism (*Denitrifying* bacteria) which uses organic matter as carbon (anabolism) and energy source (catabolism). This results in a

much higher biomass growth compared with autotrophic bacteria (5-fold higher according to Gerardi, 2002).

Among denitrifying bacteria, the most common are Achromobater, Pseudomonas, Micrococcus, Bacillus and Alcaligens. Other bacteria such as Aerobacter, Proteus, Flavobacterium are only able to convert NO_3^- to NO_2^-.

Denitrifying bacteria are facultative organisms that can use either dissolved oxygen or nitrates as source for metabolism and oxidation of organic matter. In the case of simultaneous presence of dissolved oxygen and nitrates, denitrifying bacteria use preferentially oxygen because the energy generated per unit weight of organic matter metabolized, is higher.

Therefore it is important to keep dissolved oxygen as low as possible (less than 0.3-0.5 mg/l), at least in the microenvironment surrounding the bacteria. Under anoxic conditions, denitrification reactions can be simplified as the sum of denitratation (1) and denitritation (2):

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

(2) $NO_2^- + 0.5\ CH_3OH \rightarrow 0.5\ N_2 + 0.5\ CO_2 + 0.5\ H_2O + OH^-$

Since nitrogen gas has low water solubility, it is released into the atmosphere without any environmental concern. The second reaction occurs through the formation of nitrogen oxides (NO and N_2O) which are subsequently reduced to nitrogen gas. A carbon source (shown in the above equation as methanol, CH_3OH) is required for denitrification to occur. Organic matter may be in the form of raw wastewater or external carbon source (e.g. ethanol, molasses, distillery stillage, buttermilk, methanol or acetate). Methanol has a high toxicity in humans, therefore the use of another carbon source would be preferable. When these sources are not present in the water, bacteria may depend on internal (endogenous) carbon reserves, but the nitrate removal may be lower. Removal of nitrogen is also partly due to the synthesis of new biomass and thus of organic nitrogen. This amount is about 4% of the total nitrogen removed (Nuovo Colombo - Manuale dell'Ingegnere, 2003).

The main factors which might affect the efficiency of denitrification are:

- DO; as dissolved oxygen increases, denitrification rates decreases, therefore anoxic condition should be maintained.
- presence of organic matter; the source of available carbon can influence the denitrification rate. The highest rate can be achieved by adding an easily biodegradable and assimilated carbon source, but this may implies costs for its purchase. The highest removal rates occur with the use of effluent from distillery and food industries.
- pH and alkalinity; the optimum pH is between 7.5 and 9.1, but denitrification can occur also at pH between 6 and 75. Alkalinity is produced during the process (about 3-3.5 g of alkalinity as $CaCO_3$ for each gram of NO_3^- reduced).
- temperature; it affects the growth rate of denitrifying organisms, with greater growth rate at higher temperatures. Denitrification can occur between 5 and 30°C.
- Heavy metals and organic compounds. Denitrifying organisms are generally less sensitive to toxic chemicals than nitrifiers, and recover from toxic shock loads quicker than nitrifiers.

In wastewater treatment plant, nitrification/denitrification can be performed through:

- *suspended-growth biomass processes* (e.g. conventional activated sludge, sequencing batch reactors-SBR);
- *attached-growth systems* (e.g. trickling filters, rotating biological contactors-RBC).

In suspended-growth biomass processes, several schemes and configurations can be adopted. The main distinction is the choice of a:

- *separate system configuration*, in which nitrification and denitrification are carried out in series (post-denitrification) and in distinct stages with their own clarifier and sludge recycling system. The costs are higher as two clarifiers are needed.
- *combined system configuration*, in which biomasses are mixed in a single activated sludge.

If denitrification is carried out after the nitrification (i.e. "*post-denitrification*"), an external carbon source is usually required, unless other configurations are adopted, as for instance, a post-denitrification with by-pass of part of the incoming wastewater to the anoxic tank where denitrification takes place.

A common scheme in municipal wastewater treatment plant is "*pre-denitrification*", which provides a denitrification stage followed by a nitrification-oxidation stage with oxidation of organic material and ammonia. The recirculation of nitrates provides the nitrates to the anoxic tank. This configuration requires recirculation ratios up to 4-5 times the inlet flow.

In conventional activated sludge nitrification/denitrification, the different treatment stages (i.e. denitrification, oxidation-nitrification and sedimentation) occur in separate tanks, thus requiring the presence of pipes and recirculation pumps. A valid alternative to conventional biological systems is the system *SBR (sequencing batch reactor)* which requires only one reactor (or more reactors in parallel) in which are created in succession, the proper conditions for the different reactions.

The complete cycle may consist of six main steps:

1. filling with mixing, but without aeration
2. filling with aeration;
3. filling with mixing without aeration;
4. aeration;
5. settling;
6. drawing.

The control of aeration can be based on oxidation reduction potential in the reactor. The SBR has several advantages compared to conventional activated sludge:

- flexibility of operation and possibility to control the duration of different phases;
- more compact footprint by eliminating secondary clarifier;
- no need for recirculation pipes;
- less sludge generation;

Although conventional nitrification/denitrification process has high stability and reliability, it has several drawbacks:

- high costs of the process, due to the large amount of energy required for aeration needed for nitrification;
- need for an external carbon source for denitrification;
- infeasibility to treat wastewaters with high nitrogen concentrations or low C/N ratios;
- relatively high sludge generation.

These disadvantages can be overcome through the use of innovative, sustainable and cost-effective nitrogen removal technologies, as those described in the following paragraph.

1.2.3 Innovative and sustainable technologies for biological nitrogen removal

Generally, the conventional biological nitrogen removal process is used for treating wastewaters with relatively low nitrogen concentrations (total nitrogen concentration less than 100 mg N/l (Van Hulle et al, 2010)).

Some wastewater streams such as anaerobic digester effluents, landfill leachate, and some industrial wastewaters (fertilizer industry, explosive industry, tannery industry, etc.) contain high concentrations of nitrogen, usually in the form of ammonium. If these streams are returned back to the inlet of the municipal WWTP, the result is a considerable increase in the ammonium loading in the mainstream.

Although the volumetric flow of side-streams such as the effluents from dewatering of digested sludge by centrifuges or belt presses is a small proportion of the total inflow to the WWTP (usually less than 5%), their total nitrogen load can be very high and up to 30% of the total N-load to the treatment plant (Siegrist, 1996; Janus & van der Roest, 1997; Pearce et al, 2000; Mackinnon et al, 2003; Thornton et al, 2006; Henze, 2008), with consequent impacts on the global efficiency and risk to not meet the effluent discharge standards. At Himmerfjärdsverket-Grödinge WWTP (Sweden) and Rotterdam-Dokhaven WWTP (The Netherlands) it amounts to 15% of the incoming nitrogen load (SYVAB, Himmerfjärdsverket. Available at: http://www.syvab.se/396/Vattnets-vag.html).

These stream are often highly concentrated with ammonium consequently small tank volumes may be required. In addition some of these flows has high temperature (20-35°C) (van Haandel & van der Lubbe, 2007) compared to the main treatment stream and thus bacteria activity is higher, with consequent possibility to operate with shorter solid retention times (SRT).

Removing the ammonium with a separate treatment of these side-streams, can lead to a significant improvement of the final effluent quality (Henze, 2008) and can be a valid option when existing plants require upgrading due to more stringent requirements or increased load.

Conventional biological nitrogen removal process (denitrification-nitrification) is uneconomical and complicated when treating high nitrogen contained wastewaters with low C/N ratio.

During the last decade, several new sustainable and cost-effective alternatives have been discovered and studied and their implementation can be a valid option to treat strong nitrogenous wastewaters characterized by high ammonium concentrations and low biodegradable organic matter content.

The novel processes which have been recently developed include:

- nitritation – denitritation (SHARON®);

- partial nitritation and anaerobic ammonium oxidation (ANAMMOX®) in two separate reactors (combined SHARON®-ANAMMOX® processes);
- the combination of partial nitritation and ANAMMOX® in one single reactor, also called Deammonification, or CANON (completely autotrophic nitrogen removal over nitrite), or SNAP (single-stage nitrogen removal using ANAMMOX and partial nitritation) or DEMOX;
- the coupling of denitrification and ANAMMOX® (called DENAMMOX or DEAMOX);
- Bio-augmentation (BABE®)

These novel processes are described in the following paragraphs.

1.2.4 Nitritation-Denitritation (SHARON®)

Nitritation–denitritation process over nitrite (or commonly called SHARON® process) is a more sustainable alternative to the traditional nitrification/denitrification (Van Hulle et al, 2010).

SHARON stands for Single reactor High Activity Ammonia Removal Over Nitrite. The SHARON® process was developed in the late 1990s at the Delft University of Technology by Hellinga et al (1998).

The SHARON® process is usually performed in separate reactor compartments with continuous flow. In this process, ammonium is oxidized under aerobic conditions to nitrite (Nitritation) and the produced nitrite is in turn reduced and heterotrophically denitrified to nitrogen gas under anoxic conditions by using an external carbon source (Denitritation).

The bacteria culture is a mix of Nitrosomonas and aerobic denitrifiers and the process is operated without any biomass retention. As the process functions without sludge retention there is no influence of the presence of suspended solids in the wastewater.

Ideally the reactions of the process are the following:

Nitritation:

$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2\ H_2O + 2\ H^+$

Denitritation:

$NO_2^- + 0.5\ CH_3OH \rightarrow 0.5\ N_2 + 0.5\ CO_2 + 0.5\ H_2O + OH^-$

This process requires less oxygen and less organic carbon in comparison with the traditional nitrification–denitrification. The reduction in the oxygen demand amount to 25 % and the reduction of carbon demand to approximately 40% (Fig. 3-4). The sludge generation is lower compared to the conventional denitrification/nitrification.

The main goal of this process was to arrest the autotrophic nitrification (i.e. ammonium oxidation) at nitrite by creating unsuitable conditions for subsequent oxidation process to nitrate, in order to save costs for aeration and carbon source.

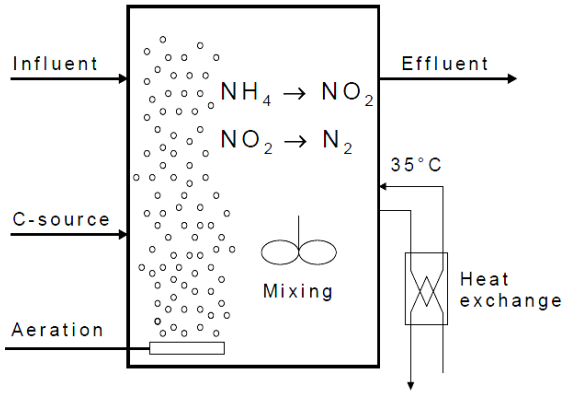

Fig. 2. Schematic representation of Nitritation/Denitritation (SHARON®) (source: Notenboom, Jacobs, van Kempen, van Loosdrecht, 2002).

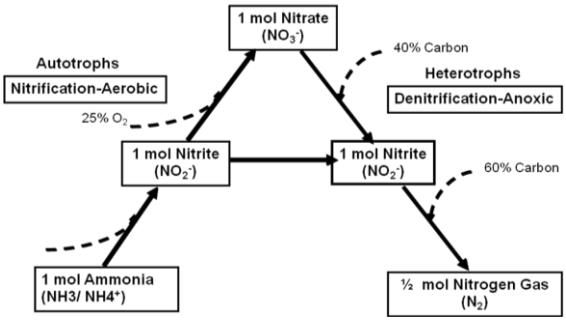

Fig. 3. Nitrification/Denitrification (source: H. D. Stensel, Sidestream treatment for nitrogen removal, 2006).

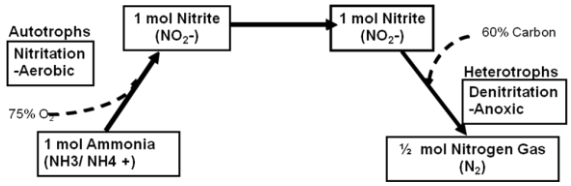

Fig. 4. Nitritation/Denitritation (source: H. D. Stensel, Sidestream treatment for nitrogen removal, 2006).

The operating variables in order to obtain a stable partial nitrification are:

- temperature;
- hydraulic retention time;
- dissolved oxygen;
- pH
- substrate and inhibitor concentration.

The process requires elevated temperatures (above 25°C), at which the maximum specific growth rate of the desired ammonium oxidizers is higher than that of the "undesired" nitrite oxidizers. At the operational temperature of 35°C, the maximum specific growth rate of nitrite oxidizers is approximately only half of the one for the ammonium oxidizers (0.5 and 1 day^{-1}, respectively) (Khin & Annachhatre, 2004).

At temperatures of 25-35 °C ammonium oxidizers have a shorter minimum required sludge age and a proper hydraulic retention time is chosen in order to wash out nitrite oxidizers and keep the ammonium oxidizers inside the reactor (Fig. 5).

Lower dissolved oxygen concentration limit the growth of Nitrite Oxidizing Bacteria (NOB) due to their lower oxygen affinity compared to AOB (Wiesmann, 1994). Thus dissolved oxygen is a key parameter of high importance.

As stated by Van Hulle et al (2007), free ammonia (NH_3) and free nitrous acid (HNO_2) concentration are the actual substrate/inhibitor for ammonium and nitrite oxidation instead of ammonium (NH_4^+) and nitrite (NO_2^-).

Nitrite oxidizers can be outcompeted at higher pH (7.5–8), because the amount of nitrous acid decreases and uncharged ammonia increases. This promotes ammonium oxidizers and suppresses nitrite oxidizers.

However, at higher concentration uncharged ammonia and nitrous acid may act as inhibitory factors. Anthonisen et al stated that ammonia oxidizers (AOB) are inhibited at NH_3 concentrations of 8–120 mg N/l and HNO_2 concentrations of 0.2–2.8 mg N/l while inhibition of nitrite oxidizing bacteria (NOB) is observed already at NH_3 concentration of 0.08–0.82 mg N/l and a HNO_2 concentration of 0.06–0.83 mg N/l. However these thresholds are dependent on bacteria adaptation. At higher pH values the free ammonia concentration is higher, limiting the growth of Nitrite Oxidizing Bacteria (NOB) due to their higher sensitivity to free ammonia inhibition than Ammonia Oxidizing Bacteria (AOB) (Anthonisen et al, 1976).

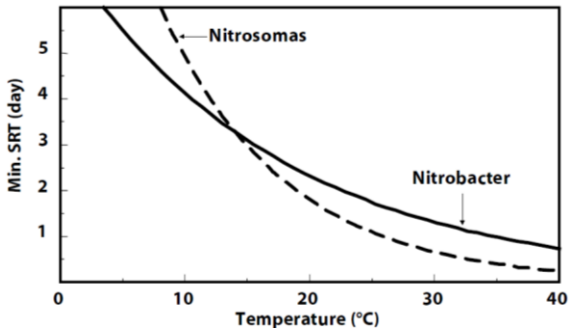

Fig. 5. Minimum residence time for ammonium and nitrite oxidizers at different temperatures (source: Notenboom et al, 2002).

As mentioned by Van Hulle (2010), some authors such as Guisasola et al (2005) and Wett & Rauch (2002) reported a reduction in ammonia oxidizing activity due to bicarbonate limitation and Nowak et al (1996) reported a reduction of nitrite oxidation at phosphate concentrations below 0.2 mg P/l and of ammonium oxidation at 0.03 mg P/l. Some pollutant resulted more inhibitory to the oxidation of nitrite than to the oxidation of ammonium. Some examples are chlorate, formic, acetic, propionic and n-butyric acid, cyanide, azide and hydrazine, bromide and chloride (Van Hulle, 2010).

The first full-scale application was built in Rotterdam-Dokhaven in 1999. Between the years 2000-2005 four full-scale application have been constructed in the Netherland with an average total N removal efficiency of 88% and in 2007 the first one installation was built in New York.

The process can also be run in a single reactor system using intermittent aeration. Fux et al, (2006) obtained 85–90% nitrogen removal by nitritation/denitritation of ammonium-rich sludge dewatering liquor in a SBR with continuous loading (loading rate of 1.2 g NH_4^+-N m^{-3} d^{-1}). High process stability was achieved at a total HRT of 1 day.

1.2.5 ANAMMOX® process

The ANAMMOX® (ANaerobic AMMonium OXidation) process is a novel and promising alternative in which ammonium is directly oxidized to dinitrogen gas using nitrite as the electron acceptor under anoxic conditions (Jetten et al, 1999). This process, although predicted more than 30 years ago (Broda, 1977) on the base of thermodynamic calculations (standard free energy values of chemical reactions), was discovered about 15 years ago, during experiments on a denitrifying pilot plant of a multi-stage wastewater treatment system at Gist-Brocades (Delft, The Netherlands) where it was noted that ammonium

disappeared from the reactor effluent at the expense of nitrate with a concomitant increase in dinitrogen gas production (Mulder et al,1995). Later it was realized that nitrite rather nitrate was the electron acceptor for this reaction.

The overall ANAMMOX reaction is (Strous et al, 1999):

$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 2.03\ H_2O + 0.066\ CH_2O_{0.5}N_{0.15}$

Ammonium is converted to dinitrogen gas with nitrite as electron acceptor in a ratio of 1:1.32, without the need of oxygen or carbon source. Small amount of nitrate are produced (about 10%). An analysis of mass balances by Strous (1998) on an enriched culture of Anammox microorganisms *(Candidatus Brocadia anammoxidans*) showed that the Anammox bacteria uses CO_2 as its carbon source to produce biomass ($CH_2O_{0.5}N_{0.15}$) and that NO_2^- not only functions as an electron acceptor for NH_4^+oxidation, but also as an electron donor for the reduction of carbon dioxide.

^{15}N-labeling experiments showed that hydroxylamine and hydrazine are formed as intermediates. The mechanism involves the partial reduction of nitrite with the formation of hydroxylamine (NH_2OH), which reacts further with ammonium to form hydrazine (N_2H_4). Hydrazine is further converted to nitrogen gas (N_2). This oxidation would give the necessary reducing equivalents for the initial reduction of nitrite (Van de Graaf, 1996; Jetten et al, 1999). The actual substrate for Anammox bacteria is NH_3 rather than NH_4^+.

Anammox bacteria were found in several wastewater treatments plants, in coastal anoxic marine sediments all over the world (e.g. Gullmarsfjorden in Sweden, Skagerrak in the North Sea, Colne Estuary National Nature Reserve in United Kingdom, Greenland Arctic Sea, Mertz Sea in Antarctica, Benguela OMZ in Namibia, Chesapeake Bay in U.S.A.) or anoxic basins (e.g. in the Black Sea and Golfo Dulce, Costa Rica). The presence and activity of Anammox bacteria have been detected in more than 30 natural freshwater and marine ecosystems all over the world (Op den Camp et al, 2006). In the sediments with low organic carbon content, Anammox accounted for 20-79% of total N_2 production (Op den Camp et al, 2006).

The anaerobic ammonium oxidation is carried out by chemolithoautotrophic bacteria belonging to the order *Planctomycetales*. Five genera of Anammox bacteria have been defined so far (Table 9).

The Anammox biomass has a brown-reddish color. These bacteria are characterized by slow maximum specific growth rate (μ=0.00648 d^{-1}) with a doubling time of 10.6 days (Strous et al, 1998; Jetten et al, 1999; van Dongen; 2001) and low biomass yield (0.11-0.13 g VSS/g NH_4^+-N) (Strous et al, 1997). This means that a low amount of sludge is produced but long start-up period (up to one year) (Trigo et al, 2006) are required to grow enough biomass if insufficient seed sludge is available.

The maximum specific nitrogen consumption rate is very high (0.82 gN/gVSS d^{-1}) as well as the affinity for the substrates ammonia and nitrite (K_s<0.1 mgN/l).

The optimum pH range is 6.7-8.3 whereas the optimum temperature is (20-43 °C) (Strous et al, 1999). Anammox activity was observed by Egli et al (2001) only between pH 6.5 and 9, with an optimum at pH 8 and a temperature optimum at 37 °C. A temperature of 45°C causes an irreversible decrease of the Anammox activity due to biomass lysis. The possibility to operate the Anammox process at lower temperature is object of study. Cema et al (2007a) studied the Anammox process at 20 °C in a successfully operating RBC (Rotating Biological Contactor).

Anammox activity is also sensitive to visible light with a decrease in activity of 30 to 50% (van de Graaf et al, 1996).

The reaction is strongly but reversibly inhibited by dissolved oxygen (Jetten et al, 2001). It was noticed that the growth of the Anammox bacteria is reversibly inhibited by low oxygen concentrations between 0.25-2% air saturation (Strous et al, 1997, Egli et al, 2001).

The reaction is irreversibly inhibited by nitrite when NO_2^--N exceeds a concentration of 70 mg NO_2^--N/l for several days (van Dongen et al, 2001). Batch-scale experimental studies on the effects of nitrite inhibition on Anammox bacteria (Bettazzi et al, 2010) showed a short-term inhibition, with more than 25% maximum nitrite removal rate decrease at concentrations higher than 60 mg NO_2^--N/l and losses of activity were detected with nitrite concentrations higher than 30 mg NO_2-N/l. Fux et al (2004) also reported serious inhibition of Anammox activity when nitrite was present at concentrations of 30-50 mg NO_2^--N/l for six days. Other authors obtained different threshold values of nitrite inhibition and there are still ongoing studies on this. It seems also that different Anammox genera show

Table 9 – Microbial species of ANAMMOX bacteria discovered up to date. (Source: Kumar & Lin, 2010; Van Hulle et al, 2010)

Genus	Species	Sources
Brocadia	Candidatus Brocadia anammoxidans	Wastewater
	Candidatus Brocadia fulgida	Wastewater
Kuenenia	Candidatus Kuenenia stuttgartiensis	Wastewater
Scalindua	Candidatus Scalindua brodae	Wastewater
	Candidatus Scalindua wagneri	Wastewater
	Candidatus Scalindua sorokinii	Seawater
	Candidatus Scalindua arabica	Seawater
Jettenia	Candidatus Jettenia asiatica	Not reported
Anammoxoglobus	Candidatus Anammoxoglobus propionicus	Wastewater

different intolerance for nitrite. The inhibition caused by free ammonia is limited and occurs only at high ammonium concentration (> several hundred mg NH_4^+-N/l) (van Haandel & van der Lubbe, 2007). For these reasons, the Anammox process must be operated under conditions of nitrite limitation.

Anammox are chemolithoautotrophs bacteria which utilize inorganic carbon as carbon source, thus the influent bicarbonate concentration is an important factor. Dexiang et al (2007) observed low Anammox activity at bicarbonate/ammonium ratios of 2.3:1.

Anaerobic ammonium oxidation is more than seven times slower than aerobic ammonia oxidation (Strous et al, 1998). Jetten et al (1999) observed that also "classical nitrifiers" *Nitrosomonas sp.* are able to oxidize ammonium under anaerobic conditions, but at a specific rate 25-fold lower than Anammox bacteria.

Many advantages can be obtained by the implementation of the ANAMMOX® process:

- No requirement for external organic carbon source
- Smaller production of excess sludge
- High nitrogen removal
- Smaller reactor footprint (up to 50% less)
- Reduction of energy demand and power consumption up to 60-90% (compared to conventional nitrification/denitrification)
- Reduction of CO_2 emission (up to 90%) because during the process bicarbonate is consumed instead of carbon dioxide produced (as in conventional denitrification). Thus this process has a much lower contribution to greenhouse effect.
- N_2O (strong greenhouse gas with a GWP = 298) is not an intermediate in the Anammox reaction.

In order to remove ammonium nitrogen successfully from wastewater using the ANAMMOX® process, a proper molar nitrite-ammonium ratio (1.32:1) is needed, but such a ratio is rarely encountered in any wastewater and thus Anammox process alone is not advisable in a WWTP but it should always be combined with a preceding aerobic partial nitritation process which can produce nitrite. Anammox process is nowadays studied in combination with other processes such as partial nitrification in one-single reactor or in two separate reactors.

1.2.6 Partial nitritation and ANAMMOX in separate reactors (2-reactor system)

This process is also called "combined SHARON®-ANAMMOX® processes" or "autotrophic nitrogen removal process". The process is run in two reactors in series. In the first aerobic reactor about 50 % of ammonium is partially nitrified to nitrite. The produced nitrite is in turn reduced to nitrogen gas through the ANAMMOX® process in a second anaerobic reactor.

The ideal goal for the first reactor would be to obtain a stable effluent suitable for the ANAMMOX® reactor (i.e. with a molar ammonium/nitrite ratio of 1.32:1 according to the stoichiometry of ANAMMOX® reaction proposed by Strous et al (1999)). In practice, however, this ratio is not produced, but it is kept closer to 1:1 in order to prevent nitrite inhibition in the second reactor by providing an excess of ammonium (Fig. 6.).

The ideal reaction in the first reactor, which produces the 50:50 mixture of nitrite and ammonium is:

$$NH_4^+ + 0.75\ O_2 + HCO_3^- \rightarrow 0.5\ NH_4^+ + 0.5\ NO_2^- + CO_2 + 1.5\ H_2O$$

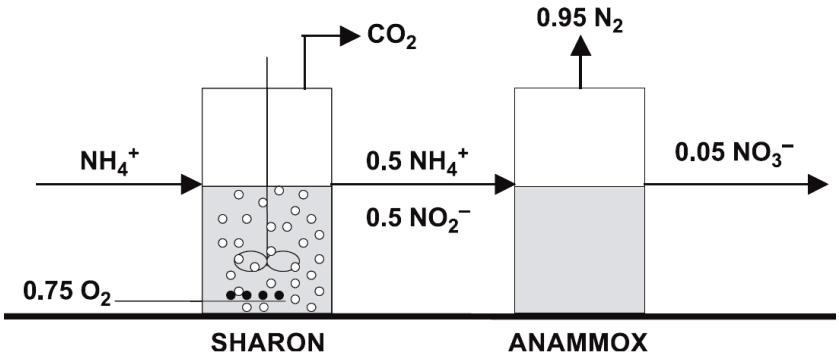

Fig. 6. Schematic representation of combined SHARON®-ANAMMOX® processes (source: Khin et al, 2004).

The operating variables for the SHARON® reactor in order to obtain a stable ANAMMOX-suited effluent are temperature, oxygen conditions, pH, hydraulic retention time and selection of the substrate availability. The sensitivities of ammonium and nitrite oxidizer towards these parameters are different (see paragraph 1.2.4).

Generally the operating conditions in the first reactor are: pH 6.6–7.0, T=30–40 °C, HRT=1 day, no sludge retention (Ahn, 2006). In case of treatment of digester effluent, no extra addition of base is necessary since digester effluent generally contain enough alkalinity.

Absence of inhibiting factors in the ANAMMOX® reactor is important for the successful operation of the combined process.

The combination of a partial nitritation and ANAMMOX® process has been studied and tested by several authors at a lab and pilot scale in recent years (Fux et al, 2002; Van Dongen et al, 2001) with nitrogen removal efficiencies over 80%.

This sustainable alternative allows achieving high saving costs in terms of aeration (40%) and carbon source (100%) respectively, compared to the conventional nitrification–denitrification processes (Van Loosdrecht & Jetten, 1998; van Dongen, 2001). The overall nitrogen removal in the combined process requires less oxygen (1.9 kg O_2/kg N instead of 4.6 kg O_2/kg N), no carbon source (instead of 2.6 kg BOD/kg N) and low sludge production (0.08 instead of approximately 1 kg VSS/kg N) (Van Loosdrecht & Jetten, 1998). Because the combined process does not require any input of external carbon source, the COD and nitrogen removal operations can be optimized and carried out separately, eliminating the need for complex compromises between COD and N-removal as in the conventional N-removal process (Jetten et al, 1997; van Dongen et al, 2001). One possible solution is the adoption of a denitrifying unit (anoxic) before the partial nitrification stage.

Compared to conventional nitrification/denitrification, the combined system partial nitritation/ANAMMOX® in two reactors reduces CO_2 emission by more than 100%, because the combined process consumes CO_2 (Van Loosdrecht & Jetten, 1997). The combined process is 90% less expensive than the conventional nitrification/denitrification processes (Dijkman & Strous, 1999).

For full scale application a CSTR or a SBR are recommended for the partial nitritation step as it is easier to manipulate the SRT (sludge retention time), whereas a biofilm or granular-based bioreactor is preferable since anammox bacteria easily form sludge granules or biofilms obtaining a high biomass concentration in the reactor (Van Hulle, 2010).

1.2.7 Partial nitritation and ANAMMOX in one single reactor (1-reactor system)

This process is called with several names: "Deammonification", "CANON" (Completely Autotrophic Nitrogen removal Over Nitrite), "SNAP" (Single-stage Nitrogen removal using the Anammox and Partial nitritation), "DEMON" or "aerobic/anoxic deammonification".

In order to avoid confusion dealing with this particular system, which is object of study of the present thesis, this process will be called Deammonification or sometimes partial nitritation-ANAMMOX in one single reactor.

Compared with partial nitrification in series, Deammonification process needs only one rector. This implies a small footprint and less investment costs. The disadvantage is the more complex control of the overall process. The two stage deammonification process has lower N_2O emission (Kampschreur et al, 2009) and avoid the risk of high toxic nitrite concentration for ANAMMOX bacteria, but needs a control of the nitrite/ammonium ratio in the inflow to the ANAMMOX reactor and has a higher consumption of alkalinity compared to the one-stage process.

Deammonification process is based on the harmonious co-existence and cooperation of aerobic (AOB) and anaerobic ammonium-oxidizing (ANAMMOX) bacteria in one single reactor. This can be established under oxygen-limited conditions to avoid inhibition of ANAMMOX bacteria by oxygen and to achieve appropriated conditions to obtain partial nitritation. In practice the main systems that can provide the favorable microaerobic conditions for the co-existence of these two bacteria species are the biofilm system (moving bed biofilm reactors, MBBR), reactors with an intermitted aeration (SBR or RBC) or granular sludge.

The ammonium oxidizers (AOB) oxidize ammonium to nitrite. Under low concentration of dissolved oxygen, the growth of nitrite oxidizing bacteria NOB (and subsequent nitrate production) is usually small due to their lower affinity to oxygen compared to AOB and for nitrite compared to ANAMMOX bacteria.

The optimal bulk oxygen concentration in the liquid in a reactor that carries out deammonification with biofilm system may be different case by case and depends mainly on different configuration of the reactors and lnfluent components. In our case, biofilm thickness and density, boundary layer thickness, the COD content of the influent and the temperature need to be considered to decide DO concentration inside the reactor (van Hulle et al, 2010).

In this process part of the ammonium is oxidized into nitrite (partial nitritation), which serves as electron acceptor for NH_4^+oxidation, and the remaining ammonium is converted to dinitrogen gas by ANAMMOX bacteria. The nitrate (NO_3^-) that is produced is primarily due to ANAMMOX bacteria. The presence or activity of nitrite oxidizers (NOB) may affect the global efficiency and a further oxidation of nitrite to nitrate should be prevented or reduced at minimum. Some operation strategies are useful for the process monitoring and are based on different growth conditions of ammonia oxidizers (AOB) and nitrite oxidizers (NOB). As described in paragraph 1.2.4, they are essentially dissolved oxygen (DO), temperature, pH value, free ammonia (FA) and sludge retention time (SRT).

Recent researches by a PhD student from KTH (Cema et al, 2010) demonstrated that the nitrite concentration was the rate-limiting factor for the simultaneous nitritation/Anammox process.

Deammonification process is an autotrophic nitrogen removal which offers a sustainable alternative for treating highly loaded nitrogen streams with an unfavorable carbon to nitrogen ratio (C/N or COD/N), such as reject water from dewatering of digested sludge. In fact during anaerobic digestion fast biodegradable organic content is converted to biogas and, as consequence, only slow biodegradable organic matter will be present in the effluent.

The overall reaction can be approximately written as Third et al (2001):

$1\ NH_4^+ + 0.85\ O_2 \rightarrow 0.13\ NO_3^- + 0.435\ N_2 + 1.4\ H^+ + 1.43\ H_2O$.

As the global process produces H^+ (or similarly consumes HCO_3^-), alkalinity is consumed. It is clear that one candidate wastewater which can be treated by this process is certainly the sludge reject water. If this process is applied to the treatment of reject water from anaerobic digester, usually these wastewaters have enough alkalinity to stand the potential decrease of pH and provide a whole stability of the process.

The first full-scale application with Deammonification process is date back to April 2001 in a moving bed reactor using Kaldnes® carriers at the WWTP of Hattingen (Germany). One reactor with a volume of 104 m^3 and two reactors with a volume of 67m^3 each with a total effective biofilm surface area of 47200 m^2 allow reaching efficiency up to 70-80%. The load is 120 kg N/d and the removal rate is 400 g N m^{-3} d^{-1}. In this case the oxygen concentration is kept below 1 mg/l.

Other full-scale plants for deammonification of reject-water from digested sludge dewatering are currently in operation in:

- Strass (Austria), treating the wastewater of 200.000 population equivalents (load up to 340 kg NH_4^+-N/d) by a sequencing batch reactor (SBR) of 500 m^3 with NH_4^+-N and TN removal efficiencies of 90% and 86% respectively (Wett, 2006).
- Glarnerland-Zurich (Switzerland) where a suspended-growth sequencing batch (SBR) reactor of 400 m^3 treats over 635 kg N/d (ammonium oxidation rates of about 500 g N m^{-3} d^{-1}) with efficiency over 90% (Joss et al, 2009)
- Rotterdam Dokhaven (The Netherlands) (620.000 p.e.) where granular sludge is used and the load is around 700 kg N/d. The reactor is compact (volume = 72 m^3) and with NH_4^+-N and total N removal efficiencies of 95% and 85% respectively. (http://www.paques.nl).

- Himmerfjärden Grödinge (Sweden) (278.000 p.e.), which was started in April 2007. Two reactors of 900 m^3 are run with intermittent aeration (40 min aerobic phase with DO set point about 3-4 mg O_2/l and 20 minutes anoxic phase) and treat 600 kg N/d (removal rates of 300 g N m^{-3} d^{-1}) (http://www.syvab.se).

1.2.8 DENAMMOX process

This process is also called DEAMOX (DEnitrifying AMmonium OXidation) and it is the coupling of denitrification and ANAMMOX® processes. Researches on this process are still ongoing. It can be applied to the treatment of wastewater with high nitrogen concentrations with high organic carbon levels, such as landfill leachate and wastewaters from digested animal waste (Van Hulle et al, 2010).

Denitrifying bacteria and Anammox bacteria do not need oxygen, therefore this process has a high potential in term of costs saving for aeration and DO control. The main issue is the co-existence of these two bacteria in a long-term perspective. As mentioned by Kumar and Lin (2010), denitrifiers have higher growth yield ($Y_{heterotrophs}$ = 0.3 gVSS/gNH_4^+-N, whereas $Y_{anammox}$ = 0.066±0.01 gVSS/gNH_4^+-N).

In wastewaters with high quantities of slowly biodegradable organic carbon such as digested liquor and landfill leachate, heterotrophic denitrifying growth is limited by the low availability of easily biodegradable organic carbon and, as consequence, denitrifiers should not be able to dominate in these systems and outcompete Anammox bacteria.

Beyond certain amounts of organic carbon, Anammox organisms may not longer be able to compete for nitrite with denitrifiers. Moreover, as reported by Van Hulle et al (2010) denitrification reaction (ΔG=−427 kJ/mol) is thermodynamically more favorable than anaerobic ammonium oxidation (ΔG=−355 kJ/mol) of Anammox bacteria.

ANAMMOX reaction produce small amounts of NO_3^- (according to the molar ratio $NO_{3\,out}^-/NH_{4\,in}^+$=0.26) and the co-existence of ANAMMOX and denitrification in one reactor could be an aid to reduce this quantities of nitrates produced in the reactor. Besides this, the denitrification produces nitrite as intermediate which can be used by the Anammox bacteria for the oxidation of ammonium. For this reasons, DENAMMOX process could be a potential valid option for simultaneous nitrogen and carbon removal as claimed by Kumar et al (2010).

Some authors state that Anammox bacteria are not longer able to compete with heterotrophic denitrifying bacteria at COD/N ratio above 2 (Chamchoi et al, 2008).

Another thing to be kept into account is that Anammox bacteria are irreversibly inhibited by low concentrations of methanol (15 mg/l) and ethanol (Güven et al, 2005). Methanol is often used to remove nitrate in a post-denitrification step.

Recently a new process called SNAD (Simultaneous partial Nitrification Anammox and Denitrification) has been developed. The main difference is the addition of the denitrification to the partial nitrification/Anammox process (Chen et al, 2009).

1.2.9 Bio-Augmentation BABE®

BABE stands for Bio-Augmentation Batch Enhanced. The main goals of this process are to remove nitrogen in the concentrated side-stream and to increase the nitrification capacity of the main activated sludge system by "seeding" it with the nitrifiers produced in the BABE reactor (van Haandel & van der Lubbe, 2007) (Fig. 7). BABE® process was developed and designed based on model simulation.

The nitrifier seed enhances nitrification rate and thus complete nitrification occurs at lower SRT. It can be a useful upgrading option for system with limited tank volume or high SRT (i.e. colder climates) and thus limited nitrification capacity.

BABE is a small reactor where nitrifying bacteria are cultivated. It is a reactor continuously inoculated with sludge from the aeration basin and fed with digester effluent. It operates at higher temperatures than the main activated reactor, thus it has a higher nitrogen removal rate.

On the next page a summary comparison of some of the innovative systems is shown (Table 10).

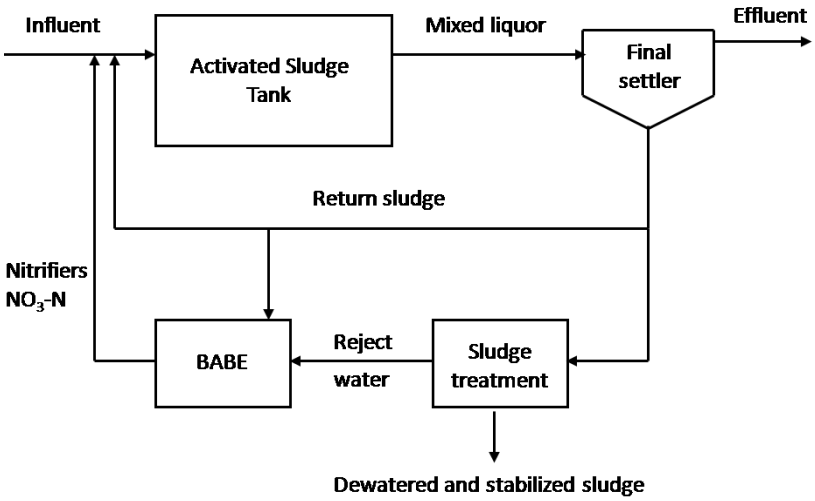

Fig. 7. BABE configuration for reject water from dewatering of digested sludge. (source: Khin et al, 2004).

Table 10 – Comparison of the innovative processes for nitrogen removal (Jetten et al 2002; Ahn, 2006)

Characteristic	Conventional nitrification/ denitrification	Nitritation/ denitritation (SHARON®)	Partial nitritation (50%) and ANAMMOX in two reactors	Partial nitritation and ANAMMOX in one single reactor
Number of reactors	2	2	2	1
Conditions	oxic/anoxic	oxic/anoxic	oxic/anoxic	oxygen limited
Oxygen requirement [gO_2/gN]	4.57 / 0	3.43 / 0	1.71 (1) / 0	1.94
% O_2 saving (2)	-	24.9 %	62.6 %	57.5 %
Alkalinity consumption [$gCaCO_3/gN$]	7.07 / -3.57	7.07 / -3.57 (3)	3.57 / 0.24	3.68
pH control	yes	none	none	none
Carbon source requirement [gCOD/gN] (4)	3.7	2.3	0	0
% reduction in carbon source requirement (2)	-	37.8 %	100 %	100 %
Main bacteria involved	Nitrifiers (AOB,NOB) / denitrifiers	AOB / denitrifiers	AOB / ANAMMOX	AOB / ANAMMOX
Biomass retention	none / none	none / none	none / yes	yes
Sludge production	high	low	low	low

(1) If the partial nitritation was carried on to 60% the oxygen requirement would be 2.06 g O_2/g N.

(2) Compared to conventional nitrification/denitrification.

(3) Alkalinity is produced in the heterotrophic denitrification and denitritation steps.

(4) Based on methanol.

1.3 Moving Bed Biofilm Reactor (MBBR) technology

1.3.1 Introduction of MBBR

The Moving Bed Biofilm Reactor technology (Fig. 8) is sometimes called "Hybrid Fixed Film Process" in case of co-presence of activated sludge. It has been developed to integrate the advantages of both biofilm systems and suspended activated sludge in one process without being restrained by their disadvantages.

It makes use of polymeric carriers, which are kept in continuous movement in the reactor by aeration or simply mixing.

Several type of carriers (Fig. 9) have been developed (e.g. Kaldnes™), with the common goal to provide optimal conditions and a large protected surface area for the biomass, which grows as a biofilm on the surfaces of them.

The MBBR technology does not require any recirculation of the sludge and saves costs for the sedimentation.

The relatively high concentration of maintained biomass allows a higher load-ing rate, which results in reduction in reactor volume or increase of treatment capability within existing basins.

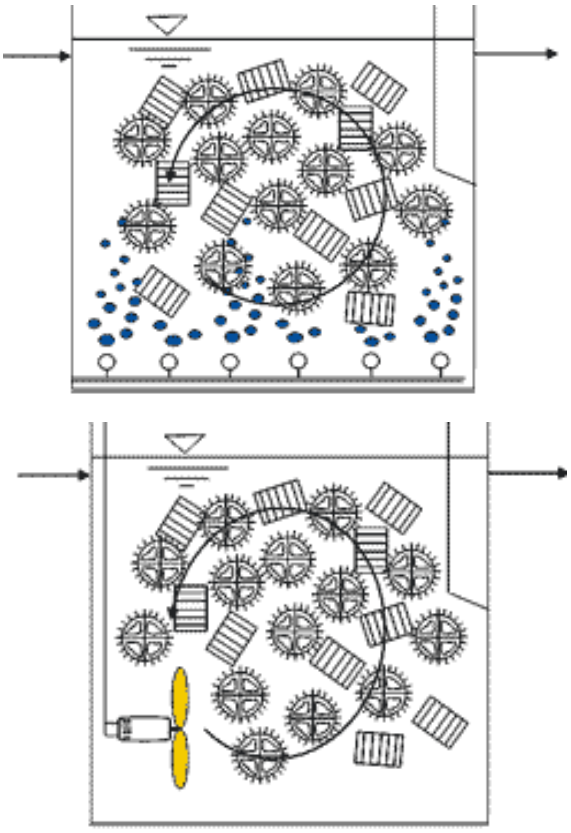

Fig. 8. MBBR - Moving Bed Biofilm Reactor with Kaldnes carriers

The main advantages of this system are:

- Higher biomass concentrations due to biofilm process and carriers with high internal surface area;
- Small reactor footprint (compact system);
- Low sludge generation;
- No sludge return;
- High ammonia removal in a single process;
- Suitable to create anoxic condition in the inner part of the biofilm;
- Possibility to use this technology to enhance or upgrade an existing system (i.e. activated sludge);
- Economical attractive and low investment costs;
- Minimal maintenance and simplicity of operation;
- No media clogging;
- Higher process stability under load variations;
- Lower sensitivity to toxic compounds;
- Customizable reactor shapes;
- Flexibility and suitability to different types of wastewater treatments.

Fig. 9. Different carrier media used inside a MBBR.

1.3.2 Advantages compared with activated and granular sludge and fixed biofilm systems

In the *activated sludge* the biomass is suspended inside the reactors as flocs. A great number of existing wastewater treatment plants makes use of this technology nowadays. However this conventional technology has several drawbacks compared to the recent technologies. It has relatively poor settling characteristics (up to 1 m/h), low permissible dry solid concentration in the aeration tank and low maximum hydraulic load of the secondary clarifier and therefore large footprints are required for the reactors and the sedimentation tanks. Moreover the biomass production is higher compared to the other systems. This technology has lower flexibility related to fluctuating loading rates and it is also vulnerable to high concentrations or shock loads of toxic compounds in the influent.

The *granular sludge* consists of microorganisms which are compacted on dense biomass granules. These granules have fast settling velocity and high biomass density. This technology is usually applied in sequencing batch reactors (SBR) which enable the separation of sludge and effluent inside the reactor itself. The high biomass retention (i.e. high sludge retention time) reduces the sludge production. The high biomass concentration biomass concentration inside the reactor (up to 10÷16 g VSS/l) makes the reactor very compact and with high biomass densities. Another advantage is the improved settling ability (sludge volume index (SVI) <50 ml/g). The sludge volume index (SVI) is the volume in milliliters occupied by 1 g of a suspension after a settling period of 30 minutes.

The diffusive processes are important for this system, as well as for the biofilm systems. Extensive shear stress in the reactor (e.g. mechanical

Table 11 – Advantages and limitations of different reactor configurations.

System	Advantages	Disadvantages
Activated Sludge	- Conventional and common process - Large surfaces	- Usually low sludge settling ability - Foaming and sludge bulking problems - High surplus biomass production - Vulnerable to shock loads or high concentrations of toxic compounds in the influent
Granular sludge	- No need for a clarifier if SBR is used. - Higher biomass retention - No sludge return - Higher settling ability - Co-existence of aerobic and anoxic microorganisms on granules - Highest rate of reaction / m^3	- More complex operation (in case of SBR) - Discontinuous discharge (in case of SBR) - Extensive shear stress may damage granules - Start up period may be long
Fixed film (RBC)	- No need for a clarifier - Low energy consumption - Alternation of oxic and anoxic conditions - Co-existence of aerobic and anoxic microorganisms on the disks	- May need maintenance
Fixed film (Trickling filters)	- No need for a clarifier	- Problems of clogging and maintenance
MBBR	- No need for a clarifier or biomass recirculation - Low sludge production - High specific surface area and higher biomass density - High biomass retention (high sludge age) - Small footprint - Possibility to upgrade existing systems - Co-existence of aerobic and anoxic microorganisms in biofilms - More robust technology and resilient bacteria population - Possibility to handle high loads or temporary limitations - Easy to operate and simple design - Low maintenance required - No problem of clogging	- Longer start up period may be required

mixing, aeration, etc.) might cause detachment of biomass from the granules.

Fixed biofilm systems use a porous medium which provides a static media (a "fixed bed") as support for the biomass film growth. The fixed bed can be made of rocks, gravel, slag, polyurethane foam, sphagnum peat moss, ceramic or plastic media (Wikipedia, 2010). Some examples of fixed film system are the trickling filters or the rotating biological contactor (RBC). The latter consists of disks which rotate slowly on a horizontal shaft and have a 40% of their surface always submerged, thus allowing an alternation of aerobic and anaerobic condition. Fixed film systems have the capacity to handle shock loads. One serious drawback of trickling filters is the clogging and the risks to have septic conditions even under moderate loading conditions.

The *Moving Bed Biofilm Reactor* technology is an attached growth biological wastewater treatment process. It combines the advantages of activated sludge and biofilm systems without being restrained by their disadvantages.

A MBBR operates continuously and it is not affected by problem of clogging that may require need for backwashing or maintenance. Compared to the fixed film system, the moving bed biofilm systems have much higher specific surface area for the biofilm. The specific surface area are 500 m^2/m^3 (Kaldnes K1 media) or 1200 m^2/m^3 (Kaldnes Flat Chip), whereas a trickling filter media has a specific surface area of 46-60 m^2/m^3 (rocks) or 90-150 m^2/m^3 (plastic) and a rotating biological contactor of about 100-150 m^2/m^3 (Weiss et al, 2005). The main reason of this difference lies in the fact that the MBBRs utilize the whole tank volume for biomass and not only the fixed bed. The carriers can occupy up to 70% of the reactor volume on a bulk volume basis. Experience has shown that mixing efficiency decreases at higher percentage fills (Weiss et al, 2005). This process is more robust compared to activated sludge because the biofilm is protected by the biocarriers design. This system has lower sensitivity and better recovery from shock loading. For example it can tolerate higher concentration of NO_2^-. The advantages of MBBR compared to activated sludge are the lower sludge production, low loss of biomass, higher process stability under load variations, no need for sludge return and lower costs.

1.3.3 Kaldnes Moving Bed™ Process

The choice of the media for the Moving Bed Biofilm Reactor is extremely important for a good performance and operation of the reactor over time.

The media should be carefully designed to have a long service life and a large protected internal surface which acts as a carrier for the biomass growth. In these experimental studies the Kaldnes Moving Bed™ process was used.

This particular technology was developed in Norway by Kaldnes Miljøteknologi AS in the late 1980s and early 1990s and it has been patented.

The Kaldnes Moving Bed™ consists of polyethylene rings (or "wheels") with a stable internal cross and a density slightly lower than water (0.95 g/cm^3) which allows easy movement of the carrier material in the completely mixed reactor. The small difference from the water density avoids negative buoyancy effects.

The type of media chosen for the operation is Kaldnes K1 media (Fig. 10) which provides a high specific internal surface area of 500 m^2/m^3. It is shaped like a cylinder with a cross inside the cylinder and fins on the outside. The shape allows a small amount of water to flow through the carrier.

The total surface area (Table 12) consists of both inner and outer surface while the protected surface area is the effective internal area where the biofilm seems to attach and grow as shown in figure 10.

As suggested by Ødegaard et al (2000) the performance of a biofilm reactor is primarily dependent upon the biofilm growth surface area ($kg_{substrate}/m^2_{biofilm\ area}/d$) in the reactor and not on the reactor volume.

In case of the one-stage partial nitritation-ANAMMOX process, it is composed by two

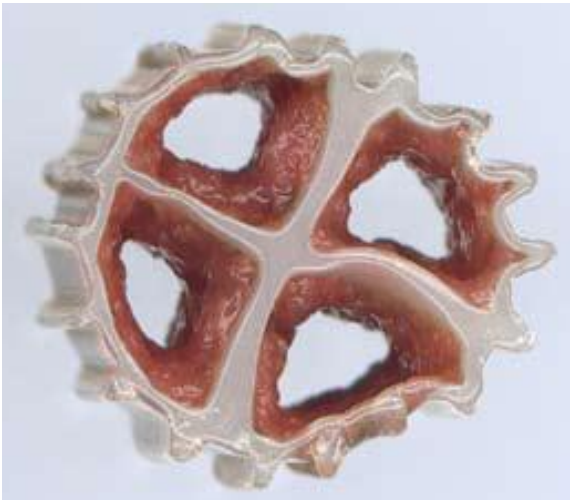

Fig. 10. Kaldnes K1 media™ (Trela et al, 2008)

Table 12 – Kaldnes K1 media™ (source: http://www.anoxkaldnes.com)

Model	Length	Diameter	Protected surface	Total surface
K1	7 mm	10 mm	500 m^2/m^3	800 m^2/m^3

main bacteria culture: the ammonium oxidizing bacteria and the Anammox bacteria.

The ammonium oxidizers are mainly located in the outer layers of the biofilm and they produce the suitable anoxic conditions for the Anammox bacteria sited in the inner layers, by reducing the dissolved oxygen concentration in the bulk liquid and providing nitrites ions necessary for the ANAMMOX reaction. The thickness of biofilm strongly influences bacteria composition; usually the oxygen diffusion is lower in thicker biofilms and therefore a larger anoxic layer is created, where more Anammox bacteria can live and be active. In biofilm systems mass transfer is usually the limiting step. As underlined by Van Hulle et al (2010), as long as ammonium concentrations outside the biofilm are much higher than the oxygen or nitrites concentrations, ammonium diffusion into the biofilm does not limit the process rate. If the nitrites produced in the outer layer are mainly consumed in the inner layer, oxygen is the main limiting factor controlling the overall rate and its bulk concentration in the liquid is crucial. A too high value may inhibit the ANAMMOX reaction and increase the oxidation of nitrite to nitrate by NOB (i.e. Nitrobacter), whereas a too low value may reduce the production of nitrite by AOB (i.e. Nitrosomonas).

As shown if Fig .11, in the outer layer ammonium is converted to nitrite by ammonium oxidizing bacteria (AOB), while Anammox bacteria are active in the inner layer. Anammox bacteria are characterized by a low growth rate and this type of attached growth system (Moving Bed Biofilm reactor) ensures that they are not washed out but are retained inside the reactor, attached at the carriers.

Other bacteria may be present in minor amounts such as nitrite oxidizing bacteria (NOB), denitrifiers (Fig. 11) and other heterotrophic bacteria.

It is difficult to suppress completely nitrite oxidizers even under oxygen-limited concentrations, because it is difficult to subtly manipulate SRT in one-stage partial nitrification-ANAMMOX process. This view is supported by the detection of nitrite oxidizers in some CANON biofilm systems.

In biofilm system (i.e. Moving Bed Biofilm Reactor), the overall process performance is strongly dependent on dissolved oxygen concentration, nitrogen-surface load (ammonium loading rate), temperature, biofilm thickness, pH and nitrite concentrations.

Simplified growth of the main bacteria involved in the partial Nitritation/ANAMMOX process (i.e. ammonia oxidizing bacteria and Anammox bacteria) could be approximately expressed with an equation based on the kinetics model developed by Michaelis-Menten or Monod:

$$\mu = \mu_{max} \frac{S}{K_S + S}$$

where μ_{max} is the maximum specific growth rate, S is the concentration of the substrate (or limiting nutrient) and K_s is the half saturation constant

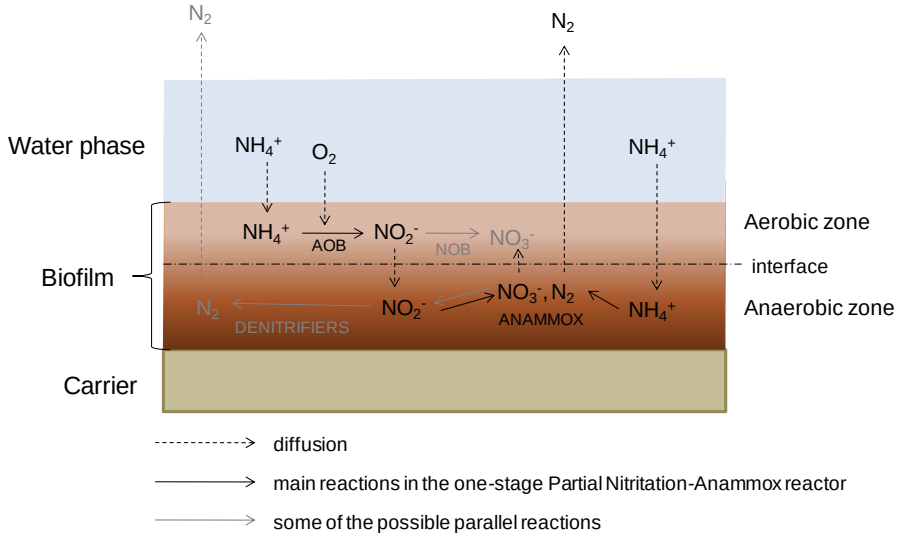

Fig. 11. Simplified scheme of the main reactions within the biofilm

which represents the substrate concentration (in mg/l) at which μ equals $\mu_{max}/2$.

The growth of ammonium oxidizer (AOB) can be expressed as (Van Hulle et al, 2007):

$$\mu = \mu_{\max} \frac{C_{NH_3}}{K_{NH_3} + C_{NH_3}} \cdot \frac{C_{O_2}}{K_{O_2} + C_{O_2}} \cdot \frac{C_{I,NH_3}}{K_{I,NH_3} + C_{I,NH_3}} \cdot \frac{C_{I,HNO_2}}{K_{I,HNO_2} + C_{I,HNO_2}}$$

where C_{NH3} is the concentration of free ammonia (which is the actual substrate), C_{O2} is the oxygen concentration and $C_{I,NH3}$ and $C_{I,HNO2}$ are the concentration of NH_3 and HNO_2 which can inhibit the process at high concentrations. All concentrations are to be considered as concentration diffused into the biofilm.

The growth of Anammox bacteria can be expressed as

$$\mu = \mu_{\max} \cdot \frac{C_{NH_3}}{K_{NH_3} + C_{NH_3}} \cdot \frac{C_{NO_2}}{K_{NO_2} + C_{NO_2}} \cdot \frac{C_{I,O_2}}{K_{I,O_2} + C_{I,O_2}} \cdot \frac{C_{I,NH_3}}{K_{I,NH_3} + C_{I,NH_3}} \cdot \frac{C_{I,HNO_2}}{K_{I,HNO_2} + C_{I,HNO_2}}$$

where $C_{I,O2}$ is the oxygen concentration considered as inhibiting factor for Anammox bacteria.

The bacteria growth rate is thus a function of many factors:

$$\mu = f(C_S, C_{e^- accept}, K_S, K_{e^- accept}, D_S, D_{e^- accept}, T, pH, C_{Inhib}, b)$$

where C_S is the substrate concentration or energy source (NH_3 for both Anammox and Nitrosomonas), $C_{e- accept}$ is the concentration of the electron acceptor (NH_3 for Anammox and O_2 for Nitrosomonas), K_S and $K_{e- accept}$ are the affinity constants for the substrate and electron acceptor respectively, D_S and $D_{e- accept}$ are the diffusion coefficients in the biofilm, T is the temperature, C_{inhib} are the inhibitory factors such as free HNO_2, NH_3, toxic compounds or O_2 for Anammox bacteria and b is the biomass decay coefficient.

Below a comparison of the main physiological parameters of different bacteria populations (Table 13) is given, although the differences that can be found among different reactors configurations.

Table 13 – Physiological parameters of different bacteria populations

Parameter	AOB	NOB	Heterotrophs	ANAMMOX
pH range	6.5-8.5 (6)	6.5-8.5 (6)	7.5-9.1 (3)	6.7-8.3 (2)
Optimum pH	7.9-8.2 (5) 7.8-8.0 (6) 7.6-7.8 (7)	7.2-7.6 (5) 7.3-7.5 (6) 7.9 (8)	6.5-7.5 (9) 7.0-7.5 (10)	8.0 (4)
T range [°C]	5-42 (11)	5-42	20-35	20-43 (2)
optimum T [°C]	25-30 (6) 35 (8)	25-30 (6) 38 (8)		37 (4)
Free energy [kJ/mol substrate]	-275 (1)	-74 (13)	-427 (12)	-357 (1)
Biomass yield [g protein / g NH_4^+-N] [molC / mol NH_4^+] [gVSS / gN]	0.1 (1) - 0.127 (17)	- - 0.056 (17)	- - 0.3 (32)	0.07 (1) 0.066 (14) 0.11-0.13 (18)
[g $COD_{biomass}$ / gN]	0.15 (15) 0.18 (17)	0.04 (15) 0.08 (17)	0.67* (17) 1.6-1.8 **	-
Aerobic rate [mmol NH_4^+ h^{-1} mg $protein^{-1}$]	12-36 (1)	-	-	0 (1)
Anaerobic rate [mmol NH_4^+ h^{-1} mg $protein^{-1}$]	0.12 (1)	-	-	3.6 (1)
Maximum specific growth rate μ_{max} [d^{-1}]	0.96 (11) 1.08 (15) 0.6-0.8 (16) 1.21 (17) 1.39 (22) 0.98 (24) 0.66-0.77 (28)	2.6 (15) 0.6-1.0 (16) 1.02 (17) 0.91 (23) 0.79 (25)	3-6 (20) 5-10 (21) 8.42 (17)	0.0648 (14)
Doubling time [d]	0.73 (1) 0.29-0.33 (27) 0.33-1.46 (33)	0.42-0.54 (27) 0.5-1.63 (33)	-	10.6 (1)

Table 13 – Physiological parameters of different bacteria populations (continued)

Affinity constant K_{NH4+} [mg/l]	0.09-46.8 (1) 0.96 (24) 0.60 (29) 0.48-1.62 (28)	n/a	n/a	0.09 (1)
Affinity constant K_{NO2-}[mg/l]	n/a	0.24 (34)	-	<0.23 (1)
Affinity constant K_{O2} [mg/l]	0.32-1.6 (1) 0.74 (9) 0.03-1.3 (17) 0.24-1.22 (28)	0.94 (24) 1.75 (9) 0.3-2.5 (17) 1.1 (31)	0.2 (26)	n/a

n/a = not applicable.
* = g biomass COD /g COD;
** = g biomass COD /g NO_3^--N

(1) Jetten et al, 2001.
(2) Strous et al, 1999.
(3) Manuale dell'Ingegnere 84 ed., 2003.
(4) Egli et al, 2001.
(5) Paredes et al, 2007.
(6) Ramadan A. E. K., 2007
(7) Holt et al, 1993.
(8) Grunditz and Dalhammar, 2001.
(9) Wang et al, 2009.
(10) Gerardi, 2002.
(11) Ahn, 2006.
(12) Van Hulle et al, 2010.
(13) http://nitrification.org/
(14) Strous et al, 1998
(15) Ahn et al, 2008.
(16) Henze, 2002.
(17) Jubany Güell I., 2007. At 25°C and pH 7.5
(18) Strous et al, 1997.
(19) Van Hulle, 2005.
(20) Henze et al, 2002. With organic matter in wastewater.
(21) Henze et al, 2002. With methanol.
(22) Calculated as:
$\mu_{max} = 2.22 \cdot 10^{11} \cdot e((-6.5 \cdot 10^4)/(8.314 \cdot (T + 273.15)) \cdot (8.21/(8.21 - 1 - 10^{|7.23 - pH|})$
[d^{-1}] at pH 7.5 and T=25°C. (Volcke et al, 2007)
(23) Calculated as:
$\mu_{max} = 4.5 \cdot 10^{11} \cdot e((-4.5 \cdot 10^4)/(8.314 \cdot (T + 273.15)) \cdot (8.21/(8.21 - 1 - 10^{|7.23 - pH|})$
[d^{-1}] at pH 7.5 and T=25°C. (Volcke et al, 2007)
(24) Van Hulle et al, 2007.
(25) Wiesmann, 1994. At 20°C.
(26) Henze et al, 2000.
(27) Philips et al, 2002.
(28) Park and Noguera, 2007.
(29) Helliga et al, 1999. At 35°C and pH 7.
(30) Brion & Billen, 1998.
(31) Zhang et al, 2008.
(32) Kumar & Lin, 2010.
(33) Prosser, 2005.
(34) Kornaros et al, 2010.

2 AIM OF THE PRESENT STUDY

This study is carried out with the main objective to better understand and evaluate the performance - on a lab and pilot-scale - of partial Nitritation and Anammox in one single reactor, which has still few full-scale installations in the world.

General aims regarding the partial nitritation/Anammox process are:

- Review literature and recent publications about innovative nitrogen removal from wastewater;
- Get familiar with laboratory-scale and pilot plant reactors operation and understand the optimal conditions for bacteria growth and an efficient nitrogen removal in the one-stage partial nitritation/Anammox process;
- Evaluation of the process performance by chemical analyses, physical parameters monitoring and biomass measurements. Perform calibrations and cleaning of the portable and on-line instruments;

With specific regard to the laboratory scale experiments:

- Check different nitrogen removal possibilities of partial nitritation/Anammox process under different nitrogen influent loads and different type of influent wastewater;
- Monitor the evolution of Anammox bacteria activity through SAA (Specific Anammox Activity) tests;

With specific regard to the pilot scale experiments:

- Try to find some correlations between physical parameters and chemical analyses results;
- Assess the evolution of Anammox bacteria (Specific Anammox Activity) in the biofilm;
- Assess the evolution of Nitrosomonas, Nitrobacter and Heterotrophic bacteria activity in the biofilm through OUR (Oxygen Uptake Rate) tests;
- Assess the Nitrate Uptake Rate (NUR) by the biofilm and its evolution;

- Compare the activities of different bacteria in the biofilm with their activity in the activated sludge.

In the next chapter material and methods used for the experimental studies are described.

In the fourth and fifth chapters the results from the lab and pilot-scale reactors respectively will be discussed and analyzed in detail.

In the last section conclusions will be drawn and recommendation for full-scale installation and further research will be presented.

3 MATERIAL AND METHODS

This chapter gives a brief introduction of Hammarby Sjöstadsverk research facility where the research studies discussed in this master thesis were carried out in the period of time between April and September (chapter 3.1).

An overview of the parallel research studies undertaken within the present thesis are shown in chapter 3.2.

The following chapters deal with the methodology and materials used for these studies, such as:

- instruments, materials and procedure used to monitor and follow the reactors operation (chapter 3.3);
- materials and methods for analytical measures such as chemical analyses and suspended solids measurements (chapters 3.4 and 3.5);
- methodology for batch test carried out on the biomass such as OUR, NUR and SAA (chapter 3.6).

3.1 Hammarby Sjöstadsverk research facility

Hammarby Sjöstadsverk is a research and demonstration facility for wastewater treatment. It was built in 2003 and it is located on top of Henriksdals WWTP, in Stockholm. Henriksdals underground WWTP is the biggest in Sweden and serves a population equivalent of 700.000.

The plant Hammarby Sjöstadsverk is owned and operated by a consortium lead by the Royal Institute of Technology (KTH) and IVL Swedish Environmental Research Institute.

The facility contributes to develop knowledge and skills in water treatment and it is used for development and demonstrations of new solutions and equipment for industries and partners and for researching and testing more sustainable and effective technologies in the wastewater purification field. The main activities at Hammarby Sjöstadsverk consist of research and development on water treatment technology and biogas production (source: IVL Swedish Environmental Research Institute).

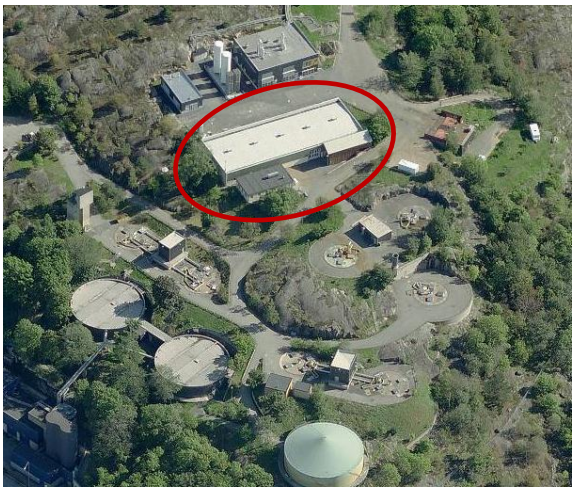

Fig. 12. Hammarby Sjöstadsverk research facility from above (source: http://sjostad.ivl.se)

At Hammarby Sjöstadsverk there are five parallel lines in pilot plant scale, three main lines with a capacity of 1-2 m^3/h (i.e. line 1: Aerobic treatment with activated sludge and biological nitrogen and phosphorous removal; line 2: Aerobic treatment with membrane bioreactor and reverse osmosis; line 3-4 combined: Anaerobic treatment with UASB, a line for sludge treatment (line 5) and an anaerobic membrane bioreactor (MBR) (line 6).

Hammarby Sjöstadsverk is also used for education, including a fair number o different degree projects and PhD thesis, and for collaboration with national and/or international research programs/projects and consultancy.

Several research projects are currently underway at the facility, including the project "*Control and optimization of the deammonification process*" under the leadership of Jozef Trela and Elzbieta Plaza, from

Fig. 13. Hammarby Sjöstadsverk research plant - part of the treatment line 1 (source: http://www.sjostadsverket.se/; photo: Per Westergård)

KTH (http://www.sjostadsverket.se/) within which this master thesis has been developed.

KTH has been conducted research on the deammonification process since 1999, leading to the first full-scale plant in Scandinavia, at Himmerfjärds WWTP, south of Stockholm, in Södertälje municipality.

The main goal of this new project is to gather new knowledge, test different operation strategies and determine the optimal parameters for an efficient nitrogen removal with deammonification process. This thesis is part of this project and it is focused on the one-step partial nitrification/Anammox process in the moving bed biofilm reactor (MBBR) with Kaldnes carriers.

3.2 Overview of the experimental strategy

An overview of different studies undertaken within the present thesis is shown in figure 14. During the period 23rd March – 25th July two different laboratory scale reactors were operated whereas the pilot plant-scale reactor was started the 27th May and followed for four months from its start-up.

The main analyses carried out and the main parameters studied in each reactor operation are briefly summarized in Table 14.

Table 14 – Experimental work and analyses carried out.

Reactor	Monitoring parameters and analytical measures	Batch Tests
Laboratory-scale reactor treating reject water diluted 1:2.5	*Inflow*: pH, conductivity, alkalinity, COD, NH_4^+-N, TOT-P, inflow rate. *Outflow/Reactor*: pH, conductivity, DO, T, alkalinity, COD, NH_4^+-N, NO_2^--N, NO_3^-N, TOT-P, TSS/VSS (biocarriers), TSS/VSS (activated sludge).	SAA
Laboratory-scale reactor treating effluent from UASB reactor and sand filtration	*Inflow*: pH, conductivity, alkalinity, COD, NH_4^+-N, TOT-P, inflow rate. *Outflow/Reactor*: pH, conductivity, DO, T, alkalinity, COD, NH_4^+-N, NO_2^-N, NO_3^-N, TOT-P, TSS/VSS (biocarriers), TSS/VSS (activated sludge & influent).	SAA
Technical-scale pilot plant reactor treating reject water from sludge dewatering after anaerobic digestion	*Inflow*: pH, conductivity, ORP, alkalinity, COD, NH_4^+-N, NO_2^--N, NO_3^-N, TOT-N, $CBOD_5$, TOT-P, inflow rate. *Outflow/Reactor*: pH, ORP, DO, T, conductivity, alkalinity, COD, NH_4^+-N, NO_2^--N, NO_3^-N, TOT-N, TOT-P, TSS/VSS (biocarriers), TSS/VSS (activated sludge & influent).	SAA, OUR, NUR

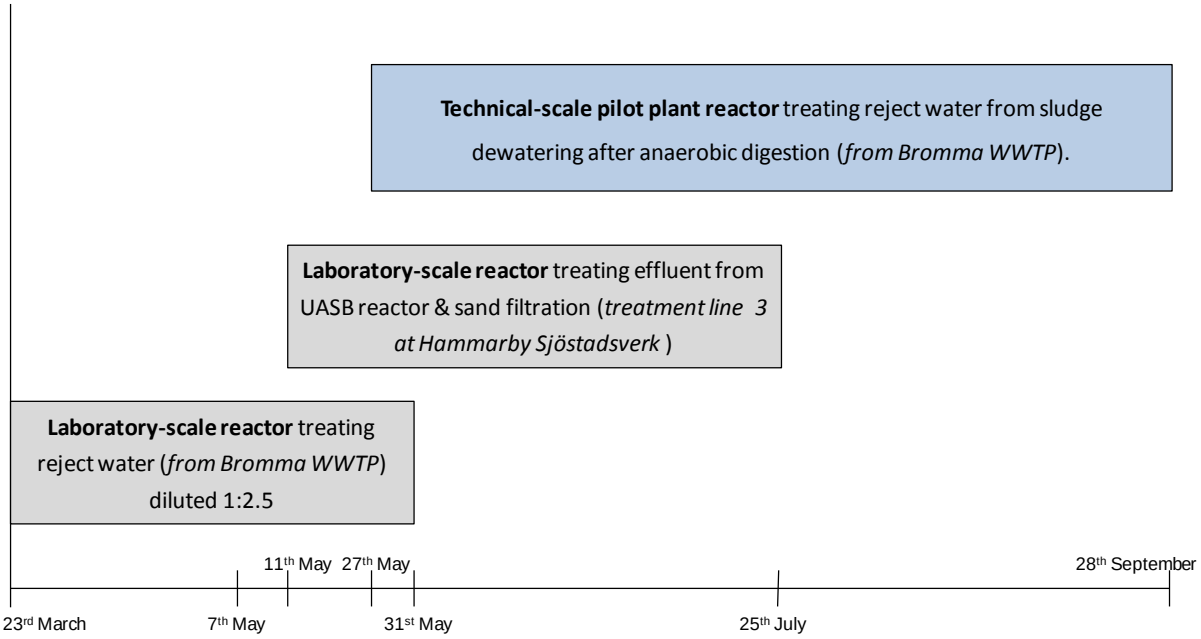

Fig. 14. Different studies on 1-stage partial Nitritation/Anammox process during the experimental work carried out at Hammarby Sjöstadsverk

3.3 Physical parameters monitoring

3.3.1 Parameters description

The physical parameters were measured to keep the reactor operation under control and in the optimum range for bacteria and biological reactions. Some of the parameters were mainly used as a monitoring tool for the conditions in the reactor but were never corrected (redox, potential, pH, conductivity), whereas other parameters (DO, inflow rate) were used to actively control the process. These parameters are briefly described below.

pH. The pH is a measure of the concentration of hydrogen ions (H^+ or H_3O^+) in a solution and it is mathematically defined as: $pH = -\log\{H^+\}$, where $\{\ \}$ denote activity. The activity is the molar concentration (expressed as mol/l) multiplied by the activity coefficient γ. For diluted solutions, activity is identical to concentration. The pH represents the degree of acidity or alkalinity of a solution and its scale ranges between 0 and 14. Its value is influenced by temperature. It is an important parameter to provide suitable conditions for bacteria and biological reactions. Constant pH values may be indicative of overall stability of the process.

Dissolved Oxygen (DO). The dissolved oxygen concentration (mg/l) is a key parameter for biological reaction and its concentration can enhance a reaction rather than another, or even inhibit a reaction as in the case of Anammox or denitrification. If dissolved oxygen in the bulk liquid is high, nitrifying bacteria can be very active and dissolved oxygen diffusion in the biofilm is higher, leading to inhibition of Anammox bacteria. Dissolved oxygen is provided by aeration, which is one of the biggest costs in wastewater biological treatments. DO concentration is influenced by the temperature (inversely proportional) and atmospheric pressure (directly proportional).

Oxidation Reduction Potential (ORP/redox). The ORP is also called redox potential or indicated as E_h. It is a measure that can be useful for determining the oxidizing or reducing conditions of a solution. It is measured in millivolts (mV) or volts (V). Reduced substances in water predominate when the redox potential is negative and oxidized substances predominate when the redox potential is positive. In biological wastewater treatment the ORP is a useful tool to assess the aerobic/anoxic/anaerobic condition in the reactor. A low and negative ORP (<200 mV) indicates anaerobic and methanogenic conditions whereas a high and positive value (above 200 mV) are usually typical of aerobic activated sludge processes. The ORP could also be used as a monitoring tool in a low dissolved oxygen wastewater treatment process (Holman and Warehem, 2000). ORP is dependent on temperature and aeration, organic substrate and activity of microorganisms in the reactor.

Conductivity. The electrical conductivity of a solution is a measure of the ionic activity in term of its capacity to transmit current. It is proportional to the total amount of ions, their valence and temperature. It is usually measured in μS/cm or mS/cm. In these studies it was used as an easy and direct monitoring parameter as indicator of process performance and ammonium removal. As suggested by Szatkowska et al (2007c) and Levlin (2007), these monitoring parameters can be used for wastewater treatment that causes changes in total salt concentration (and thus in conductivity) as in the case of partial nitrification and Anammox process where the main ions – NH_4^+ and HCO_3^- – are converted to CO_2 and N_2.

Temperature. The temperature was another parameters for bacteria activity and rates of biological reactions. It was kept constant at about 25°C during the reactors operations. Temperature of the system may be a problem in cold areas during winter season and could represents a cost if heat need to be supplied to the system.

Inflow rate. The inflow rate (l/d) is closely related to the nitrogen loading rate and determines the hydraulic retention time at a fixed volume of the reactor. A too high or too low inflow rate can significantly influence the efficiency of the whole process. The inflow rate was accurately checked manually with a graduated cylinder, by measuring the volume of the influent wastewater after 3 minutes.

3.3.2 Measurements in laboratory-scale studies

Measurements of physical parameters in laboratory-scale studies were carried out manually three times per week if possible. The instruments which were used are listed below:

- pH: WTW pH 330i with WTW SenTix 41 probe;
- DO: Hach Lange HQ 30d flexi together with Hach Lange LDO (Dissolved Oxygen Luminescent) 101 sensor and/or YSI Model 57 Oxygen Meter with YSI 5905 BOD probe.
- Conductivity: WTW Cond 330i with WTW tetraCon 325 sensor;

- Temperature: WTW Cond 330i with WTW tetraCon 325 sensor or Hach Lange HQ 30d flexi together with Hach Lange LDO (Dissolved Oxygen Luminescent) 101 sensor or a digital thermometer with precision ± 0.1 °C.

The pH was calibrated once a month, whereas the conductivity meter was calibrated only at the beginning of the experimental studies (as it was advised a calibration every six months). The DO meter was calibrated each measurement. The ORP was not measured in the lab-scale studies.

3.3.3 Measurements in pilot plant-scale studies

Regarding the pilot plant-scale reactor, the parameters were all measured online and data were logged every 10 seconds. The control boxes installed were two Cerlic BB2 central units (Fig. 15) equipped with one Cerlic pHX sensor, two Cerlic ReX sensors (one for inflow and one inside the reactor) and one Cerlic O2X Dissolved Oxygen sensor (Fig. 16). The conductivity was measured by Dr Lange Analon Cond 10 Conductivity Monitor unit. The DO concentration and the air flow supplied were controlled by a Samson Trovis 6493 Compact PID Controller with an electropneumatic actuator Samson type 3372 and valve Samson type 3321. The temperature was controlled by a Jumo dTRON 316 microprocessor PID controller. A float was used to monitor the water level for safety reasons: if the flow falls below a certain level, then the heater would stop.

Regarding the DO, only the air calibration was done, whereas the conductivity sensors were calibrated with air and in few occasions with standard solution 10 mS/cm. In the last six weeks it was not possible to do the calibration with standard solution anymore, probably because of their usage. The Cerlic O2X sensor, however, do not need a frequent calibration, as it is written in the manual (once every six month). The inflow rate was checked between once and thrice per week.

Fig. 15. Online measurements and control panel for the pilot plant-scale reactor.

3.4 Chemical analyses

Regarding the laboratory scale reactors, the chemical analyses were usually per-formed once per week for both inflow and outflow and within a time equal to the hydraulic retention time of the reactor. Regarding the pilot plant operation the in-flow was analyzed once per week and the outflow twice per week, in order to monitor the performance more frequently. The two chemical analyses on the outflow were usually performed

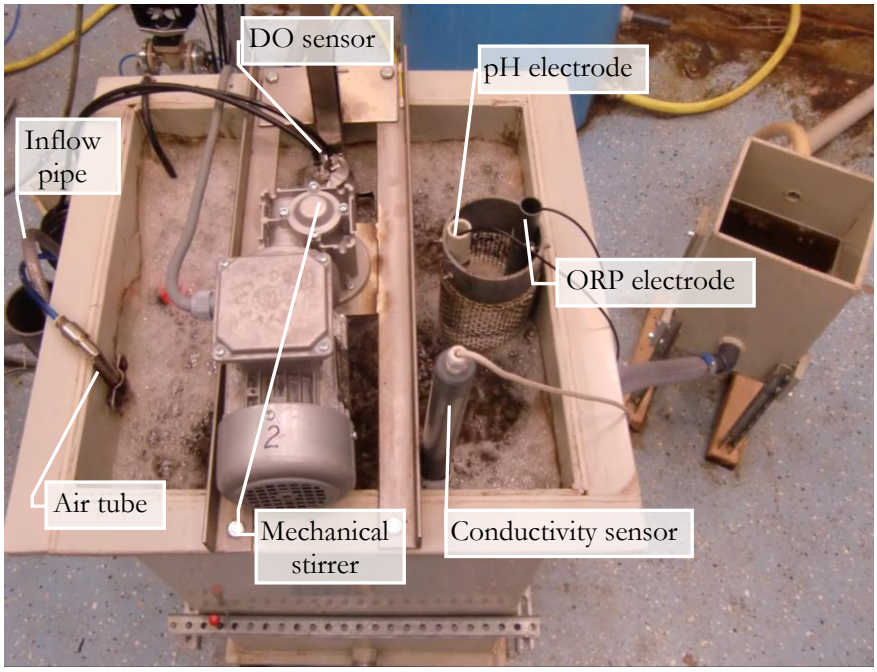

Fig. 16. Monitoring instruments for the pilot plant-scale reactor.

Fig. 17. Dr. Lange Cuvette Tests used for chemical analyses.

two and four days after the measurement on the inflow to the reactor.

The samples were taken from the outflow tank/vessel after 20-30 minutes having emptied it and filtered soon afterwards.

Dr. Lange Cuvette Tests (Fig. 17) were used for the chemical analyses. The samples were filtered with Schleicher & Schuell membrane filters 0.45 μm (mixed cellulose ester). If the chemical parameters were outside the measuring range, dilution with distilled water was done. In a few occasions samples were filtered, frozen and analyzed the following day. The cuvettes were evaluated with the Dr Lange XION 500 spectrophotometer. Hach Lange Thermostat LT200 was used for COD, TOT-N and TOT-P measurements.

The measurements on unfiltered samples were done after having mixed carefully the samples. The pipettes were checked regularly.

The cuvettes used are listed below:

- NH_4^+-N LCK 305, 1-12 mg/l
- NH_4^+-N LCK 303, 2-47 mg/l
- NH_4^+-N LCK 302, 47-130 mg/l
- NO_2^--N LCK 342, 0.6-6 mg/l
- NO_3^--N LCK 339, 0.23-13.50 mg/l
- NO_3^--N LCK 340, 5-35 mg/l
- TOT-N LCK 338, 20-100 mg/l
- TOT-P LCK 350, 2-20 mg/l
- Acid Capacity $K_{S\ 4.3}$ LCK 362, 0.5-8.0 mmol/l
- COD LCK 314, 15-150 mg O_2/l
- COD LCK 514, 100-2000 mg O_2/l

The $CBOD_5$ was measured only once on the influent reject water to the pilot reactor according to the procedure described in Standard Method 5210 B (5-day BOD Test). The bottles used were 286 ml capacity and no seeding was needed. The only difference is that, among the reagents NH_4Cl was not added because ammonium was already present in the reject water and $MgCl_2$ was added instead of $MgSO_4$. pH was adjusted to 7.2 with sulfuric acid (H_2SO_4) and ATU was used as nitrification inhibitor. Dilutions were prepared in graduate cylinders.

3.5 Suspended solids measurements

Suspended solids measurements were carried out in order to estimate the total and the volatile suspended solids contents for both the biofilm developed on the carriers and the liquid inside the reactor. The suspended solids were measured using filters with pore size 1.6 μm, because due to problem with purchase was not possible to carry out the analyses with filters with pore size 0.45 μm. The filtration was done by a vacuum filter. The digital scale used for weighting was Acculab LA-series with a precision of ± 0.1 mg. The micro-glass fiber filters used were Munktell MG A type. The aluminum plates used did not show any loss of weight after having been left for 40 minutes at high temperatures. In one occasion a box of filters show a decrease of weight, especially at high temperatures (-0.06 %). A sort of calibration was done on eight filters in order to estimate the average weight loss and correct the results afterwards.

3.5.1 Total and volatile suspended solids as biofilm

The total suspended solids (TSS) and the volatile suspended solids (VSS) content of the biofilm were estimated averaging the results from 4 sample rings taken randomly from inside the reactor according to the procedure described in APPENDIX I.

3.5.2 Total and volatile suspended solids in the influent and inside the reactor

The total suspended solids (TSS) and the volatile suspended solids (VSS) were measured according to Standard methods 2540 D (Total Suspended Solids Dried at 103-105°C) and 2540 E (Fixed and Volatile Solids Ignited at 550°C) as described in APPENDIX I.

The measurement of VSS (or sometimes indicated as MLVSS – Mixed Liquor Volatile Suspended Solids) is a rough approximation of the amount of organic matter present in the solid fraction. In presence of activated sludge it can be an estimation of the biomass concentration. However this conventional suspended solid measurement includes both the living biomass

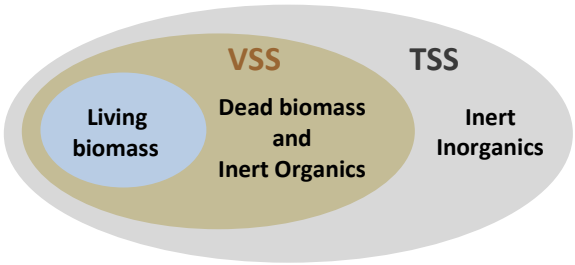

Fig. 18. Suspended solids classification (modified from Whalen, 2007).

and the dead biomass and inert organics as drawn in figure 18.

New molecular techniques such as fluorescent in situ hybridization (FISH), RNA analysis, DAPI staining, and ATP analysis make possible to measure directly and quantify the metabolically active fraction of the activated sludge mixed liquor.

3.6 Batch tests

Suspended solids measurements were carried out in order to Batch tests were performed in order to assess the activity of different bacteria populations present in the reactor and measure the Anammox activity (SAA), the oxygen and nitrate uptake rates (OUR and NUR). With regard to the pilot plant-scale reactor, which was followed for a longer period of time, the aim was also to assess the presence of any trend in the bacterial activity.

3.6.1 Specific Anammox Activity (SAA) test

The SAA test has the aim to evaluate the Anammox bacteria activity. The tests were performed according to the methodology described by Dapena-Mora et al (2007). These batch tests are based on the measurement of the increment of pressure inside a closed volume, proportional to the production of nitrogen gas by ANAMMOX bacteria which use nitrite and ammonium as their substrates (Strous et al, 1999):

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 2.03\ H_2O + 0.066\ CH_2O_{0.5}N_{0.15}$$

The analyses were carried out in vials with a total volume of 38.0 ml and a volume of liquid of 25.0 ml, sealed tightly with rubber caps. The gas phase was therefore equal to 13.0 ml.

Each vial was filled with phosphate buffer solution (0.14 g/l KH_2PO_4 and 0.75 g/l K_2HPO_4) and 15 Kaldnes rings, which have been previously washed twice with buffer solution (Fig. 19). The total volume of phosphate buffer solution and the 15 rings was always equal to 24 ml,

Fig. 19. Vial with 15 Kaldnes carriers after SAA analysis.

in order to have a constant gas phase. The initial pH value was about 7.8.

The vials were closed and oxygen in the liquid phase was removed by supplying N_2 gas for about three minutes by means of a needle inserted through the septum. Another needle was used to remove the gas excess from the vials.

concentrations of NH_4^+-N and NO_2^--N inside the bottles were 70 mg N/l. This concentration is not inhibiting according to Strous et al (1999) and Dapena-Mora et al (2007).

Then the vials were tightly closed and the pressure was equalized to the atmospheric one with another needle. From that moment the test was started. The pressure in the headspace was monitored with a time frequency depending on the biomass activity (usually 30 minutes) by means of a pressure transducer (Centrepoint Electronics model PSI. 5) that measures the overpressure in a range from 0 to 5 psi. The duration of the test was about 2 hours. The final pH value was measured only in the first test and it was between 8 and 8.08, thus in the optimal range for the Anammox activity. In the first months the SAA test on the rings from the pilot plant reactor was performed at both 25°C and 35°C. Regarding the lab-scale studies SAA was carried out only at 35°C.

The total amount of N_2 gas produced was calculated from the overpressure measured in the headspace of each vial by using the ideal gas law equation (Dapena-Mora et al, 2007). Ammonium, nitrite and nitrate concentrations removed from the liquid phase (or produced, in the case of nitrate) were not measured in these experiments. The accuracy of the test was estimated by Dapena-Mora et al (2007) which measured the

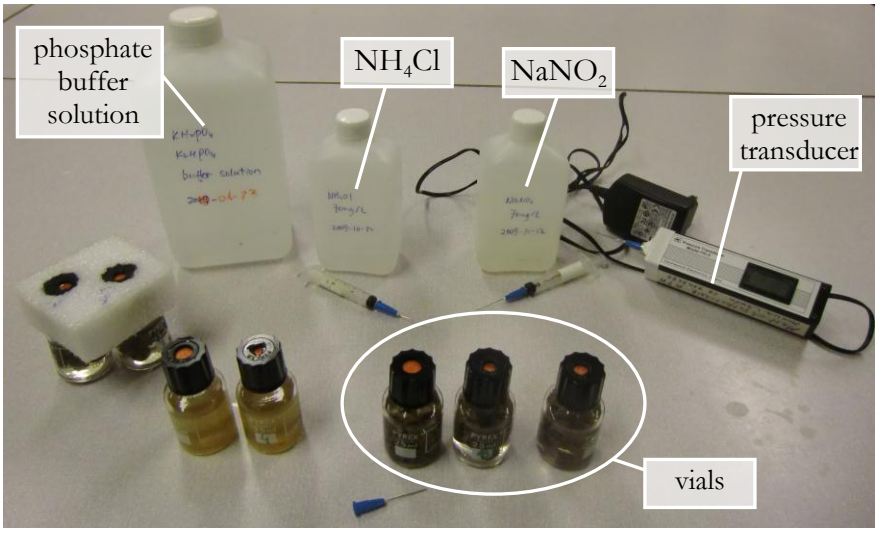

Fig. 20. SAA material and instruments.

average errors from the balances and resulted lower than 7%. Analysis of the produced biogas composition by Dapena-Mora et al (2007) indicated that more than 99% of the produced gas was N_2.

The main inconvenient which may occur is the nitrogen gas leakage from the cap if it has been worn out by use. In those cases, the linearity of the curves (pressure vs. time) obtained from the experimental measures, show a fall or a sharp change of slope and the data from those particular tests are not reliable.

A potential limitation of SAA test and its results might be related to the possibility of denitrifiers to produce nitrogen gas N_2 (and other gases such as nitrous oxide N_2O and nitric oxide NO) by consuming NO_3^- (that is produced by Anammox bacteria) or NO_2^- and the COD stored by the biomass, as electron donor. If this takes place, the estimation of the nitrogen gas production might be slightly overestimated.

The values of pressure measured by the pressure transducer were given in mV. These values were converted to mmHg by multiplying by a factor equal to 2.65 based on a calibration.

The correctness of this value was later checked

Table 15 – Calculations for SAA tests on the biocarriers.

Result	Unit	Formulas
N_2 gas production rate (dN_2/dt)	$\frac{mol\ N_2}{\min}$	$\frac{dN_2}{dt} = \frac{\alpha \cdot V_G}{R \cdot T}$
SAA (Specific Anammox Activity)	$\frac{g\ N_2}{m^2\ d}$	$SAA = \frac{\frac{dN_2}{dt} \cdot 28}{S_{biofilm}} \cdot 60 \cdot 24$
SAA (Specific Anammox Activity)	$\frac{g\ N_2}{g\ VSS\ d}$	$SAA = \frac{\frac{dN_2}{dt} \cdot 28}{X} \cdot 60 \cdot 24$

α = slope of the pressure increase inside the vial plotted versus time (atm/min);

V_G = volume of the gas phase (0.013 l), calculated by subtracting the volume of liquid with 15 biocarriers (25 ml) from the total volume of the vial (38 ml):

R = ideal gas constant 0.0820575 (atm l mol^{-1} K^{-1});

T = temperature (K);

28 = molecular weight of N_2 (g N/mol);

60 and 24 = unit conversion factors from min to days.

$S_{biofilm}$ = surface area of 15 biocarriers = $7.00935 \cdot 10^{-3}$ m^2, calculated as the product of the specific area of Kaldnes media and the volume occupied by 15 rings (calculated by proportion on the base of the measurement that 107 rings occupy 100 ml):

$$S_{biofilm} = 500 \frac{m^2}{m^3} \cdot V_{15rings}$$

X = grams of biomass attached on 15 rings;

Table 16 – Calculations for SAA tests on the activated sludge.

Result	Unit	Formulas
N_2 gas production rate (dN_2/dt)	$\frac{mol\ N_2}{\min}$	$\frac{dN_2}{dt} = \frac{\alpha \cdot V_G}{R \cdot T}$
SAA (Specific Anammox Activity)	$\frac{g\ N_2}{g\ VSS \cdot d}$	$SAA = \frac{\frac{dN_2}{dt} \cdot 28}{X \cdot V_L} \cdot 60 \cdot 24$

α = slope of the pressure increase inside the vial plotted versus time (atm/min);

V_G = volume of the gas phase (0.013 l), calculated by subtracting the volume of liquid with 15 biocarriers (25 ml) from the total volume of the vial (38 ml):

R = ideal gas constant 0.0820575 (atm l mol^{-1} K^{-1});

T = temperature (K);

28 = molecular weight of N_2 (g N/mol);

60 and 24 = unit conversion factors from min to days.

X = biomass concentration inside the vial (g VSS/l);

V_L = volume of the liquid phase in the vial (approximately 18.97 ml). It has been calculated as difference between 25 ml and the equivalent volume occupied by carriers, based on the measurement that 4 l of rings occupy approximately a volume of 1.72 l.

and verified.

The pressure expressed in mmHg was then converted to atm by dividing by 760. The N_2 gas production rate $\left(\frac{dN_2}{dt}\right)$ was calculated through the ideal gas equation $PV = nRT$ and assuming a zero order kinetic $\frac{dn}{dt} = k$ for nitrogen gas production. This hypothesis can be considered valid because of the high initial concentrations of nitrogen, the short duration of the experiment (about 2 hours) and the experimental results which showed that the pressure data plotted versus time were aligned on a straight line.

The calculations used to estimate the SAA on the biocarriers are summarized in Table 15.

In one occasion SAA analysis was carried out on the activated sludge. In that case the vials used were the same ones (38 ml) and the liquid phase consisted of 24 ml of liquid from the reactor (activated sludge), 0.5 ml of NH_4Cl and 0.5 ml of $NaNO_2$. No buffer solution was added and no pH value was measured in that experiment. The SAA test was performed at 25°C.

N_2 gas production rate and the SAA calculations for the activated sludge (Table 16) have been calculated similarly but referred to the biomass concentration inside the vial expressed as (g VSS/l).

3.6.2 Oxygen Uptake Rate (OUR) test

The OUR test has the aim to assess qualitatively and quantitatively ammonia- and nitrite-oxidizing bacteria (AOB and NOB) as well as heterotrophic activity. The tests were performed on the base of the methodology described by Gut et al (2005).

The principle of OUR test is to monitor the rate of dissolved oxygen uptake by bacteria and selectively inhibit different bacterial populations during the test (Fig. 21).

The dissolved oxygen was measured by YSI Model 57 Oxygen Meter with YSI 5905 BOD probe and data were recorded every second by TESTO® Comfort-Software 2004 v 3.4. The batch test was performed in a glass bottle with a volume of 1.56 l. The bottle was filled with reject water (characterization is shown in paragraph 5.1.2.) previously diluted approximately 1:10 in order to have a NH_4^+-N initial concentration of about 100 mg/l. This value was measured before starting the test. The bottle was placed in the water bath until the temperature measured had reached about 25°C. Then the bottle was vigorously shaken or, alternatively, air was supplied in the reject water to reach a DO concentration over 6.5-7 mg/l. The bottle was placed in the water bath and on a magnetic stirrer, in order to assure a proper mixing of the liquor. At this point, the Kaldnes carriers, washed with the same diluted reject water, were rapidly inserted into the bottle. 107 Kaldnes carriers were used for the test, which correspond to a volume of approximately 100 ml. Larger amounts of Kaldnes carriers were rejected in order to avoid the risk to damage too much the biofilm bacteria culture activity by the tests. The Kaldnes carriers were usually taken directly from the reactor about one hour before the test and kept in diluted reject water. The reactor vessel was completely closed,

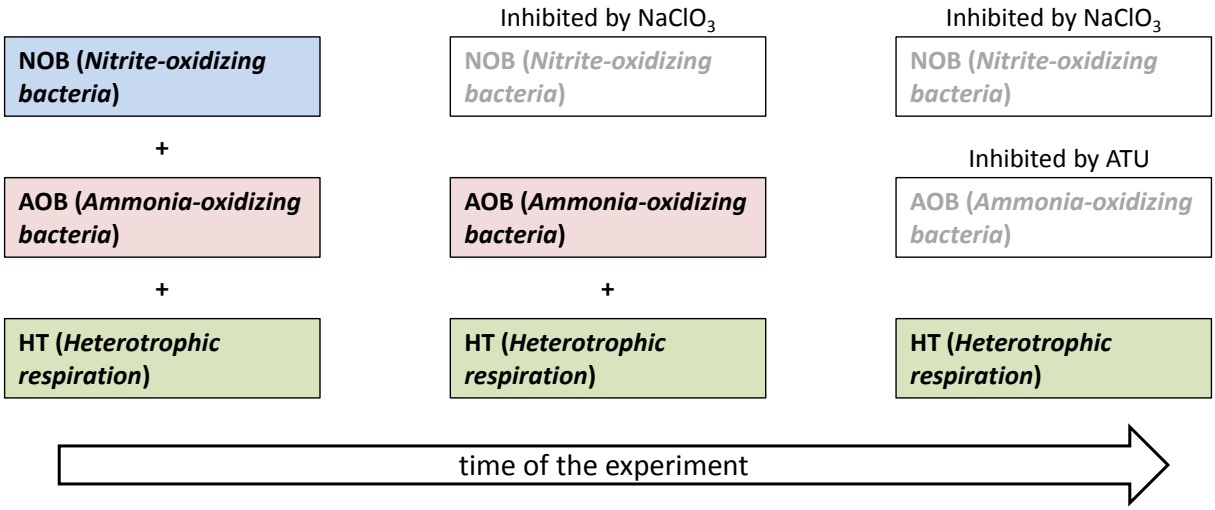

Fig. 21. Selective inhibitions of bacteria populations.

with rubber corks and parafilm, in order to avoid air intrusion in the bottle, and test was started.

First, the total oxygen uptake was measured.

After about 4-5 minutes and depending on test progress, 4 ml of sodium chlorate ($NaClO_3$) (solution 131.4 mg/100 ml) were added to the mixed liquor in order inhibit NO_2^--N oxidation by NOB. The final concentration in the bottle was about 32.6 μM. This concentration was lower than other values described in the literature studies. A ClO_3 concentration above 1 mM inhibits completely the NO_2^--N oxidation to NO_3^--N (Peng and Zhu., 2006; Xu et al, 2010). Surmacz-Górska et al (1996) suggested a concentration of 17 mM $NaClO_3$ for NOB inhibition. Belser & Mays (1980) observed that 10 mM $NaClO_3$ does not affect AOB, which are inhibited by sodium chlorite $NaClO_2$. However, according to Yang J. and Zubrowska M. (personal information, not published) which carried out some OUR tests on a parallel pilot reactor in Hammarby Sjöstadsverk on biocarriers with the same origin, the results with the concentration used in this thesis were not different from the results obtained with the higher concentration that is mentioned in literature.

After about 5-6 minutes, 6 ml of Allylthiourea (ATU) ($C_4H_8N_2S$) (solution 390 mg/100 ml) were added to the liquor. The final concentration in the bottle was about 132.9 μM, which is higher than the one suggested by the concentration suggested in the 21st edition of Standard Methods for measuring CBOD (about 57.4 μM). Probably a lower concentration was sufficient to fully inhibit nitrification.

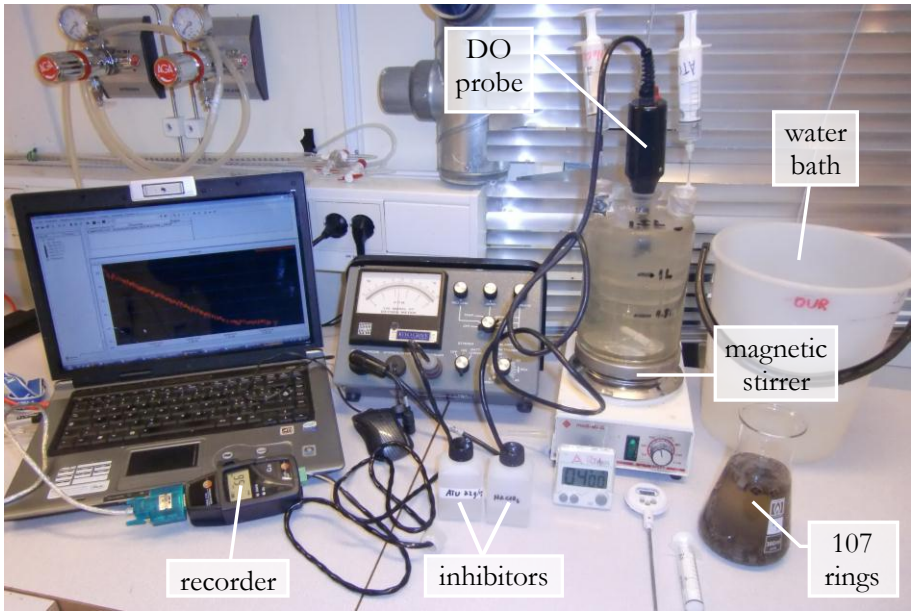

Fig. 22. Material and equipment for OUR tests

Table 17 – Calculations for OUR tests on the biocarriers.

Result	Unit	Formulas
Dissolved oxygen uptake rate (dO_2/dt)	$\frac{g\ O_2}{m^2 \cdot d}$	$\frac{dO_2}{dt} = \frac{-\alpha_i \cdot V_L}{S_{biofilm}} \cdot \frac{60 \cdot 60 \cdot 24}{1000}$
OUR - Nitrobacter	$\frac{g\ O_2}{m^2 \cdot d}$	$(OUR)_{NOB} = \left(\frac{dO_2}{dt}\right)_{AOB+NOB+HT} - \left(\frac{dO_2}{dt}\right)_{AOB+HT}$
OUR - Nitrosomonas	$\frac{g\ O_2}{m^2 \cdot d}$	$(OUR)_{AOB} = \left(\frac{dO_2}{dt}\right)_{AOB+HT} - \left(\frac{dO_2}{dt}\right)_{HT}$
OUR - Heterotrophs	$\frac{g\ O_2}{m^2 \cdot d}$	$(OUR)_{HT} = \left(\frac{dO_2}{dt}\right)_{HT}$

α_i – slope of the dissolved oxygen concentration decrease inside the bottle plotted versus time (mg O_2 l^{-1} s^{-1}). Subscript "i" indicates the slope of the respective phase of the test (AOB+NOB+HT, HT+AOB or HT). The values of the three slopes are the averages of the three OUR tests performed;

V_L = volume of the liquid phase (about 1.517 l) calculated by subtracting from the total volume of the bottle (1.56 l), the equivalent volume of liquid displaced by 107 Kaldnes biocarriers (calculated by a simple proportion, on the base of the measurement that 4 l of biocarriers occupy approximately an equivalent volume of water of 1.72 l). The volume of the liquid phase V_L was slightly different during the three steps of the test because of the stepwise additions of inhibitors (4 ml and 6 ml); This was kept into account in the calculations and the volumes are approximately 1.507 l, 1.511 l and 1.517 l;

$S_{biofilm}$ = surface area of 107 biocarriers = 0.05 m^2, calculated as the product of the specific area of Kaldnes media and the volume occupied by 107 rings (i.e. 100 ml): $S_{biofilm} = 500 \frac{m^2}{m^3} \cdot V_{107\,rings} \cdot 10^{-6}$;

60, 60 and 24 = unit conversion factors from seconds to days;

1000 = unit conversion factors from mg to g.

The difference between the total OUR and the one after $NaClO_3$ addition was identified as the oxygen uptake due to NOB (nitrite oxidizers), whereas the difference between the OUR with $NaClO_3$ and the OUR after addition of ATU was attributed to the oxygen consumption by AOB (ammonium oxidizers). Ultimately, the OUR measured in the presence of two chemicals represented the oxygen uptake of the heterotrophs (HT).

The inhibitors were added by means of two needles inserted in the rubber corks. The pH was measured manually at the beginning and the end of a couple of tests and it was between 8.05 and 8.25. The temperature was maintained around 25 °C during the whole test. Three tests were performed in order to obtain more reliable results. The value in output from the recorder

was in mV and it was later converted to mg O_2/l on the basis of the calibration done before starting that specific OUR test.

As underlined by Gut et al (2005), a limitation of this method is the impossibility to distinguish between the oxygen consumption for substrate oxidation and endogenous respiration of heterotrophic bacteria.

The dissolved oxygen uptake rate (OUR) was calculated by linear regression from the slope of the three curves (line segments) of the oxygen uptake plotted versus time. The calculations used to estimate the oxygen uptake rate (OUR) by different bacterial populations on the biocarriers are shown in Table 17.

In one occasion OUR test was carried out on the activated sludge in order to have a term of comparison. In that case, the bottle was filled with fresh activated sludge directly taken from the reactor. The bottle was shaken vigorously and 9 ml NH_4HCO_3 were added to raise the NH_4^+-N concentration up to 100 mg/l. Then the data logging was started. The duration of the test was shorter. The first inhibitor ($NaClO_3$) was added after 2 minutes and ATU about 1.5 minutes later. The concentrations added were the same. The oxygen uptake rate was calculated as shown in Table 18.

3.6.3 Nitrate Uptake Rate (NUR) test

The NUR test has the aim to assess the NO_3^- removal rate from the liquor. The bacteria responsible for nitrate removal are essentially denitrifying bacteria. However Anammox bacteria (which may use the nitrite produced during denitrification) can act in the opposite direction, leading to an underestimation of the nitrate removal rate by denitrifiers.

The test was performed in a 1.5 l plastic container, which was filled with 1 l of reject water diluted with tap water (50-75% reject water and 50%-25% tap water), in order to have a slightly lower initial pH and an initial COD concentration

Table 18 – Calculations for OUR tests on the activated sludge.

Result	Unit	Formulas
Specific dissolved oxygen uptake rate (dO_2/dt)	$\frac{g\ O_2}{g\ VSS \cdot d}$	$\frac{dO_2}{dt} = \frac{-\alpha_i}{X} \cdot 60 \cdot 60 \cdot 24$
OUR - Nitrobacter	$\frac{g\ O_2}{g\ VSS \cdot d}$	$(OUR)_{NOB} = \left(\frac{dO_2}{dt}\right)_{AOB+NOB+HT} - \left(\frac{dO_2}{dt}\right)_{AOB+HT}$
OUR - Nitrosomonas	$\frac{g\ O_2}{g\ VSS \cdot d}$	$(OUR)_{AOB} = \left(\frac{dO_2}{dt}\right)_{AOB+HT} - \left(\frac{dO_2}{dt}\right)_{HT}$
OUR - Heterotrophs	$\frac{g\ O_2}{g\ VSS \cdot d}$	$(OUR)_{HT} = \left(\frac{dO_2}{dt}\right)_{HT}$

α_i = slope of the dissolved oxygen concentration decrease inside the bottle plotted versus time (mg O_2 l^{-1} s^{-1}). Subscript "i" indicates the slope of the respective phase of the test (AOB+NOB+HT, HT+AOB or HT). The values of the three slopes are the averages of the three OUR tests performed;

X = biomass concentration inside the bottle (mg VSS/l); the biomass concentration inside the bottle was slightly different during the test because of the stepwise dilutions made (9 ml NH_4HCO_3, 4 ml $NaClO_3$ and 6 ml ATU). These changes were kept into account in the calculations and the VSS concentration was recalculated according to the new volume of liquid;

60, 60 and 24 = unit conversion factors from seconds to days;

of about 350-450 mg O_2/l. The container was placed in the water bath until the temperature measured had reached about 25°C and on a submersible magnetic stirrer in order to assure a proper mixing of the liquor.

Afterwards, nitrogen gas (N_2) was supplied into the liquor to decrease the dissolved oxygen concentration below 0.5 mg/l and the container was covered by parafilm. At this point, the Kaldnes carriers, washed with the same diluted reject water, were put into the container. 400 ml of Kaldnes carriers were used for the test. The Kaldnes carriers were usually taken directly from the reactor about one hour before starting the test and kept in diluted reject water in order to prevent oxygen diffusion into the biofilm.

Then 10 ml $NaNO_3$ solution (6 g $NaNO_3$/100 ml) were added in order to reach 100 mg/l NO_3^--N in the container. After having waited about one minute that the solution was spread in the liquor evenly, the first sample was taken and filtered with 0.45 µm filter. A filter with pore size 1.6 µm was used to prevent rapid clogging of the
filter with smaller pores size. Nitrogen gas was supplied during the whole test under parafilm, in order to avoid oxygen diffusion into the liquor. The duration of the test was about 4 hours and 5 sample were taken in total (one each hour). COD and NO_3^--N were analyzed at the end of the test.

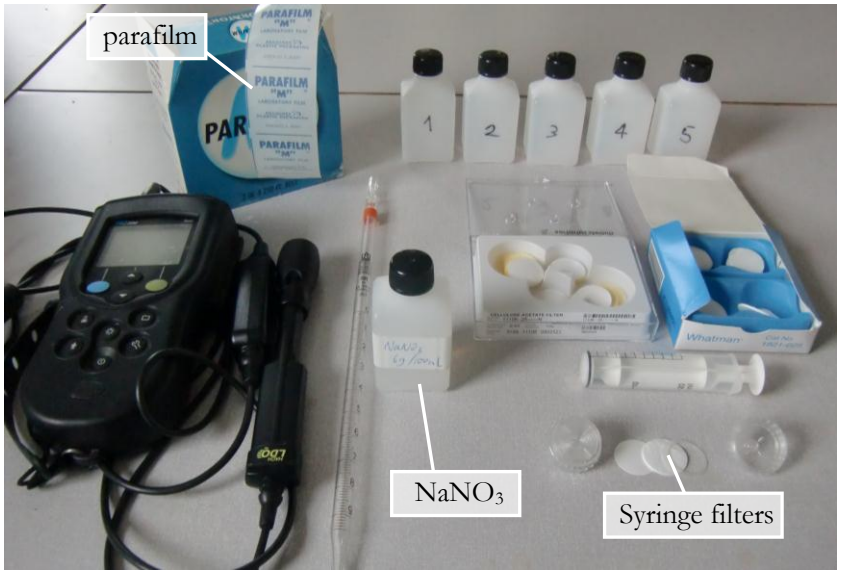

Fig. 22. Material and equipment for NUR tests

Table 19 – NUR calculations for the biocarriers and activated sludge.

Result	Unit	Formulas
NUR (biocarriers)	$\frac{g\ NO_3^- - N}{m^2 \cdot d}$	$NUR = \frac{-\alpha \cdot V_L}{S_{biofilm}} \cdot 1000 \cdot 60 \cdot 24$
NUR (activated sludge)	$\frac{g\ NO_3^- - N}{g\ VSS \cdot d}$	$NUR = \frac{-\alpha}{X} \cdot 60 \cdot 24$

α = slope of the nitrate concentration consumption inside the container plotted versus time (mg NO_3^--N l^{-1} min^{-1});

V_L = volume of the liquid phase equal to 1l.

$S_{biofilm}$ = surface area of 400 ml biocarriers = 0.2 m^2, calculated as the product of the specific area of Kaldnes media and the volume of the rings: $S_{biofilm} = 500 \frac{m^2}{m^3} \cdot 400 \cdot 10^{-6}$;

X = biomass concentration inside the container (mg VSS/l);

60 and 24 = unit conversion factors from min to days;

1000 = deriving from conversion from mg to g and from ml to m^3.

As the decrease of COD was found to be low, only the first and last samples were analyzed for COD. NO_2^--N initial concentration in the liquor was close to zero. The pH was measured at the beginning and the end of the test on a couple of occasions and it ranged between 8 and 8.9.

The dissolved nitrate uptake rate (NUR) was calculated by linear regression from the slope of the curve (straight line) of the nitrate uptake plotted versus time.

In one occasion NUR test was carried out on the activated sludge in order to have a term of comparison. In that case the container was filled with 1 l of activated sludge directly taken from the reactor. The container was placed in the water bath at 25°C and nitrogen gas (N_2) was supplied into the liquor to decrease the dissolved oxygen concentration below 0.5 mg/l. The container was covered by parafilm and the magnetic stirred assured proper mixing. There was no need to increase the NO_3^--N concentration as it was already 104 mg/l. The pH was measured at the beginning and the end of the test and ranged between 8 and 8.7.

4 LABORATORY-SCALE STUDIES

The laboratory-scale studies were carried out during the first four months of thesis work (period April-June). These studies were conducted in order to get practice with the whole one-stage deammonification process, investigate the factors influencing the process performance and assess different possibilities of treatment.

Two different laboratory reactors were run:

- a laboratory scale reactor treating diluted reject water (cfr. paragraph 4.1)
- a laboratory scale reactor treating the effluent of the upflow anaerobic sludge blanket (UASB) reactor (cfr. Paragraph 4.2).

4.1 Laboratory-scale reactor treating diluted reject water

The laboratory-scale reactor was started on 23rd March in the chemical labora-tory of the research facility Hammarby Sjöstadsverk and run for two months until the pilot plant scale reactor in the facility was started.

4.1.1 Reactor operation and experimental set-up

The laboratory-scale system consisted of one single reactor filled with ap-proximately 40% of Kaldnes™ carriers. The reactor was simply a plastic bucket, open at the top (Fig. 24). The outflow of the reactor consisted of an overflow system. The reactor had a volume of 9.345 L and it was filled with about 3.9 L of Kaldnes rings (model K1) with a specific internal surface area of 500 m^2/m^3. The effective volume of liquid in the reactor was 7.685 L.

The temperature was kept at about 25°C by an electric water heater thermostat. The stirring was assured by two electric submersible aquarium water pumps located at the basis of the reactor in order to avoid as much as possible sedimentation of Kaldnes rings. Oxygen was supplied continuously by aeration through air stone connected to the aeration pipe. The reactor was usually covered by aluminum paper in order to avoid possible effect of light and keep biocarriers in the dark and it was also wrapped in insulating material to prevent heat loss and reduce electricity consumption for heating.

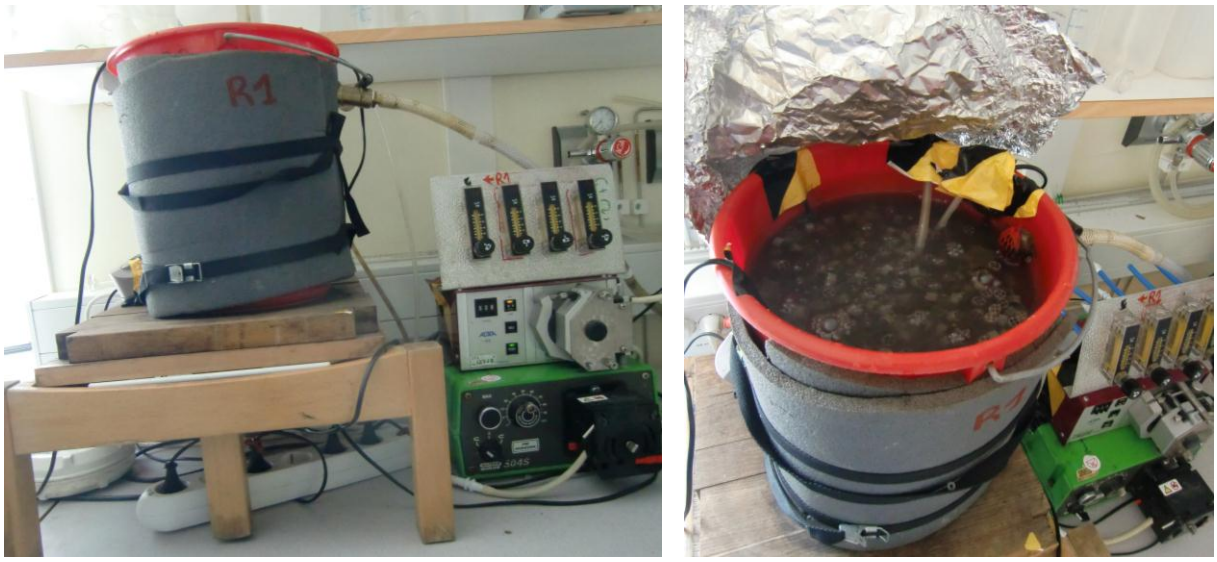

Fig. 24. Laboratory scale reactor treating diluted reject water.

The hydraulic retention time (HRT) was kept at two days and the inflow rate was checked three times per week in order to make sure the inflow rate was maintained constant at about 3.78 l/d and to prevent the occurrence of a decrease in the inflow rate due to deposition of suspended solids in the inflow pipe of small diameter.

The operational parameters are summarized in Table 20.

The reactor was working as Continuous Stirred-Tank Reactor (CSTR) and no sludge recycling was provided. The Kaldnes™ carriers used for the reactor start-up are shown in figure 25. They were brought in January, from Himmerfjärden Wastewater Treatment Plant where deammonification process is carried out using biocarriers with a biofilm composed by Anammox and Nitrosomonas bacteria. Before the use for the laboratory scale studies here described, they have been kept in anoxic conditions into a vessel for two months as they were a "surplus" from another reactor that was started in January, 2010.

The supernatant used as inflow was periodically taken from a storage tank in Hammarby Sjöstadsverk and it was brought from Bromma WWTP. The inflow vessel in the laboratory, where the influent to the reactor was stored, was refilled with about 20-30 liters two-three times per week.

The reactor feed consisted of reject water from sludge dewatering after anaerobic digestion which was diluted 1:2.5 with tap water in order to have an ammonia nitrogen (NH_4^+-N) concentration of about 400 mg/l. Nitrate (NO_3^-) and nitrite (NO_2^-) concentrations were very low and negligible.

The physical and chemical parameters of the influent to the reactor are summarized in Table 21. Unlike the pilot plant reactor described in chapter 5, the total nitrogen, the suspended solids in the influent and in the reactor have not been measured in this laboratory study.

Table 20 – Operational parameters for the laboratory-scale reactor.

Parameter	Unit	Value
Hydraulic Retention Time (HRT)	day	2.04
Reactor Volume (liquid)	l	7.69
Flow rate	l/d	3.78
Kaldnes carriers	l	3.9
Temperature	°C	25.4

Fig. 25. Biocarriers used for laboratory-scale reactor start-up.

Table 21 – Characterization of the influent diluted reject water 1:2.5.

Parameter	Unit	Mean±S.D.	Measurements
Ammonium NH_4^+	mg/l NH_4^+-N	399.1 ± 27.7	12
Alkalinity	mmol/l K_s 4.3	36.5 ± 16.4	9
	g/l $CaCO_3$	1.82 ± 0.82	
Alk/NH_4^+-N	mol Alk / mol N	1.06 ± 0.09	8
$COD_{soluble}$	mg O_2/l	311 ± 48	8
COD_{tot}	mg O_2/l	496 ± 73	7
COD/ NH_4^+-N	-	0.79 ± 0.12	8
Total Phosphorus (unfilt.)	mg/l	2.10 ± 0.91	5
Conductivity	mS/cm	3.07 ± 0.27	39
pH	-	8.19 ± 0.38	38

4.1.2 Analytical measurements and sampling procedures

The physical parameters (pH, T, DO, conductivity) in the reactor and the inflow rate were measured manually three-four times per week in order to provide good and stable conditions for bacteria and evaluate process efficiency with the operating conditions.

The flow was kept constant during the whole study but ammonia nitrogen in the influent showed slight variations in concentration.

The chemical analyses were performed once per week for both inflow and outflow and within two days of each other. Between the inflow and outflow analyses the tank containing the influent diluted supernatant was not refilled in order not to change its composition between the two measurements.

The main compounds and parameters monitored were NH_4^+-N, NO_2^-N, NO_3^-N, COD (filtered 0.45μm and unfiltered) and alkalinity. The decrease of ammonia nitrogen in the reject water over two days was negligible and only once it was found to be higher (about 8.1 %) but that was probably due to an analysis error because the following times it was negligible (about 1%). The outflow samples were taken from a small outflow container after about 30-40 minutes having emptied it.

4.1.3 Results and discussion

Operational conditions

The results from measurements of physical parameters in the reactor are shown in the chart in figure 26. The average values over the months of operation are shown in Table 22 whereas the raw table with all the physical parameters measured is included in the APPENDIX II.

The dissolved oxygen was the most sensitive and problematic parameter to keep stable and sometimes its concentration was not evenly distributed in different parts of the reactor. This was mainly due to the rather punctual aeration system which was not the most suitable. Moreover the absence of an online control system on the aeration supply device made it hard to regulate the aeration and keep the dissolved oxygen at a constant concentration without variations. Several times it was needed to decrease aeration in order to bring back the pH to higher values and to prevent further oxidation to nitrate and other times there was the need to increase aeration to enhance nitritation. This was done according to the results from the chemical analyses on the outflow and the pH value measured in the reactor. High dissolved oxygen concentration effects seemed to be reversible and they caused only accumulation of nitrates in the reactor, resulting in a couple of days of bad performance with higher concentrations of nitrates in the outflow.

The pH was found to be strongly dependent on the dissolved oxygen concentration in the reactor.

Table 22 – Physical parameters in the laboratory scale reactor.

Parameter	Unit	Mean±S.D.	Measurements
pH	-	7.30 ± 0.54	35
DO	mg O_2/l	1.13 ± 0.77	35
T	°C	25.45 ± 0.20	34
Conductivity	mS/cm	1.19 ± 0.49	33

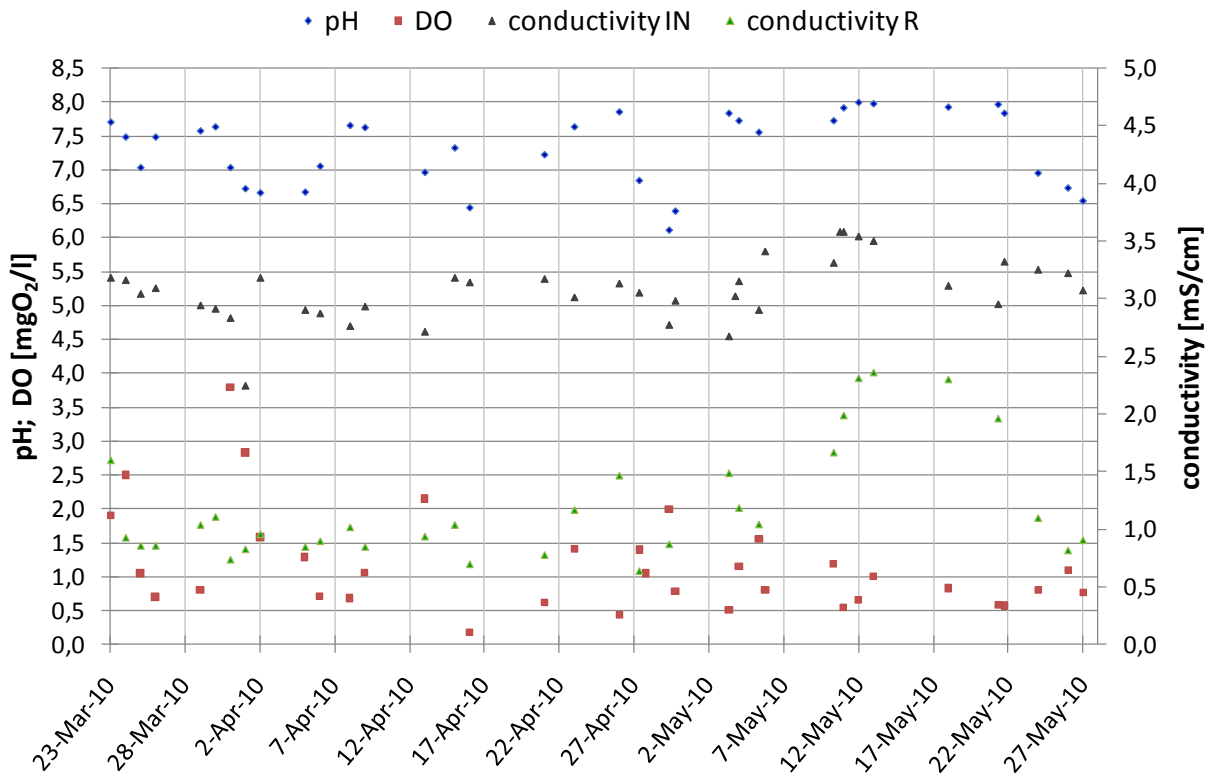

Fig. 26. Physical parameters in the reactor: pH, DO and conductivity.

If the dissolved oxygen concentration was too high, then, as a consequence, the pH tended to decrease inevitably, due to an enhanced nitrification. During the 6th week of operation, for example, the pH dropped down to 6.1 due to a too high aeration.

The conductivity was used as a useful secondary monitoring tool for the indication of the process performance.

The temperature was slightly higher (about 26-27°C) during the last two weeks of operation.

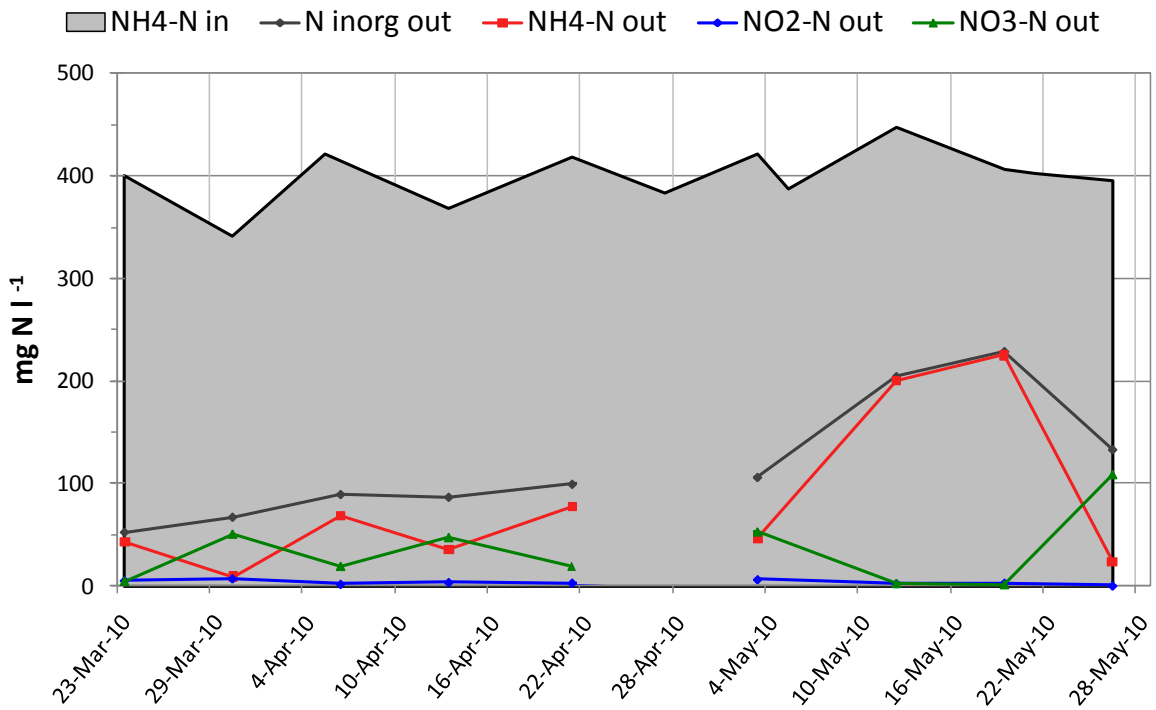

Fig. 27. Results from the chemical analyses on inorganic nitrogen forms.

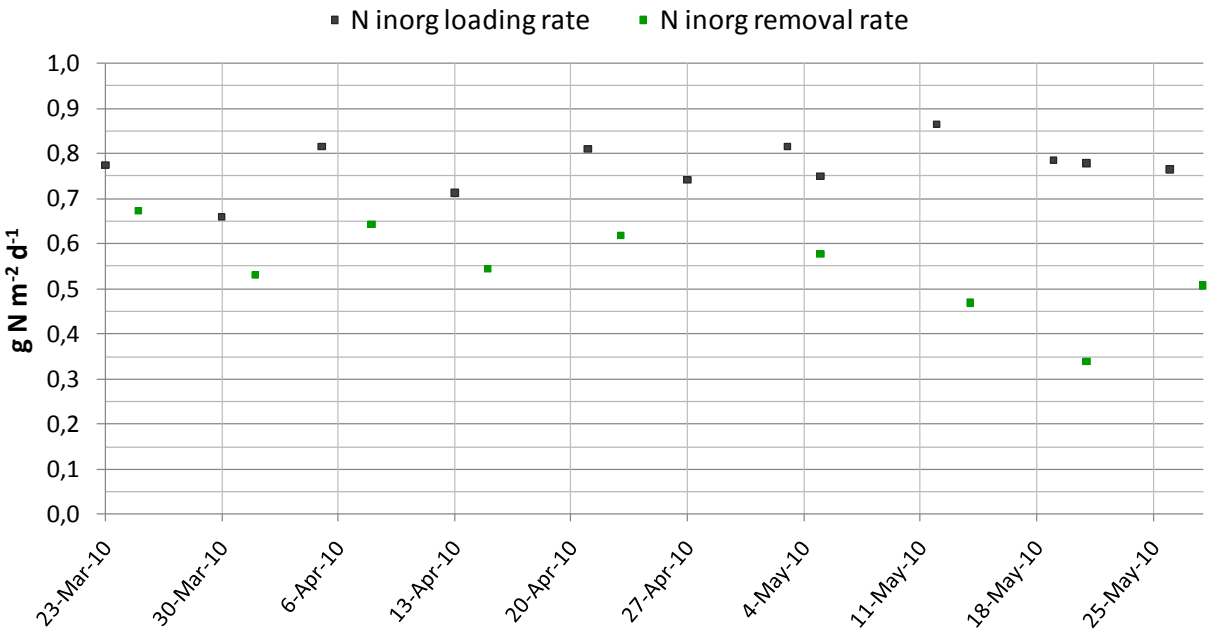

Fig. 28. Nitrogen loading and removal rates.

Analyses of nitrogen compounds in the outflow and their dependence on operational parameters

As discussed before, the results from the chemical analyses on the outflow where strongly related to the conditions in the reactor, especially the dissolved oxygen. The results are presented in figure 27.

During the 2nd week (29th March - 4th April) the ammonium nitrogen in the outflow was very low (9.0 mg/l) as well as the alkalinity (1.26 mmol/l), in contrast to nitrate nitrogen levels which were high (50.7 mg/l). An alternation of periods of consumption of ammonium and production of nitrate was observed during the following weeks. This was due to the sensitivity of the air supply device and the strong dependence of the partial nitritation/Anammox process on the dissolved oxygen concentration in the reactor.

The chemical analyses were not performed on the 6th week (26th April - 2nd May) because conditions

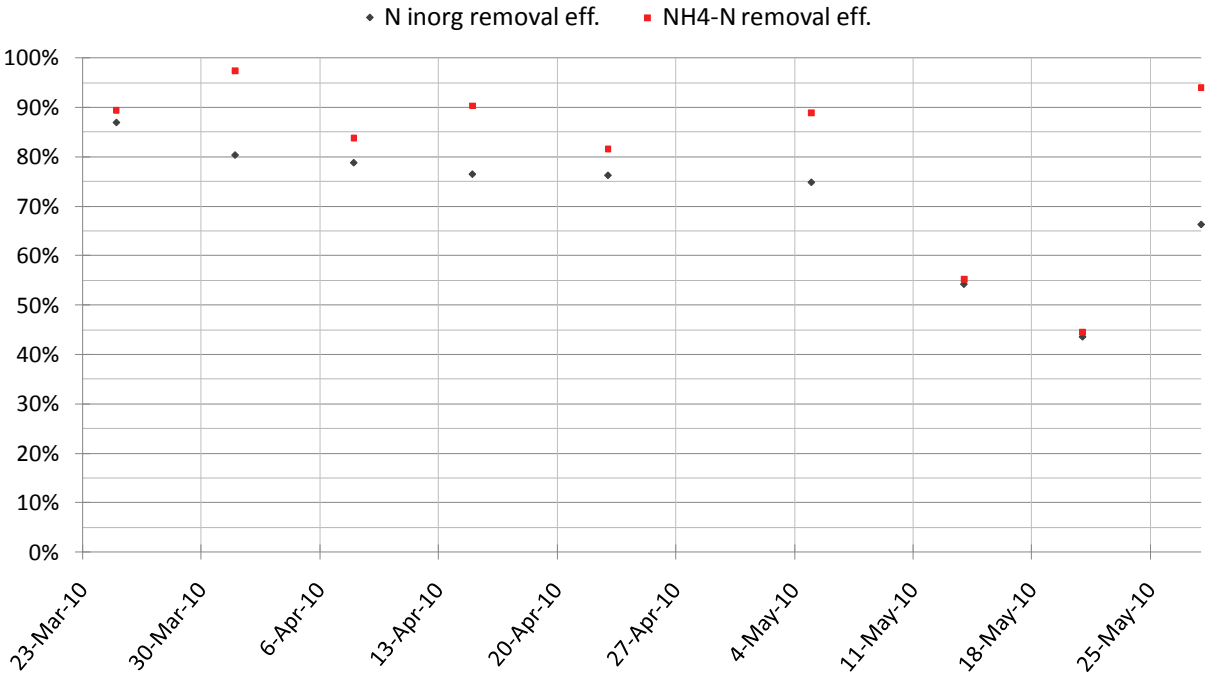

Fig. 29. Removal efficiencies for inorganic nitrogen and ammonia nitrogen.

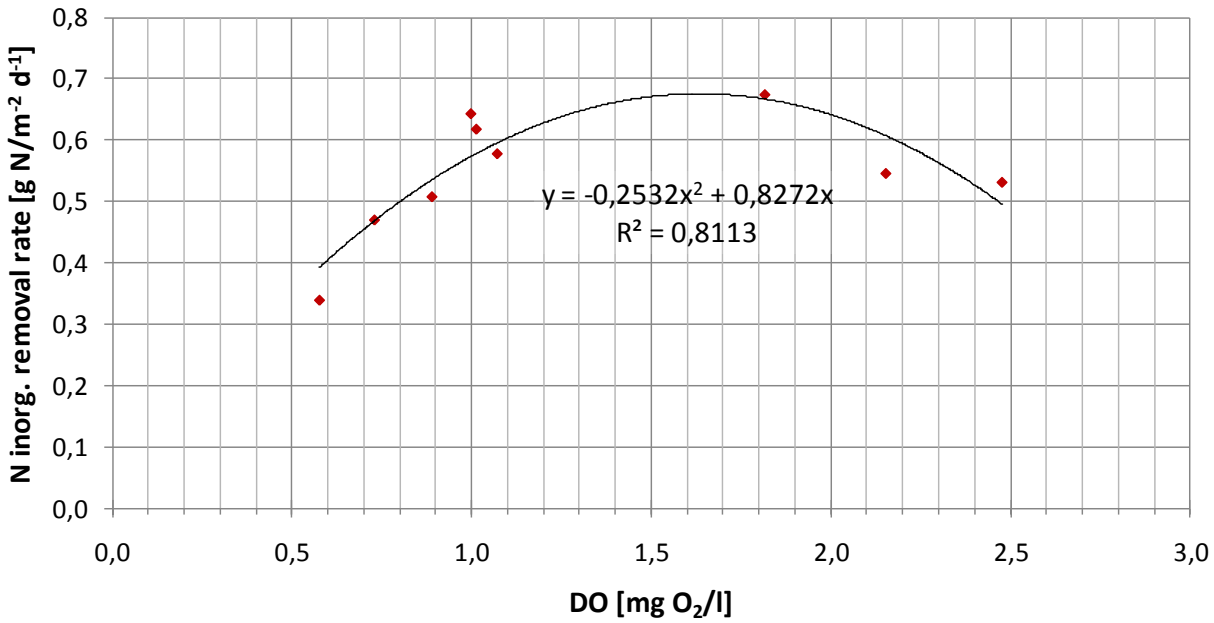

Fig. 30. Dissolved oxygen concentration influencing the nitrogen removal rate.

of the reactor were out of optimum range for bacteria and low process efficiency was expected. A too high aeration caused a pH drop down to 6.1 (due to acidification from nitrification process) and a very low alkalinity content of the liquid in the reactor (0.52 mmol/l).

During 8th and 9th week (10th – 23rd May) low dissolved oxygen concentrations (about 0.7 mg O_2/l) limited the nitritation and Anammox processes and ammonium was only partly converted. In these two weeks, high pH (about 7.9), high conductivity (2.18 mS/cm) and high alkalinity in the effluent (21.7 mmol/l) were noticed.

A better view and analysis can be obtained from the charts with the nitrogen load and nitrogen removal rates (Fig. 28) and the chart with the efficiencies of NH_4^+-N removal and inorganic nitrogen removal (Fig. 29).

As the dissolved oxygen was the most important parameter, an evaluation on its relations with nitrogen removal efficiencies has been done (Fig. 30).

The values of dissolved oxygen used are the averages of available data from two or three days before chemical analyses on the outflow were done. A fairly good interpolation of the data was found with parabolic equation with intercept set equal to zero (which means that for values of dissolved oxygen equal to zero, the nitrogen removal is zero). From a first derivative calculation, the maximum of the parabolic fit was found to be at 1.63 mg O_2/l. Unfortunately no value between 1.2 and 1.7 mg O_2/l is available. Proba-

Table 23 – COD, Alkalinity and conductivity.

Parameter	Unit	Mean±S.D.	Samples
COD_{tot} removed	mg/l	292.4 ± 76.4	5
COD_{tot} removal	%	60.0 ± 5.7	5
$COD_{soluble}$ out	mg/l	167.1 ± 17.4	6
$COD_{soluble}$ removed	mg/l	132.8 ± 62.0	6
$COD_{soluble}$ removal	%	41.9 ± 13.1	6
$COD_{tot\ removed}$ / $N_{inorg\ removed}$	-	0.80 ± 0.24	4
$COD_{soluble\ removed}$ / $N_{inorg\ removed}$	-	0.34 ± 0.15	5
Alkalinity consumed	mmol/l K_s 4.3	27.5 ± 5.1	5
	g/l $CaCO_3$	1.37 ± 0.25	
Alkalinity $_{consumed}$ / NH_4^+-N $_{removed}$	mol Alk/mol N	1.07 ± 0.14	5
	g $CaCO_3$ / g NH_4^+-N	3.83 ± 0.49	5
Conductivity removed	mS/cm	1.85 ± 0.43	33

bly dissolved oxygen of about 1.5 mg O_2/l could have given slightly higher efficiencies. However this was not proved because the laboratory reactor was stopped at the end of May and this relationship was studied later.

Calculations on COD removals, alkalinity consumptions and conductivity decrease are shown in Table 23. The results showed in the table have been obtained averaging the data from each single week. The calculations include only the first six weeks of operation, during which more stable conditions were maintained.

The ratio $COD_{removed}/N_{inorg\ removed}$ ranged between 0.54-1.17 and 0.15-0.59 considering the COD unfiltered (COD_{tot}) and filtered 0.45 μm ($COD_{soluble}$) respectively.

Measurements of volatile suspended solids content in the biofilm of Kaldnes carriers

In this study, measurements on the biomass were carried out only on the biofilm of the Kaldnes rings, in order to assess the growth on them. The results (Fig. 31) show that no growth was observed in the first two months.

Unlike the pilot plant scale reactor, only one analysis on the activated sludge and suspended solids was carried out. The reason lay in the interest devoted to the biofilm, where Anammox bacteria are supposed to be present and active, and the activity of the Anammox bacteria during the reactor operation. The result of the measurement, performed on the 21st May, gave the following results: TSS = 905 mg/l and VSS = 780 mg/l, which is a relatively high for a MBBR. A reason may be a not very good mixing from the two electric submersible aquarium water pumps and thus a lower content of suspended solids leaving the reactor through the overflow system. Another cause could be the detachment of biomass from the rings, as suggested from the last measurement. However this hypothesis was not verified by next measurements.

On the basis of the measurements of the 21st May, the total amount of suspended biomass in the reactor has been compared with the biomass attached on the rings on the same day.

$$VSS_{biofilm} = 10.85 mg/ring \cdot 4173 rings = 45277.1 mg$$

$$VSS_{act.sludge} = 780 mg/l \cdot 7.69 l = 5998.2 mg$$

where:

10.85 mg/ring = measurement of biomass on the biocarriers (21st May);

4173 rings = estimated number of rings in the reactor, on the basis that 107 biocarriers occupy 100 ml and the reactor was filled with 3.9 l of rings.

Few days before the study on the reactor was finished, the biomass in the activated sludge accounted for the 11.07% of the total biomass estimated in the reactor.

Results from Specific Anammox activity (SAA)

The Anammox bacteria activity was followed by weekly measurements (SAA tests) in order to assess whether there was an increase in Anam-

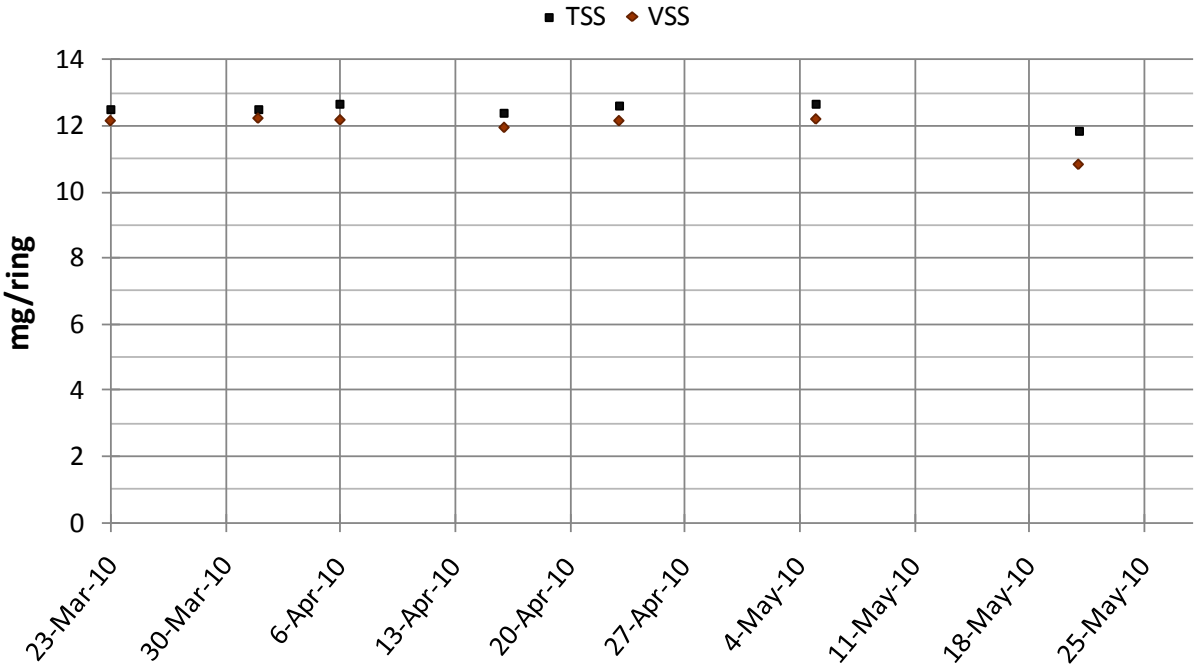

Fig. 31. No signs of biofilm growth.

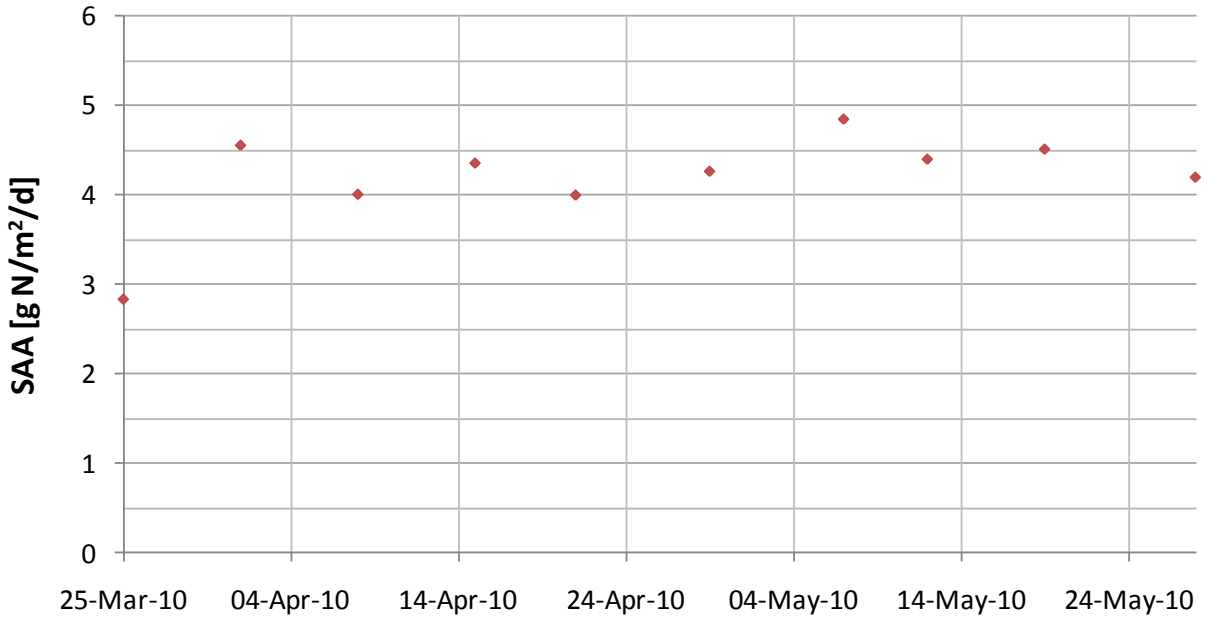

Fig. 32. Activity of Anammox bacteria in the biofilm (SAA test at 35°C)

mox bacteria activity during operation or not and trying to quantify it. In total 10 series of SAA analyses have been carried out. The tests were all performed at 35°C and had a duration of about 120 minutes. The experimental data are included in APPENDIX II. The results are summarized in figure 32.

No particular increase of Anammox bacteria activity was noticed during the two months of reactor operation.

An average value of 4.3 g N m^{-2} d^{-1} can be assumed for the SAA tests carried out at 35°C.

4.1.4 Conclusions

Some conclusions can be drawn on the basis of the obtained results on this laboratory scale reactor. They are briefly summarized below:

- Dissolved oxygen is the key parameter for a good efficiency and the overall stability of the partial nitrification/Anammox process;
- An optimum DO concentration of 1.5-1.6 mg O_2/l was probably the more appropriate for this reactor;
- A better system for DO control based on a PID controller is strongly recommended in order to avoid too high or too low aeration periods and control the set point of dissolved oxygen concentration in the reactor. An aeration system which bases the air supply on the oxygen consumption could be more effective than a system which supply a constant air flow, as it was in this case;
- Conductivity could be an useful monitoring tool for the indication of the process performance and the ammonia consumption between the inflow and the outflow;
- The low nitrite concentration in the reactor suggests that nitrite was probably the limiting factor for Anammox bacteria;
- The alkalinity/nitrogen in the inflow (1.06 mol Alk/mol NH_4^+-N) was suitable to stand the decrease in pH induced from nitrification during the reactor operation;
- The COD/NH_4^+-N in the influent was low (about 0.79) and therefore suitable for the partial nitritation/Anammox process;
- A removal efficiency of about 80% of inorganic nitrogen has been achieved;
- SAA results did not show any particular evidence of increase/decrease in Anammox bacteria activity.

4.2 Laboratory-scale reactor treating diluted reject water

The laboratory-scale reactor was started on 11th May in the chemical laboratory of the research facility Hammarby Sjöstadsverk and it was run for two months until mid-July. This laboratory reactor was started in order to evaluate the biological treatment of the effluent from the treatment line 3 at Hammarby Sjöstadsverk research facility, through the deammonification process (partial nitrification and Anammox in one single reactor).

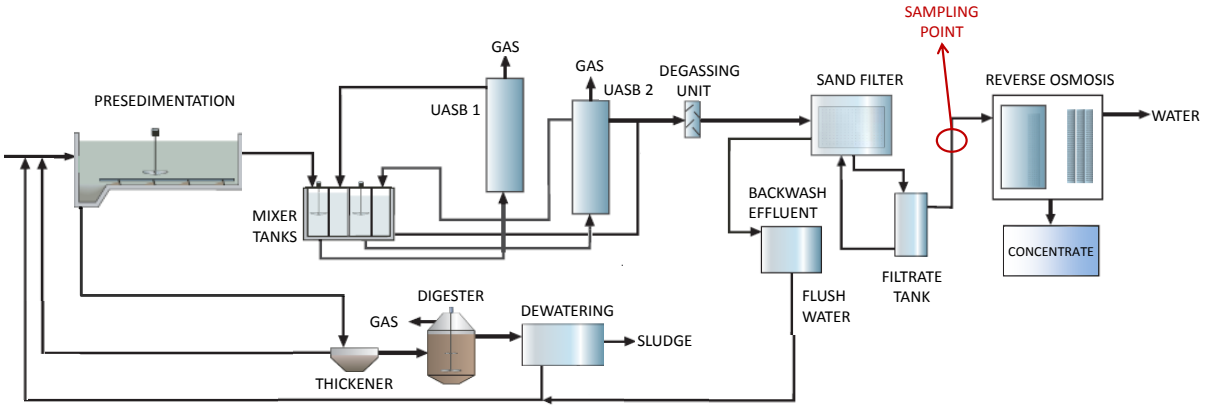

Fig. 33. Treatment Line 3 – Anaerobic treatment with UASB and sand filtration (modified from:

4.2.1 Treatment Line 3 - Anaerobic treatment with UASB

The treatment line 3 at Hammarby Sjöstadsverk is outlined in figure 33. The process line consists of a pre-sedimentation step of the sewage water which has already been preliminary treated by bar screens and grit chamber.

The primary sedimentation is followed by an anaerobic treatment with two UASB (Upflow Anaerobic Sludge Blanket) reactors run in series with granules and biogas production. The main purpose is to reduce the COD content and produce a valuable energy product (biogas). The two mixing chambers have the purpose to mix the incoming wastewater with the recirculation from the UASB reactors. The effluent from the reactors is sent to a degassing unit to separate the residue gas and then to a slow sand filter for solids and biomass separation. The filtrate water undergoes a further polishing step for nutrients removal consisting of reverse osmosis. However in those months no reverse osmosis was carried out and the effluent was discharged directly. Nowadays reverse osmosis technology is still expensive and this laboratory reactor was run with the goal to make a preliminary assessment of the possibility of using the deammonification process as polishing step for nitrogen removal. The influent wastewater to the laboratory-scale reactor was taken downstream the sand filtration as showed by the red circle. The filtered water is used as backwash water for the regeneration of the sand bed filter. The backwash effluent is then sent back to the inlet of the treatment line. The primary sludge from the pre-sedimentation (mainly solid particles) is thickened, digested anaerobically (for COD removal and biogas production) and dewatered. The removed water is then sent back to the precipitation and flocculation step.

4.2.2 Characterization of the effluent from sand filter after anaerobic treatment with UASB

The influent to the laboratory scale reactor was periodically taken from the tap of the treatment line 3 located downstream the sand filtration and stored in a tank of about 200 L. From this tank, the wastewater was brought manually to the adjacent chemical laboratory to refill the inflow vessel with about 15-20 liters, two-three times per week. Nitrate (NO_3^-) and nitrite (NO_2^-) were

Table 24 – Characterization of the influent diluted reject water 1:2.5.

Parameter	Unit	Mean±S.D.	Measurements
Ammonium NH_4^+	mg/l NH_4^+-N	43.7 ± 3.2	10
Alkalinity	mmol/l K_s 4.3	5.68 ± 0.28	8
	g/l $CaCO_3$	0.284 ± 0.014	
Alk/NH_4^+-N	mol Alk / mol N	1.80 ± 0.09	8
$COD_{soluble}$	mg O_2/l	53.4 ± 2.9	7
COD_{tot}	mg O_2/l	90.1 ± 25.7	7
COD/ NH_4^+-N	-	1.22 ± 0.09	7
Total Phosphorus (unfilt.)	mg/l	5.2 ± 0.8	8
Conductivity	mS/cm	0.772 ± 0.027	31
pH	-	7.83 ± 0.30	32

measured once and the results were NO_3^--N = 0.176 mg/l and NO_2^--N < 0.2 mg/l, therefore very low and negligible. The physical and chemical parameters of the influent to the reactor are summarized in Table 24. Unlike the pilot plant reactor described in chapter 5, the total nitrogen has not been measured in this laboratory scale study.

The only measurement of suspended solids was performed with filters with a pore size of 1.6 μm and the result was very low and less than 3 mg/l for both total and volatile suspended solids.

4.2.3 Laboratory-scale reactor configuration and experimental set-up

The laboratory-scale reactor was filled with approximately 40% of Kaldnes™ carriers. The reactor (Fig. 34) was simply a plastic bucket open at the top and slightly smaller but however similar to the one described in paragraph 4.1. The reactor was filled with about 3.17 L of Kaldnes rings (model K1) with a specific internal surface area of 500 m^2/m^3. The effective volume of liquid in the reactor, measured with the biocarriers inside it, was 6.74 L.

The temperature was kept at about 25°C by an electric water heater thermostat. The stirring was provided by two electric submersible aquarium water pumps located at the basis of the reactor in order to avoid sedimentation of Kaldnes rings and provide a good mixing of the liquid inside the reactor.

Oxygen was not supplied because the organic matter and ammonium nitrogen of the incoming wastewater was rather low and the monitored dissolved oxygen concentration was around 1 mg/l and anyway it was never found to be less than 0.35 mg/l. It is likely that part of the dissolved oxygen utilized by bacteria derived from the action of refilling the inflow tank in the laboratory. The reactor was usually covered by aluminum paper in order to avoid possible effect of light and keep biocarriers in the dark and it was also wrapped in insulating material to prevent heat loss and reduce electricity demand for heating.

The hydraulic retention time (HRT) was chosen to be kept at one day, with the purpose of increasing the load, because the ammonium nitrogen in the influent was lower (about only 44 mg/l) compared to the laboratory scale reactor described in paragraph 4.1 (HRT = 2 days and NH_4^+-N = 400 mg/l). As it will be stressed in the discussion of the results, a shorter hydraulic retention time would have been preferable. An explanation for the choice of this hydraulic retention time and not a lower one was mainly due to practical problems such as the small size of the inflow tank in the chemical laboratory and the unsuitable and too light inlet system.

The inflow rate was checked about two times per week in order to make sure the inflow rate was maintained constant. In the first six week of operation the effluent from UASB digester and sand filtration had a higher turbidity and higher content of suspended solids. However, since the 25th June, the incoming wastewater prior to treatment was clearer and with a lower turbidity. Despite this, the flow was found to be slightly higher or lower in some occasions, but however this never happened the day before the chemical analyses on the inflow and between the inflow and outflow chemical analyses. For example, on the 7th June, the inflow rate was decreased for one day, on the 20th June no inflow to the reactor was provided (probably since one or two days before) and lastly the 3-4 days prior to the 13th July no inflow to the reactor was supplied (owing to a my short return to Italy due to academic reasons).

Fig. 34. Laboratory scale reactor treating effluent supernatant from Line 3.

The reactor was run as Continuous Stirred-Tank Reactor (CSTR) and no sludge recycling was provided. The outflow consisted of an overflow system. The operational parameters are summarized in Table 25.

The origin of the Kaldnes™ carriers used for the reactor start-up is here below explained. The biocarriers were the same as those ones used for the laboratory scale reactor described in paragraph 4.1 and showed in figure 25. The main difference is that during the period between the 23rd March and 7th May they had been used for a trial study to assess the consequence of Anammox and partial nitritation process on raw sewage water after sedimentation. Unfortunately that trial study turned to be a process with characteristics of heterotrophic denitrification and biofilm might have undergone a slight change in composition or activity. After that trial they have been stored for four days in supernatant from dewatering of anaerobic digester sludge, diluted with tap water, before being used for the new laboratory scale reactor described in this chapter.

4.2.4 Analytical measurements and sampling procedures

The physical parameters (pH, T, DO, conductivity) in the reactor and the inflow rate were measured manually three-four times per week when possible. The chemical analyses were usually performed once per week for both inflow and outflow and within one day of each other. Between the inflow and outflow analyses the tank containing the influent wastewater was not refilled in order to not change its composition between the two measurements.

The main compounds and parameters monitored were NH_4^+-N, NO_2^-N, NO_3^-N, COD (filtered 0.45µm and unfiltered) and alkalinity. The outflow samples were taken from a small outflow container after about 20-30 minutes having emptied it.

Table 25 – Operational parameters for the laboratory-scale reactor.

Parameter	Unit	Value
Hydraulic Retention Time (HRT)	day	1.00
Reactor Volume (liquid)	l	6.74
Flow rate	l/d	6.73
Kaldnes carriers	l	3.17
Temperature	°C	26.77

4.2.5 Results and discussion

Operational conditions in the reactor

The results from measurements of physical parameters in the reactor are shown in the chart in figure 35.

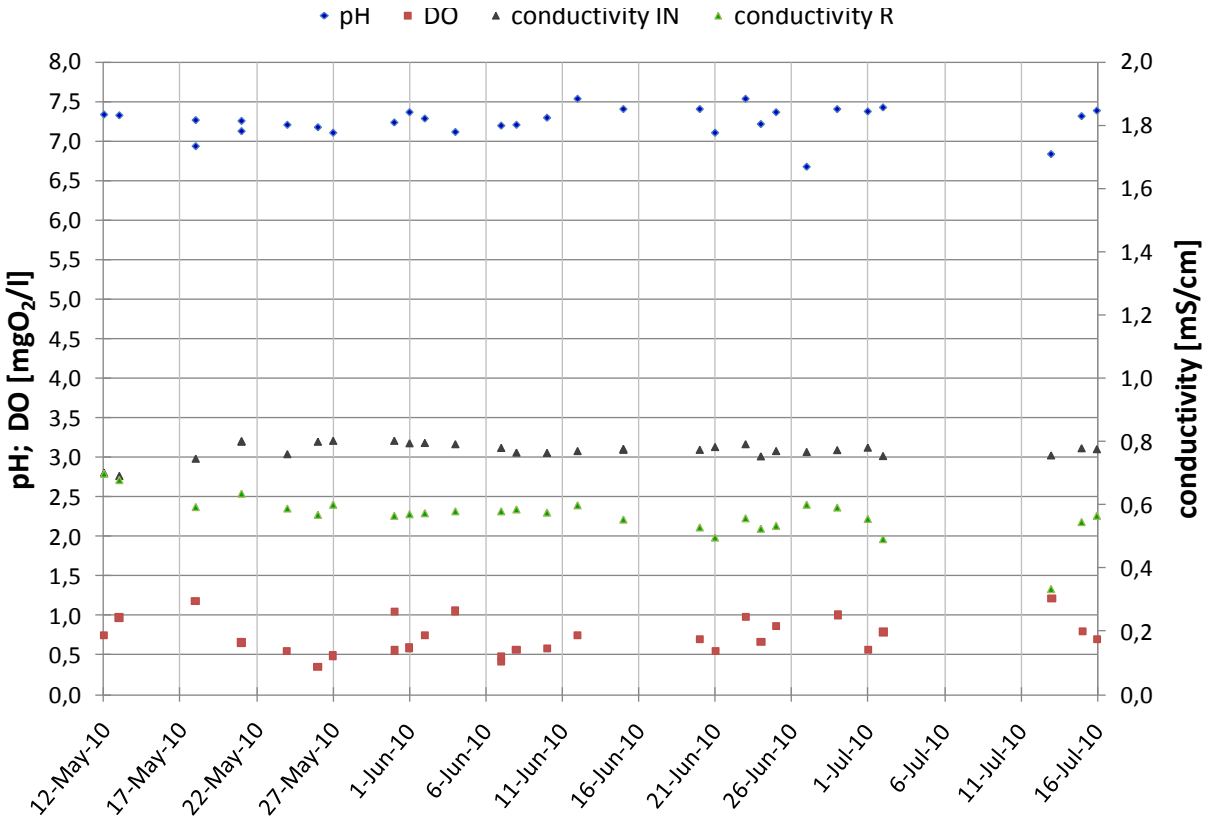

Fig. 35. Physical parameters in the reactor: pH, DO and conductivity.

Table 26 – Physical parameters in the laboratory scale reactor.

Parameter	Unit	Mean±S.D.	Measurements
pH	-	7.24 ± 0.19	30
DO	mg O_2/l	0.74 ± 0.23	28
T	°C	26.77 ± 1.67	28
Conductivity	mS/cm	0.57 ± 0.06	29

The average values of the parameters monitored over the two months of operation are shown in Table 26, whereas the raw table with all the physical parameters measured is included in the APPENDIX III.

The pH and the dissolved oxygen (DO) were easily kept stable during the whole operation. The ratio alkalinity-ammonia nitrogen of the influent to the reactor was sufficient to contrast pH drops and acidification from the process. pH values never dropped below 6.5.

The conductivity removal was fairly constant and equal to 0.22 mS/cm. The average temperature was slightly higher than the previous laboratory reactor and it was around 26-27 °C, except the period around the 7th week (20th June - 2nd July) with a temperature of nearly 29°C. After those days the heater was stopped because there was no need of heating, due to warmer temperatures.

Nitrogen removal performance and efficiency evaluation

The results from chemical analyses on the outflow are shown in figure 36.

After three weeks of operation the ammonia nitrogen was almost completely depleted with one day of retention time. An increase in nitrate nitrogen in the outflow was noticed. No explanations has been found for the high value of nitrate in the outflow measured on the 4th June, as the pH was 7.11 and not particularly low, the dissolved oxygen concentration was just slightly higher than usual (i.e. 1.06 mg O_2/l) as well as the alkalinity (1.46 mmol/l) in the reactor which was not particularly low that day if compared to the other weeks.

The nitrite NO_2 was almost certainly the limiting substrate for Anammox bacteria and the hydraulic retention time seemed to be too high for this reactor operation, which resulted in a full depletion of nitrite in the reactor and a full conversion of ammonium and nitrite to nitrate.

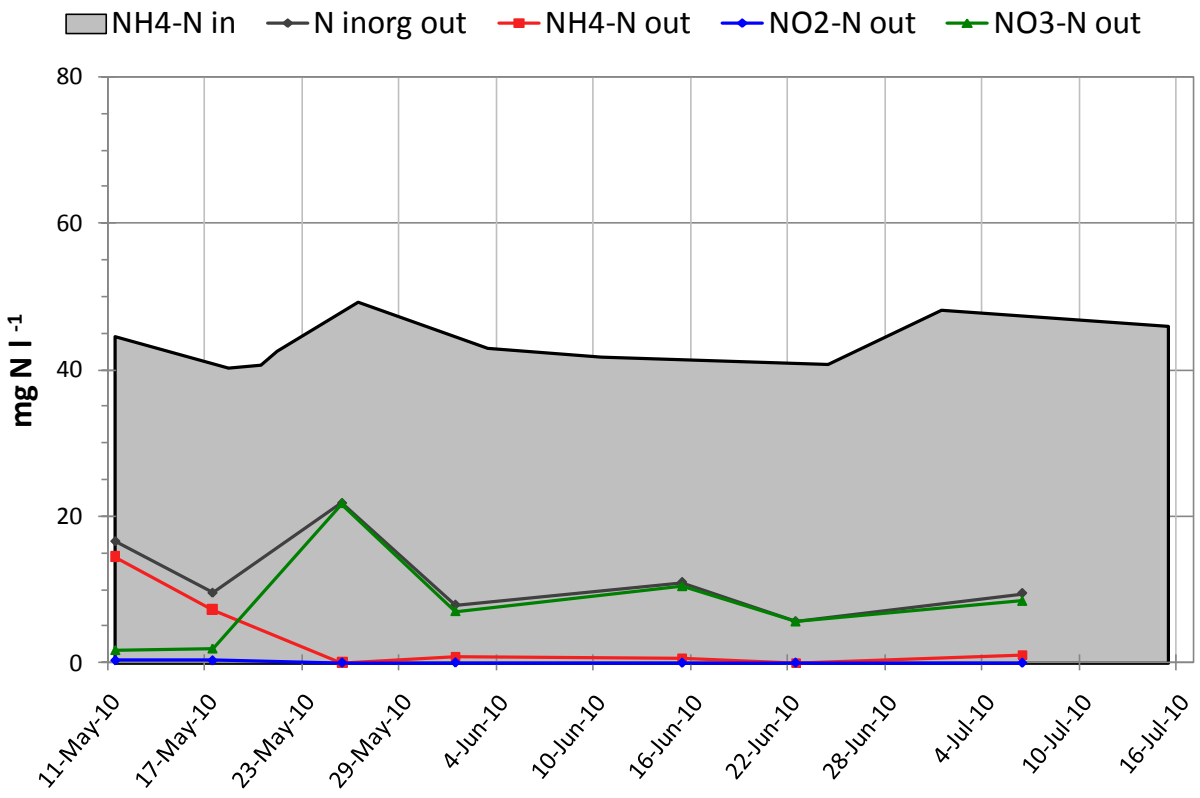

Fig. 36. Chemical analyses results on the inorganic nitrogen forms.

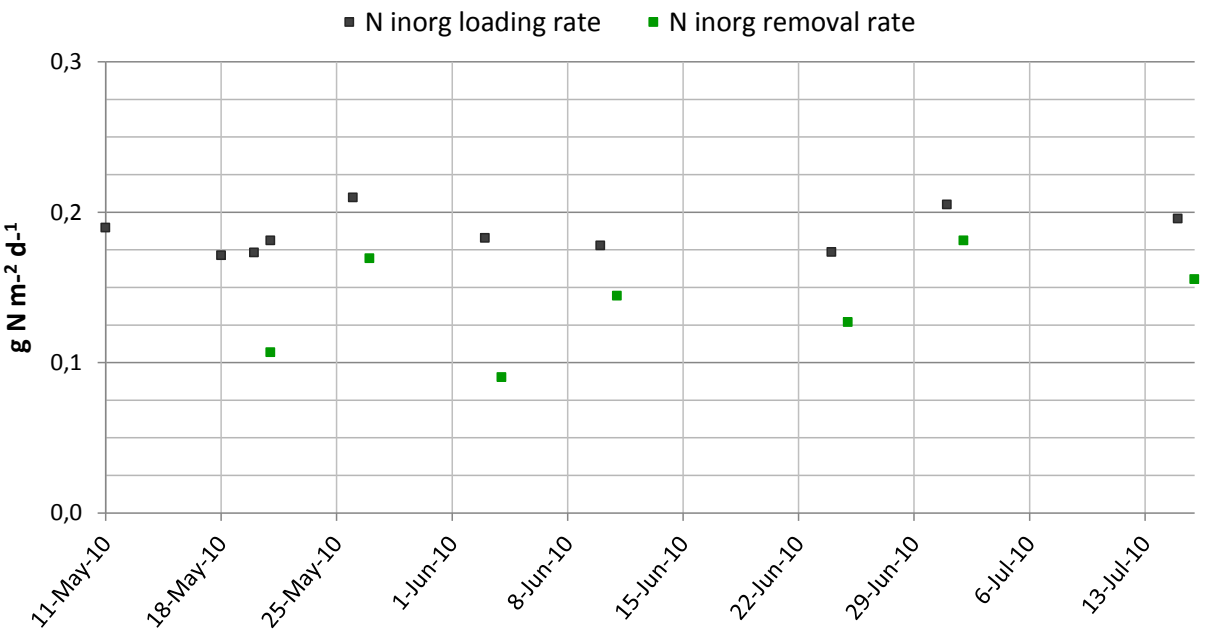

Fig. 37. Nitrogen loading and removal rates.

Unfortunately, unlike the pilot plant reactor operation, no measurements of redox potential were done for a better understanding of the oxidizing conditions inside the reactor.

Nitrogen load and nitrogen removal rates are shown in figure 37, whereas the efficiencies of NH_4^+-N removal and inorganic nitrogen removal are summarized in figure 38. The nitrogen loading and removal rates have been calculated according to APPENDIX I.

Considering the period 25th May – 16th July and excluding the results from the chemical analyses performed on the 4th June, the ratio between the nitrogen loading rate and removal rate was 80.6 %.

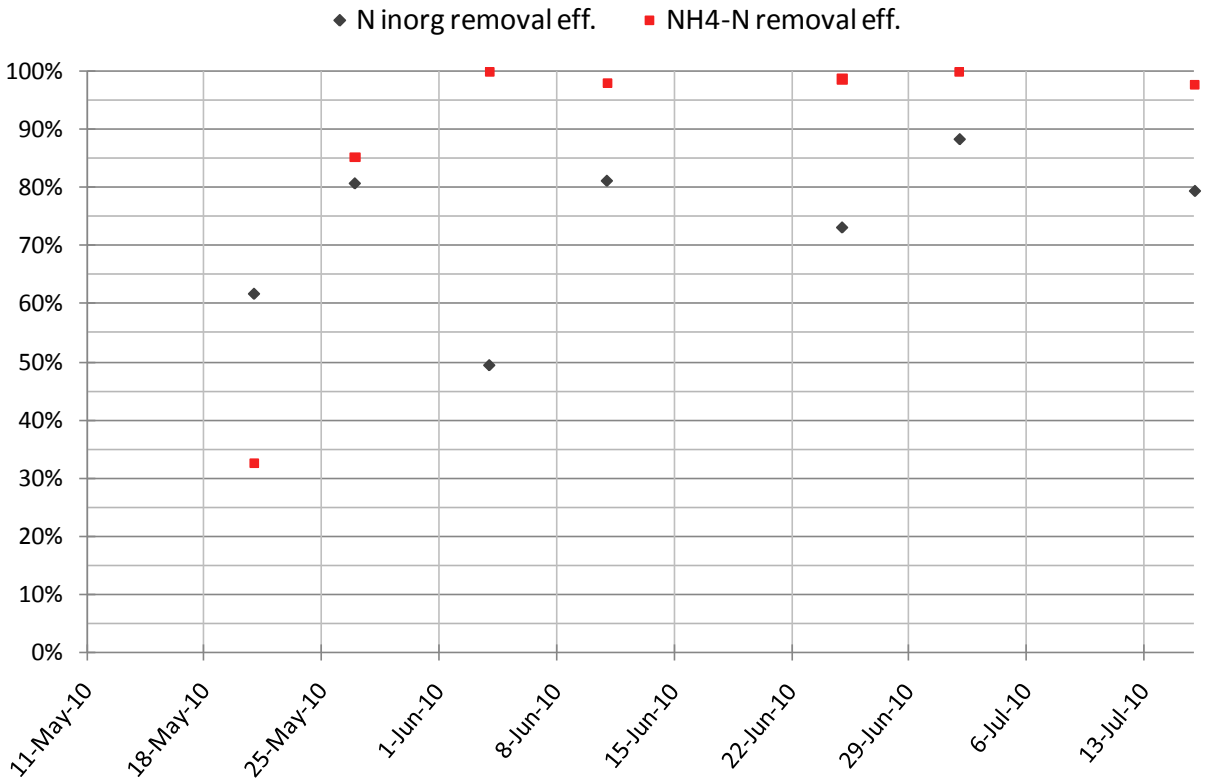

Fig. 38. Removal efficiencies for inorganic nitrogen and ammonia nitrogen.

Table 27 – COD, Alkalinity and conductivity (data from 20th May).

Parameter	Unit	Mean±S.D.	Samples
COD_{tot} removed	mg/l	7.0 ± 20.1	5
COD_{tot} removal	%	11.9 ± 27.0	5
$COD_{soluble}$ out	mg/l	47.7 ± 4.0	6
$COD_{soluble}$ removed	mg/l	5.9 ± 5.7	6
$COD_{soluble}$ removal	%	10.7 ± 9.9	6
$COD_{tot\ removed}$ / $N_{inorg\ removed}$	-	0.23 ± 0.60	5
$COD_{soluble\ removed}$ / $N_{inorg\ removed}$	-	0.17 ± 0.21	6
Alkalinity consumed	mmol/l K_s 4.3	3.81 ± 1.12	7
	g/l $CaCO_3$	0.19 ± 0.06	
Alkalinity $_{consumed}$ / NH_4^+-N $_{removed}$	g $CaCO_3$ / g NH_4^+-N	4.59 ± 0.77	7
Conductivity removed (1)	mS/cm	0.17 ± 0.15	33

Calculations on COD removed, alkalinity consumptions and conductivity decrease are shown in Table 27.

The results have been obtained averaging the data from each single week.

The ratio $COD_{removed}/N_{inorg\ removed}$ was about 0.2. The COD consumption was very low for COD measured both on samples unfiltered (COD_{tot}) and filtered 0.45µm ($COD_{soluble}$). The low consumption of COD might have been due to a low content of biodegradable organic substance. However the total influent COD was already low and less than 100 mg O_2/l. The low COD concentration might have been a limiting factor for heterotrophic denitrifying bacteria activity. On one occasion (25th June) an increase of unfiltered COD between outflow and inflow was measured. A reason could be found in the sampling procedure or perhaps to a not perfectly homogeneous conditions in the reactor. The ratio alkalinity consumed and NH_4^+-N removed was about 4.6 g $CaCO_3$/g NH_4^+-N and perhaps

slightly high for the one-stage deammonification process, but however, no pH drop below 6.6 were observed.

From the chart of conductivities in figure 39, it is possible to have a confirmation of the results obtained from the chemical analysis on the outflow. The start-up period for this reactor, before achieving a stable nitrogen removal was about two weeks (11th May – 26th May)

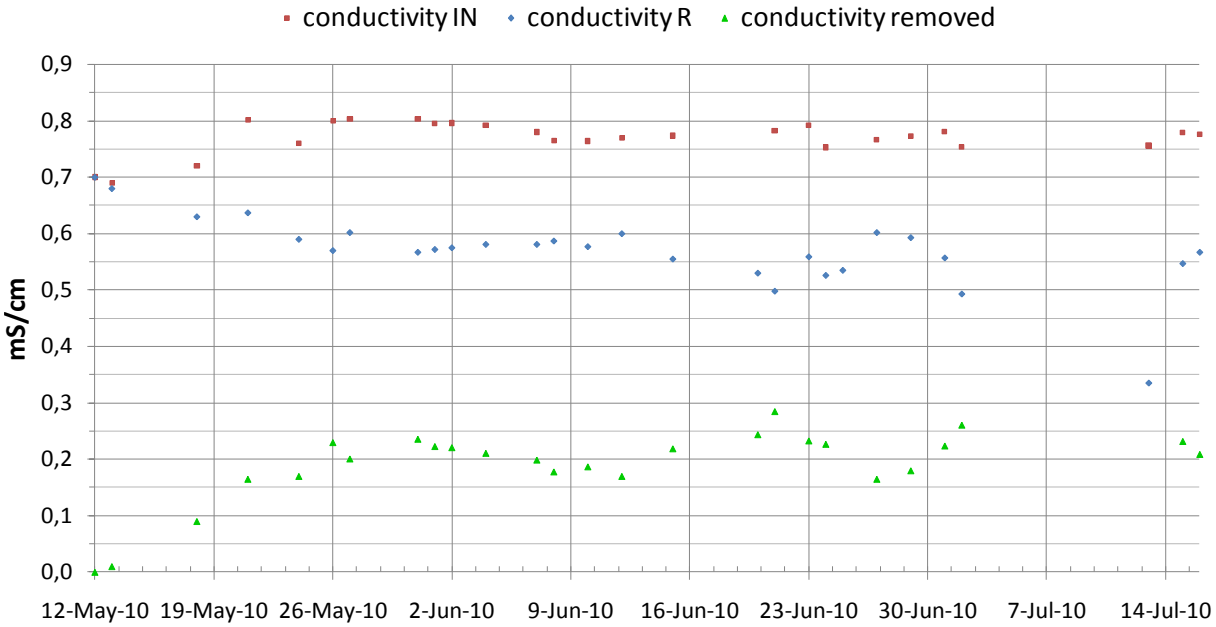

Fig. 39. Conductivity decrement in the reactor.

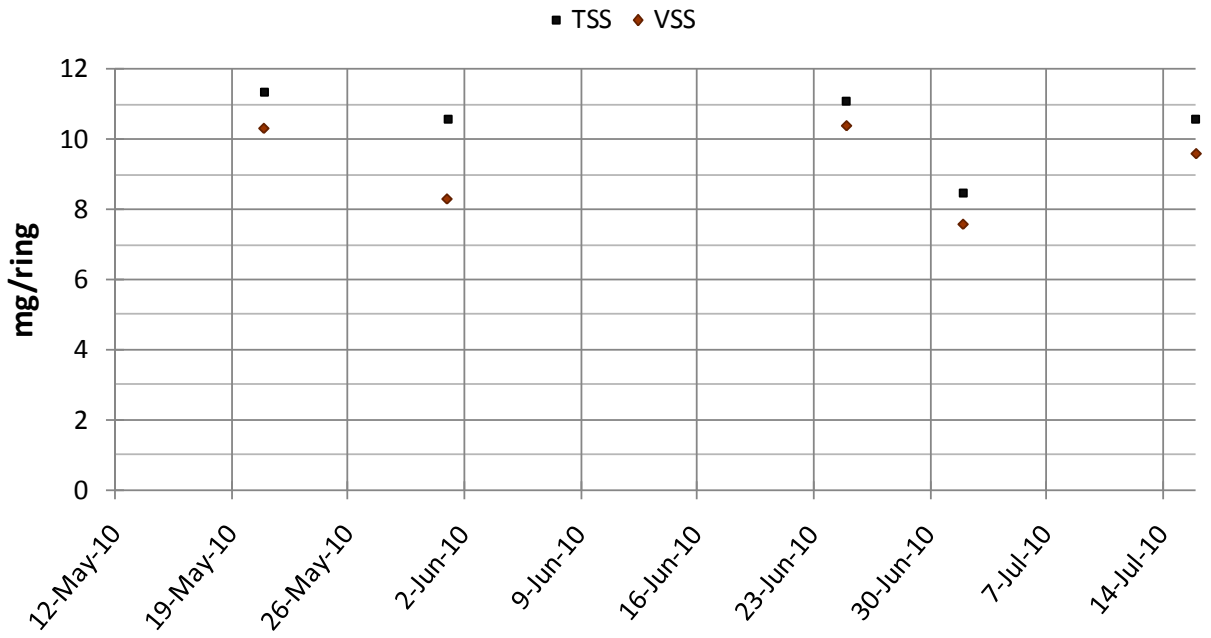

Fig. 40. Suspended solids as biofilm. No particular sign of growth or decay.

Measurements of volatile suspended solids content of the biofilm and as activated sludge

In this study, measurements on the biomass were carried out on the biofilm of the Kaldnes rings, in order to assess the volatile solids in the biofilm, and since the 1st June, also on the volatile suspended solids in the reactor as an estimate of the biomass concentration in the activated sludge.

The results from the measurements on the biocarriers (Fig. 40) did not show any particular trend of growth or decrease of the biomass content.

Only four measurements on the suspended solids were done (Fig. 42). Apart from the measurement on the 1st June, the following three measurements gave similar results between 103 and 112 mg VSS/l.

On the basis of the measurements of the 1st and 25th June, 2nd and 16th July, the total amount of suspended biomass in the reactor has been compared with the biomass attached on the rings on the same days. The results are summarized in Table 28.

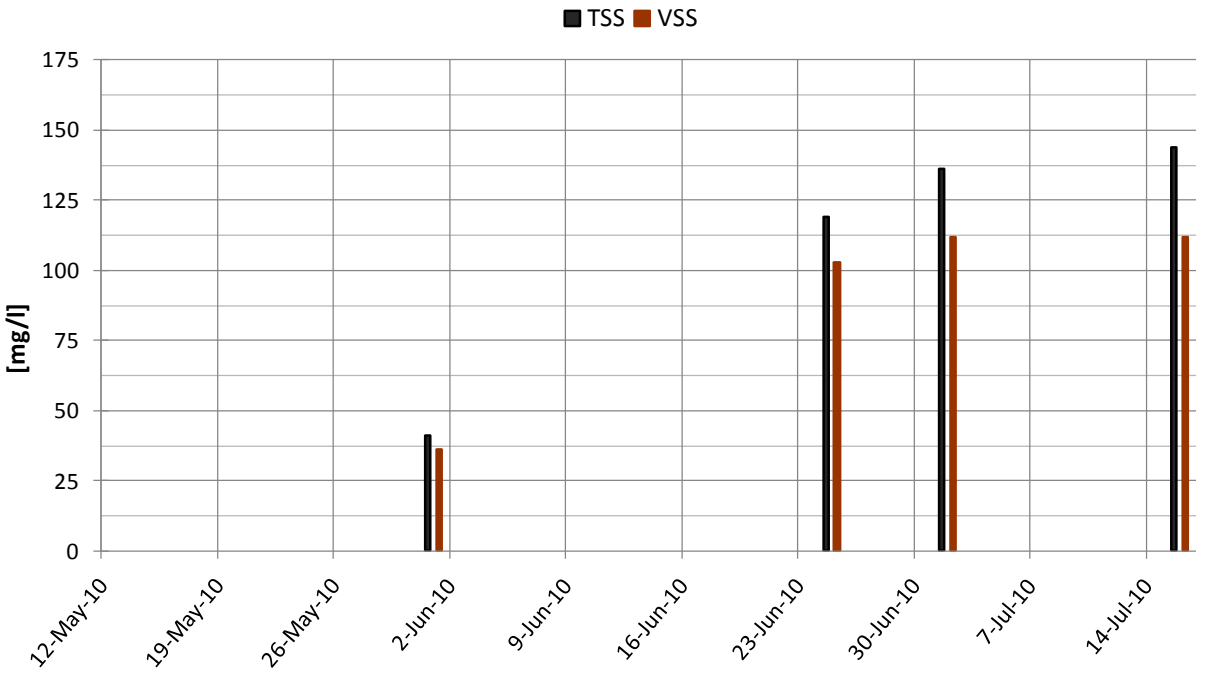

Fig. 42. Total and volatile suspended solids in the reactor

Table 28 – Comparison between biomass attached on the carriers and as activated sludge.

Parameter	Unit	Result	Period
$VSS_{biofilm}$ (1)	mg VSS	28152.8	1st June
$VSS_{act.\ sludge}$ (2)	mg VSS	244.3	1st June
% $VSS_{act.\ sludge}$	%	0.86 %	1st June
$VSS_{biofilm}$ (1)	mg VSS	31177.2	25th June – 16th July
$VSS_{act.\ sludge}$ (2)	mg VSS	734.7	25th June – 16th July
% $VSS_{act.\ sludge}$	%	2.35 %	25th June – 16th July

(1) and (2), calculated according to APPENDIX I.

Results from Specific Anammox activity (SAA)

The Anammox bacteria activity was followed by weekly measurements (SAA tests) in order to evaluate the Anammox bacteria activity during this operation at lower nitrogen load and check whether there was a decrease or not. In total 6 series of SAA analyses have been carried out. The tests were all performed at 35°C and had a duration of 70-80 minutes. The experimental data are included in APPENDIX III. The results are summarized in figure 43. The chart shows a gradual reduction of Anammox bacteria activity. A plausible reason of this decrement may be the load that was too low and not suitable for Anammox bacteria growth over time. The average SAA from the last five analyses is 3.8 g N m^{-2} d^{-1}.

4.2.6 *Possibility to treat supernatant from UASB with deammonification process*

The main challenge for the partial nitritation/Anammox process run in reactors with a low concentration of nitrogen in the influent is related to the long-term stability of the process. High ammonium removal efficiency can be easily achieved as showed from the graphs, but a higher load is strongly recommended for a good and stable operation. Unfortunately, due to practical reasons, this was not possible in this study. The main problem lies in the fact that Anammox bacteria have a low maximum specific growth rate (about 0.065 d^{-1}) and, as consequence, a too low ammonia nitrogen load can limit the growth of Anammox bacteria and their decay rate might exceed their growth rate. Unfortunately this reactor was not run for a long term, but only for two months and the results from suspended solids on the biocarriers did not show any clear trend. The results from SAA analyses seem to show a decrease in activity of Anammox biomass attached on the rings.

However, if a short hydraulic retention time was provided, and therefore a higher inflow load to the reactor (>0.7-0.8 g N m^{-2} d^{-1}), this technology might represent an effective treatment of the effluent from UASB reactor over time. A possibility to overcome this issue for the partial nitrification/Anammox step could be to treat a stream which has been previously concentrated by technology such as ion exchange or reverse osmosis (downstream degradation of organic matter and particles removal) but, nevertheless, these tech-

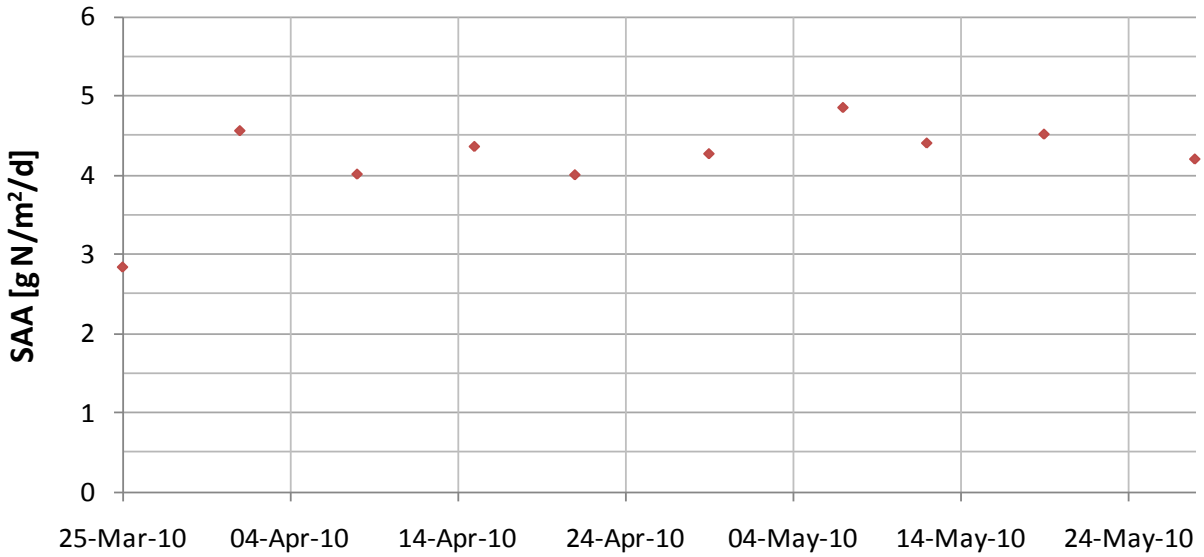

Fig. 43. Activity of Anammox bacteria in the biofilm (SAA test at 35°C)

nologies might increase costs for the treatment and frequent regenerations might be required if a large volume is treated and the economic and operational convenience of these alternative solutions is questionable. More studies are therefore needed to achieve an effective partial nitritation/Anammox process which can fully replace nitrification and denitrification or can be implemented in the main treatment line of a municipal WWTP.

Another problem that should not be overlooked is that the lower temperature of the process (< 25°C used for this experimentation) compared to the temperature of the reject water from the sludge treatment line can significantly reduce nitrogen removal efficiencies.

5 Single Partial Nitritation/Anammox Pilot Plant Reactor

5.1 Pilot plant reactor operation

The most interesting and useful results in these studies were obtained for the pilot plant reactor. The technical scale pilot plant reactor was started on the 27th May with the intention to study the performance of the partial nitritation and Anammox process in one single reactor over a rather long period of time under stable conditions. It was installed at Hammarby Sjöstadsverk research station in Stockholm. This master thesis evaluates the performance of the pilot reactor over the first four months (27th May – 28th September) at a temperature of 25°C and a load of about 3.4 g $N/m^2/d$.

5.1.1 Pilot plant reactor design

The technical-scale pilot plant reactor (Fig. 44) was designed as a continuous stirred and aerated Moving Bed Biofilm Reactor (MBBR) with Kaldnes™ carriers (model K1). The biocarriers used in this study were brought from Himmerfjärden Wastewater Treatment Plant (SYVAB Company) where deammonification process was carried out using biocarriers with a biofilm composed by Anammox and Nitrosomonas bacteria.

There was no need for any long start-up period because growth of biomass on the media was already adequate as shown on Fig. 45.

The reactor was continuously fed with supernatant from sludge dewatering after anaerobic digestion. It is assumed that it was operating as a CSTR. The reject water used in the operation came from Bromma Wastewater Treatment Plant which serves the north-western parts of Stockholm area. The reactor was run without any sludge recirculation.

Fig. 44. The one-stage pilot plant scale reactor for partial Nitritation/Anammox.

5.1.2 Reject water characterization

The reject water had a high content of ammonium of about 1000 mg/l, high alkalinity (about 3700 mg/l $CaCO_3$) and low content of biodegradable organic matter. Nitrate and nitrite concentrations were almost zero.

Fig. 45. Biocarriers used for pilot reactor start-up.

Table 29 – Characterization of the reject water from sludge dewatering after anaerobic digestion at Bromma WWTP.

Parameter	Unit	Mean±S.D.	Measurements
Ammonium NH_4^+	mg/l NH_4^+-N	963.3 ± 75.9	21
Nitrite NO_2^-	mg/l NO_3^--N	< 0.1	5
Nitrate NO_3^-	mg/l NO_2^--N	2.0 ± 0.3	5
Alkalinity	mmol/l K_s 4.3 g/l $CaCO_3$	74.2 ± 6.3 3.71 ± 0.31	18
Alk/NH_4^+-N	mol Alk / mol N	1.08 ± 0.09	18
Total Nitrogen (soluble)	mg/l	987.7 ± 89.8	12
Total Nitrogen (unfilt.)	mg/l	1142.7 ± 107.0	3
$COD_{soluble}$	mg O_2/l	611 ± 115	17
COD_{tot}	mg O_2/l	1137 ± 179	15
COD/ NH_4^+-N	-	0.63 ± 0.10	17
Total Phosphorus (soluble)	mg/l	1.63	1
Total Phosphorus (unfilt.)	mg/l	9.31 ± 1.64	3
Conductivity	mS/cm	9.31 ± 0.69 (1)	online
pH	-	8.43 ± 0.05	13
ORP	mV	-469.6 ± 88.0 (1)	online
Total Suspended Solids (TSS)	mg/l	335.5 ± 51.6 (2)	11
Volatile Suspended Solids (VSS)	mg/l	272.1 ± 62.4 (2)	11

(1) The mean value has been calculated from daily averages of the online measurements.
(2) Determined by filtration 1.6 μm.

The main characteristics of the reject water from Bromma WWTP are shown in Table 29. The high alkalinity makes this stream suitable for the deammonification process. The carbon to nitrogen ratio (expressed as the soluble ratio COD/NH_4^+-N) is about 0.63 and low enough for a potential good performance of the whole process.

The reject water was delivered periodically, according to the required use by the pilot reactor

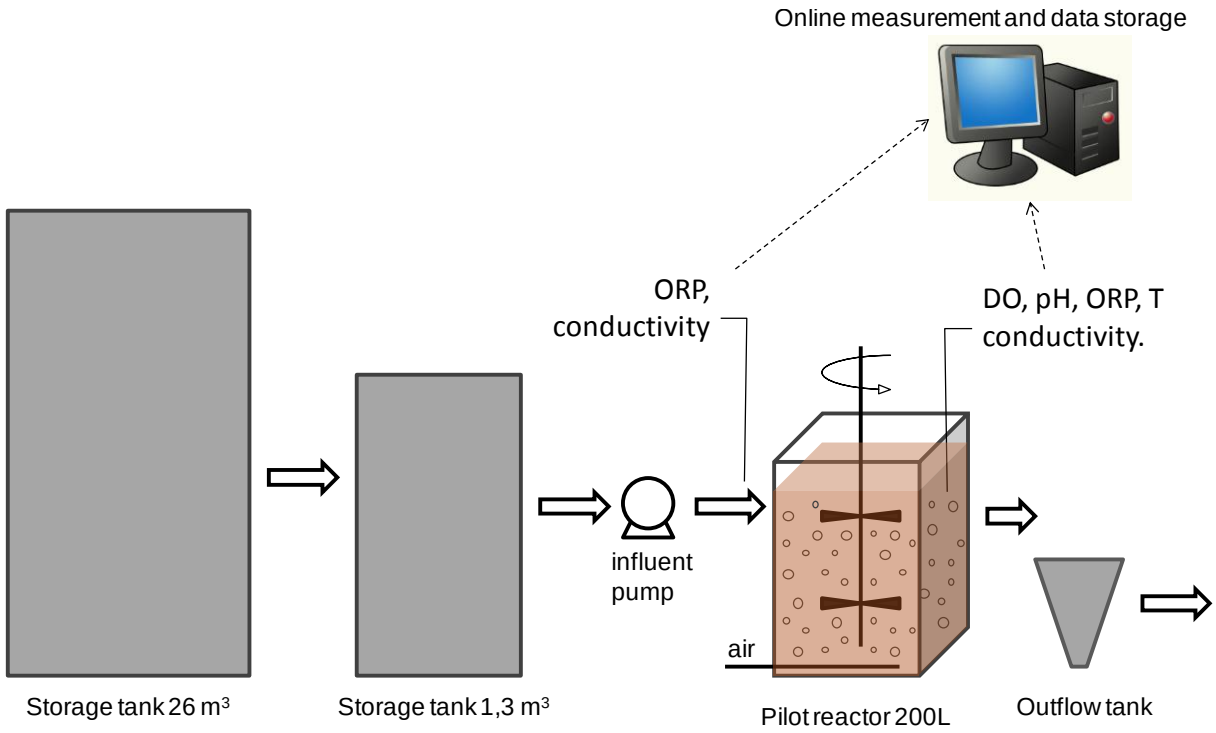

Fig. 46. Simplified scheme of the pilot scale reactor.

and other parallel installations, and stored in a big tank of 26 m^3 which was not stirred.

From this tank the reject water was regularly pumped to a smaller tank of 1.3 m^3 which was not stirred either and pumped continuously to the reactor by means of a volumetric pump, for the treatment of the supernatant (Fig. 46).

5.1.3 Operational strategy

The reactor had a working volume of 200 l which was filled with 80 l (about 40%) of Kaldnes rings with a specific internal surface area of 500 m^2/m^3. The effective volume of liquid in the reactor was about 166 l and it was measured at the beginning of the operation, after biocarriers introduction. The hydraulic retention time was measured using 166 l as volume divided by the inflow rate: $HRT = \frac{V}{Q} = \frac{166l}{l/day}$ [day].

The volume in the reactor was kept constant during the whole study. An appropriate mixing was provided by stirring and air supply. The aeration was located at the bottom of the reactor and the two-bladed stirrers were located, respectively, at about one-third and two-third of the working height of the reactor.

In the first two months the operational strategy was based on the flow rate, which was checked on a regular basis. The flow was kept at around 144 l/d, regardless of the concentration of ammonia in the inflow, with the exception of the first 18 days during which the flow was lower (about 101 l/d).

In the last two months the reactor operation was based on the operational strategy of maintain a constant ammonium surface load (ASL) of slightly more than 3.4 mg N m^{-2} day^{-1} and the pump rate was periodically set depending on the ammonia nitrogen concentration in the influent. The reason of this choice was to minimize changes in substrate (ammonium) loading rate. This resulted in an average flow rate of 135.8 l/d, but the ammonium in the reject water in this period was slightly higher. The hydraulic retention time at the higher load was kept constant at about 1.20 day (28.4 hours). In a couple of occasions the flow had been noticeably different from the average value and this was due to no inflow at the inlet (13th July between 8.30 and 17.30; 8th August the whole day), non-delivery of the reject water (23rd the whole day until the 24th 10 a.m.) and halving of the inflow rate to keep enough reject water in the tank (between 17th afternoon and 21th September morning). In these occasions the dissolved oxygen set point was decreased.

Except this the reactor was run in a stable way and without any kind of particular problem. During the whole study the reactor was heated up and the temperature was maintained at an average value of 25°C, varying between 24.4°C and 25.5°C. This range is below the optimum temperature of ANAMMOX bacteria and the operational temperature of the SHARON process, therefore a reduction of the efficiency can be expected. However running the process at a temperature of 25°C has the clear advantage of saving electricity needed for the heating, with the possibility to exploit the heat content in the supernatant from anaerobic digestion.

The DO concentration in the reactor was the main operational parameter that was varied in order to provide the suitable conditions for the process and bacteria activity. The dissolved oxygen in the reactor was automatically maintained constant at the value set in the control panel through air insufflations by means of a stainless steel sparger tube with minute perforations and located at the bottom of the reactor. The inflow rate was the parameter that was changed in order to maintain a constant load of ammonia nitrogen to the reactor. No pH adjustments and needs of chemicals have ever been required, and this is a good and promising sign for a long-term stability of the one-stage process.

Table 30 – Operational parameters for the laboratory-scale reactor.

Parameter	Unit	Value
Reactor Total volume	l	approx 200
Reactor Volume (liquid)	l	166
Kaldnes carriers	l	80

5.1.4 Measurements and experimental procedure

Physical parameters in the reactor such as pH, T, ORP, DO, conductivity were measured automatically every 10 seconds. These values have been corrected according to a calibration based on about twenty data because it was found that during the data logging the values of the parameters were slightly lowered compared to the ones showed in control panel in front of the reactor. The correctness of this calibration had been later verified.

The hydraulic retention time was measured manually as well as the pH of the reject water in inflow.

The chemical analyses on the main compounds of interest were usually carried out once per week

for the inflow and twice per week for the outflow, if possible. It was assumed that the decrease of ammonia nitrogen in the reject water over one week was negligible. Its average decrease was about 2.3% per week. The outflow samples were taken from the outflow settling tank after about 20 minutes having emptied it.

5.2 Results and discussion

5.2.1 Physical parameters

Most of the pH variations were a direct consequence of voluntary changes in DO set point with the aim to try to increase the efficiency of the process (i.e. in case of a higher nitrates concentration in the outflow, the dissolved oxygen concentration was decreased). The involuntary pH fluctuations were mostly the consequence of short anoxic periods (due to problems with DO-meter in few occasions) or momentary stopping of the inflow. However these incidents were quite rare. Temporary anoxic conditions resulted in an increase of the pH, whereas stopping of inflow (without change of dissolved oxygen concentration in the reactor) caused a decrease of pH.

The average values are shown in Table 31 and the variations over time are shown in figure 47.

A detailed table of the physical parameters is included in APPENDIX IV.

Some days the data logger was not recording and those data are missing, but however the process was monitored by reading the value from the control panel during working hours.

Several periods can be observed from the chart. The first period (27th May-12th June) was characterized by a lower inflow rate (100.8 l/d) in order to avoid a too high load for the biomass and perform a softer start-up. The DO was adjusted manually until the 6th June by turning the DO valve and the dissolved oxygen concentration was read on the control panel. The second period (13th June-22nd July) was the more unstable; the redox potential showed high values with great variations and pH varied between 6.5 and 7.4. However the chemical analyses in this period did not give any noticeable decrease in process efficiency. The flow rate was about 144-152.6 l/d, except a problem with the pump on the 13th July. In the third period (23rd July-22nd August) the pH

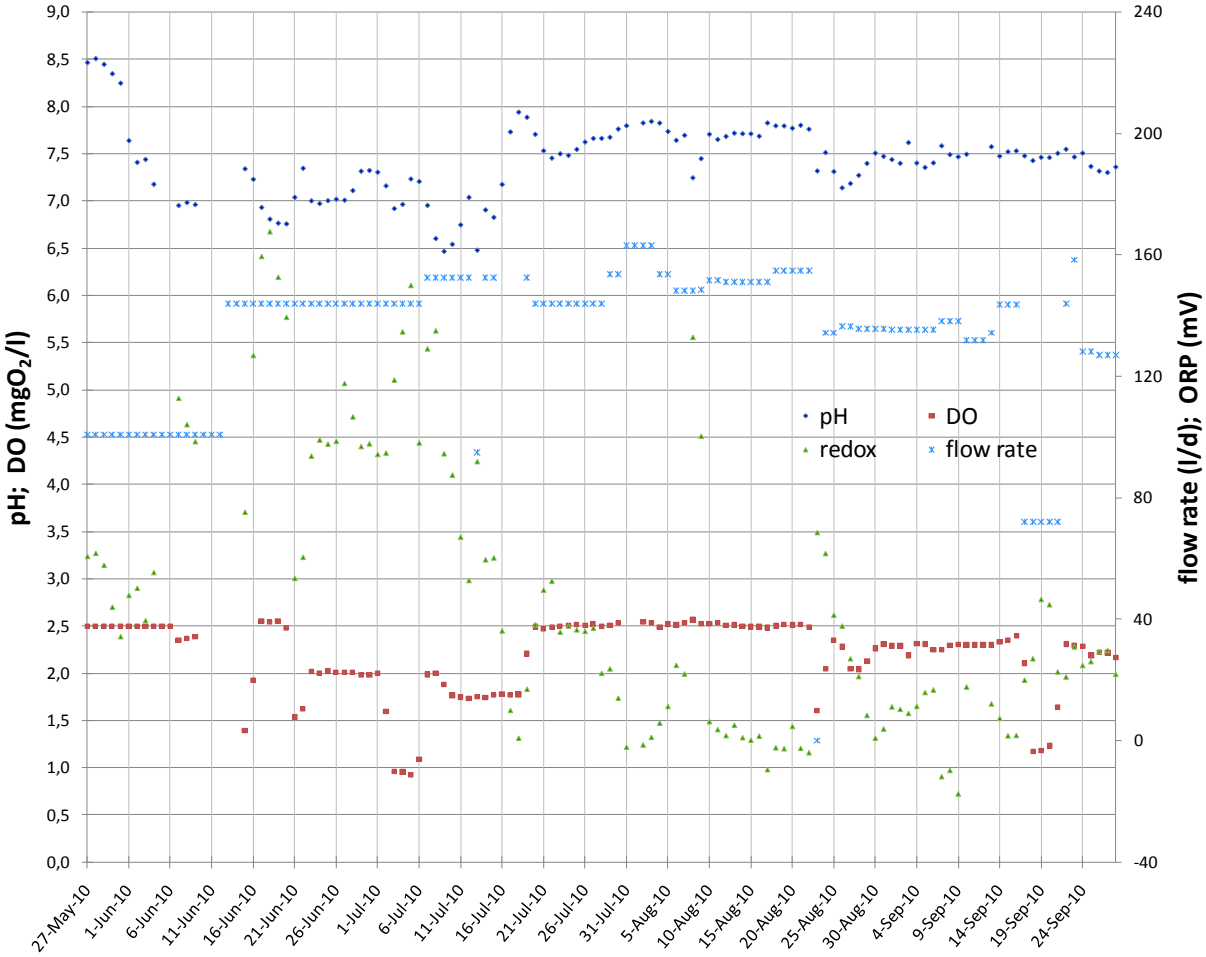

Fig. 47. Daily averages of the main physical and chemical parameters.

value was maintained between 7.3 and 7.8 and the dissolved oxygen at 2.5 mg/l. The reactor worked under stable conditions. The sharp increase of pH in between period 2 and 3 may have been due to a too high inflow rate. The fourth period (24th August-28th September) was also stable (with the exception of a necessary reduction in the inflow rate for some days due to external causes), but the pH was slightly lower (7.4 and 7.5) as well as the DO (2.3 mg O_2/l).

5.2.2 Biomass analyses. Total and volatile suspended solids and biofilm growth

Measures of total and volatile suspended solids were carried out on the activated sludge and on the biofilm, to assess the presence of any growth of the biofilm. No quantitative evaluation of biofilm thickness was carried out because of the sometimes strong difference between biofilm on the carriers and irregularity of the growth. FISH method was performed by my colleague and PhD student Jingjing Yang at Delft University of Technology.

FISH (Fluorescence In Situ Hybridization) analysis

FISH analyses done by J. Yang and M.K. Winkler (2010) at Delft University of Technology (The Netherland) on some sample carriers taken from the pilot plant reactor in July showed the coexistence of Nitrosomonas and ANAMMOX bacteria in the biofilm with only a small amount of Nitrobacter. The ANAMMOX bacteria are mostly belonging to the species "Candidatus Brocadia fulgida". No quantitative information about the different bacteria populations were communicated.

FISH method is applied to detect selectively specific groups of microorganisms in a mixture with others (e.g. the biofilm) and their spatial distribution, by using their specific 16S rRNA sequence. This technique exploits differences between the ribosomal gene sequences of different bacteria. By using specific rRNA-targeted oligonucleotide probes is possible to visualize single species, whole genera of bacteria or even phyla and domains (Amann et al, 1995 cited in Arshad, 2008, p.31).

Total and Volatile Suspended solids in the influent and activated sludge

The measurements were carried out with 1.6 µm filters glass fiber filters. Unfortunately it was not possible to measure the volatile suspended solids content on not-cellulose 0.45 µm filters because of delay with the purchase.

The suspended solids (volatile and not) in the *influent reject water* showed wide variations mainly depending on the time passed since the delivery of the new reject water from Bromma WWTP, because of the progressive sedimentation in the tanks the following day after the delivery. A likely average estimate of total suspended solids in the influent can be 461.1 ± 565.5 mg/l, with 84.6% of volatile solids content (389.9 ± 393.2 mg/l).

The *activated sludge* concentration inside the reactor was estimated by the measurement of the volatile suspended solids concentration from a mixed sample taken from the reactor (MLVSS Mixed Liquor Volatile Suspended Solids, or simply VSS). As suggested by many authors the use of VSS measurement as a measure of biomass

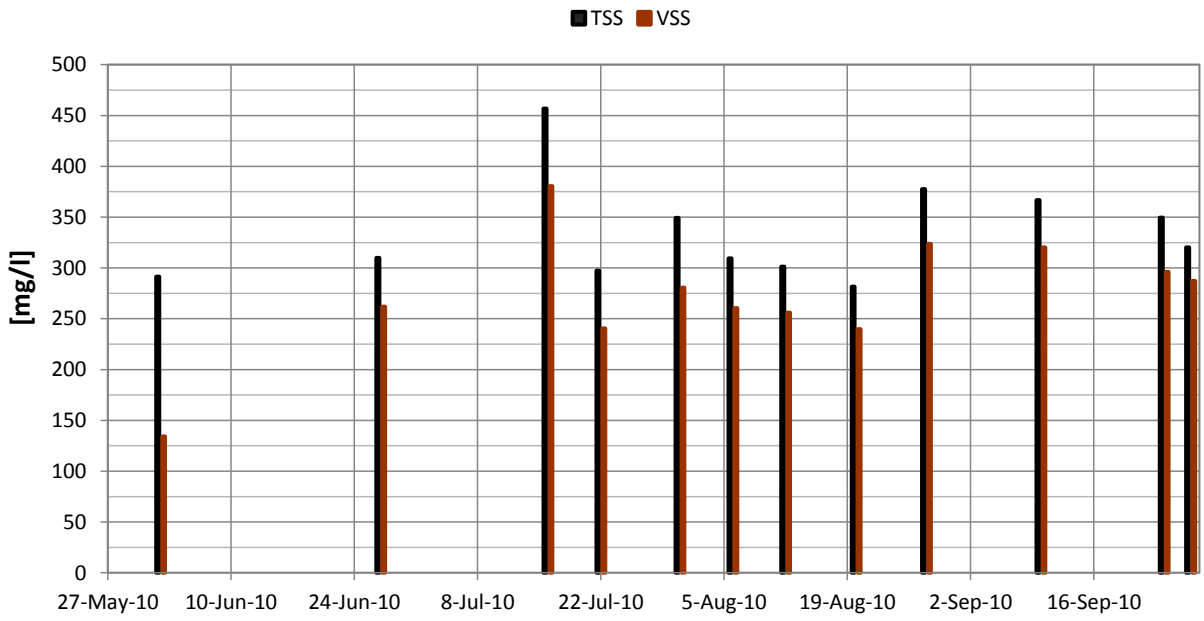

Fig. 48. Activated sludge in the reactor (filtered 1.6 µm).

is convenient but includes both endogenous and inert volatile solids in the activated sludge. New molecular techniques such as fluorescent in situ hybridization (FISH), RNA analysis, DAPI staining, and ATP analysis make possible to measure directly and quantify the metabolically active fraction of the activated sludge mixed liquor.

A likely average estimate of total suspended solids can be 334.3 ± 49.4 mg/l, with an 82% of volatile solids content (273.5 ± 59.6 mg/l). A chart is shown in figure 48.

No explanation has been found for the increment measured on the 16th July except that a higher inflow rate or a wrong measurement, whereas the mea-surement on the 28th August was close to the 23rd August, when the supernatant fed to the reactor was denser and containing a higher load of suspended solids as it was at the bottom of the tank. Detailed tables of the suspended solids measurements are included in the APPENDIX IV.

Measurements on total suspended solids were carried out also with filters with pores size of 0.45 μm and the resulting concentrations are slightly higher. The average on total suspended solids measurements is 347.5 ± 71.0 mg/l (based on a total of 8 measurements during the four months).

Bacteria growth as biofilm

Measurements of the volatile suspended solids on the carriers were carried out in order to assess whether there was any biofilm growth or not. The results are shown in figure 49.

The measurements (expressed in mg/ring) show an increase in the volatile solids content on the carriers, especially from the end of July. The lower result from the measurement on the 20th August could be related to the random sampling of the four carriers taken from reactor and used to obtain an estimate of the volatile solids content on a single ring.

The reason of the decrease from the high percentage of volatile solids measured on the 5th day of operation (99.3%) to the lower average percentage measured during next months of reactor operation (85.3 ± 2.2 mg/l) was not found except as a direct consequence of two different types of reject water with which biocarriers were fed prior to reactor start up and during pilot reactor operation.

Surprisingly, the growth observed on the biocarriers (+38.6% and about 4.8 mg/ring between the 22nd July and 10th September) resulted only in a slight increase of process efficiency (+5.2% calculated as inorganic nitrogen removed in g N/m^2 /d as comparison between the periods 18th June-20th July and 23rd July-26th September). This could be due to an increased diffusion resistance for substrates (NO_2^- and NH_4^+) through the thicker biofilm, or to an overestimation of the metabolically active biomass by VSS measurements. Unfortunately no results from microbiology analyses on the grown biofilm are available in order to monitor and to assess quantitatively the composition of the new biofilm. The increase of biomass attached on the carriers was observed together with a decrease in the biomass

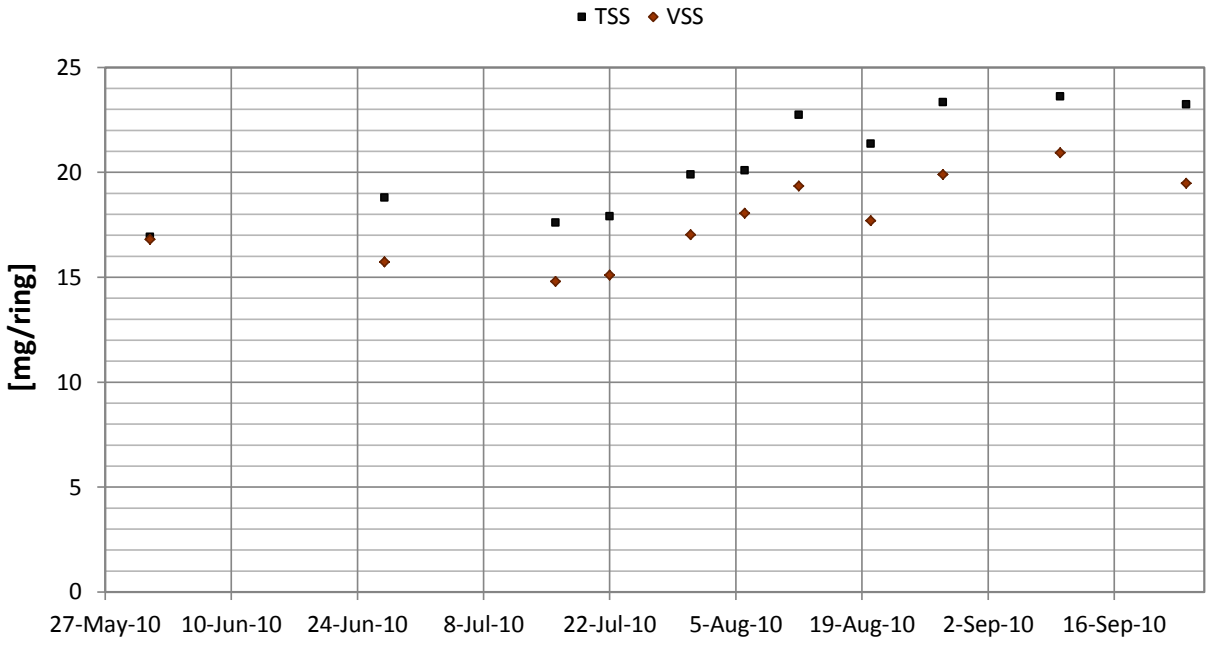

Fig. 49. Biomass attached on the Kaldnes carriers (filtered 1.6 μm).

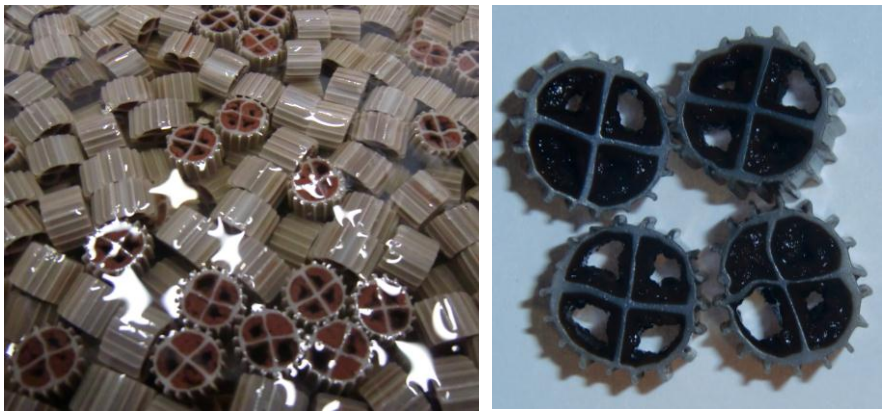

Fig. 50. Comparison of the biocarriers before starting the pilot reactor (left) and after 106 days of operation (right).

concentration in the activated sludge. During that period the mixer was working at about 27 rpm. Probably this rotational speed was suitable for bacteria growth and did not show effect of detachment of the biomass from the carriers. At the beginning of September (around the 3rd September) the mixing was increased to 50 rpm.

According to results of the 10 measurements carried out on the biomass attached on the rings and the corresponding suspended biomass in the reactor carried out on the same days, the activated sludge accounted for the 2.9% ± 0.8% of the biomass attached on the rings (both expressed as mg VSS).

This apparently small amount of bacteria may actually "cooperate" with Anammox biofilm on Kaldnes rings, by removing dissolved oxygen from the liquid and partly convert ammonium to nitrite needed by Anammox bacteria, as stated by Cema et al (2007b).

A visual comparison of the biofilm developed on the Kaldnes carriers is shown below in figure 50. The photos were taken on the start-up day (27th May) and on the 10th September. As it can be seen the growth of the biofilm was quite irregular among the Kaldnes media and a lower thickness of the biofilm on some rings might have been a direct consequence of detachment phenomena due to the mechanical stirring or aeration on those carriers supporting a high quantity of biomass.

5.2.3 Reactor performance evaluation and chemical analyses results

The raw results from chemical analyses on the inflow and outflow concentrations of the one-stage pilot plant reactor for partial nitritation and Anammox process are shown in figure 51. Detailed tables with all the chemical analyses results for both inflow and outflow are included in the APPENDIX IV.

The reactor performance during the four months was satisfying. High nitrogen removal was achieved and ammonium was greatly reduced as well as total nitrogen concentration, with a hydraulic retention time of only about 1.16 ± 0.15 days (i.e. 27.9 hours), calculated from the end of the first period with a lower inflow rate.

Two main phases can be identified from the chemical analyses results. The first one that goes until the 23rd July, during which the nitrate concentrations in the outflow were slightly higher and with a mean value of 117.7 ± 23.8 mg/l. This was probably due to a dissolved oxygen concentration in the bulk liquid slightly higher than necessary and thus part of the produced nitrite was oxidized to nitrate by Nitrobacters.

During the last two months with a constant load of 3.4 g N m^{-2} d^{-1} the average nitrate nitrogen concentration in the outflow was 82.9 ± 13.6 mg/l but an opposite slight increase of ammonium nitrogen in the outflow was noticed. This was equal to 76.0 ± 17.1 mg NH_4^+-N/l instead of 62.3 ± 13.9 mg NH_4^+-N/l of the previous period.

Perhaps a possible explanation of the reduced production of nitrate in the second period might be a lesser activity for Nitrobacter at pH=7.6-7.7 compared to pH=7, even though the average DO concentrations during this period were slightly higher than the previous period and ranging between 2.3 and 2.5 mg/l.

The nitrite concentration in the reactor were rather low and maintained constant at a mean value of NO_2^--N = 7.3 ± 1.8 mg/l. It is likely that nitrite nitrogen was the limiting factor for Anammox bacteria.

The nitrate in the outflow was not so high if compared with the global reaction for the deammonification process with a stoichiometric coefficient of 0.13, as showed on the next page.

$1\ NH_4^+ + 0.85\ O_2 \rightarrow 0.13\ NO_3^- + 0.435\ N_2 + 1.4\ H^+ + 1.43\ H_2O$.

The calculation can be performed on the last two months of operation. Considering the average inflow concentration of NH_4^+-N = 975.5 mg/l, which corresponds to NH_4^+ = 1254.2 mg/l and 69.92 mmol NH_4^+/l, about 69.92·0.13 = 9.06 mmol NO_3^-/l should be expected in the outflow. In the last two months of operation the nitrate nitrogen in outflow was NO_3^-N = 89.2 mg/l which corresponds to NO_3^- = 395.0 mg/l and 6.37 mmol NO_3^-/l, that is less than expected, but must be kept into account the untreated ammonium (NH_4^+-N = 76.0 mg/l = 5.43 mmol NH_4^+/l) and the nitrite in outflow (NO_2^--N = 7.9 mg/l = 0.56 mmol NO_2^--N /l).

Looking at the Fig. 52, removal efficiencies of 95%, 85% and 83% for NH_4^+-N, inorganic nitrogen, and Total Nitrogen (TN) respectively, have been achieved by partial nitritation/ANAMMOX process in one single reactor.

The ammonia nitrogen removal efficiency has been found to be higher when the hydraulic retention time and/or the dissolved oxygen concentration were higher than predefined reactor conditions for those specific days.

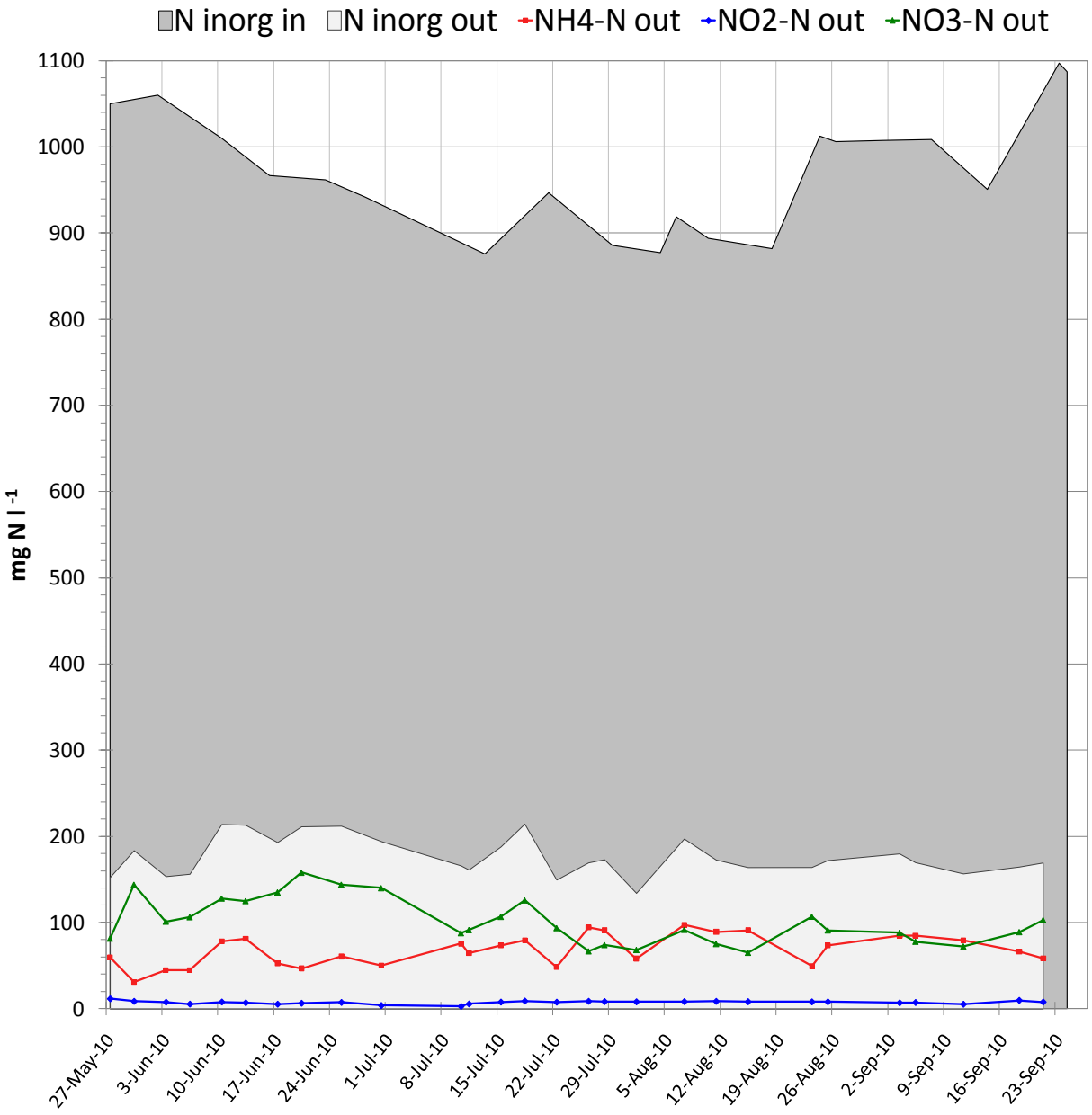

Fig. 51. Inorganic forms of nitrogen in the inflow and outflow during the four months of evaluation of the process in the pilot plant reactor.

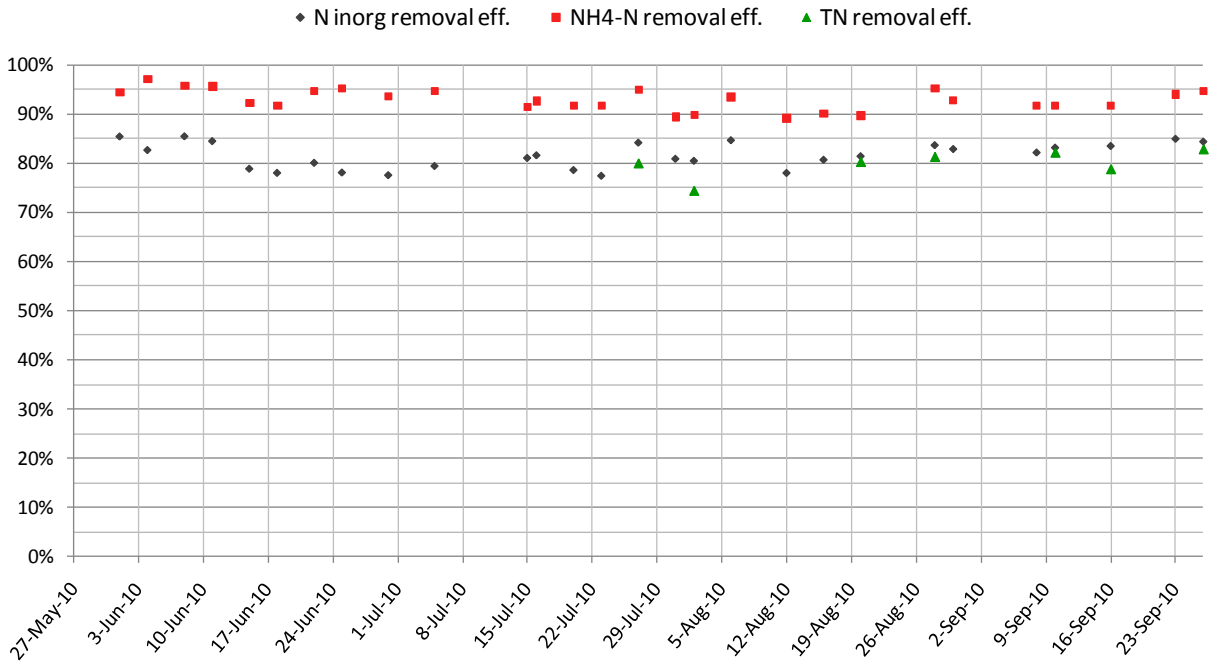

Fig. 52. Removal efficiencies for inorganic nitrogen (sum of NH_4^+-N, NO_3^--N and NO_2^--N), ammonia nitrogen (NH_4^+-N) and total nitrogen (TN, sum of inorganic and organic nitrogen).

Efficiencies have been calculated according to APPENDIX I.

During the last days of study on pilot reactor performance, the three removal efficiencies analyzed reached their highest values throughout all the experimental period, if we exclude the first days of reactor operation at a lower ammonium load. The general trend was slight positive and

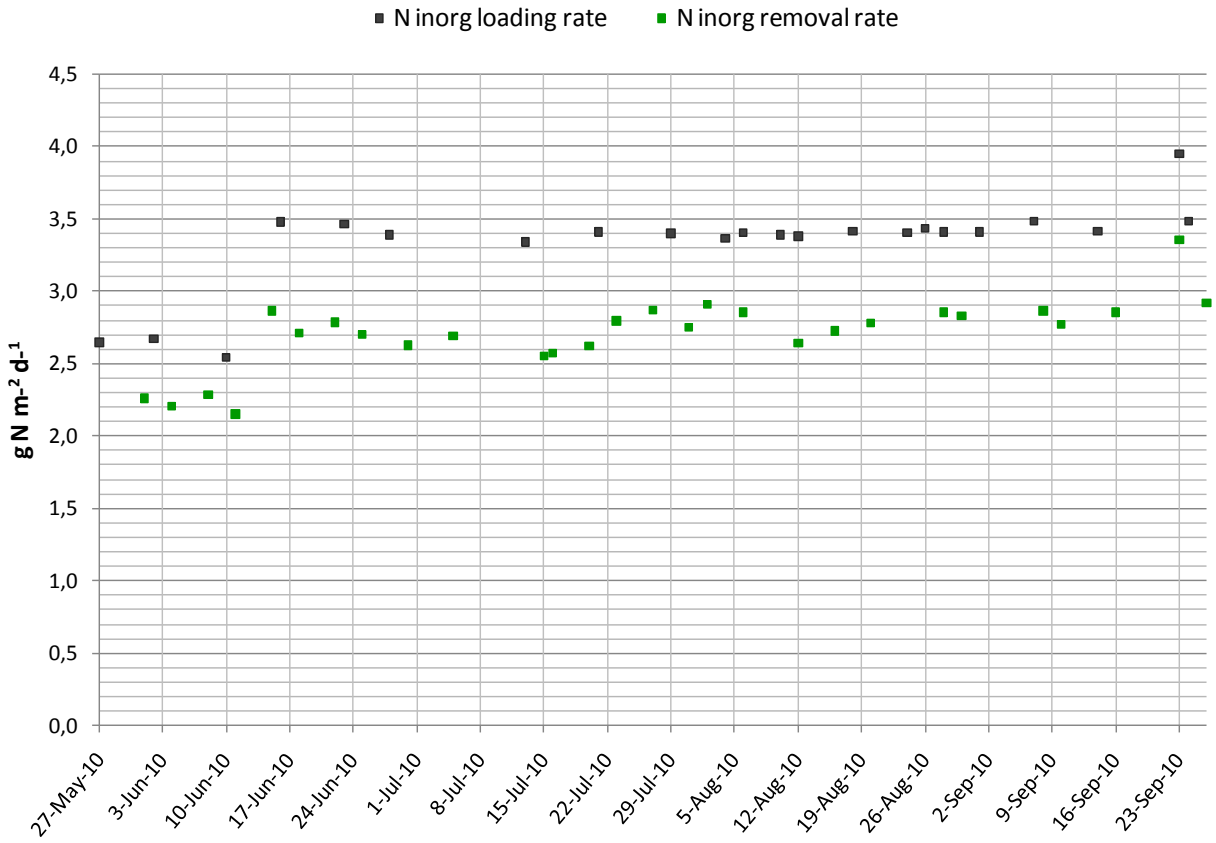

Fig. 53. Nitrogen loading rate (ASL) and nitrogen removal rate during the four months of pilot reactor operation.

Table 32 – ASL, inorganic nitrogen loading and removal rates.

Parameter	Unit	Mean±S.D	Period
Loading rate (ASL)	g N m^{-2} d^{-1}	2.62 ± 0.07	27th May-12th June
Loading rate	mg N l^{-1} d^{-1}	631.6 ± 16.0	27th May-12th June
Removal rate	g N m^{-2} d^{-1}	2.25 ± 0.29	27th May-12th June
	mg N l^{-1} d^{-1}	542.6 ± 26.8	27th May-12th June
Ratio (loading rate)/(removal rate)	%	86.0	27th May-12th June
Loading rate (ASL)	g N m^{-2} d^{-1}	3.44 ± 0.13	13th June-28th Sept.
Loading rate	mg N l^{-1} d^{-1}	819.1 ± 95.3	13th June-28th Sept.
Removal rate	g N m^{-2} d^{-1}	2.78 ± 0.16	13th June-28th Sept.
	mg N l^{-1} d^{-1}	667.2 ± 76.8	13th June-28th Sept.
Ratio (loading rate)/(removal rate)	%	80.1	13th June-28th Sept.
Loading rate (ASL)	g N m^{-2} d^{-1}	3.45 ± 0.14	21st July-28th Sept.
Loading rate	mg N l^{-1} d^{-1}	839.5 ± 60.2	21st July-28th Sept.
Removal rate	g N m^{-2} d^{-1}	2.85 ± 0.16	21st July-28th Sept.
	mg N l^{-1} d^{-1}	692.8 ± 58.9	21st July-28th Sept.
Ratio (loading rate)/(removal rate)	%	82.7	21st July-28th Sept.

efficiencies might have been even higher if the reactor performance had been analyzed for a couple of weeks more.

Considering the last two months the average efficiencies were: 92%, 82.5% and 80% for NH_4^+-N, inorganic nitrogen and TN respectively.

Decreases in efficiencies due to the flow rate increase and thus ammonium loading rate rise from 2.6 g N m^{-2} d^{-1} to about 3.4 g N m^{-2} d^{-1} were noticed. The load was changed on the 13th June. Comparing the first two weeks of operation (influent load of 2.62 g N m^{-2} d^{-1}) and the last two weeks of June (3.44 g N m^{-2} d^{-1}), efficiencies decreased by -2.37% and -7.19% for inorganic nitrogen and NH_4^+-N respectively (Fig. 52). After a couple of weeks bacteria adaptation to the new load was observed and probably in the last month bacteria could have been ready to treat a higher load, but this was not studied in this thesis.

A better evaluation of the process can be conducted by analyzing the nitrogen loading rate and the nitrogen removal rate (Fig. 53).

The loading and removal rates have been calculated according to APPENDIX I. The loading

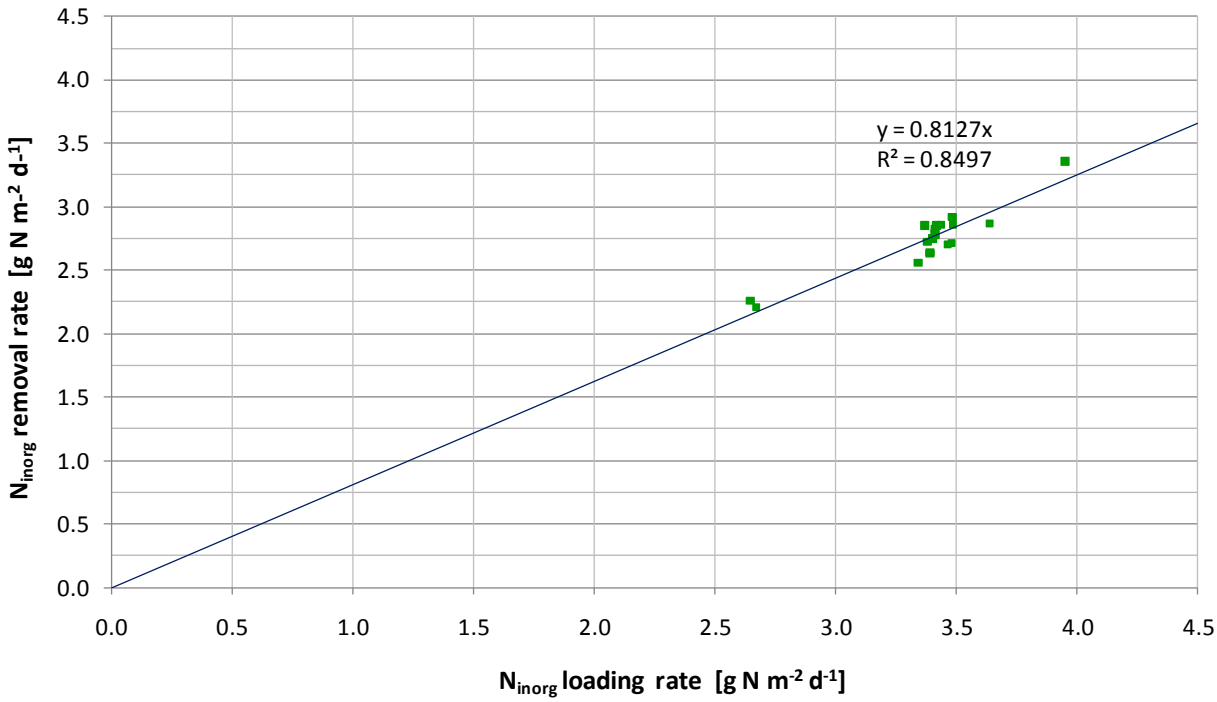

Fig. 54. Rough estimate of nitrogen removal rate at higher ASL

Table 33 – COD, Alkalinity and conductivity.

Parameter	Unit	Mean±S.D.	Samples
COD_{tot} removed	mg/l	462.7 ± 182.7	11
COD_{tot} removed	%	41.1 ± 12.0	11
$COD_{soluble}$ out	mg/l	350.6 ± 42.6	13
$COD_{soluble}$ removed	mg/l	246.1 ± 106.7	11
$COD_{soluble}$ removed	%	39.9 ± 12.0	11
$COD_{tot\ removed}$ / $N_{inorg\ removed}$	-	0.61 ± 0.26	11
$COD_{soluble\ removed}$ / $N_{inorg\ removed}$	-	0.32 ± 0.14	11
Alkalinity consumed	mmol/l K_s 4.3	67.6 ± 7.6	13
	g/l $CaCO_3$	3.38 ± 0.38	
Alkalinity $_{consumed}$ / NH_4^+-N $_{removed}$	g $CaCO_3$ / g NH_4^+-N	3.88 ± 0.34	13
Conductivity removed	mS/cm	7.32 ± 0.67	online

rate (g N m^{-2} d^{-1}) is sometimes called ASL (ammonium surface load). A summary table on loading rate and nitrogen removal rate for three different period is shown in Table 32.

The first period (27th May-11th June) includes the first two weeks at lower loading rate, the second one is the whole period at higher load (13th June - 28th September) and the third one is the period (21th July - 28th September) where a more stable operation and higher efficiencies were obtained.

As expected, the removal rate efficiency at the loading rate of 3.4 g N m^{-2} d^{-1} was lower compared to the previous one at the loading rate of 2.6 g N m^{-2} d^{-1}, but it was anyway above 80%.

A simplistic modeling approach (Fig. 54) tries to predict the possible nitrogen removal rates at higher ammonium loading rates. However at a too high loading rate, efficiency will drop due to the shorter hydraulic retention time. However only two main group of nitrogen loading rate were tested (2.6 and 3.4 g N m^{-2} d^{-1}) with the addition of a single point during which a higher loading rate was provide for two days (22-23 September). In the chart the intercept of the fitted line was set equal to zero.

Comparisons and average results of COD removals, alkalinity consumptions and conductivity decrement for the period at load of about 3.4 g N m^{-2} d^{-1} are shown in Table 33.

The COD removal [%] calculated as $1-\frac{(COD)_{out}}{(COD)_{in}}$ had been lower during the last month compared to the previous months (29.3 % instead of 47.9 % for the COD unfiltered and 31.0 % instead of 45.0 % for the COD filtered 0.45 μm). Plausible hypothesis could be changes in biodegradable organic matter content in the reject water from dewatering of digested sludge from Bromma WWTP, a reduced activity or concentration of denitrifiers in the reactor or a higher sedimentation in the reject water tank prior to treatment.

The ratio $COD_{removed}/N_{inorg\ removed}$ ranged between 0.22-1.01 and 0.14-0.55 considering the COD unfiltered and filtered 0.45 μm respectively.

The drop of conductivity between inflow and in the reactor is due to ammonia oxidation to nitrite and nitrogen gas and alkalinity consumption in the nitritation and Anammox processes.

The decrement of conductivity between inflow and the outflow is shown in figure 55.

The COD in the outflow was fairly constant and ranging between 300 and 400 mg O_2/l (Fig. 56).

The curve of the soluble COD removed over time seems to be parallel to the curve showing the concentration of soluble COD in the influent supernatant, whereas the soluble COD in the reactor was rather constant.

A possible explanation might be that the biodegradable organic matter was a limiting factor for heterotrophic bacteria and the percentage of COD removed (about 40% as shown in Table 33) was only the biodegradable fraction. This hypothesis could be confirmed by the $CBOD_5$ measurement of the 14th September (the procedure adopted is briefly described in chapter 3.4) determined on the influent reject water which gave the result of:

$$CBOD_5 = \frac{D_1 - D_2}{0.01} = 173\ \text{mg/l},$$

where:

D_1= DO of diluted sample immediately after preparation (8.94 mg/l);

D_2= DO of diluted sample after 5 d incubation (7.21 mg/l);

0.01 = 1% dilution of the sample;

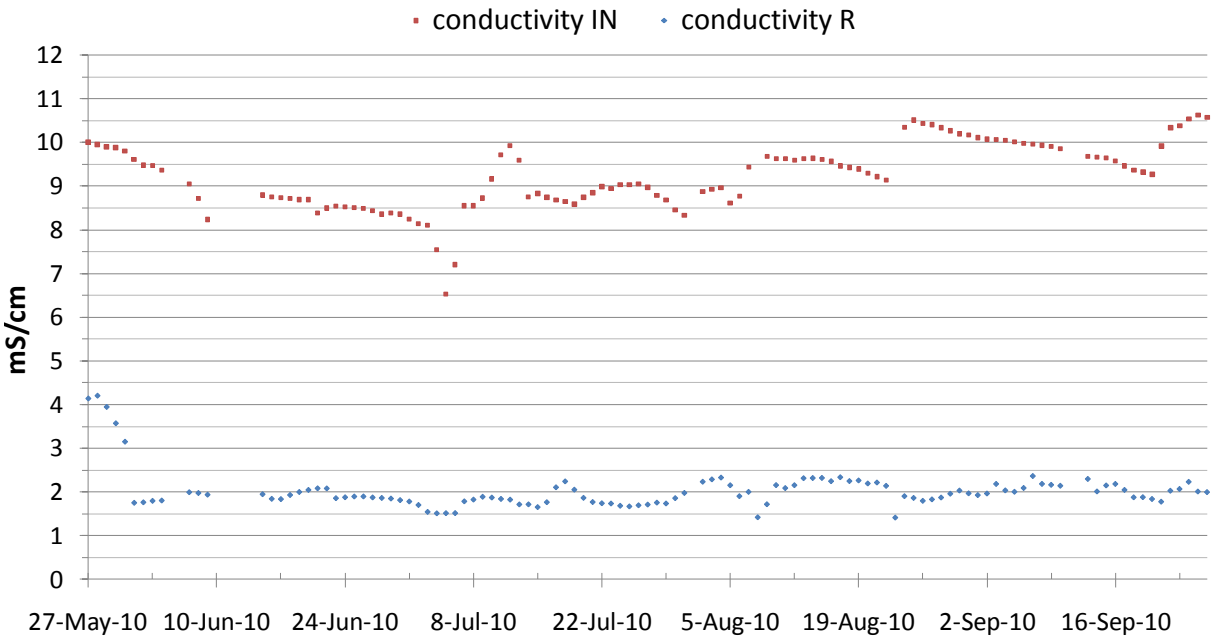

Fig. 55. Conductivity decrement in the reactor (about 78.7 %).

Unfortunately the dilution did not result in a DO uptake of at least 2 mg/l and the temperature during the measurement reached 21.5 °C for a couple of hours. The other dilution chosen (0.5%) gave a too high and not reliable result probably due to a small presence of air bubbles in the upper part of the bottle.

The DO uptake for the dilution water blank was 0.18 mg/l, thus less than 0.2 mg/l.

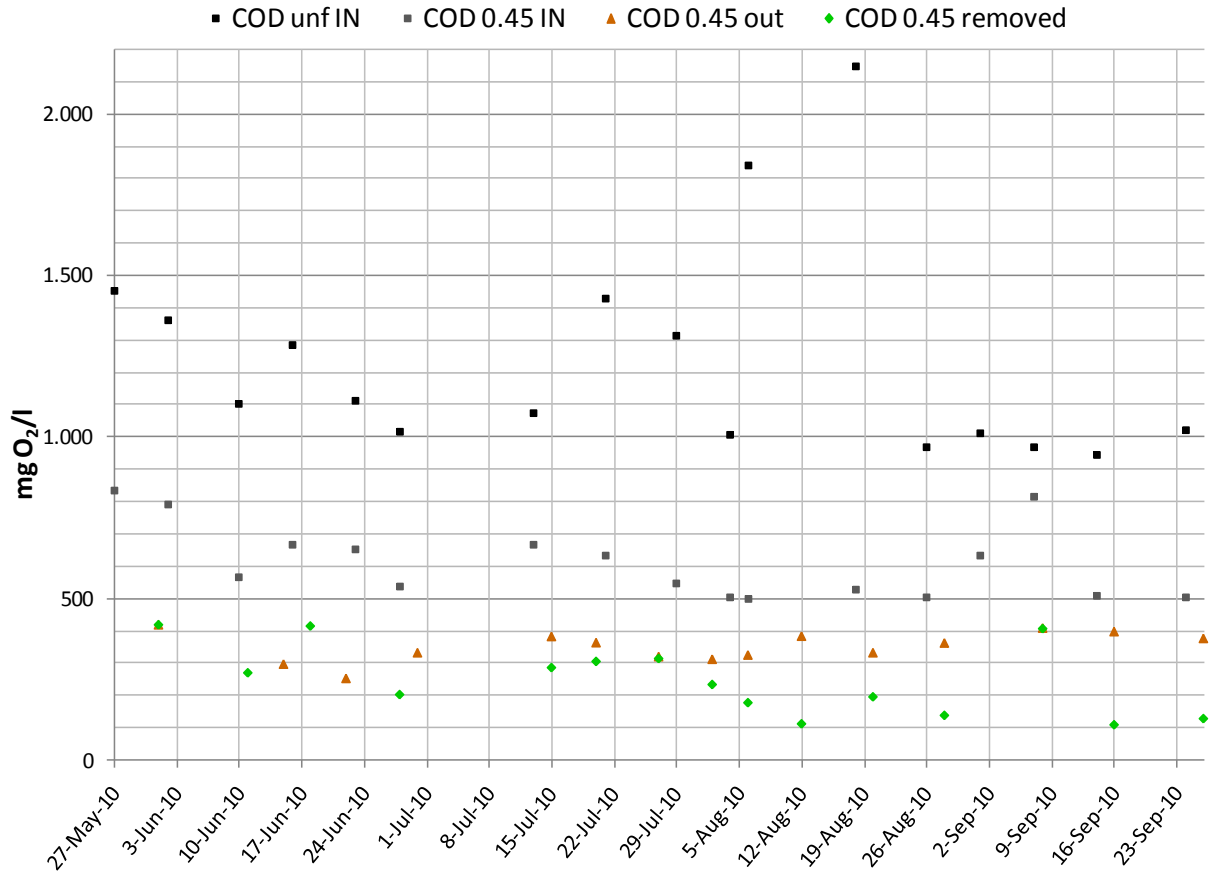

Fig. 56. COD prior to treatment, COD in the outflow and COD removed.

The result from $CBOD_5$ measurement represents the 34.1% of the soluble COD (0.45 μm) measured the same day on the same reject water. However that week, the COD consumption was only the 21.5 % of the soluble COD in the inflow.

The free ammonia (NH_3) and free nitrous acid (HNO_2) concentrations in the reactor have been approximately calculated using the equilibria at 25°C:

$NH_{3(aq)} + H_2O \leftrightarrow NH_4^{+}{}_{(aq)} + OH^{-}{}_{(aq)}$,

$K_b = \frac{[NH_4^+][OH^-]}{[NH_3]} = 1.78 \cdot 10^{-5}$;

$HNO_{2(aq)} + H_2O \leftrightarrow NO_2^{-}{}_{(aq)} + H^{+}{}_{(aq)}$,

$K_a = \frac{[NO_2^-][H^+]}{[HNO_2]} = 4.57 \cdot 10^{-4}$;

From the concentration of ammonium and nitrite in outflow, the average values of pH on that specific day of outflow measurement and the equilibrium constants the results obtained are:

- Free ammonia NH_3 = (1.51 ± 1.05) mg/l with a maximum value of 3.87 mg/l and a minimum of 0.29.
- Free nitrous acid HNO_2 = (0.0024 ± 0.0016) mg/l with a maximum value of 0.0079 mg/l.

It is likely that nitrite oxidizing bacteria (NOB) might have been inhibited to some extent by free ammonia (crf. paragraph 1.2.4). The concentrations calculated above might have been slightly lower because the results have been calculated with molar concentrations without activity corrections.

Total soluble phosphorus in the outflow (filtered 0.45 μm) was measured only once (27th July and under stable conditions) and the result was about 0.7 mg/l (with an inflow concentration of about 1.6 mg/l).

Some relationships and correlations have been studied and are here below presented (Fig. 57-60). The following parameters showed a good correlation in spite of possible small errors of measurement or calibration and/or interferences from changes in other parameters.

The conductivity was found to be a good parameter to monitor the performance of the process and the ammonia nitrogen removal. Its advantage is that it can be easily measured giving an immediate result. Most of the times, the lowest values of conductivity were observed together with higher ammonia nitrogen removal (Fig. 57) and low NH_4^+-N concentration in outflow.

High conductivity removal was often associated to a higher consumption of alkalinity (Fig. 58-59). This is related to the hydrogen carbonate (HCO_3^-) consumption as consequence of nitrification and Anammox reactions and the production of hydrogen ions.

Lower values of pH and pH drops were found together with decrements of alkalinity of the liquor in the reactor (Fig. 60). A consumption of alkalinity, as a consequence of HCO_3^- removal, leads to a lower buffer capacity and ability of the liquor to withstand pH drops. pH drops in the reactor can be provoked by higher DO concentrations (and thus an enhanced nitrification which produces H^+) or a lower inflow rate (and thus a lower incoming of HCO_3^- and alkalinity).

Very often low values of pH were observed together with high redox potential (ORP) as showed in figure 61.

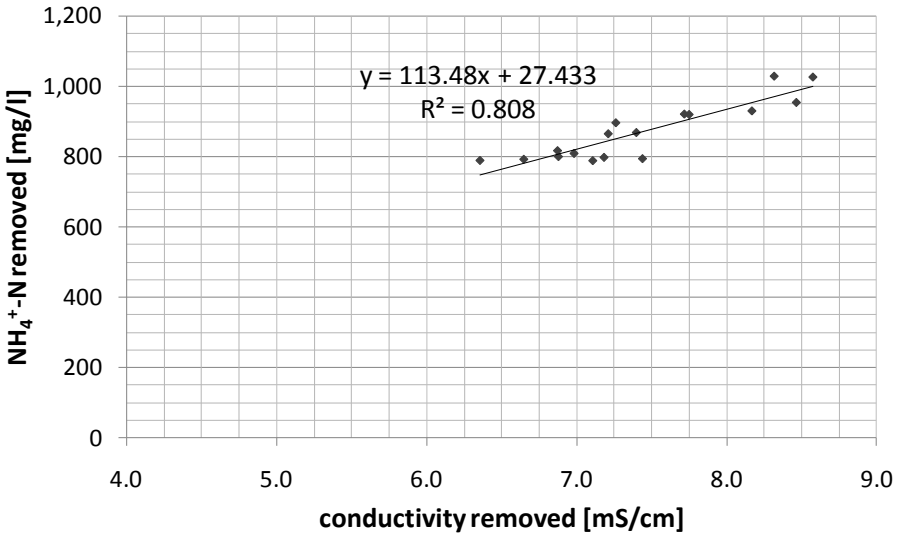

Fig. 57. Conductivity removed and NH_4^+-N removed

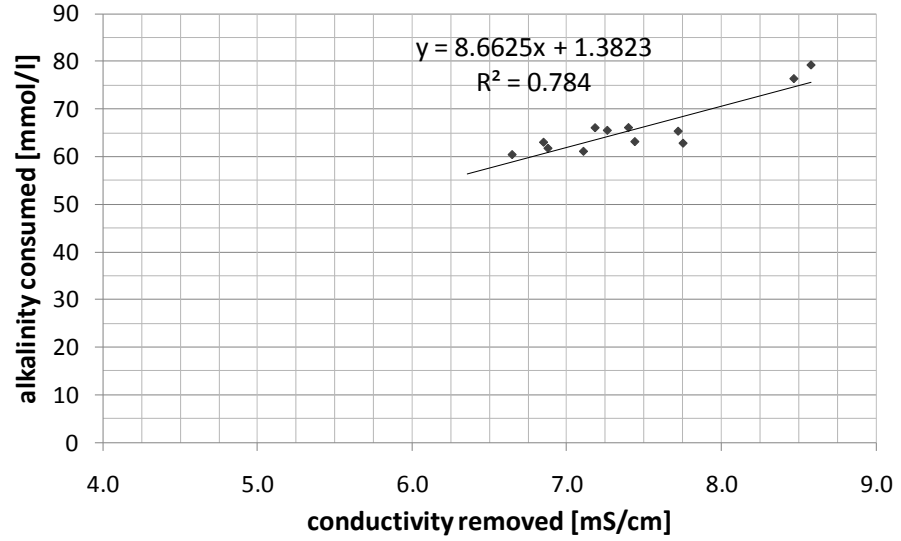

Fig. 58. Conductivity removed and alkalinity consumption

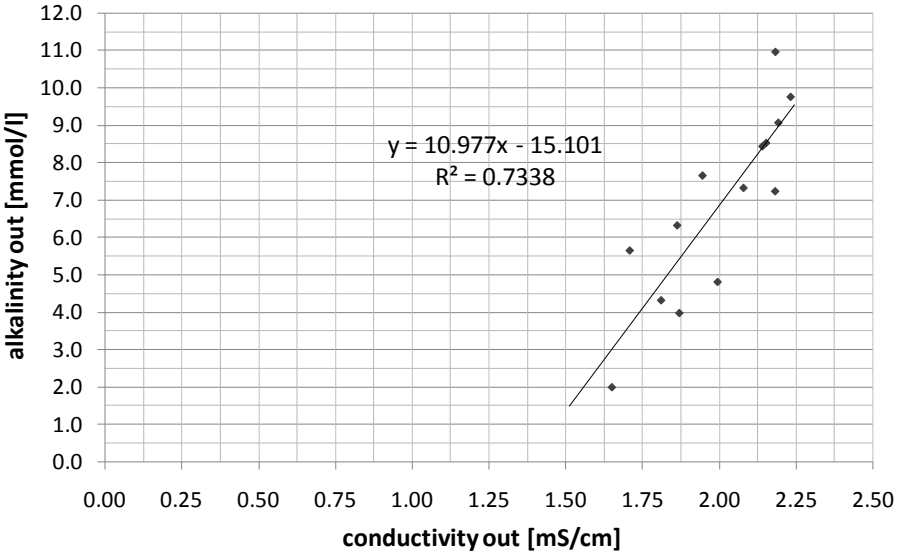

Fig. 59. Conductivity and alkalinity in out-flow

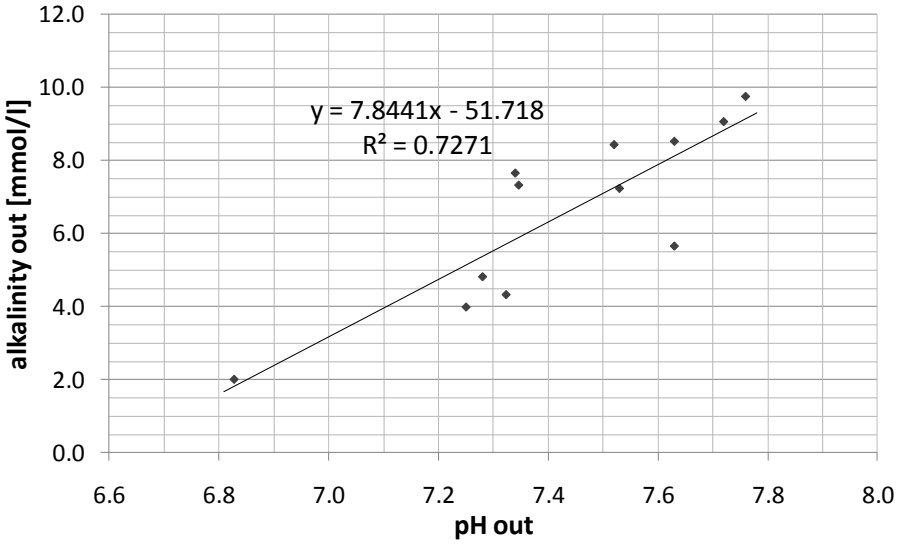

Fig. 60. pH in the reactor and alkalinity in outflow

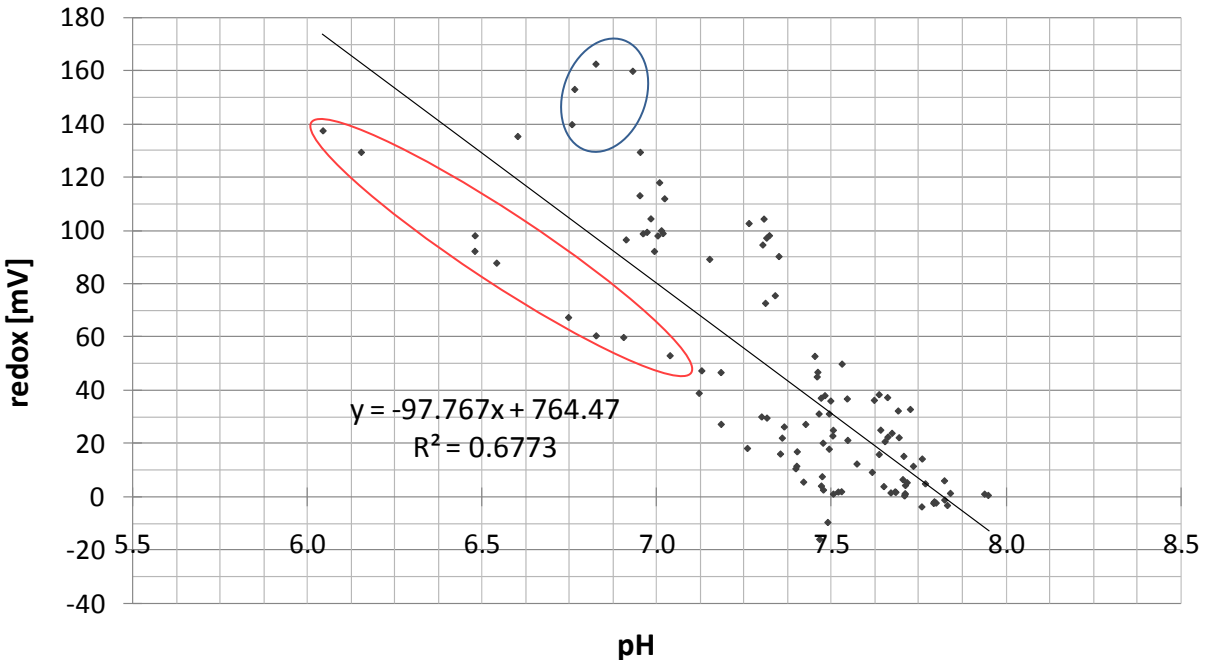

Fig. 61. pH and redox (ORP) conditions in the reactor.

The main reason lies in the fact that both of these two parameters are strongly dependent on the DO concentration in biological treatment. Oxygen is a strong oxidizer in waters, which therefore raises the redox potential and oxidizes ammonium and nitrite through nitrification process by Nitrosomonas and Nitrobacters, leading to a decrease of the pH. The values of these two parameters – ORP and pH – within the reactor are also influenced by the incoming flow of reject water which tends to lower the redox potential and contrast the pH decrease. The reject water prior to treatment has, in fact, basic and reducing conditions with a negative and low ORP (around -470 mV) and high pH value (8.43).

The redox potential in the reactor may also be influenced by other reductants/oxidants compounds concentrations such as NO_3^-, SO_4^{2-} (oxidants) or NH_4^+, NH_3, organic matter, HS^-, Fe^{2+}, Mn^{2+} (reductants).

For example in figure 61, the nine points marked in red and ranging between ORP value of 52.8 and 137.2, belong to the period 9th July – 15th July during which the chemical analyses results on the 15th July gave lower values of DO (1.77 mg/l) and NO_3^--N (87.6 mg/l) compared to other chemical analyses performed with similar high values of ORP. For instance the period 17-20 June, whose values are marked in blue, had higher DO and NO_3^--N concentrations. According to the measurements on the 15th June and 22nd June, NO_3^-N was expected to be in the range 125-135 mg/l or even higher, because DO concentration was around 2.53 mg/l and higher than the previous and following days. Thus higher DO and NO_3^--N concentration resulted in a higher ORP.

5.2.4 Evaluation of biomass activity

Three different kinds of batch tests (OUR, SAA, NUR) were performed in order to evaluate and monitor trends and changes in activity of different bacteria populations on the carriers during the four months of pilot reactor operation. The batch tests were carried out according to the methodology described in chapter 3.6. In the last days of study on pilot reactor a set of batch test was carried out on the activated sludge, in order to have a term of comparison with the bacterial activity in the biofilm.

Oxygen Uptake Rate (OUR)

The oxygen uptake rate (OUR) tests (cfr. chapter 3.6.2) aimed to evaluate the oxygen consumption by nitrifying bacteria (AOB, mainly Nitrosomonas spp., and NOB, mainly Nitrobacter spp.) and heterotrophic bacteria. A higher oxygen uptake rate by nitrifying bacteria reflects higher nitrification rate and ammonia consumption.

In total 9 series of OUR tests were performed during the first four months of pilot plant-scale reactor operation. The data from the tests are shown in APPENDIX V. The results are summarized in figure 62. The oxygen uptake rates were calculated according to the formulas described in chapter 3.6.2.

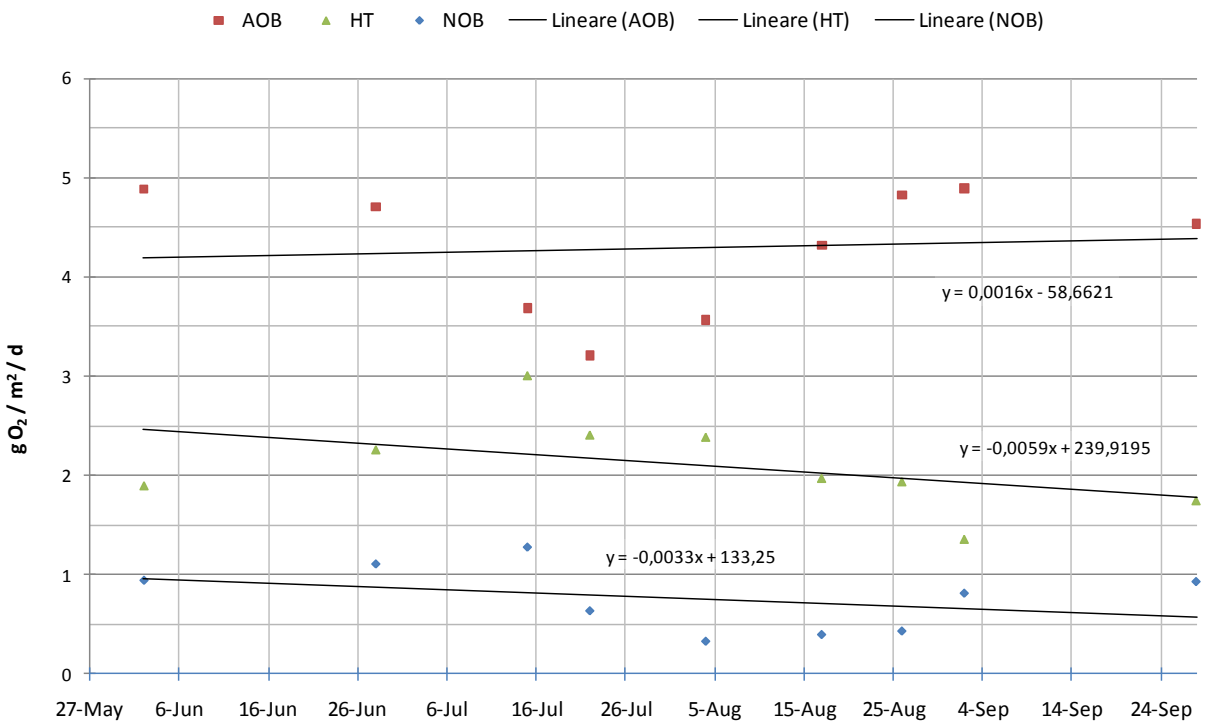

Fig. 62. Results from OUR tests on the biocarriers during the period June-September 2010

The pH was measured manually at the beginning and the end of a couple of tests and it decreased from about 8.25 to 8.05. The diluted reject water had a NH_4^+-N initial concentration of 99.7 ± 3.4 mg/l and the tests were started with a DO concentration above 6.5 mg/l. NO_2^--N and NO_3^--N concentrations were almost zero.

Analyzing the results from OUR tests during these four months of pilot reactor operation, a general increase of ammonia oxidizing bacteria (AOB) was noticed at the expense of nitrite oxidizing bacteria (NOB) and heterotrophic bacteria (indicated as HT). This trend in the biofilm activity was in the right direction for a good performance of the partial nitritation/Anammox process.

A total increase in Nitrosomonas bacteria activity was observed during August. The higher activity of Nitrosomonas reflects the biomass growth on the biocarriers observed during August by VSS measurements. During that period of time, the pH in the reactor was about 7.6, the DO concentration mostly between 2.4-2.5 mg/l and the ORP average value was lower than 20 mV.

The decrease of Nitrosomonas activity observed during July could have been due to a lower DO concentration in the pilot plant reactor during the first three weeks of July (DO about 1.7-1.8 mg/l).

The lower COD consumption during the last two months of operation is evidenced by a decrease in heterotrophic activity.

Despite that, three objections could be raised about the results from these OUR tests. Firstly, the concentration of sodium chlorate ($NaClO_3$), the inhibitor of NOB, was lower than the concentration reported in literature which was found to fully inhibit Nitrobacter spp. This might have led to an overestimation of AOB (calculated as difference of DO consumption rate between the second and third phase of the test) and an underestimation of NOB (calculated as difference of DO consumption rate between the first and second phase of the test). Secondly, the test was started with a NO_2^- concentration almost zero, and the rate of nitrification by NOB was probably limited to some extent by the oxidation of NH_4^+ by AOB. This probably led to an underestimation of NOB. However nitrite limiting conditions are also present inside the pilot-scale reactor. Moreover at the beginning of July, FISH analysis confirmed that the Nitrobacter spp. bacteria were few in the biofilm, and the results from OUR tests seem to confirm that. Thirdly the rather high pH above 8 was higher than the real conditions in the reactor, and Nitrobacter might have been inhibited by free ammonia to some extent.

If the results are then expressed as specific oxygen uptake rate (SOUR) (gO_2 $gVSS^{-1}$ d^{-1}), by using the measurements of the biomass attached on the rings which were obtained in the days close to the date of OUR tests and estimate the

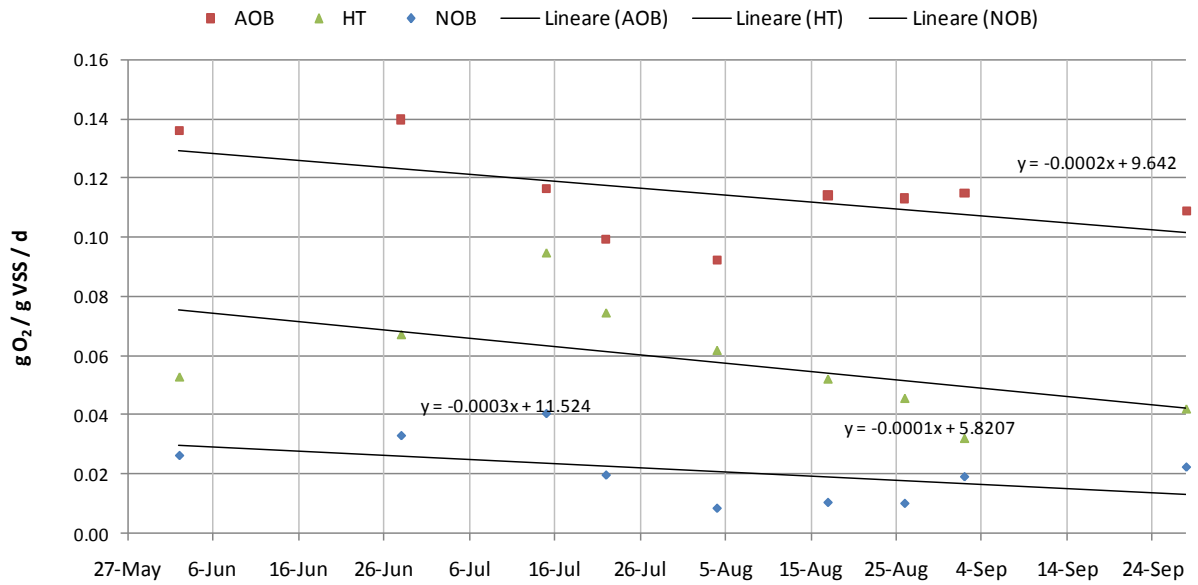

Fig. 63. Specific Oxygen Uptake Rate (SOUR) of the biofilm during the period June-September 2010

concentration in the bottle for OUR test, the chart is slightly different (Fig. 63).

The chart shows a rather similar decrease in activity for all the bacteria population. A likely reason can be the increase of the biofilm thickness, and therefore a higher resistance to the diffusion of substrates and dissolved oxygen within the biofilm, or the presence of a small amount of death biomass not metabolically active. That means for example that total activity of Nitrosomonas bacteria has basically increased because of the observed growth of Nitrosomonas bacteria in the biofilm with time, but their specific activity per unit of weight of biomass seems to have slightly decreased, probably because of the reasons explained above.

During the last days of the studies on the pilot plant reactor, on the 27th September, the OUR test was performed on the activated sludge and compared to the OUR results obtained on the 28th September. The specific oxygen consumption rates are summarized in Table 34.

The pH of the liquid from the reactor, and thus the pH value of this series of three tests was between 7.3-7.5. The test was performed on fresh activated sludge taken directly from the reactor.

Table 34 – OUR test results performed on activated sludge

OUR (AOB)	OUR (NOB)	OUR (HT)
g O_2 g VSS^{-1} d^{-1}	g O_2 g VSS^{-1} d^{-1}	g O_2 g VSS^{-1} d^{-1}
3.2781	1.0571	0.2716

A comparison with the OUR test carried out on the 28th September on the biocarriers was done (Table 36). The principle was to recalculate the results from the OUR tests on the rings as g O_2 g VSS^{-1} d^{-1} (Table 35) and then adjust both the results obtained inside the bottle of 1.56 l with activated sludge and biocarriers to the reactor configuration that is 80 l of biocarriers in 166 l of liquid. The results obtained from the tests on the activated sludge (calculated as g O_2 g VSS^{-1} d^{-1}) have been simply multiplied by the VSS concentration of the activated sludge in the reactor on that day (284.74 mg VSS/l - average on three measurements). The results were finally compared as g O_2 m^{-3} d^{-1}.

The comparison between the activity of AOB, NOB and heterotrophs in the activated sludge and the biocarriers (Table 36) confirms what the theory suggested. Ammonium oxidizers (AOB) are mainly attached on the carriers, whereas the nitrite oxidizers (NOB) are more active in the activated sludge. The heterotrophic bacteria (among which the heterotrophic denitrifying bacteria) are present and active to a greater extent in the biofilm rather than in the activated sludge.

Few objections can be raised to this comparison. The biomass concentration in the activated sludge depends strongly on the hydraulic retention time of the period prior to the test. A higher HRT may lead to a higher washing out of the biomass from the reactor. Moreover the biomass present as activated sludge is more sensitive to shock loads or variations in the reactor.

Table 35 – Recalculations of OUR results obtained for the Kaldnes rings.

Result	Unit	Formulas
Specific dissolved Oxygen Uptake Rate (dO_2/dt)	$\frac{g\ O_2}{g\ VSS \cdot d}$	$\frac{dO_2}{dt} = \frac{-\alpha_i}{X} \cdot 60 \cdot 60 \cdot 24$
Dissolved oxygen uptake rate ($d[O_2]/dt$)	$\frac{g\ O_2}{m^3 d}$	$\frac{d[O_2]}{dt} = \frac{dO_2}{dt} \cdot \frac{19.47 mg/ring \cdot 85600 rings}{166 l}$

α_i = slope of the dissolved oxygen concentration decrease inside the bottle plotted versus time (mg O_2 l^{-1} s^{-1}). Subscript "i" indicates the slope of the respective phase of the test (AOB+NOB+HT, HT+AOB or HT). The values of the three slopes are the averages of the three OUR tests performed;

X = concentration of the biomass attached on the 107 rings inside the bottle (mg VSS/l), based on the concentration of VSS on each ring measured on the 24th September (i.e. 19.47 mg/ring). The biomass concentration inside the bottle was then adjusted to the stepwise dilutions made (4 ml NaClO3 and 6 ml ATU);

60, 60 and 24 = unit conversion factors from seconds to days;

19.47 mg/ring = biomass attached on the rings measured on the 24th September;

85600 rings = estimate of the total number of carriers inside the 166 l of liquid of the pilot reactor, calculated by proportion and based on the measurement that 1070 carriers occupy a volume of 1 l and the reactor was filled with 80 l of carriers;

166 l = volume of liquor in the reactor.

Table 36 – OUR – comparison between biofilm and activated sludge

Object of the OUR test	OUR (AOB)	OUR (NOB)	OUR (HT)
Activated sludge (inside reactor)	g O_2 m^{-3} d^{-1}	g O_2 m^{-3} d^{-1}	g O_2 m^{-3} d^{-1}
	933.40	300.99	77.32
Biofilm (inside reactor)	g O_2 m^{-3} d^{-1}	g O_2 m^{-3} d^{-1}	g O_2 m^{-3} d^{-1}
	1092.32	223.39	421.08
Ratio biofilm / A.S. (inside reactor)	1.17	0.74	5.45

Although the ammonia nitrogen concentration was nearly the same, the COD was probably different; in one case a dilution 1:10 was done for the test on the biocarriers, whereas in the other case the liquid was directly taken from the reactor, which had a higher COD concentration but with a likely lower percentage of biodegradable content.

Specific Anammox activity (SAA)

The main objective of the Specific Anammox Activity (SAA) tests (cfr. chapter 3.6.1) was to monitor the Anammox activity during the four months of pilot plant-scale reactor operation.

In total 14 SAA analyses were performed (10 at a temperature of 25°C and 4 at 35°C). The data from the tests are shown in APPENDIX V.

The results are summarized in figure 64.

The tests carried out during the four months of operation show a general increase of Anammox bacteria activity over time. During the last two months of operation the Anammox activity was higher and about 4 g N m^2 d^{-1}. A total increase in activity of 34.3 % was noticed from the test at 25°C, between the start and the end of the study on the pilot reactor.

The tests carried out at a higher temperature (35°C), closer to the optimum temperature of Anammox bacteria, showed a steeper increase in activity over time compared to the tests carried out at the reactor operating temperature of 25°C.

If the results are then expressed as Specific Anammox Activity per grams of biomass attached as biofilm on 15 rings (by using the measurements on suspended volatile solids carried out on days close to the SAA analyses), the SAA does not show any increase and has an average value of 0.0965 gN gVSS d^{-1} (Fig. 65).

A SAA test was carried out on the suspended activated sludge taken directly from reactor and the result is showed in APPENDIX V.

The Anammox activity resulted to be 0.1203 g N g VSS^{-1} d^{-1}. This value has been transformed to g N m^{-3} d^{-1} by multiplying by the volatile suspended solids concentration in the reactor on that day (280.73 mg/l) and compared to the result obtained on the biofilm adjusted to the reactor characteristic (80 l of rings and 166 l of liquor) and expressed as g N m^{-3} d^{-1}. The procedure is very similar to the one used for OUR test before.

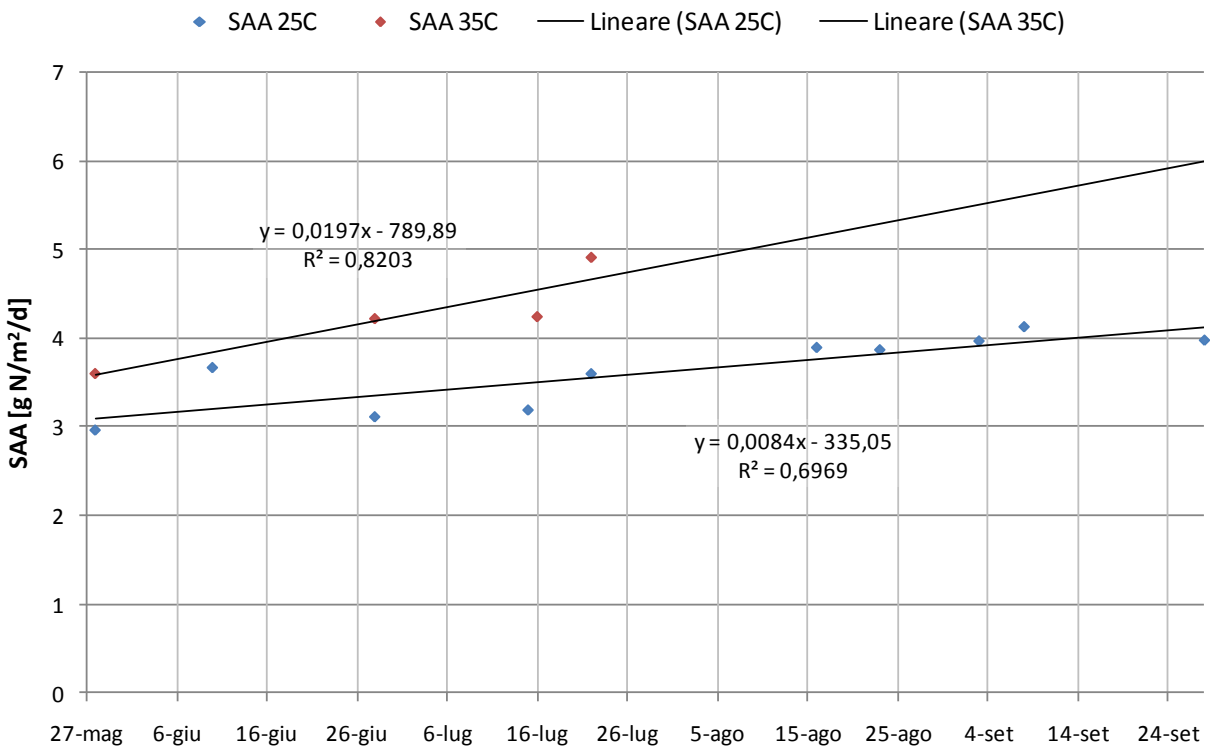

Fig. 64. Activity of Anammox bacteria attached on the biocarriers during the period June-September 2010

From Table 37 is possible to verify that almost all the activity of bacteria is concentrated in the biofilm (>96.6%).

However this value could be even higher because in the test on activated sludge denitrifiers were able to have access to the COD (contained in the liquor from the reactor), to the substrate (70 mg/l NO_2^--N and about 100 mg/l NO_3^--N) and probably to a variety of other micronutrients which were not present in the synthetic liquid used for the SAA test on the biofilm.

Nitrate Uptake Rate (NUR)

NUR test was carried out to assess the NO3-removal rate from the liquor. The method is described in chapter 3.6.3. In total 10 NUR tests were per-formed on the biocarriers during the

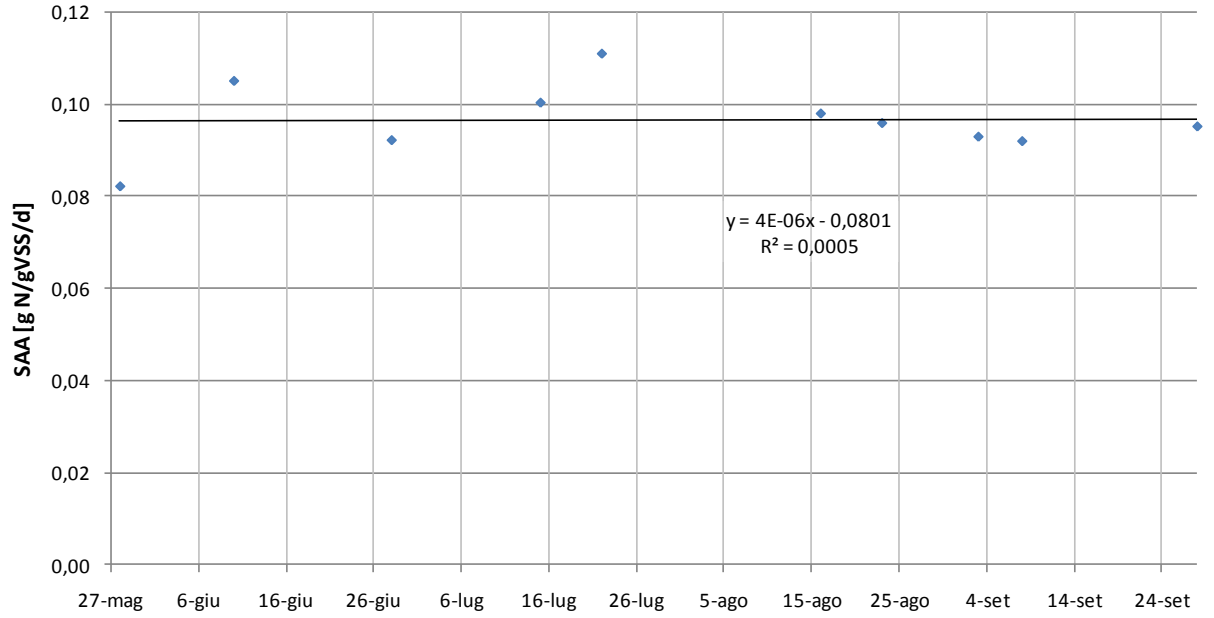

Fig. 65. Specific Anammox Activity (25°C) of the biofilm during the period June-September 2010

Table 37 – SAA – comparison between biofilm and activated sludge

Object of the OUR test	Unit	SAA
Activated sludge (inside reactor)	g O_2 m^{-3} d^{-1}	33.77
Biocarriers (inside reactor)	g O_2 m^{-3} d^{-1}	956.01
% activity by biocarriers (inside reactor)	%	96.59

four months of evaluation of the pilot plant-scale reactor.

The experimental data from the tests are shown in APPENDIX V, while the results are summarized in figure 66.

Parallel to NUR test, COD analyses were carried out and are presented below.

NUR tests results does not show any particular tendency for the nitrate up-take rate by the denitrifiers in the biofilm. The low result obtained on the 26th August may be due to the problems occurred on the 23rd August when the reactor could not be fed for one day. A part from this value, the average Nitrate Uptake Rate in this four month was approximately 0.84 g N m^2 d^{-1}.

Reduction in COD removal was observed during the last two months (Fig. 66 and Fig. 56).

In some cases the COD removal was even negative and it was observed an increase in COD between the beginning and the end of the NUR test. A plausible reason of this may be an increase of soluble COD due to solubilization of COD initially present in insoluble form, that is the condition which underlies the process of anaerobic fermentation. The decrease in NUR during July is similar to the one reported by OUR tests. In August an increase in nitrate uptake rate was observed, consistent with the increase of volatile solids on the biocarriers during those weeks.

No explanations were found for the low value measured on 26th August, if not because of the problems occurred on the 23rd August when no inflow was provided to the reactor or a wrong result from the test.

The last test (26th September) was followed by measurements of inorganic nitrogen forms (three samples for NH_4^+-N and NO_2^--N and five, as usual, for NO_3^--N). After about 3 hours and 30 minutes, a decrease of 4.0% of NH_4^+-N was noticed (i.e. from 748 mg/l to 718 mg/l), while the removal of NO_3^--N was 25.0% (i.e. from 101.2 mg/l to 75.9 mg/l). The ammonia nitrogen decrement might have been caused by ammonia stripping phenomena due to nitrogen gas supply during the whole test (the pH was about 8.25), or to the consumption by Anammox bacteria which might have been used, under anoxic conditions, the nitrite produced during the denitrification process, although the kinetic rate for the conversion of NO_2^- to N_2, is usually higher than the for NO_3^- to NO_2^-. The NO_2^--N was less than 0.15 mg/l during the whole test.

Thus a possible limitation of NUR test carried out on reactors with a partial nitrita-

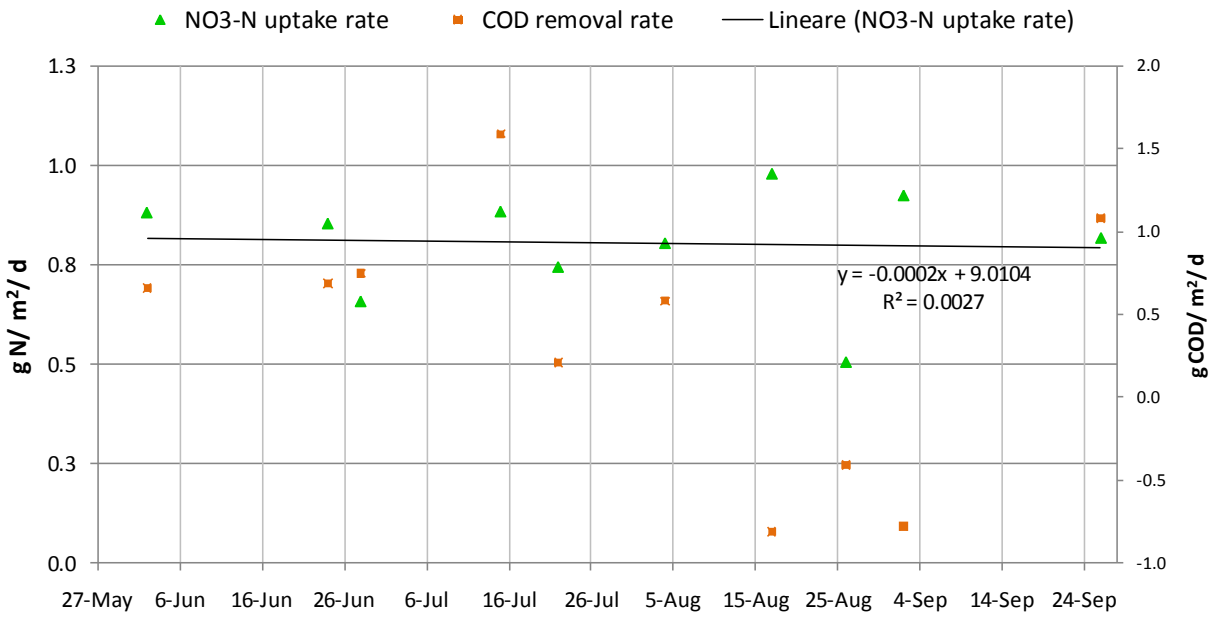

Fig. 66. Results from NUR tests on the biofilm during the period June-September 2010

Table 38 – Recalculations from NUR results on the biocarriers.

Result	Unit	Formulas
Specific Nitrate Uptake Rate (dNO_3^--N/dt)	$\frac{g\ N}{g\ VSS \cdot d}$	$\frac{dN}{dt} = \frac{-\alpha}{X} \cdot 60 \cdot 24 = 0.0196$
Dissolved Nitrate Uptake Rate (d[O_2]/dt)	$\frac{g\ N}{m^3 d}$	$\frac{d[N]}{dt} = \frac{dN}{dt} \cdot \frac{19.47 \cdot 85600}{166l} = 197.17$

α = average slope of the nitrate concentration decrease plotted versus time (mg N l^{-1} min^{-1});

X = mg VSS of 400 ml of carriers inside 1 l container. The concentration of VSS on each ring measured on the 24th September (i.e. 19.47 mg/ring) was calculated by 107 and 4, based on the measurement that 107 carriers occupy a volume of 100 ml. $X = \frac{19.47 mg/l \cdot 107 \cdot 4}{1l}$;

60 and 24 = unit conversion factors from seconds to days;

19.47 mg/ring = biomass attached on the rings measured on the 24th September;

85600 rings = estimate of the total number of carriers inside the 166 l of liquid of the pilot reactor, calculated by proportion and based on the measurement that 1070 carriers occupy a volume of 1 l and the reactor was filled with 80 l of carriers;

166 l = volume of liquor in the reactor.

tion/Anammox process is that the nitrate uptake rate by denitrifying bacteria may be slightly underestimated because of the opposite action exerted by Anammox bacteria which produce nitrates and may reduce the result of nitrate uptake rate by denitrifiers.

On the 27th September the NUR test was performed on fresh activated sludge taken directly from the reactor and compared to the NUR results obtained on the 26th September. The nitrate uptake rate (NUR) was found to be 0.139 g N $gVSS^{-1}$ d^{-1} while the soluble COD increased from 380 mg O_2/l to 393 mg O_2/l.

A comparison with the NUR test carried out on the 26th September on the biocarriers was made as g N m^{-3} d^{-1}. The principle used to compare the result is the same used previously for OUR test and it is shown in Table 38.

The correspondent NUR on the activated sludge has been multiplied by the VSS concentration of the activated sludge used for the test (i.e. 296.18 mg VSS/l):

$$0.139 \frac{g\ N}{g\ VSS\ d} \cdot 296.18 mg VSS/l = 41.16 \frac{g\ N}{m^3 d}$$

The comparison shows a ratio $NUR_{biocarriers}/NUR_{act.\ sludge}$ = 4.79, therefore the nitrate uptake is mostly carried out within the biofilm, where anoxic conditions are favorable to the nitrate uptake by denitrifiers. However this ratio could actually be higher, because the negligible concentration of Anammox bacteria in the activated sludge played a minimal role in the production of nitrate during NUR test compared to the NUR test carried out on the biocarriers where Anammox bacteria are present and might produce a small percentage of NO_3^- by using the NO_2^-.produced during denitrification.

6 CONCLUSIONS

These studies were carried out to investigate and evaluate the partial nitritation/Anammox technology in moving bed biofilm reactors (one-reactor system). Literature review and experimental work carried out in this thesis confirmed the sustainability and the potential advantages of the partial nitritation/Anammox as a viable option for the treatment of ammonium-rich wastewaters.

Two laboratory-scale reactors and a pilot plant-scale reactor were studied. The laboratory scale studies allowed understanding the parameters involved in the process and directly examine their influence on the process performance. Conclusions and findings concerning the laboratory-scale reactors are given in chapter 4. The pilot plant-scale reactor, which was evaluated for a longer period, allowed establishing a stable partial nitritation/Anammox process with good and promising results. The main conclusions given below are largely related to this reactor.

The following conclusions can be stated:

- By varying and adjusting carefully operational parameters such as DO concentration, temperature and HRT (i.e. inflow rate) is possible to obtain high and stable efficiency of the whole process.
- Efficiencies of 95%, 85% and 83% for NH_4^+-N, inorganic nitrogen, and Total Nitrogen re-

spectively have been simultaneously achieved in the one-stage partial nitritation/Anammox Moving Bed Biofilm Reactor filled with about 40% of Kaldnes rings and with an influent load of 3.4 g N m^{-2} d^{-1}. The maximum removal rate at this loading rate was about 2.9 g N m^{-2} d^{-1}.

- DO is a key parameter and the oxygen-limiting conditions must be provided for a good performance of the process. A too high dissolved oxygen concentration resulted in a temporarily accumulation of nitrates in the reactor, but its effects seemed to be reversible. An average DO concentration in the reactor of 2.2 mg/l was used in this study, providing good results.
- Conductivity is a good parameter which can be easily used to monitor the performance of the process and the NH_4^+ removal. It gives an immediate result without the need to perform analyses.
- pH and, to a slightly lower extent, ORP can give useful information about the conditions in the reactor and the adequacy of the dissolved oxygen concentration provided by aeration; moreover, a low pH and high ORP were usually noticed together with higher NO_3^--N concentration in the reactor.
- An on-line control of physical parameters with particular regard to DO and pH is advisable for a continuous and real-time monitoring of the process.
- A PID controller for aeration may be important to avoid peak concentrations of dissolved oxygen, especially for research purpose.
- Ratios such as COD/N and Alkalinity/N in the wastewater prior to treatment are extremely important for the stability of the process. A too high COD might enhance denitrifiers growth which could outcompete Anammox bacteria on a long-term scale. A too low alkalinity may not be sufficient to cope with the general decrease in pH of the partial nitritation/Anammox process.
- NO_2^- was the limiting factor for the Anammox bacteria in the one-stage partial nitritation/Anammox reactor and its concentration inside the reactor was only the 11.6% the concentration on NH_4^+.
- Anammox bacteria are strongly influenced by temperature and dissolved oxygen inhibition. A higher temperature can result in higher Anammox bacteria activity; however at a temperature of 25°C it was already possible to obtain a satisfying nitrogen removal.
- If the reactor is run at 25°C, the heat deriving from the anaerobic digestion of the sludge can be exploited, with the advantage to save costs for the heating.
- The coexistence of aerobic and anaerobic ammonium oxidizers (i.e. Nitrosomonas and Anammox) within the biofilm was confirmed by FISH analyses. A smaller percentage of Nitrobacter was found to be present within the biofilm.
- An increase of biofilm thickness was observed on the biocarriers (+38.6% between the 22nd July and 10th September) together with a moderate increase of process efficiency (+5.2%) and a decrease in biomass concentration as activated sludge.
- The suspended biomass in the reactor accounted for about 2.9% of the total biomass in the reactor (activated sludge + biomass attached on the Kaldnes rings). However the small percentage of aerobic activated sludge with nitrifying bacteria may be important for removing dissolved oxygen from the liquor and converting NH_4^+ to NO_2^- and thus provide good conditions for anaerobic bacteria (Anammox) in the biofilm.
- Batch tests such as SAA, OUR and NUR can give useful information about evolution of bacteria activity over time. In this thesis, these tests have been carried out on the Kaldnes rings from the pilot plant-scale reactor for a period of four months.
- Results from the tests are highly dependent on proper execution of tests. Test duration should be kept as constant as possible between different tests. In a couple of tests (NUR and especially SAA tests) a decrease in the experimental curves slope was noted for duration longer than about 90 and 180 minutes for SAA and NUR tests respectively.
- OUR tests carried out on the Kaldnes rings showed a total increase in Nitrosomonas activity and a decrease in activity for Heterotrophs and Nitrobacter.
- Oxygen Uptake Rates (OUR) (at 25°C) of the biofilm attached on the rings were estimated of about 4.3 g O_2 m^{-2} d^{-1} for Nitrosomonas, 2.1 g O_2 m^{-2} d^{-1} for Heterotrophs and 0.8 g O_2 m^{-2} d^{-1} for Nitrobacters.
- The calculated ratios between the OUR (g O_2 m^{-3} d^{-1}) by the biofilm and the OUR

($g\ O_2\ m^{-3}\ d^{-1}$) by the activated sludge were 1.17 for Nitrosomonas, 5.45 for Heterotrophs and 0.74 for Nitrobacters.

- SAA tests carried out on the Kaldnes rings at 25°C showed a constant increase in Anammox activity (+ 34.3 %) with a maximum value of 4.1 $g\ N\ m^{-2}\ d^{-1}$.
- The Anammox bacteria activity is almost entirely concentrated in the biofilm (>96.5%).
- A sufficiently high nitrogen loading rate is required for a stable partial nitritation/Anammox process in order to not limit the slow growth rate of Anammox bacteria. If the load is too low the decay rate might exceed the Anammox bacteria growth rate.
- NUR results showed a slight decrease in nitrate uptake rate by the biofilm, probably due to a decrease activity of denitrifiers. The average value was 0.84 $g\ N\ m^{-2}\ d^{-1}$.
- The calculated ratio between the NUR ($g\ N\ m^{-3}\ d^{-1}$) by the biofilm and the NUR ($g\ N\ m^{-3}\ d^{-1}$) by the activated sludge was 4.79.

7 SUGGESTIONS FOR FULL-SCALE IMPLEMENTATION AND FUTURE RESEARCH

In this chapter proposal for the implementation of partial nitrification/Anammox process using the moving bed biofilm technology are discussed both as an upgrading option for existing WWTP (chapter 7.1) and as an alternative for nitrogen removal from leachate (chapter 7.2) or other stream with high content of organic matter. A brief discussion is given about the possibility to use the studied process within the main treatment line (chapter 7.3). A last section (chapter 7.4) deals with future research which is needed to understand and generally improve the scientific knowledge about these innovative wastewater treatment options.

7.1 Partial nitrification/Anammox in municipal WWTPs

The pilot plant scale reactor seemed to work well with the volume of carriers used for the operation and the influent reject water from dewatering of the anaerobically digested sludge which fed the reactor. High efficiencies, above 80%, were reached. Removal efficiencies of 95% for NH_4^+-N and 85% for inorganic nitrogen have been achieved simultaneously. Based on the results obtained during the last two months of study on the pilot reactor and despite the large nitrogen removal achieved from the high initial concentration of the reject water (974.3 mg/l NH_4^+-N), average concentrations of 76.8 mg/l NH_4^+-N, 7.82 mg/l NO_2^--N and 83.4 mg/l NO_3^--N were still present in the effluent. These values are above the requirements for discharge, thus a further treatment is needed.

An example of municipal WWTP (Fig. 67) consists of a primary treatment to remove solids, a secondary treatment to reduce the organic content and nutrients (phosphorus and nitrogen) and finally the treatment and handling of sludge (anaerobically digested). In Fig 67 the secondary treatment is depicted as a biological treatment with enhanced biological phosphorus removal (e.g. Johannesburg system). The partial nitrification/Anammox reactor in a full scale WWTP can be located downstream the sludge treatment line. In most cases, the supernatant from the dewatering of the digested sludge is suitable to undergo partial nitritation/Anammox process.

The small but highly concentrated side stream with a relatively high temperature and through the Moving Bed Biofilm Reactor (MBBR) technology make it possible to have a treatment within a tank with a smaller and compact footprint. By upgrading an existing WWTP with the partial nitritation/Anammox reactor large advantages in term of costs and sustainability can be obtained compared to the recirculation of the reject water from sludge dewatering directly to the inlet of the WWTP without further treatment. Economical and environmental benefits can be gained, if compared to the conventional nitrification/denitrification. For instance, by treating the side stream of the effluent from sludge dewatering by partial nitritation/Anammox technology, a lower nitrogen load (-15-20%) is supplied to the main treatment line, a lower aeration and additional carbon source are needed and less CO_2 emissions are produced from the whole WWTP.

Several options can be adopted for the partial nitritation/Anammox process in one-single reactor. Here below (Fig. 68) an example of a possible interesting configuration is illustrated.

Two MBBRs in series with bypass of part of reject water allow achieving a large reduction of the incoming nitrogen load. The reactors could also work in parallel if are equally loaded with the clarified effluent from the anoxic tank where denitrification takes place in order to consume the nitrates (NO_3^-) produced by Anammox and Nitrobacters in partial nitritation/Anammox

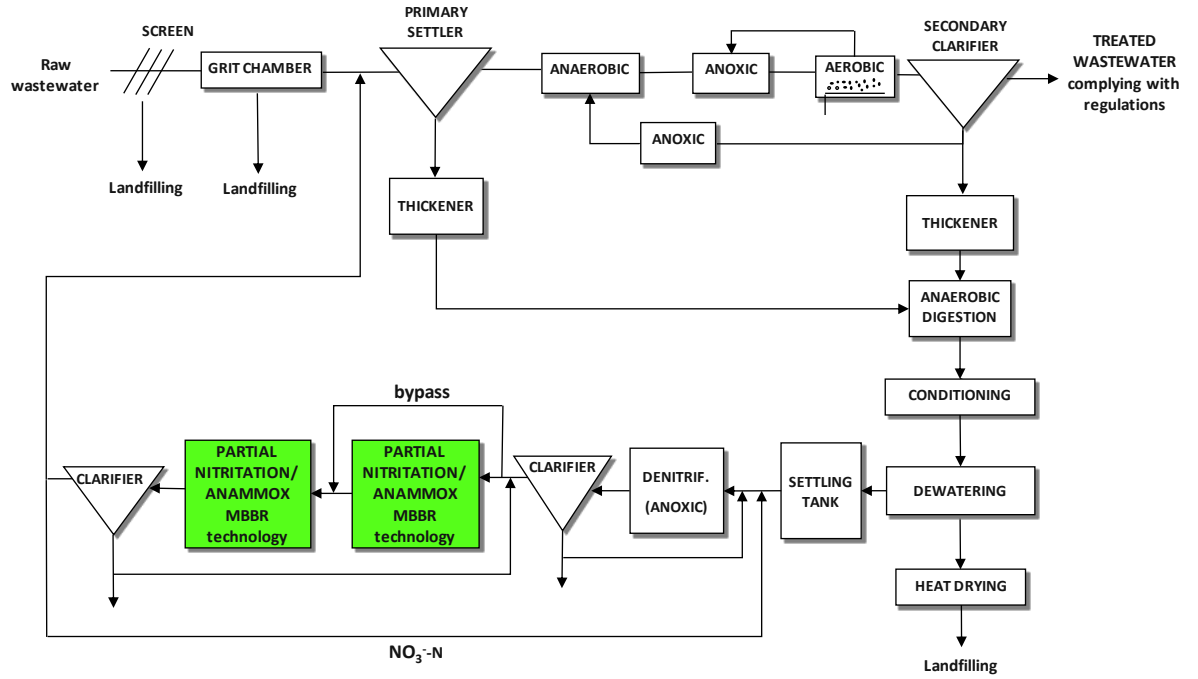

Fig. 67. A general overview of treatments in a municipal WWTP. Two MMBRs in series with bypass and pre-denitrification could be implemented in the sludge line.

reactors. The treated effluent can be recirculated back to the main treatment line or discharged if it meets the requirements for discharge. The denitrifiers and the sludge from the partial nitritation/Anammox reactors can be used to seed the denitrification and nitrification units of the main stream treatment line or otherwise sent back to the primary settler.

7.2 Partial nitrification/Anammox for leachate treatment

The partial nitrification/Anammox MBBR could also be used for treatment of landfill leachate with low biodegradable content or after COD removal. Particular regard should be paid to the presence of high concentrations of inhibiting substances or nitrification inhibitors (e.g. heavy metals), which if present to some extent may slow down or inhibit the whole process. A chemical precipitation for metal removal is usually carried out before the biological treatment.

A biological anaerobic treatment could be applied prior to the biological treatment with partial nitritation/Anammox in MBBRs as shown in the overview of the line of treatment in figure 69.

The biogas produced is a valuable product and the higher temperature from the anaerobic digestion can improve the process efficiencies. However, the most suitable option for the choice of a treatment line must always be based on a case-specific decision.

The effluent from biological processes should be able to meet requirements for nitrogen, but further treatment might be needed to comply discharge requirements for other pollutants.

Similar streams with high COD concentrations, and especially the effluents from food industries

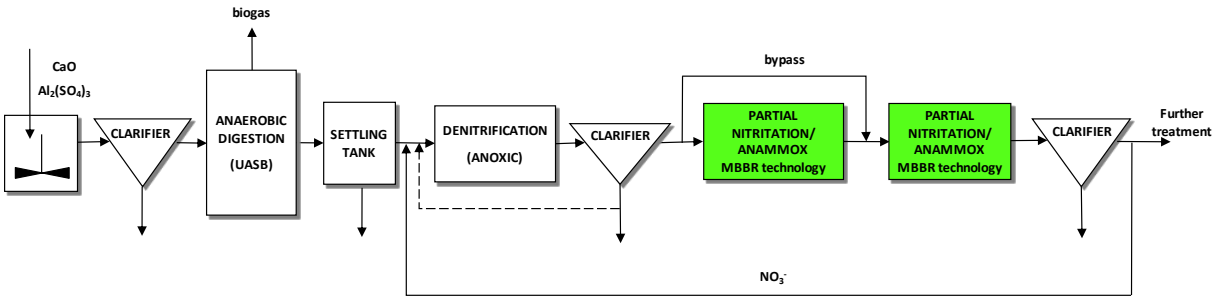

Fig. 68. Anaerobic treatment, pre-denitrification and partial nitritation/Anammox reactor for biological treatment of leachate.

which have a high biodegradable fraction of COD, need a previous degradation of organic matter by means of an anaerobic digestion, if a partial nitritation/Anammox process is chosen as option for the nitrogen removal.

7.3 Future research

Future research and studies are still needed to fully understand this novel technology and increase the scientific knowledge for its future full-scale installations.

A couple of directions for future research have emerged from this study:

- Long term coexistence of denitrifiers and Anammox bacteria and the stability of the process must be further investigated, with special regard to the implementation of DEAMOX systems.
- Intermitted aeration in Moving Bed Biofilm Reactors or Sequencing Batch Reactors must be further studied in order to compare the performance of different operational strategies with partial nitritation and Anammox process.
- More research on acclimation of bacteria populations responsible for partial nitritation and Anammox process to lower temperatures could provide a thorough understanding of the perspectives to run this process at low temperatures.
- More research is needed to fully understand and provide models for the diffusion of substrates in biofilm systems and the degree of influence of the biofilm thickness.
- More studies are needed to study the slow-down in the biological kinetics due to the mass transfer limitation inside the biofilm. The kinetics could be evaluated in batch tests varying concentrations of substrates in the bulk liquid and the biofilm thickness. Different shape of the carriers should be studied and compared in order to contain and minimize this problem.
- More research should be conducted towards faster start-up strategies for Anammox-based systems.
- Studies about N_2O emission from the treatment are important in order to reduce and minimize the impacts from the emission of this strong greenhouse gas. In particular aeration strategy or gas recirculation should be investigated.
- A detailed evaluation of the consequences of the possibility to seed the main treatment line with the produced sludge from the side stream treatment are interesting in order to evaluate the extent of the benefits.
- Online monitoring for nitrogen compounds (NH_4^+, NO_3^-, NO_2^-) might be of great interest for a monitoring and real-time control of the process performance, without the need of chemical analyses.
- COD fractionation (e.g. inert, biodegradable, readily biodegradable, slowly biodegradable, etc. fractions of COD) can give useful information about the wastewater which has to be treated and about the COD removed by the process.
- Further improvements and alternatives should be investigated with the common aim to reduce costs (especially aeration) of the conventional nitrification and denitrification treatment.
- More research is needed in order to fully replace traditional nitrification and denitrification with new innovative and sustainable technologies for nitrogen removal. It will be a challenge to make partial nitrification/Anammox process (or similar novel processes) suitable for the treatment of wastewater with lower nitrogen concentrations and low temperatures.

8 REFERENCES

Ahn, Y.H., Hwang, I.S. & Min, K.S. 2004. ANAMMOX and partial denitritation in anaerobic nitrogen removal from piggery waste. Water Science and Technology. 49(5-6):145-53.

Ahn, Y.H., 2006. Sustainable nitrogen elimination biotechnologies: A review. Process Biochemistry 41:1709–1721.

Ahn, J.H., Yu, R. & Chandran, K. 2008. Distinctive microbial ecology and biokinetics of autotrophic ammonia and nitrite oxidation in a partial nitrification bioreactor. Biotechnology and Bioengineering 100(6):1078-87.

Amann, R.I. 1995. In situ identification of micro-organisms by whole cell hybridisation with rRNA-targeted nucleic acid probes. Molecular microbial ecology manual vol. 3.3.6. Dordrecht, The Netherlands: Kluwer Academic Publishers. 1-15.

American Academy of Pediatrics Committee on Nutrition. 1970. Infant methemoglobinemia: the role of dietary nitrate. Pediatrics. 46(3):475-478.

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Srinath, E.G. 1976. Inhibition of nitrification by ammonia and nitrous acid. Journal of Water Pollution Control Federation. 48:835-852.

Arshad, I. 2008. Determination of the heterotrophic and autotrophic active biomass during activated sludge respirometric batch assays using molecular techniques. Doctoral Thesis. Durban University of Technology.

Belser, L. & Mays, E. 1980. Specific inhibition of nitrite oxidation by chlorate and its use in assessing nitrification in soils and sediments. Applied and Environmental Microbiology. 39:505–510.

Bettazzi, E., Caffaz, S., Vannini, C., Lubello, C. 2010. Nitrite inhibition and intermediates effects on Anammox bacteria: A batch-scale experimental study. Process Biochemistry 45:573–580.

Bilstad, T. 1995. Nitrogen separation from domestic wastewater by reverse osmosis. Journal of Membrane Science. 102:93-102.

Bricker, S.B., Clement, C.G., Pirhalla, D.E., Orlando, S.P., Farrow, D.R.G. 1999. National estuarine eutrophication assessment: effects of nutrient enrichment in the nation's estuaries. NOAA, National Ocean Service, Special Projects Office and the National Centers for Coastal Ocean Science. Maryland: Silver Spring. 71 p.

Broda, E. 1977. Two kinds of lithotrophs missing in nature. Zeitschrift fur Allgemeine Mikrobiologie. 17: 491-493.

Buddhavarapu, S. & Inniss, E.C. 2006. Activated sludge characterization by ORP profiles. Proceedings of Texas Water 2006 Annual Water Conferencee. 124-130.

Cema, G. 2010. Comparative study on different Anammox systems. Doctoral Thesis, Stockholm: KTH.

Cema, G., Wiszniowski, J., Zabczynski, S., Zablocka-Godlewska, E., Raszka, A., Surmacz-Górska, J. 2007a. Biological nitrogen removal from landfill leachate by deammonification assisted by heterotrophic denitrification in a rotating biological contactor (RBC). Water Science and Technology 55(8-9):35-41.

Cema, G., Plaza, E. & Surmacz-Górska, J. 2007b. Activated sludge and biofilm in the Anammox reactor – Cooperation or competition? Proceedings of Polish-Swedish seminars, Cracow March 17-18, 2005. Integration and optimisation of urban sanitation systems. Eds: Plaza E., Levlin E. Report 13: 129-138.

Chamchoi, N., Nitisoravut, S. & Schmidt, J.E. 2008. Inactivation of ANAMMOX communities under concurrent operation of anaerobic ammonium oxidation (ANAMMOX) and denitrification, Bioresource Technology. 99:3331–3336.

Chen, H., Liu, S., Yang, F., Xue, Y., Wang, T. 2009. The development of simultaneous partial nitrification, ANAMMOX and denitrification (SNAD) process in a single reactor for nitrogen removal. Bioresource Technology. 100:1548–1554.

Clesceri, L.S. ; Greenberg, A.E. ; Trussell, R.R. 1989. Standard Methods for the Examination of Water and Wastewater 17th ed. American Public Health Association.

Comly, H.H. 1945. Cyanosis in infants caused by nitrates in well water. Journal of the American Medical Association. 129:112-116.

Dapena-Mora, A., Fernández, I., Campos, J.L., Mosquera-Corral, A., Mendez, R., Jetten, M.S.M. 2007. Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production. Enzyme and Microbial Technology. 40:859-865.

Degenaar, A.P., Mudaly, D.D, Manganyi, A., Bux, F. 2000. An evaluation of volatile suspended solids as a true measure of metabolic activity in activated sludge. WISA 2000 Biennial Conference. Sun City, South Africa. 28 May - 1 June 2000. 9 p.

Dexiang, L., Xiaoming, L., Qi, Y., Guangming, Z., Liang, G., Xiu, Y. 2007. Effect of inorganic carbon on anaerobic ammonium oxidation enriched in sequencing batch reactor. Journal of Environmental Sciences. 20:940–944.

Dijkman, H. & Strous, M. 1999. Process for ammonia removal from wastewater. Patent PCT/NL99/00446.

Dosta, J., Galí Serra, A., Benabdallah El-Hadj, T., Macé, S., Mata-Álvarez, J. 2007. Operation and model description of a sequencing batch reactor treating reject water for biological nitrogen removal via nitrite, Bioresource Technology. 98(11): 2065-2075.

Dosta, J., Fernández, I., Vázquez-Padín, J., Mosquera-Corral, A., Campos, J.L., Mata-Álvarez, J., Méndez, R. 2008. Short- and long-term effects of temperature on the Anammox process. Journal of Hazardous Materials 154:688-693

Egli, K., Fanger, U., Alvarez, P.J.J., Siegrist, H., Van der Meer, J., Zehnder, A.J.B. 2001. Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate. Archives of Microbiology 175:198-207 .

Ehrig, H.J. 1989. Leachate quality, In: Christensen, T. H., Cossu, R., Stegmann, R. (edited) Sanitary landfilling: Process, technology and environmental impact. London: Academic Press Limited. 213-230.

Fux, C., Velten, S., Carozzi, V., Solley, D., Keller, J. 2006. Efficient and stable nitritation and denitritation of ammonium-rich sludge dewatering liquor using an SBR with continuous loading, Water Research. 40:2765-2775.

Fux, C., Marchesi, V., Brunner, I., Siegrist, H., 2004. Anaerobic ammonium oxidation of ammonium-rich waste streams in fixed-bed reactors. Water Science and Technology. 49(11-12):77-82.

Fux, C, Boehler, M, Huber, P, Brunner, I, Siegrist, H. 2002. Biological treatment of ammonium-rich wastewater by partial nitritation and subsequent anaerobic ammonium oxidation (Anammox) in a pilot plant. Journal of Biotechnology. 99:295-306.

Galì Serra, A. 2006. Optimisation of biological nitrogen removal proceses to treat reject water from anaerobic digestion of sewage sludge. Doctoral Thesis, Universitat de Barcelona.

Ganigué, R., Gabarro, J., Sànchez-Melsió, A., Ruscalleda, M., López, H., Vila, X., Colprim, J., Balaguer, M.D. 2009. Long-term operation of a partial nitritation pilot plant treating leachate with extremely high ammonium concentration prior to an anammox process. Bioresource Technology. 100:5624-5632.

Gerardi, M. H. 2002. Nitrification and denitrification in the activated sludge process. New York: John Wiley and Sons, Inc. 193 p.

Ghyoot, W. & Verstraete, W. 2000. Reduced sludge production in a two-stage membrane assisted bioreactor. Water Research. 34(1):205-215.

Grunditz, C. & Dalhammar, G. 2001. Development of nitrification inhibition assays using pure cultures of nitrosomonas and nitrobacter Water Research. 35(2):433-440

Guisasola, A., Petzet, S., Baeza, J.A., Carrera, J., Lafuente, J. 2007. Inorganic carbon limitations on nitrification: Experimental assessment and modelling, Water Research. 41:277-286.

Guisasola, A., Jubany, I., Baeza, J.A., Carrera, J., Lafuente, J. 2005. Respirometric estimation of the oxygen affinity constants for biological ammonium and nitrite oxidation. Journal of Chemical Technology and Biotechnology. 80:388-396.

Gustafsson, J.P., Gunnar, J., Simonsson, M., Nilsson, I. 2007. Soil and water chemistry, Stockholm: KTH. 168 p.

Gut, L., Plaza, E. & Hultman, B. 2007. Oxygen uptake rate (OUR) tests for assessment of nitrifying activities in the deammonification system. Proceedings of Polish-Swedish seminars, Cracow March 17-18,

2005. Integration and optimisation of urban sanitation systems. Eds: Plaza E., Levlin E. Report 13: 119-128.

Gut, L., 2006. Assessment of a partial nitritation/Anammox system for nitrogen removal. Doctoral Thesis. Stockholm: KTH.

Gut, L., Plaza, E., Dlugolecka, M., Hultman, B. 2005. Partial nitritation process assessment. Vatten 61:175-182. Lund.

Gut, L. & Plaza, E. 2004: Laboratory-scale study on treatment of high strength ammonium wastewater. Proceedings of a Polish-Swedish seminar, Wisla October 25-28, 2003. Integration and optimisation of urban sanitation systems. Eds: Plaza E., Levlin E., Hultman B. Report N 11: 101-111.

Güven, D., Dapena, A., Kartal, B., Schmid, M.C., Maas, B., van de Pas-Schoonen, K.T, Sözen, S., Mendez, R., Op den Camp, H.J.M, Jetten, M.S.M., Strous, M., Schmidt, I. 2005. Propionate oxidation by and methanol inhibition of anaerobic ammonium-oxidizing bacteria, Applied and Environmental Microbiology. 71:1066-1071.

Halling-Sørensen, B. & Jørgensen, S.E. 1993. The removal of nitrogen compounds from wastewater. Studies in Environmental Science 54. Amsterdam: Elsevier Science Publishers B.V. 443 p.

Hellinga, C., Schellen, A.A.J.C., Mulder, J.W., Van Loosdrecht, M.C.M., Heijnen, J.J. 1998. The Sharon process: an innovative method for nitrogen removal from ammonium-rich waste water. Water Science and Technology. 37(9):135-142.

Hellinga, C., Van Loodsdrecht, M.C.M. & Heijnen, J.J. 1999. Model based design of a novel process for nitrogen removal from concentrated flows. Mathematical and Computer Modeling of Dynamical System. 5(4): 351-371.

Henze, M, Gujer, W. & Van Loosdrecht, M. 2000. Activated sludge models ASM1, ASM2, ASM2D and ASM3. Scientific and technical report n. 9. London: IWA Publishing.

Henze, M., Harremoës, P., Arvin, E., la Cour Jansen, J. 2002. Wastewater treatment. Biological and chemical processes 3rd ed. Berlin Heidelberg: Springer.

Henze, M., van Loosdrecht, M., Ekama, G., Brdjanovic, D. 2008. Biological wastewater treatment. Principles, modelling and design. London: IWA Publishing. 500 p.

Hernando, Z. & Martínez, S. 2010. Evaluation of Deammonification process performance for supernatant treatment. Master Thesis. Stockholm: KTH.

Hippen, A., Helmer, C., Kunst, S., Rosenwinkel, K.-H., Seyfried, C.F. 2001. Six years' practical experience with aerobic/anoxic deammonification in biofilm systems. Water Science and Technology. 44(2-3):39-48.

Holman, J.B. & Warehem, D.G. 2000. ORP as a monitoring tool in a low dissolved oxygen wastewater treatment process. Journal of Environmental Engineering. 129(1):52-58.

Holt, J. G. & Hendricks Bergey, D. 1993. Bergey's Manual of Determinative Bacteriology 9th ed. USA: Lippincott Williams and Wilkins.

Hultman, B. & Plaza, E. 2009. Wastewater treatment - new challenges. Proceedings of a Polish-Swedish-Ukrainian seminar, Stockholm, Sweden, September 23 – 25, 2009. Research and application of new technologies in wastewater treatment and municipal solid waste disposal in Ukraine, Sweden and Poland. Eds: Plaza E., Levlin E. Report 16: 1-11.

Janus, H.M. & van der Roest, H.F. 1997 Don't reject the idea of treating reject water. Water Science and Technology 35(10):27-34.

Jardin, N., Thöle, D. & Wett, B. 2006. Treatment of Sludge Return Liquors: Experiences from the Operation of Full-Scale Plants. Proceedings of the Water Environment Federation WEFTEC 2006. 19:5237-5255.

Jetten, M.S.M., Horn, S.J. & van Loosdrecht, M.C.M. 1997. Towards a more sustainable wastewater treatment system, Water Science & Technology. 35(9):171-180.

Jetten, M.S.M., Strous, M., van de Pas-Schoonen, T., Schalk, J., van Dongen, U.G.J.M., van de Graaf, A.A., Logemann, S., Muyzer, G., van Loosdrecht, M.C.M., Kuenen, J.G. 1999. The anaerobic oxidation of ammonium, FEMS Microbiology Reviews 22:421-437.

Jetten, M.S.M., Wagner, M., Fuerst, J., van Loosdrecht, M., Kuenen, G., Strous, M. 2001. Microbiology and application of the anaerobic ammonium oxidation ('anammox') process. Current Opinion in Biotechnology. 12:283-288

Jetten, M.S.M., Schmid, M., Schmidt, I., Wubben, M., van Dongen, U., Abma, W., Sliekers, O., Revsbech, N., Beaumont, H.J.E., Ottosen, L., Volcke, E., Laanbroek, H.J., Campos-Gomez, J.L., Cole, J., van Loosdrecht, M., Mulder, J.W., Fuerst, J., Richardson, D., van de Pas, K., Mendez-Pampin, R., Third, K., Cirpus, I., van Spanning, R., Bollmann, A., Nielsen, L.P., Op den Camp, H., Schultz, C., Gundersen, J., Vanrolleghem, P.A., Strous, M., Wagner, M., Kuenen, J.G. 2002. Improved nitrogen removal by application of new nitrogen-cycle bacteria. Reviews in Environmental Science and Biotechnology. 1:51-63.

Jorgensen, T.C. & Weatherley, L.R. 2003. Ammonia removal from wastewater by ion exchange in the presence of organic contaminants. Water Research. 37:1723-1728.

Joss, A., Salzgeber, D., Eugster, J., Knig, R., Rottermann, K., Burger, S., Fabijan, P., Leumann, S., Mohn, J., Siegrist, H. 2009. Full-Scale Nitrogen Removal from Digester Liquid with Partial Nitritation and Anammox in One SBR. Environmental Science and Technology. 43(14):5301-5306.

Jubany Güell, I. 2007. Operation modelling and automatic control of complete and partial nitrification of highly concentrated ammonium wastewater. Doctoral Thesis. Bellaterra: Universitat autònoma de Barcelona.

Jørgensen, S.E. 1979. Industrial waste water management. Studies in Environmental Science 5. Amsterdam: Elsevier Science Publishing Company. 388 p.

Kalyuzhnyia, S., Gladchenkoa, M., Mulderb, A., Versprillec, B. 2006. DEAMOXNew biological nitrogen removal process based on anaerobic ammonia oxidation coupled to sulphide-driven conversion of nitrate into nitrite. Water Research 40:3637-3645.

Kampschreur, M.J., Tan, N.C.G., Kleerebezem, R., Picioreanu, C., Jetten, M.S.M., van Loosdrecht, M.C.M. 2008. Effect of dynamic process conditions on nitrogen oxides emission from a nitrifying culture. Environmental Science and Technology. 42(2):429-435.

Kampschreur, M. J., Poldermans, R., Kleerebezem, R., van der Star, W. R. L., Haarhuis, R., Abma, W.R., Jetten, M.S.M., van Loosdrecht M.C.M. 2009. Emission of nitrous oxide and nitric oxide from a full-scale single-stage nitritation-anammox reactor. Water Science and Technology. 60(12):3211-3217.

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

Kornaros, M., Dokianakis, S. N. & Lyberatos G. 2010. Partial nitrification/denitrification can be attributed to the slow response of nitrite oxidizing bacteria to periodic anoxic disturbances. Environmental Science and Technology. 44(19):7245-7253

Kumar ,M. & Lin, J.G. 2010. Co-existence of anammox and denitrification for simultaneous nitrogen and carbon removal-Strategies and issues. Journal of Hazardous Materials. 178:1-9.

Köz Utku. 2007. Modeling & simulation of reject water treatment at Sjölunda WWTP with WEST. Master Thesis, Lund University.

Mackinnon, I.D.R., Barr, K., Miller, E., Hunter, S., Pinel, T. 2003. Nutrient removal from wastewaters using high performance materials. Water Science and Technology. 47(11):101-107.

Mahimairaja, S. & Bolan, N.S. 2004. Problems and prospects of agricultural use of distillery spentwash in India. Third Australian and New Zealand Soil Science Societies Joint Conference. Sydney, Australia. 5-9 December 2004.

Malovanyy, A., 2009. Monitoring and application of anammox process in one stage deammonification system. Master Thesis. Stockholm: KTH.

Manuale dell'Ingegnere, 2003. Nuovo Colombo 84ª ed. Hoepli, Italy.

Marsalek, J., Sztruhar, D., Giulianelli, M., Urbonas, B. 2004. Enhancing urban environment by environmental upgrading and restoration. NATO Science Series, IV Earth and Environmental Sciences. Dordrecht, The Netherlands: Kluwer Academic Publishers. 43:289-306.

Middlebrooks, E.J. 1968. Wastes from the preservation of wood. Journal of the Sanitary Engineering Division American Society of Civil Engineers. SA1(5785):41

Miladinovic, N. & Weatherley, L.R. 2008. Intensification of ammonia removal in a combined ion-exchange and nitrification column. Chemical Engineering Journal. 135(1-2):15-24.

Mulder, A., van de Graaf, A.A., Robertson, L.A., Kuenen, J.G. 1995. Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. FEMS Microbiology Ecology. 16:177-184.

Mulder, J.W. Duin, J.O.J., Goverde, J., Poiesz, W.G., van Veldhuizen, H.M., van Kempen, R., Roeleveld, P. 2006. Full-scale experience with the SHARON process through the eyes of the operators. Proceedings of the Water Environment Federation. WEFTEC. 67:5256-5270.

Mulder, J.W., Van Loosdrecht, M.C.M., Hellinga, C, Van Kempen, R. 2001. Full-scale application of the SHARON process for treatment of rejection water of digested sludge dewatering. Water Science and Technology. 43(11):127-134.

Murat, S., Insel, G., Artan, N., Orhon, D. 2006. Performance evaluation of SBR treatment for nitrogen removal from tannery wastewater, Water Science and Technology. 53(12):275-284.

Notenboom, G.J., Jacobs, J.C., Van Kempen, R., Van Loosdrecht, M.C.M. 2002. High rate treatment with SHARON process of waste water from solid waste digestion. 3rd International Symposium Anaerobic Digestion of Solid Wastes. Munich/Garching, Germany. 18 - 20 September 2002. IWA Publishing.

Nowak, O., Svardal, K. & Kroiss, H. 1996. The impact of phosphorus deficiency on Nitrification - case study of a biological pretreatment plant for rendering plant effluent. Water Science and Technology. 34(1-2):229-236.

Op den Camp, H.J.M., Kartal, B., Guven, D., van Niftrik, L.A.M.P., Haaijer, S.C.M., van der Star, W.R.L., van de Pas-Schoonen, K.T., Cabezas, A., Ying, Z., Schmid, M.C., Kuypers, M.M.M., van de Vossenberg, J., Harhangi, H.R., Picioreanu, C., van Loosdrecht, M.C.M., Kuenen, J.G., Strous, M., Jetten, M.S.M. 2006. Global impact and application of the anaerobic ammonium-oxidizing (anammox) bacteria. Biochemical Society Transactions. 34:174-178.

Paredes, D., Kuschk, P., Mbwette, T. S. A., Stange, F., Müller, R. A., Köser, H. 2007. New Aspects of Microbial Nitrogen Transformations in the Context of Wastewater Treatment – A Review. Engineering in Life Sciences. 7(1):13-25

Park, H.D. & Noguera, D.R. 2007. Characterization of two ammonia-oxidizing bacteria isolated from reactors operated with low dissolved oxygen concentrations. Journal of Applied Microbiology. 102(5):1401–1417

Pearce, O., Clark, T. & Ndombasi, P. 2000. Ammonnia removal from sludge liquors. Thames Water Plc, Research and Technology Report.

Peng, Y. & Zhu, G. 2006. Biological nitrogen removal with nitrification and denitrification via nitrite pathway. Applied Microbiology and Biotechnology. 73:15–26.

Plaza, E., Trela, J., Gut, L., Löwén, M., Szatkowska, B. 2003. Deammonification process for treatment of ammonium rich wastewater. Proceedings of a Polish-Swedish seminar, Gdansk, 23-25 March 2003. Integration and optimisation of urban sanitation systems. Eds: Plaza E., Levlin E., Hultman B. Report 10: 77-87.

Plaza, E., Trela, J. & Hultman, B. 2009. Swedish experience with deammonification process in biofilm system. Proceedings of a Polish-Swedish-Ukrainian seminar, Lviv Ukraine, October 26-28, 2006. Research and application of new technologies in wastewater treatment and municipal solid waste disposal in Ukraine, Sweden and Poland. Eds: Plaza E., Levlin E. Report 14: 31-40.

Philips, S., Wyffels, S., Sprengers, R., Verstraete, W. 2002. Oxygen-limited autotrophic nitrification/denitrification by ammonia oxidisers enables upward motion towards more favourable conditions. Applied Microbiology and Biotechnology. 59(4-5):557-566.

Piccinini, S., Fabbri, C. & Sassi, D. 2007. Speciale reflui - Azoto nei liquami, i metodi per rimuoverlo supplemento suinicoltura. 11:24-32.

Prosser, J.I. 2005. Nitrification. Encyclopedia of Soils in the Environment. Ed. Hillel D. Oxford: Elsevier Ltd. 31-39

Ramadan, A.E.K.. 2007. Removal of ammonia and phenol from industrial wastewater, 11th International Water Technology Conference (IWTC) Sharm El-Sheikh, Egypt. 15-18 March 2007. 949-971

Rodhe, W. 1969. Crystallization of eutrophication concepts in North Europe. Eutrophication, Causes, Consequences, Correctives. National Academy of Sciences, Washington D.C. 50-64.

Rols, J. L. & Goma, G. 1997. Reduction of sludge production during biological wastewater treatment. International Symposium Environmental Biotechnology. Ostenda. 71-74.

Renou, S., Givaudan, J.G., Poulain, S., Dirassouyan, F., Moulin, P. 2008. Landfill leachate treatment: Review and opportunity. Journal of Hazardous Materials. 150: 468-493.

Rosenwinkel, K.H. & Cornelius, A. 2005. Deammonification in the moving bed process for the treatment of wastewater with high ammonia content. Chemical Engineering & Technology. 28(1):49-52.

Sarkar, A.K. 1990. Phosphate cement-based fast setting binders. Journal American Ceramic Society Bulletin. 69:234-238.

Schmid, M., Walsh, K., Webb, R., Rijpstra, W.I.C., van de Pas-Schoonen, K., Verbruggen, M.J., Hill, T., Moffett, B., Fuerst, J., Schouten, S., Sinninghe Damsté, J.S., Harris, J., Shaw, P., Jetten, M., Strous, M. 2003. Candidatus "Scalindua brodae", sp. nov., Candidatus "Scalindua wagneri", sp. nov., two new species of anaerobic ammonium oxidizing bacteria. Systematic and Applied Microbiology. 26:529-538.

Schuiling, R.D. & Andrade, A., 1999. Recovery of struvite from calf manure. Environmental Science & Technology. 20:765-768.

Seyfried, C.F., Hippen, A., Helmer, C., Kunst, S., Rosenwinkel, K.H. 2001. One-stage deammonification: nitrogen elimination at low costs. Water Science and Technology: Water Supply 1(1):71-80.

Shin, H.S. & Lee S.M. 1997. Removal of nutrients in wastewater by using magnesium salts. Environmental Technology 19:283-290.

Siegrist, H. 1996. Nitrogen removal from digester supernatant-comparison of chemical and biological methods. Water Science and Technology. 34(1-2):399-406.

Strous, M., Van Gerven, E., Kuenen, J.G., Jetten, M. 1997. Effects of aerobic and microaerobic conditions on anaerobic ammonium-oxidizing (Anammox) sludge. Applied and Environmental Microbiology. 63:2446–2448.

Strous, M., Heijnen, J.J., Kuenen, J.G., Jetten, M.S.M. 1998. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Applied Microbiology and Biotechnology. 50(5):589-596.

Strous, M., Kuenen, J. G. & Jetten, M. 1999. Key physiological parameters of anaerobic ammonium oxidation. Applied Microbiology and Biotechnology. 65:3248-3250.

Surmacz-Górska, J., Gernaey, K., Demuynak, C., Vahrollegnem, P., Verstraete, W. 1996. Nitrification monitoring in activated sludge by oxygen uptake rate (OUR) measurements. Water Research. 30(5):1228-1236.

Swedish Environmental Protection Agency. 2000. Environmental quality criteria. Lakes and watercourses. Swedish Environmental Protection Agency, Report 5050. Stockholm. 102 p.

Szatkowska, B. 2007a. Performance and control of biofilm systems with partial nitritation and anammox for supernatant treatment. Doctoral Thesis. Stockholm: KTH.

Szatkowska, B., Cema, G., Plaza, E., Trela, J., Hultman, B. 2007b. A one-stage system with partial nitritation and Anammox processes in the moving-bed biofilm reactor. Water Science and Technology. 55(8-9):19-26.

Szatkowska, B., Trela, J. & Plaza, E. 2007c. Partial nitritation/anammox and CANON - Nitrogen removal systems followed by conductivity measurements. Proceedings of Polish-Swedish seminars, Cracow March 17-18, 2005. Integration and optimisation of urban sanitation systems. Eds: Plaza E., Levlin E. Report 13: 109-117.

Szatkowska, B., Plaza, E., Trela, J., Bakowska, A. 2004. Influence of dissolved oxygen concentration on deammonification process performance. Proceedings of a Polish-Swedish seminar, Wisla October 25-28, 2003. Integration and optimisation of urban sanitation systems. Eds: Plaza E., Levlin E., Hultman B. Report 11: 121-131.

Third, K.A., Sliekers, A.O., Kuenen, J.G., Jetten, M.S.M. 2001. The CANON system (Completely Autotrophic Nitrogen-removal Over Nitrite) under ammonium limitation: interaction and competition between three groups of bacteria. Systematic and Applied Microbiology. 24(4):588-596.

Thornton, A., Pearce, P. & Parsons, S.A. 2007. Ammonium removal from digested sludge liquors using ion exchange. Water Research. 41(2):433-439.

Trela, J., Plaza, E., Hultman, B., Cema, G., Bosander, J., Levlin, E. 2008. Utvärdering av enstegsprocess för deammonifikation. Rapport Nr 2008-18. Stockholm.

Trigo, C., Campos, J.L., Garrido, J.M., Méndez, R. 2006. Start-up of the Anammox process in a membrane bioreactor. Journal of Biotechnology., 126(4):475-487

Trimmer, M., Nicholls, J.C. & Deflandre, B. 2003. Anaerobic Ammonium Oxidation measured in sediments along the Thames Estuary, United Kingdom. Applied and Environmental Microbiology. 69(11):6447-6454.

Vandaele, S., Bollen F., Thoeye, C., November, E., Verachtert, H., Van Impe, J.F. 2000. A comparison of SBR and SBBR for nitrogen removal out of ammonia rich sludge liquors and problems encountered. Proceedings of the 2nd International Symposium on Sequencing Batch Reactor Technology. London: IWA Publishing. 1:67-74.

van de Graaf, A.A., De Bruijn, P., Robertson, L.A., Jetten, M.S.M, Kuenen, J.G. 1996. Autotrophic growth of anaerobic ammonium-oxidizing microorganisms in a fluidized bed reactor. Microbiology. 142:2187-2196.

van Dijk, L. & Roncken, G.C.G. 1997. Membrane bioreactors for wastewater treatment: the state of the art and new developments. Water Science and Technology. 35:35-41.

van Dongen, L.G.J.M., Jetten, M.S.M. & van Loosdrecht, M.C.M. 2001. The Combined Sharon/Anammox Process - A sustainable method for N-removal from sludge water. STOWA report 2001. London: IWA Publishing.

van Dongen, U., Jetten, M.S.M. & van Loosdrecht, M.C.M. 2001. The SHARON®-Anammox® process for treatment of ammonium rich wastewater. Water Science and Technology. 44(1):153-160.

Van Haandel, A.C. & van der Lubbe, J.G.M. 2007. Handbook biological waste water treatment. Design and optimisation of activated sludge systems. Leidschendam, The Netherland: Quist Publishing. 537 p.

Van Hulle, S.W.H., Vandeweyerb, H.J.P., Meesschaertc, B.D., Vanrolleghema, P.A., Dejansb, P., Dumoulinb, A. 2010. Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams. Chemical Engineering Journal 162:1-20.

Van Hulle, S.W.H., Volcke, E.I.P., Teruel, J.L., Donckels, B., van Loosdrecht, M.C.M., Vanrolleghem, P.A. 2007. Influence of temperature and pH on the kinetics of the SHARON nitritation process. Journal of Chemical Technology and Biotechnology. 82(5):471-480.

Van Hulle, S. 2005. Modelling, simulation and optimization of autotrophic nitrogen removal processes. Doctoral Thesis. Universiteit Gent.

van Kempen, R, Mulder, J.W., Uijterlinde, C.A., van Loosdrecht, M.C.M. 2001. Overview: full scale experience of the SHARON process for treatment of rejection water of digested sludge dewatering. Water Science and Technology. 44:145-152.

van Loosdrecht M.C.M., Jetten M.S.M. 1998. Microbiological conversions in nitrogen removal. Water Science Technolpgy 38:1-7.

van Loosdrecht, M.C.M., Jetten, M.S.M. 1997. Method for treating ammonia-comprising wastewater. Patent PCT/NL97/00482.

Vázquez-Padín, J., Fernádez, I., Figueroa, M., Mosquera-Corral, A., Campos, J.L., Méndez, R. 2009. Applications of Anammox based processes to treat anaerobic digester supernatant at room temperature. Bioresource Technology 100:2988-2994.

Vymazal, J. 2010. Water and nutrient management in natural and constructed wetlands. Vymazal (ed). Dordrecht, The Netherlands: Springer. 375 p.

Wang, L.K., Shammas, N.K. & Hung, Y.T. 2009. Advanced Biological Treatment Processes. Totowa, New York:Humana Press. p.738

Weiss, J.S., Alvarez, M., Tang, C., Horvath, R.W., Stahl, J.F. 2005. Evaluation of Moving Bed Biofilm Reactor technology for enhancing nitrogen removal in a stabilization pond treatment plant. Proceedings of the 78th annual water environment federation technical exposition and conference. Washington D.C., 29 October - 2 November. Alexandria, Virginia: Water Environment Federation. 3889-3904.

Wett, B. 2006. Solved upscaling problems for implementing deammonification of rejection water. Water Science Technolpgy. 53(12):121-128.

Wett, B. & Rauch, W., 2002. The role of inorganic carbon limitation in biological removal of extremely ammonia concentrated wastewater, Water Research. 37:1100-1110.

Wett, B., Rostek, R., Rauch, W., Ingerle, K. 1998. pH controlled reject water treatment. Water Science and Technology. 37 (12):165-172.

Whalen, P. 2007. Reducing waste biosolids production through wastewater treatment process optimization using ATP monitoring technology. Moving Forward,Wastewater Biosolids Sustainability: Technical, Managerial, and Public Synergy. Moncton/New Brunswick, Canada. 24-27 June 2007. 319-325.

WHO. 1999. Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. Eds: Chorus I. and Bartram J. London: E & FN Spon.

WHO. 2000. Monitoring bathing waters. A practical guide to the design and implementation of assessments and monitoring programmes. Eds: Bartram J. and Rees G. London: E & FN Spon.

Wiesmann, U. 1994. Biological nitrogen removal from wastewater. Advance in Biochemical Engineering. Ed. Fletcher A. Berlin: Springer-Verlag. 113-154.

Xu, Z.Y., Zeng, G.M., Yang, Z.H., Xiao, Y., Cao, M., Sun, H.S., Ji, L.L., Chen, Y. 2010. Biological treatment of landfill leachate with the integration of partial nitrification, anaerobic ammonium oxidation and heterotrophic denitrification. Bioresource Technology 101:79-86.

Xu, G., Xu, X., Yang, F., Liu, S. 2010. Selective inhibition of nitrite oxidation by chlorate dosing in aerobic granules. Journal of Hazardous Materials.

Zala, S.L., Ayyer, J. & Desai, A.J. 2004. Nitrate removal from the effluent of a fertilizer industry using a bioreactor packed with immobilized cells of Pseudomonas stutzeri and Comamonas testosteroni. World Journal of Microbiology and Biotechnology. 20(7):661-665.

Zhan, X., Healy, M.G. & Li, J. 2008. Nitrogen removal from slaughterhouse wastewater in a sequencing batch reactor under controlled low DO conditions. Bioprocess and Biosystems Engineering. 32(5): 607-614

Zhang, L., Zheng, P., Tang, C., Jin, R. 2008. Anaerobic ammonium oxidation for treatment of ammonium-rich wastewaters. Journal of Zhejiang University Science B. 9(5):416-426

Ødegaard, H., Gisvold, B. & Strickland, J. 2000. The influence of carrier size and shape in the moving bed biofilm process. Water Science and Technology. 43(4-5):383-391.

Other references:

http://ec.europa.eu/environment/water/water-nitrates/pdf/eutrophication.pdf

http://ec.europa.eu/environment/water/index_en.htm

http://www.anoxkaldnes.com/Eng/c1prodc1/mbbr.htm

http://campi.kth.se/unik-miljoforskning-pa-sjostadsverket-1.55135

http://en.wikipedia.org

http://www.kaldnes.com/process.html

http://www.lwr.kth.se/english/researchgroups/WaterResources/Sjostad.htm

http://www.paques.nl/en/anammox_nitrogen_removal

http://www.syvab.se/396/Vattnets-vag.html

http://www.sjostadsverket.se/

Lecture notes of the course: "Water and waste handling" (KTH)

Lecture notes of the course: "Reduction of wastewater treatment contribution to global warming" (KTH)

Lecture notes of the course Environmental Dynamics/Chemical Processes (KTH)

Lecture notes of the courses "Ingegneria sanitaria ambientale" and "Complementi di ingegneria sanitaria ambientale" (Politecnico of Turin)

APPENDIX I – DESCRIPTION OF PROCEDURES FOR ANALYSES AND MEASUREMENTS

Total and volatile suspended solids as biofilm

The procedure followed was:

1. The filter and the aluminum plate were weighted before the filtration;
2. The biomass was carefully removed from the carriers by using a needle and distilled water;
3. The removed biomass was filtered with a micro glass fiber filters with pore size 1.6 μm;
4. The biomass retained on the filter was evaporated at 105°C over night. The increase in weight of the filter represents the total suspended solids (TSS);
5. The residue from the point 4) was ignited to constant weight at 550°C for 40 minutes. The remaining solids represents the fixed total, dissolved, or suspended solids while the weight loss on ignition is the volatile solids (VSS).

The total suspended solids (TSS) were calculated as:

$$\text{mg TSS/ring} = \frac{A-B}{4}$$

where:

A = weight of filter and aluminum plate + dried residue [mg];

B = weight of filter and aluminum plate [mg];

4 = number of rings.

The volatile suspended solids (VSS) were calculated as:

$$\text{mg VSS/ring} = \frac{A-B}{4}$$

where:

A = weight of filter and aluminum plate + residue before ignition [mg];

B = weight of filter and aluminum plate + residue after ignition [mg];

4 = number of rings.

The ash content was calculated as:

$$\%\ \text{ash} = \frac{TSS-VSS}{TSS}\cdot 100$$

Total and volatile suspended solids in the influent and inside the reactor

The procedure followed was:

1. The filter and the aluminum plate were weighted before the filtration;
2. A certain amount of mixed sample was filtered with a micro glass fiber filters with pore size 1.6 μm;
3. The residue retained on the filter was evaporated at 105°C over night. The increase in weight of the filter represent the total suspended solids (TSS);
4. The residue from the point 4) was ignited to constant weight at 550°C for 40 minutes. The remaining solids represent the fixed total, dissolved, or suspended solids while the weight loss on ignition is the volatile solids (VSS).

The total suspended solids (TSS) were calculated as:

$$\text{mg TSS/l} = \frac{(A-B)\cdot 1000}{sample\ volume, ml}$$

where:

A = weight of filter and aluminum plate + dried residue [mg];

B = weight of filter and aluminum plate [mg];

The volatile suspended solids (VSS) were calculated as:

$$\text{mg VSS/l} = \frac{(A-B)\cdot 1000}{sample\ volume, ml}$$

where:

A = weight of filter and aluminum plate + residue before ignition [mg];

B = weight of filter and aluminum plate + residue after ignition [mg];

The ash content was calculated as:

$$\%\ \text{ash} = \frac{TSS-VSS}{TSS}\cdot 100$$

Estimate of the total suspended biomass and the total biomass as biofilm

The suspended biomass was calculated as :

$$VSS_{act.sludge} = (mgVSS/l)\cdot V_R$$

where:

mg VSS/l = measurement of suspended biomass;

V_R = volume of liquid in the reactor;

The total biomass attached on the Kaldness rings in the reactor was calculated as:

$$VSS_{biofilm} = (mgVSS/ring)\cdot Nrings$$

where:

mg VSS/ring = measurement of average biomass attached on the Kaldnes rings;

N_{rings} = estimated number of rings in the reactor, on the basis that 107 biocarriers occupy 100 ml and the reactor was filled with rings whose total volume was known because measured before starting the study.

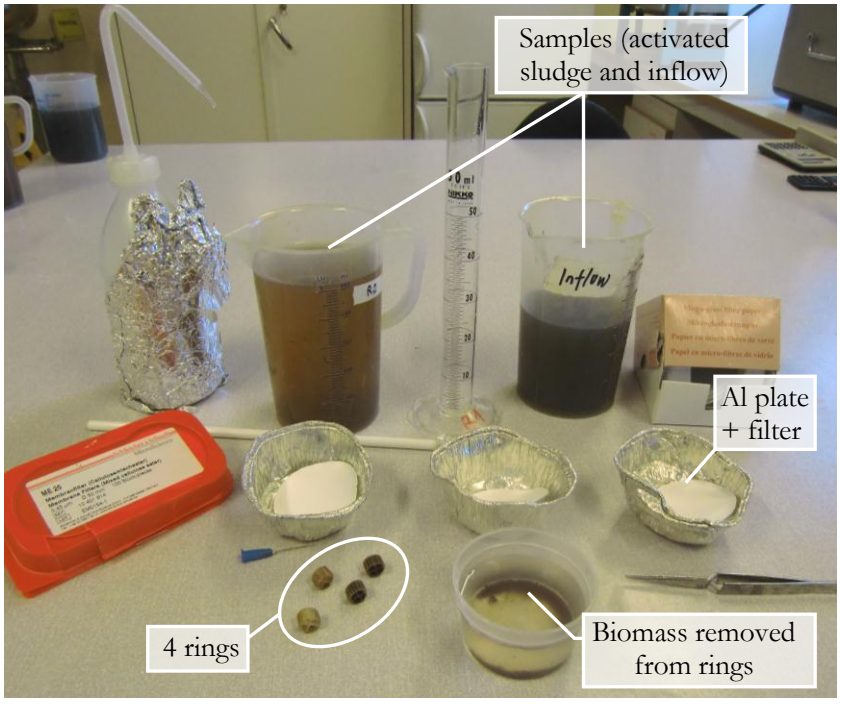

Fig. 1A. Material for the measurements of suspended solids in the influent, attached on the rings and inside the reactor.

Efficiencies for Lab-scale and Pilot Plant-scale reactors

$$\eta_{NH_4^+-N} = \frac{\left(NH_4^+ - N\right)_{in} - \left(NH_4^+ - N\right)_{out}}{\left(NH_4^+ - N\right)_{in}};$$

$$\eta_{N_{inorg}} = \frac{\left(NH_4^+ - N\right)_{in} - \left(N_{inorg}\right)_{out}}{\left(NH_4^+ - N\right)_{in}};$$

$$\eta_{TN} = \frac{\left(TN\right)_{in} - \left(TN\right)_{out}}{\left(TN\right)_{in}}.$$

Nitrogen loading and removal rates

$$\text{Loading rate [g N m}^{-2}\text{ d}^{-1}] = \frac{\left(N_{inorg}\right)_{in} \cdot \frac{Q}{1000}}{\frac{V_{rings}}{1000} \cdot 500\frac{m^2}{m^3}} \cdot 60 \cdot 24$$

$$\text{Removal rate [g N m}^{-2}\text{ d}^{-1}] = \frac{\left(N_{inorg}\right)_{in} - \left(N_{inorg}\right)_{out} \cdot \frac{Q}{1000}}{\frac{V_{rings}}{1000} \cdot 500\frac{m^2}{m^3}} \cdot 60 \cdot 24$$

where:

N_{inorg} = inorganic nitrogen [mg/l] in inflow $(N_{inorg})_{in}$, essentially NH_4^+-N, or outflow $(N_{inorg})_{out}$;

Q = inflow rate [ml/min], kept constant between inflow and outflow chemical analysis and measured manually;

V_{rings} = bulk volume of biocarriers in the reactor (Lab-scale reactor treating diluted reject water: 3.9 l; Lab-scale reactor treating effluent from UASB reactor: 3.17 l; Pilot-plant scale reactor: 80 l;

500 m^2/m^3 = specific internal surface area of Kaldnes media (model 1);

1000 = unit conversion factors mg - g, ml - l and l - m^3;

60 and 24 = unit conversion factors from min to days.

The loading and removal rates could also be expressed as mg N l^{-1} d^{-1}, but this is usually less common for biofilm systems. In this case they could be calculated as:

$$\text{Loading rate [mg N l}^{-1}\text{ d}^{-1}] = \frac{\left(N_{inorg}\right)_{in} \cdot \frac{Q}{1000}}{166l} \cdot 60 \cdot 24$$

$$\text{Removal rate [mg N l}^{-1}\text{ d}^{-1}] = \frac{\left[\left(N_{inorg}\right)_{in} - \left(N_{inorg}\right)_{out}\right] \cdot \frac{Q}{1000}}{166l} \cdot 60 \cdot 24$$

where:

166 l = liquid volume in the reactor (Lab-scale reactor treating diluted reject water: 7.69 l; Lab-scale reactor treating effluent from UASB reactor: 6.74 l; Pilot-plant scale reactor: 166 l);

1000 = unit conversion factors ml - l;

APPENDIX II – DATA FROM LAB-SCALE REACTOR TREATING DILUTED SUPERNATANT

Table 1A – Chemical analyses on the inflow to the reactor

Date	Alkalinity (mmol/l) (0,45µm)	NH4-N (mg/l) (0,45µm)	TOT-P (mg/l) unfiltr	COD (mg/l) (0,45µm)	COD (mg/l) unfiltr	N load ($gN/m^2/d$)
23/03/2010	-	400	-	-	-	0,7748
30/03/2010	79,9	341	-	284	-	0,6605
05/04/2010	31,4	421	-	247	452	0,8154
13/04/2010	29,5	368	-	257	355	0,7128
21/04/2010	30,1	418	1,82	298	576	0,8096
27/04/2010	33,9	383	0,75	358	527	0,7418
03/05/2010	33,9	421	2,07	382	497	0,8154
05/05/2010	-	387	-	-	-	0,7496
12/05/2010	31,5	447	-	-	-	0,8658
19/05/2010	31,5	406	3,04	326	540	0,7864
21/05/2010	-	402	-	-	-	0,7786
26/05/2010	26,6	395	2,82	337	526	0,7651

Table 2A – Chemical analyses on the outflow from the reactor

Date	Alkalinity (mmol/l) (0,45µm)	NH4-N (mg/l) (0,45µm)	NO2-N (mg/l) (0,45µm)	NO3-N (mg/l) (0,45µm)	TOT-P (mg/l) unfiltr.	COD (mg/l) (0,45µm)	COD (mg/l) unfiltr	Ninorg removal ($gN/m^2/d$)
23/03/2010	-	-	-	-	-	-	-	-
25/03/2010	7,16	42,4	5,23	4,45	-	141	-	0,6739
30/03/2010	-	-	-	-	-	-	-	-
01/04/2010	1,26	8,97	7,2	50,7	-	178	-	0,5310
08/04/2010	6,35	68,3	1,81	19	-	196	197	0,6428
15/04/2010	4,88	35,6	3,51	47,3	-	158	166	0,5454
23/04/2010	8,14	77	2,96	19,16	-	168	187	0,6176
29/04/2010	0,522	-	-	-	-	158	191	-
05/05/2010	1,62	46,6	6,405	52,8	-	171	204	0,5776
14/05/2010	23,72	200	2,41	2,13	-	202	-	0,4696
21/05/2010	19,72	225,2	2,92	0,96	4,14	197	415	0,3388
28/05/2010	0,668	23,6	0,191	109,2	2,56	187	314	0,5075

Table 3A – Physical parameters

Date	Influent		Inside reactor				Effluent		Remark
	pH	Cond (mS/cm)	pH	Cond (mS/cm)	DO (mg/l)	Temp (C)	pH	Cond (mS/cm)	
23/03/2010	7,38	3,18	7,70	1,60	1,90	24,6	-	-	
24/03/2010	7,66	3,16	7,48	0,93	2,50	24,5	7,90	1,24	Decreased aeration
25/03/2010	7,96	3,04	7,03	0,86	1,05	24,5	7,42	0,87	
26/03/2010	8,26	3,09	7,48	0,86	0,70	24,8	7,64	0,82	
29/03/2010	8,49	2,94	7,57	1,04	0,80	25,1	7,46	1,07	
30/03/2010	8,49	2,91	7,63	1,11		24,4	7,62	1,07	Increased aeration
31/03/2010	8,50	2,83	7,03	0,74	3,80	25,2	7,19	0,72	Decreased aeration
01/04/2010	8,57	2,24	6,72	0,83	2,83	25,3	6,80	0,88	Decreased aeration
02/04/2010	8,42	3,18	6,66	0,96	1,58	24,8	6,60	0,96	Inflow rate was too low. Increased inflow rate. Changed position stirrers.
05/04/2010	8,36	2,90	6,67	0,85	1,29	24,2	6,54	0,87	Decrease aeration (1,77->0,80).
06/04/2010	8,47	2,87	7,05	0,90	0,71	24,5	7,04	0,88	
08/04/2010	8,85	2,76	7,65	1,02	0,68	26,2	-	-	Increase aeration
09/04/2010	8,48	2,93	7,62	0,85	1,05	25,8	-	-	
13/04/2010	8,83	2,71	6,96	0,94	2,15	25,6	-	-	Decrease aeration
15/04/2010	8,32	3,18	7,32	1,04		24,4	-	-	Increased aeration. Cleaned pipes.
16/04/2010	8,02	3,14	6,44	0,70	0,18	24,5	-	-	Decreased aeration
21/04/2010	8	3,17	7,22	0,78	0,62	25,2	-	-	New DO-meter for measurement. Calibrated pH-meter.
23/04/2010	8,28	3,01	7,63	1,17	0,32	24,6	7,78	1,11	Parameters checked before new inflow. Problem with DO, not evenly distributed. (high close to the aeration (>2,5mg/l)). Changed many times.
26/04/2010	8,10	3,13	7,85	1,47	0,43	24,2	-	-	Increased aeration. Added 28 rings.
27/04/2010	7,86	3,05	6,84	0,64	1,40 1,05	26,1	-	-	Decreased aeration (1,40->1,05). Decreased inflow (2,85ml/min -> 2,57 ml/min)
29/04/2010	- 8,11	2,77 2,98	6,11 6,39	0,87	2,00 0,79	27,4	-	-	Decreased aeration. Inflow rate was slightly lower.
03/05/2010	8,48 8,30	2,67 3,02	7,83	1,49	0,51	27,2 26,0	7,80	1,33	Inflow rate was 2,541 l/min.
04/05/2010	8,27	3,15	7,72	1,19	1,3 1,0	24,0	7,60	1,13	Decreased aeration.
05/05/2010	8,47 8,35	2,90 3,41	7,55	1,05	1,55 0,80	25,0	7,58	0,97	Decreased aeration. Inflow rate was 2,6 ml/min.
07/05/2010	-	-	-	-	-	-	-	-	New inflow (but dilution 1:2 on 7/5/2010).
10/05/2010	8,36 7,18	3,31 3,58	7,72	1,67	1,19	24,2	7,43	1,56	Inflow rate was too low.
11/05/2010	7,26	3,58	7,91	1,99	0,54	25,4	-	-	
12/05/2010	7,78	3,54	7,99	2,31	0,65	25,0	7,86	2,27	Increased aeration
13/05/2010	7,79	3,50	7,97	2,36	1,00	25,4	7,84	2,30	
18/05/2010	8,05	3,11	7,92	2,30	0,83	27,8	7,92	1,77	T high (probably due to warm days). Increased slightly aeration.
21/05/2010	8,22 8,26	2,95 3,32	7,96 7,83	1,96	0,58 0,57	29,2	8,00	1,90	Calibrated pH meter. Flow was too low (1,87 ml/min). T high (warm days). Increased aeration (changed air stone that supply air). DO was not evenly distributed.
24/05/2010	8,17	3,25	6,95	1,1	0,80	26,2	7,03	1,30	
26/05/2010	8,07	3,22	6,73	0,82	1,10	26	6,96	0,79	
27/05/2010	8,34	3,07	6,54	0,908	0,77	26	-	-	

Table 4A – TSS & VSS as biofilm on the carriers (filtered 1.6 µm)

Date	Biocarriers	Empty plate + filter (g)	After 105 °C (g)	After 550 °C (g)	**TSS (mg/ring)**	**VSS (mg/ring)**	Ash (%)
23/03/2010	4	1,0604	1,1104	1,0618	12,50	12,15	2,80%
01/04/2010	4	1,0606	1,1106	1,0617	12,50	12,23	2,20%
06/04/2010	4	0,8887	0,9392	0,8905	12,63	12,18	3,56%
16/04/2010	4	0,8984	0,948	0,9002	12,40	11,95	3,63%
23/04/2010	4	0,8918	0,9421	0,8935	12,58	12,15	3,38%
29/04/2010	4	0,8908	0,9565	0,8925	16,43	16,00	2,59%
05/05/2010	4	0,8987	0,9492	0,9004	12,63	12,20	3,37%
21/05/2010	4	0,9020	0,9493	0,9059	11,83	10,85	8,25%

Table 5A – TSS & VSS in the activated sludge (filtered 1.6 µm)

Date	Sample Volume (ml)	Empty plate + filter (g)	After 105 C (g)	After 550 C (g)	**TSS (mg/l)**	**VSS (mg/l)**	Ash (%)
21/05/2010	61	20	1,0696	1,0877	1,0721	905,0	780,0

SAA tests

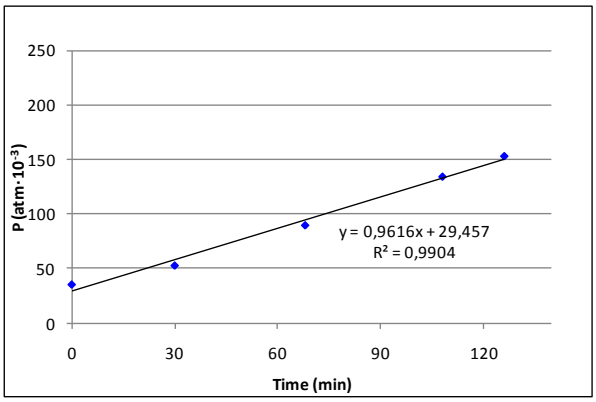

Fig. 2A – 25 March 2010 (35°C)

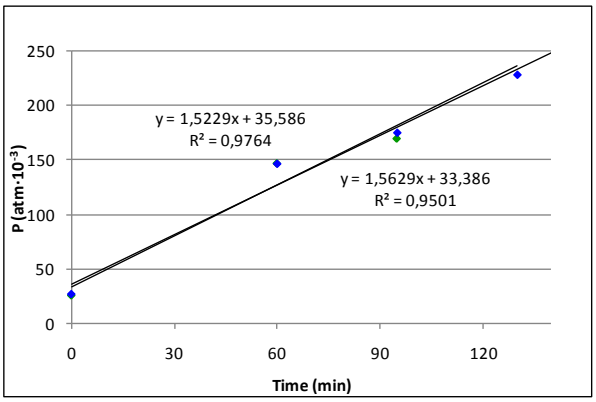

Fig. 3A – 1 April 2010 (35°C)

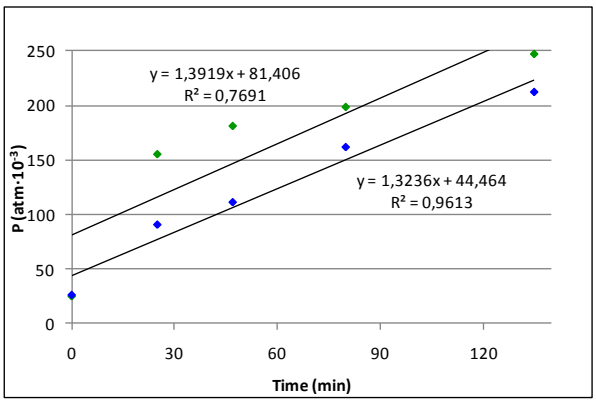

Fig. 4A – 8 April 2010 (35°C)

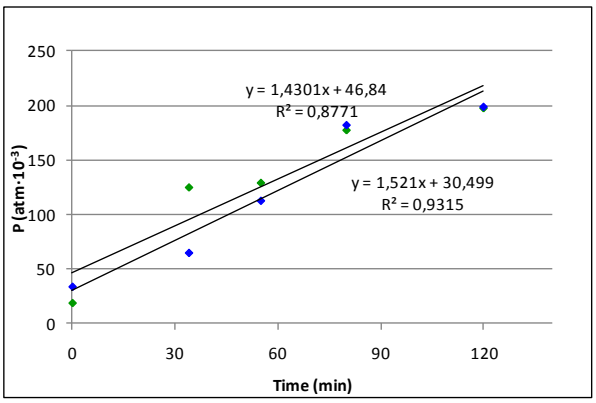

Fig. 5A – 15 April 2010 (35°C)

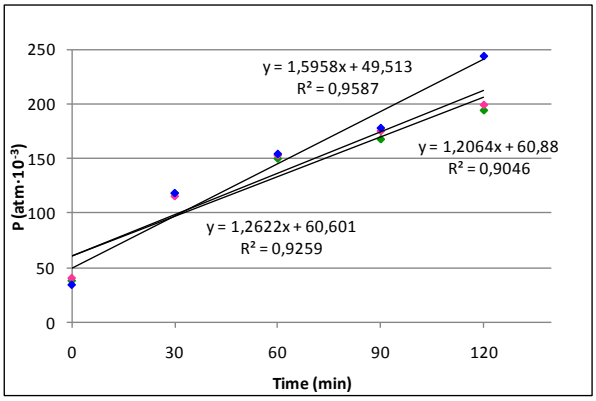

Fig. 6A – 21 April 2010 (35°C)

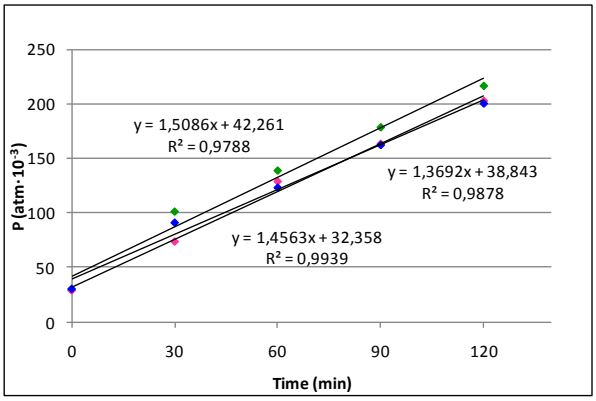

Fig. 7A – 29 April 2010 (35°C)

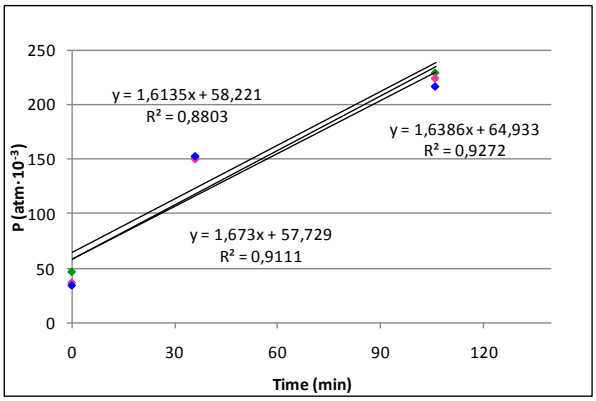

Fig. 8A – 7 May 2010 (35°C)

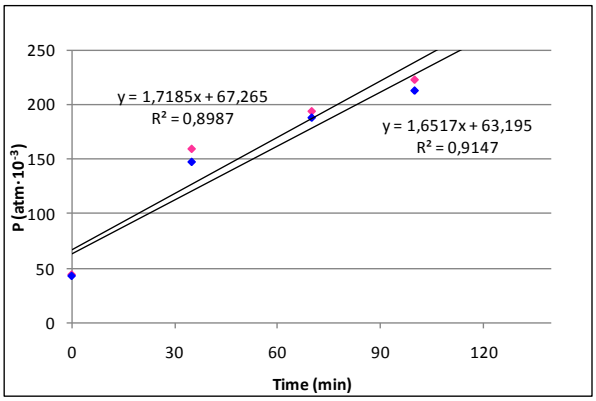

Fig. 9A – 12 May 2010 (35°C)

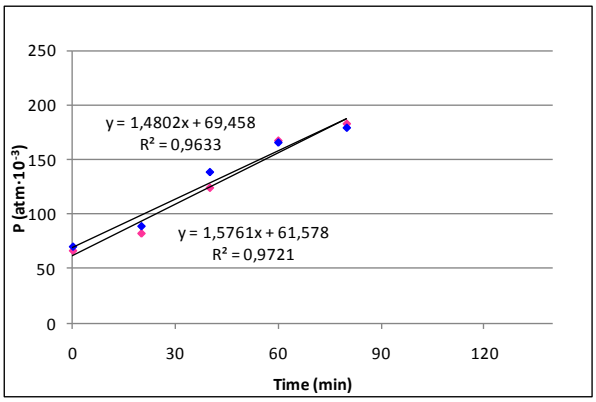

Fig. 10A – 19 May 2010 (35°C)

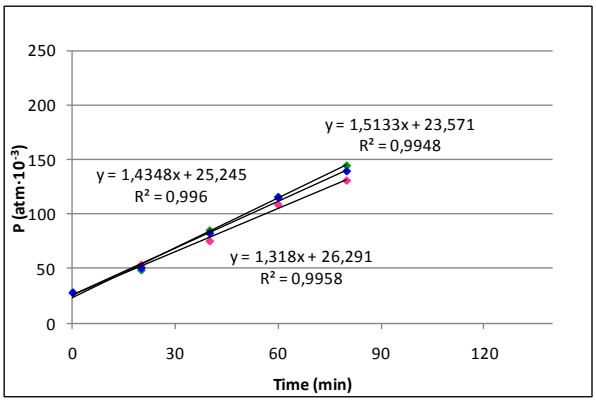

Fig. 11A – 28 May 2010 (35°C)

Table 6A – SAA results

Date	SAA (35°C) ($gN/m^2/d$)
25/03/2010	2,844
01/04/2010	4,563
08/04/2010	4,015
15/04/2010	4,364
21/04/2010	4,007

Date	SAA (35°C) ($gN/m^2/d$)
29/04/2010	4,273
07/05/2010	4,855
12/05/2010	4,407
19/05/2010	4,519
28/05/2010	4,206

APPENDIX III – DATA FROM LAB-SCALE REACTOR TREATING EFFLUENT FROM ANAEROBIC TREATMENT WITH UASB

Table 7A – Chemical analyses on the inflow to the reactor

Date	Alkalinity (mmol/l) (0,45µm)	NH4-N (mg/l) (0,45µm)	TOT-P (mg/l) unfiltr	COD (mg/l) (0,45µm)	COD (mg/l) unfiltr	N load ($gN/m^2/d$)
11/05/2010	5,83	44,6	3,45	52,5	107	0,1897
18/05/2010	-	40,3	-	-	-	0,1714
20/05/2010	5,23	40,7	4,98	57,6	59,8	0,1731
21/05/2010	-	42,6	-	-	-	0,1812
26/05/2010	5,83	49,3	5,79	55,9	63	0,2096
03/06/2010	5,33	43	5,7	52,3	70	0,1829
10/06/2010	5,59	41,8	5,6	50,6	126	0,1777
24/06/2010	5,72	40,8	5,8	49,7	98,5	0,1735
01/07/2010	6	48,2	5,4	55,3	106,5	0,2050
15/07/2010	5,93	46	5,07	-	-	0,1956

Table 8A – Chemical analyses on the outflow from the reactor

Date	Alkalinity (mmol/l) (0,45µm)	NH4-N (mg/l) (0,45µm)	NO2-N (mg/l) (0,45µm)	NO3-N (mg/l) (0,45µm)	TOT-P (mg/l) unfiltr.	COD (mg/l) (0,45µm)	COD (mg/l) unfiltr	Ninorg removal ($gN/m^2/d$)
21/05/2010	3,35	14,5	0,32	1,71	5,32	42,4	33,6	0,1068
27/05/2010	2,82	7,26	0,304	1,92	5,35	54,2	-	0,1693
04/06/2010	1,46	0,042	0,016	21,7	5,1	47,3	49	0,0903
11/06/2010	1,86	0,811	0,034	7,01	4,78	48,2	120	0,1443
25/06/2010	1,6	0,552	0	10,4	7,69	48,9	125	0,1269
02/07/2010	0,7	0	0	5,6	5	45	98	0,1812
16/07/2010	1,2	1,05	0	8,4	4,43	-	-	0,1554

Table 9A – Physical parameters

Date	Flow	HRT	Influent		Inside reactor				Effluent		Remarks
	(ml/min)	(days)	pH	Cond (mS/cm)	pH	Cond (mS/cm)	DO (mg/l)	Temp (°C)	pH	Cond (mS/cm)	
12/05/2010	4,56	1,0264	7,52	0,700	7,33	0,700	0,75	26	7,68	0,690	Increase pump
13/05/2010	4,70	0,9959	7,5	0,690	7,32	0,680	0,98	26,4	7,53	0,690	
18/05/2010	3,83	1,22	7,76	0,720	7,26	0,630	1,19	25,4	7,46	0,626	Flow was too low. Some rings were floating.
			7,17	0,771	6,93	0,559					
21/05/2010	4,68	1,00	7,43	0,802	7,12	0,637	0,66	27,2	7,61	0,626	Calibrated pH meter. Flow OK.
			7,61	0,800	7,25						
24/05/2010			7,5	0,760	7,20	0,590	0,55	25,2	2,27	0,620	
26/05/2010			7,72	0,800	7,17	0,570	0,35	24,7	7,37	0,600	
27/05/2010				0,803	7,10	0,602	0,49	24,4	7,71	-	
31/05/2010	4,83	0,9684	8,13	0,803	7,23	0,567	1,05	25,5	7,48	0,591	Calibrated pH meter. Decreased inflow rate.
							0,56				
01/06/2010			8,13	0,795	7,36	0,572	0,59	25,9	-	-	Inflow stopped for 2h. Refilled tank. Changed stirrer.
02/06/2010	4,93	0,9488	8,13	0,796	7,28	0,575	0,75	24,1	7,70	0,571	Calibrated pH meter. Decreased inflow rate
04/06/2010	4,6	1,0175	8,28	0,792	7,11	0,581	1,06	26,8	7,39	0,587	Increased inflow rate. Calibrated pH-meter.
			8,32								
07/06/2010			8	0,780	7,19	0,581	0,42	26,7	7,66	0,570	Decreased inflow (because no inflow from the line 3).
			8,12				0,48				
08/06/2010	4.68	1,0001	7,47	0,765	7,20	0,587	3,20	26,9	-	-	Decrease T. Now inflow again (h 19). New inflow from line3.
							0,57				
10/06/2010	5,03	0,9299	7,85	0,764	7,29	0,577	0,59	25,6	7,77	0,582	Decreased pump
12/06/2010			7,77	0,770	7,53	0,600	0,75	25,4	7,74	0,590	
15/06/2010	4,68	1,0001	8,03	0,774	7,40	0,555	-	25,2	-	-	Good mixing. DO inflow 0,95.
			7,86	0,777							
20/06/2010	4,17	1,1233	-		7,40	0,530	4,00	29,2	-	-	Inflow was finished. Filter was turned upside-down. Good mixing. DO too high! Added 22 rings and took 14 out. Added 1-1,5 l new inflow in the reactor. Inflow too low. Increased inflow rate.
			8,03	0,774			0,70				
21/06/2010	-	-	7,96	0,783	7,10	0,498	0,55	29,3	7,09	0,500	
23/06/2010	-	-	8,03	0,792	7,53	0,559	0,98	29,4	7,43	0,563	

24/06/2010	-	-	7,76	0,753	7,21	0,526	0,67	29,3	7,20	0,557	
25/06/2010	4,93	0,9488	- 8,17	0,770	7,36	0,535	0,87	29,1	7,89	0,542	Decreased T. Decreased inflow rate. New inflow very clear.
27/06/2010	5,27	0,8887	8,11 -	0,767	6,67	0,602	3,40 -	28,5	7,19	0,596	Decreased T. DO in the inflow tank about 1,90 mg/l. Changed membrane for DO-meter. Decreased pump.
29/06/2010	4,64	1,0087	7,94	0,773	7,40	0,593	1,01	28,9	7,35	0,600	Decrease pump. HRT is after having set the pump.
01/07/2010	-	-	7,85	0,781	7,37	0,557	0,57	28,4	7,4	0,560	
02/07/2010	-	-	7,65	0,754	7,42	0,493	0,79	28,1	7,45	0,500	Stopped heater.
13/07/2010	4,62	1,0131	7,2	0,756	6,83	0,335	1,21	26,5	6,9	0,350	Increase pump. HRT is after having set the pump. No inflow since a couple of days.
15/07/2010	-	-	7,86	0,779	7,31	0,547	0,8	25,8	7,34	0,600	
16/07/2010	-	-	7,79	0,776	7,38	0,567	0,7	25,7	7,4	0,598	Decrease inflow rate

Table 10A – TSS & VSS as biofilm on the carriers (filtered 1.6 µm)

Date	Biocarriers	Empty plate + filter (g)	After 105 °C (g)	After 550 °C (g)	**TSS (mg/ring)**	**VSS (mg/ring)**	Ash (%)
21/05/2010	4	0,8981	0,9434	0,9021	11,33	10,33	8,83%
01/06/2010	4	1,6760	1,7182	1,6850	10,55	8,30	21,33%
25/06/2010	4	1,7048	1,7492	1,7076	11,10	10,40	6,31%
02/07/2010	4	1,7013	1,7352	1,7049	8,48	7,58	10,62%
16/07/2010	4	1,7019	1,7443	1,7059	10,60	9,60	9,43%

Table 11A – TSS & VSS in the activated sludge (filtered 1.6 µm)

Date	Sample Volume (ml)	Empty plate + filter (g)	After 105 C (g)	After 550 C (g)	**TSS (mg/l)**	**VSS (mg/l)**	Ash (%)
01/06/2010	80	1,7038	1,7071	1,7042	41,25	36,25	12,12%
25/06/2010	100	1,6698	1,6817	1,6714	119,00	103,00	13,45%
02/07/2010	50	1,6650	1,6718	1,6662	136,00	112,00	17,65%
16/07/2010	50	1,6657	1,6729	1,6673	144,00	112,00	22,22%

Table 12A – SAA results

Date	SAA (35°C) ($gN/m^2/d$)
12/05/2010	5,726
19/05/2010	4,058
28/05/2010	3,815
23/06/2010	4,015
01/07/2010	3,687
15/07/2010	3,420

SAA tests

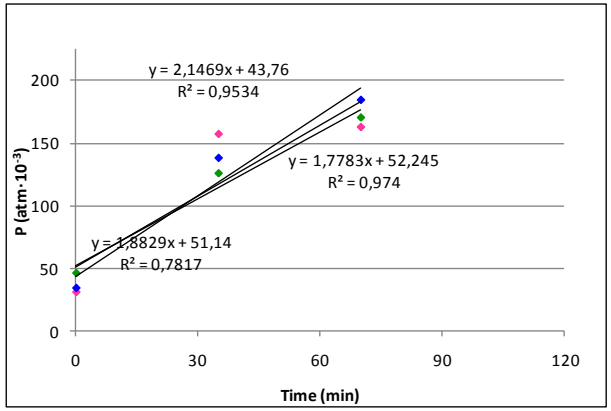

Fig. 12A – 12 May 2010 (35°C)

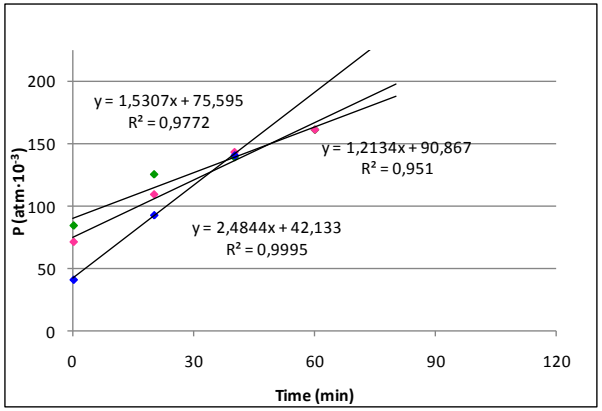

Fig. 13A – 19 May 2010 (35°C)

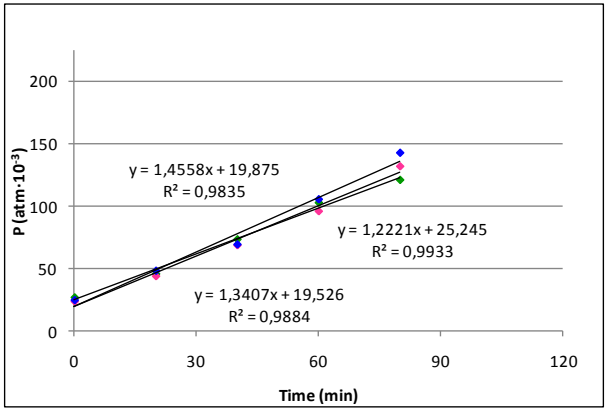

Fig. 14A – 28 May 2010 (35°C)

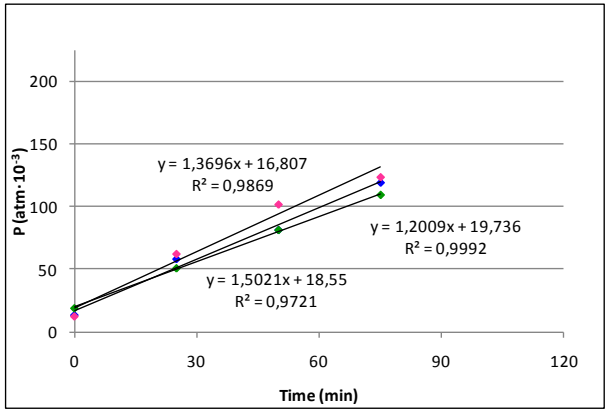

Fig. 15A – 23 June 2010 (35°C)

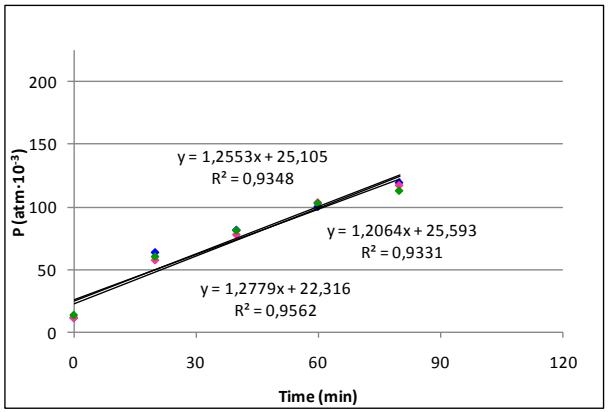

Fig. 16A – 1 July (35°C)

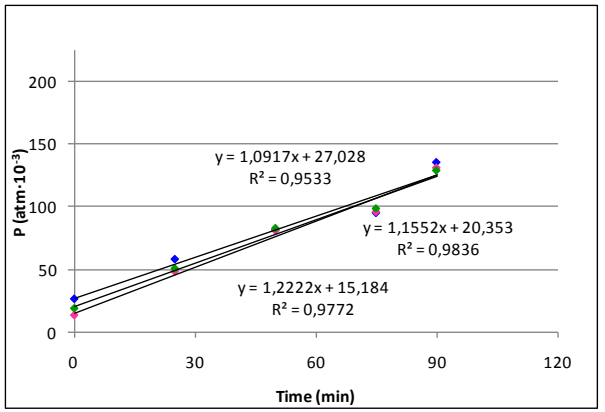

Fig. 17A – 15 July 2010 (35°C)

APPENDIX IV –DATA FROM PILOT PLANT-SCALE REACTOR

Table 13A – TSS & VSS in the influent reject water (filtered 1.6 µm)

Date	Sample Volume (ml)	Al plate + filter (g)	After 105°C (g)	After 550°C (g)	**TSS (mg/l)**	**VSS (mg/l)**	Ash (%)
01/06/2010	30	1,7450	1,7591	1,7451	470,0	466,7	0,71%
27/06/2010	50	1,6663	1,6875	1,6671	424,0	408,0	3,77%
16/07/2010	35	1,6672	1,6856	1,6684	525,7	491,4	6,52%
22/07/2010	25	1,6636	1,6687	1,6637	204,0	200,0	1,96%
31/07/2010	25	1,6827	1,6946	1,6838	476,0	432,0	9,24%
06/08/2010	17	1,6864	1,7222	1,6968	2105,9	1494,1	29,05%
12/08/2010	22	1,6862	1,6901	1,6867	178,9	155,0	13,32%
20/08/2010	40	1,6862	1,6912	1,6866	125,9	115,3	8,42%
28/08/2010	35	1,6848	1,6919	1,6852	203,9	191,8	5,93%
10/09/2010	45	1,6851	1,6914	1,6858	140,8	124,7	11,42%
24/09/2010	20	1,6857	1,6900	1,6858	216,8	210,6	2,84%

Table 14A – TSS & VSS in the influent reject water (filtered 0.45 µm)

Date	Sample Volume (ml)	Al plate + filter (g)	After 105°C (g)	After 550 °C (g)	**TSS (mg/l)**	**VSS (mg/l)**	Ash (%)
31/07/2010	10	1,6603	1,6664	-	610,0	-	-
06/08/2010	8	1,6604	1,6792	-	2350,0	-	-
12/08/2010	10	1,6654	1,6674	-	195,2	-	-
20/08/2010	9	1,6672	1,6700	-	305,9	-	-
28/08/2010	9	1,6617	1,6655	-	416,7	-	-
10/09/2010	7	1,6657	1,6686	-	407,5	-	-

Table 15A – TSS & VSS as biofilm on the carriers (filtered 1.6 µm)

Date	Biocarriers	Empty plate + filter (g)	After 105 °C (g)	After 550 °C (g)	**TSS (mg/ring)**	**VSS (mg/ring)**	Ash (%)
01/06/2010	4	1,7335	1,8012	1,734	16,9	16,8	0,74%
27/06/2010	4	1,6623	1,7375	1,6746	18,8	15,7	16,36%
16/07/2010	4	1,662	1,7324	1,6732	17,6	14,8	15,91%
22/07/2010	4	1,6627	1,7343	1,6739	17,9	15,1	15,64%
31/07/2010	4	1,7557	1,8353	1,7672	19,9	17,0	14,45%
06/08/2010	4	1,7337	1,8141	1,7419	20,1	18,0	10,16%
12/08/2010	4	1,7242	1,8152	1,7378	22,7	19,3	14,91%
20/08/2010	4	1,7307	1,8162	1,7454	21,4	17,7	17,16%
28/08/2010	4	1,7191	1,8125	1,7329	23,3	19,9	14,74%
10/09/2010	4	1,7263	1,8208	1,7371	23,6	20,9	11,39%
24/09/2010	4	1,7199	1,8129	1,7350	23,2	19,5	16,20%

Table 16A – TSS & VSS in the activated sludge (filtered 1.6 µm)

Date	Sample Volume (ml)	Empty plate + filter (g)	After 105 C (g)	After 550 C (g)	**TSS (mg/l)**	**VSS (mg/l)**	Ash (%)
02/06/2010	35	1,7382	1,7484	1,7437	291,4	134,3	53,92%
27/06/2010	50	1,6783	1,6938	1,6807	310,0	262,0	15,48%
16/07/2010	20	1,6800	1,6891	1,6815	456,7	380,6	16,68%
22/07/2010	25	1,6796	1,6870	1,6810	297,4	240,5	19,14%
31/07/2010	25	1,6788	1,6875	1,6805	349,4	280,6	19,69%
06/08/2010	25	1,6818	1,6895	1,6830	309,4	260,5	15,79%
12/08/2010	27	1,6811	1,6892	1,6823	301,3	256,1	15,01%
20/08/2010	25	1,7167	1,7237	1,7177	281,6	239,7	14,87%
28/08/2010	25	1,7163	1,7257	1,7176	377,6	323,7	14,26%
10/09/2010	26	1,6631	1,6726	1,6643	366,6	320,2	12,67%
24/09/2010	25	1,7031	1,7118	1,7044	349,5	296,0	15,30%
27/09/2010	averages on 5 measures on activated sludge for batch test (OUR, NUR) carried out on 27th Sept.				320,2	287,3	10,26%

Table 17A – TSS & VSS in the activated sludge (filtered 0.45 µm)

Date	Sample Volume (ml)	Empty plate + filter (g)	After 105 C (g)	After 550 C (g)	**TSS (mg/l)**	**VSS (mg/l)**	Ash (%)
27/06/2010	20	1,6579	1,6622	-	217,4	-	
31/07/2010	15	1,7004	1,7058	-	360,0	-	
06/08/2010	15	1,6495	1,6546	-	342,9	-	
12/08/2010	15	1,6500	1,6550	-	336,2	-	
20/08/2010	15	1,6536	1,6577	-	276,3	-	
28/08/2010	12	1,6522	1,6573	-	428,7	-	
10/09/2010	12	1,6654	1,6699	-	371,1	-	
24/09/2010	13	1,6587	1,6618	-	242,1		

Table 18A – Chemical analyses on the reject water in inflow to Pilot Plant scale reactor

Date	Flow (ml/min)	Alkalinity (mmol/l) (0.45µm)	COD (mg/l) (0.45µm)	COD (mg/l) (1.6 µm)	COD (mg/l) unfilt	TOT-P (mg/l) (0.45µm)	TOT-P (mg/l) unfilt	TN (mg/L) (0.45µm)	TN (mg/L) unfilt	NH_4^+-N (mg/l) (0.45µm)	NO_2^--N (mg/l) (0.45µm)	NO_3^--N (mg/l) (0.45µm)	N_{inorg}	N load ($gN/m^2/d$)
27/05/2010	70	74,9	836	1011	1452		-	-		1050	0	0	1050,0	2,6460
02/06/2010	70	63,4	791	872	1359		-	-		1060	0	0	1060,0	2,6712
10/06/2010	70	70,6	567	-	1103		8,38	-		1010	-	-	1010,0	2,5452
16/06/2010	100	75,0	667	-	1286		-	970		965	0,084	1,62	966,7	3,4802
23/06/2010	100	77,0	650	-	1110		8,35	1094		960	0,084	1,62	961,7	3,4622
28/06/2010	100	88,8	535	-	1015		-	-		940	0,084	1,62	941,7	3,3901
13/07/2010	66	68,0	668	-	1074		-	913		874	0,084	1,62	875,7	3,3417
21/07/2010	100	71,1	634	-	1429		-	-		945	0,084	1,62	946,8	3,4084
29/07/2010	106,7	70,1	546	-	1313	1,63	11,2	916	1030	884	0,084	1,62	885,7	3,4011
04/08/2010	106,7	67,9	503	-	1007	-	-	913	-	875,5	0,084	1,63	877,2	3,3685
06/08/2010	103,0	73,2	496	-	1840	-	-	947	1155	917	0,032	1,84	918,9	3,4073
10/08/2010	105,3	71,6				-	-	863	-	892	0,032	1,84	893,9	3,3896
18/08/2010	107,5	70,1	528	-	2146	-	-	947	-	880	0,032	1,84	881,9	3,4128
24/08/2010	93,3	81,8				-	-	-	-	1010	0,113	2,37	1012,5	3,4020
26/08/2010	94,8	80,3	501	-	967	-	-	1100	1243	1003,6	0,113	2,37	1006,1	3,4348
01/09/2010	94,0		632	-	1012	-	-	-	-	1005,0	0,113	2,37	1007,5	3,4093
07/09/2010	96,0	73,7	814	-	966	-	-	-	-	1006,0	0,113	2,37	1008,5	3,4855
10/09/2010	91,7							1082,4		-	-	-		
14/09/2010	99,8	73,3	507	-	946	-	-	976,8	-	948,2	0,113	2,37	950,7	3,4168
23/09/2010	100,0									1095	0,011	2,15	1097,2	3,9498
24/09/2010	89,0	84	505		1020			1130		1085	0,011	2,15	1087,2	3,4833

Table 19A – Chemical analyses on the outflow from Pilot Plant scale reactor

Date	Flow (ml/min)	Alkalinity (mmol/l) (0.45µm)	COD (mg/l) (0.45µm)	COD (mg/l) (1.6 µm)	COD (mg/l) unfilt	TOT-P (mg/l) (0.45µm)	TOT-P (mg/l) unfilt	TN (mg/L) (0.45µm)	TN (mg/L) unfilt	NH_4^+-N (mg/l) (0.45µm)	NO_2^--N (mg/l) (0.45µm)	NO_3^--N (mg/l) (0.45µm)	N_{inorg}	N removal ($gN/m^2/d$)
01/06/2010	70	8,62	419	509	616	-	-	-	-	59,5	11,50	81,3	152,3	2,2622
04/06/2010	70	-		-	-	-	-	-	-	30,9	8,54	144,0	183,4	2,2089
08/06/2010	70	7,72		-	-	-	-	-	-	44,8	7,58	101,0	153,4	2,2847
11/06/2010	70	-		-	-	-	-	-	-	44,5	5,42	106,2	156,1	2,1518
15/06/2010	100	7,65	298	-	778	-	-	-	-	78,0	7,78	128,0	213,8	2,8664
18/06/2010	100	-		-	-	-	-	-	-	81,0	6,89	125,0	212,9	2,7137
22/06/2010	100	7,32	254	-	760	-		-		52,5	5,32	135,0	192,8	2,7860
25/06/2010	100	-		-	-	-	8,78	-	630,0	46,7	6,38	158,0	211,1	2,7023
30/06/2010	100	4,32	333	-	514	-	-	-	-	60,5	7,30	144,0	211,8	2,6277
05/07/2010	100	-		-	-	-	-	-	-	50,2	3,80	140,0	194,0	2,6917
15/07/2010	106	2,00	383	-	469	-	-	162,0	-	75,5	2,76	87,6	165,9	2,5554
16/07/2010	106	-		-	-	-	-	-	-	64,3	5,56	91,2	161,1	2,5727
20/07/2010	106	6,32	364	-	609	-	-	164,4	-	73,4	7,70	106,6	187,7	2,6254
23/07/2010	100	-		-	-	-	-	-	-	79,5	8,88	125,8	214,2	2,7956
27/07/2010	100	5,65	321	-	670	0,735	5,57	183,4	202,4	48,3	7,59	93,5	149,4	2,8706
31/07/2010	113,3	-		-	-	-	-	-	-	94,2	8,58	66,5	169,3	2,7511
02/08/2010	113,3	9,75	313	-	595	-	-	233,4	390,0	90,9	8,28	73,8	173,0	2,9080
06/08/2010	103,0	-	326	-	631	-	-	-	-	58,0	8,04	68,0	134,0	2,8538
12/08/2010	105,0	8,52	384	-	603	-	-	195,5	232,5	97,2	8,25	91,5	197,0	2,6428
16/08/2010	105,0	-		-	-	-	-	-	-	89,1	8,52	75,0	172,6	2,7264
20/08/2010	107,5	9,06	333	-	641	-	-	186,9	-	90,9	8,16	64,8	163,9	2,7787
28/08/2010	94,2	3,98	363	-	785	-	-	206,0	226,8	49,2	7,96	106,8	164,0	2,8548
30/08/2010	94,2	-		-	-	-	-	-	-	73,2	7,96	90,8	172,0	2,8277
08/09/2010	96,0	10,95	409	-	580	-	-	-	-	84,8	6,81	88,1	179,7	2,8644
10/09/2010	91,7	8,43			-			193,5		84,6	7,23	77,7	169,5	2,7687

14/09/2010	99,8													
16/09/2010	99,8	7,23	398	-	645	-	-	207,0		79,2	5,23	72,1	156,5	2,8542
23/09/2010	100,0									66,0	9,64	88,8	164,4	3,3578
26/09/2010	88,3	4,81	377	-	749	-	-	194,4		58,5	7,80	102,9	169,2	2,9169

Table 20A – Physical paramenters – daily average from on-line measurements on Pilot Plant scale reactor

Date	Flow (ml/min)	Flow (l/day)	HRT (days)	Inflow			Inside Reactor					Remarks
				pH	ORP (mV)	Cond (mS/cm)	pH	DO (mg/L)	ORP (mV)	Cond (mS/cm)	T (°C)	
27/05/2010	70	100,8	1,65		-500,9	10,005	8,46	2,5	60,8	4,145	25,2	DO set point (2,5 mg/l) controlled manually.
28/05/2010	70	100,8	1,65		-496,1	9,956	8,51	2,5	61,8	4,209	25,2	
29/05/2010	70	100,8	1,65		-532,5	9,900	8,45	2,5	57,9	3,951	25,2	
30/05/2010	70	100,8	1,65		-530,9	9,884	8,35	2,5	44,1	3,575	25,2	
31/05/2010	70	100,8	1,65		-491,4	9,805	8,25	2,5	34,4	3,152	25,2	
01/06/2010	70	100,8	1,65		-462,1	9,609	7,64	2,5	47,9	1,749	25,2	
02/06/2010	70	100,8	1,65		-400,9	9,479	7,41	2,5	50,3	1,760	25,2	
03/06/2010	70	100,8	1,65		-507,2	9,468	7,44	2,5	39,6	1,794	25,2	
04/06/2010	70	100,8	1,65		-458,0	9,371	7,18	2,5	55,5	1,801	25,2	Calibration pH-meter, redox-meter (INflow and Reactor), conductivity meter (IN and R). DO set point (2,5 mg/l) controlled manually.
05/06/2010	70	100,8	1,65					2,5			25,2	DO set point (2,5 mg/l) controlled manually.
06/06/2010	70	100,8	1,65					2,5			25,2	
07/06/2010	70	100,8	1,65		-338,0	9,053	6,95	2,35	112,9	1,992	25,01	DO controlled automatically. DO set point 2,4 mg/l. Installed on-line measurement for T.
08/06/2010	70	100,8	1,65		-496,0	8,723	6,98	2,37	104,2	1,974	24,99	
09/06/2010	70	100,8	1,65		-546,7	8,235	6,96	2,39	98,6	1,935	25,00	
10/06/2010	70	100,8	1,65	8,37								New reject water. Calibration pH-meter, redox-meter (IN and R), conductivity meter (IN and R).
11/06/2010	70	100,8	1,65									
12/06/2010	70	100,8	1,65									

13/06/2010	100	144	1,15									DO set point 2,0 mg/l
14/06/2010	100	144	1,15									DO set point 1,4 mg/l
15/06/2010	100	144	1,15		-223,0	8,797	7,34	1,39	75,4	1,945	25,05	
16/06/2010	100	144	1,15		-224,3	8,755	7,23	1,93	127,0	1,840	25,01	DO set point 2.3 mg/l
17/06/2010	100	144	1,15		-299,4	8,738	6,93	2,55	159,6	1,833	24,97	
18/06/2010	100	144	1,15		-376,4	8,719	6,81	2,55	167,8	1,928	25,00	
19/06/2010	100	144	1,15		-497,8	8,691	6,77	2,55	152,8	1,997	24,98	
20/06/2010	100	144	1,15		-515,2	8,694	6,76	2,48	139,6	2,045	24,99	
21/06/2010	100	144	1,15		-302,1	8,387	7,04	1,53	53,5	2,081	24,98	Calibrationd DO-meter. DO set point 1,5 mg/l
22/06/2010	100	144	1,15		-409,6	8,500	7,35	1,62	60,5	2,079	24,99	DO set point 2,0 mg/l. Calibration Redox (IN), Cleaning conductivity meter (IN and R), pH-meter, redox-meter (R).
23/06/2010	100	144	1,15		-462,3	8,542	7,00	2,02	93,8	1,855	24,97	
24/06/2010	100	144	1,15		-532,7	8,522	6,97	2,00	99,2	1,876	24,97	
25/06/2010	100	144	1,15		-524,2	8,506	7,00	2,02	97,8	1,894	24,95	
26/06/2010	100	144	1,15		-527,1	8,493	7,02	2,01	98,7	1,894	24,98	
27/06/2010	100	144	1,15		-445,9	8,435	7,01	2,01	117,8	1,872	24,96	
28/06/2010	100	144	1,15		-495,5	8,362	7,11	2,01	106,8	1,860	24,93	Calibration pH-meter, DO-meter, redox-meter (IN and R), conductivity-meter (IN and R). Increased pH probably due to anoxic period (25 min) during DO calibration.
29/06/2010	100	144	1,15		-528,7	8,383	7,32	1,98	97,0	1,846	24,92	
30/06/2010	100	144	1,15		-522,5	8,360	7,32	1,98	97,9	1,810	24,91	
01/07/2010	100	144	1,15		-531,3	8,251	7,30	2,00	94,4	1,779	24,96	
02/07/2010	100	144	1,15		-517,1	8,143	7,16	1,60	94,9	1,699	24,93	Add water in reject water (until 5/7). Decreased DO set point to 1 mg/l
03/07/2010	100	144	1,15		-526,1	8,113	6,92	0,96	118,9	1,540	24,92	
04/07/2010	100	144	1,15		-393,2	7,543	6,96	0,95	134,8	1,507	24,93	
05/07/2010	100	144	1,15		-477,6	6,530	7,23	0,92	150,1	1,511	24,93	
06/07/2010	100	144	1,15		-355,2	7,206	7,21	1,09	98,2	1,512	24,94	New reject water. DO set back to 2mg/l. Calibration conductivity.-meter (R).
07/07/2010	106	152,6	1,09		-515,1	8,552	6,95	1,99	129,2	1,782	24,93	
08/07/2010	106	152,6	1,09		-544,5	8,554	6,60	2,00	135,1	1,823	24,92	

09/07/2010	106	152,6	1,09		-548,2	8,728	6,47	1,88	94,6	1,888	24,92	DO set point 1.8 mg/l.
10/07/2010	106	152,6	1,09		-555,1	9,167	6,54	1,77	87,6	1,869	24,87	
11/07/2010	106	152,6	1,09		-559,6	9,718	6,75	1,75	67,2	1,838	24,89	
12/07/2010	106	152,6	1,09		-558,8	9,924	7,04	1,73	52,8	1,822	24,84	
13/07/2010	66	95,04	1,75			9,596	6,48	1,75	92,0	1,712	24,89	Inflow pipe was block since 8.30 until 17.30. pH dropped down to 5,22 and redox increased up to 215mV.
14/07/2010	106	152,6	1,09		-491,9	8,759	6,91	1,74	59,7	1,713	24,86	
15/07/2010	106	152,6	1,09		-497,9	8,834	6,83	1,77	60,3	1,650	24,85	
16/07/2010					-465,6	8,747	7,17	1,78	36,2	1,762	24,89	Calibration pH-meter, redox-meter (R), conductivity-meter (R). Cleaning conductivity meter (IN), Redox-meter (IN) and DO-meter.
17/07/2010					-485,9	8,690	7,73	1,77	10,0	2,105	24,90	
18/07/2010					-438,0	8,646	7,94	1,77	0,9	2,241	24,91	
19/07/2010	106	152,6	1,09		-452,8	8,589	7,89	2,21	17,1	2,051	24,90	DO setpoint 2,5 mg/L
20/07/2010	100	144	1,15		-477,0	8,743	7,70	2,49	38,3	1,863	24,90	New reject water.
21/07/2010	100	144	1,15		-550,2	8,854	7,53	2,47	49,7	1,766	24,90	
22/07/2010	100	144	1,15		-542,6	8,993	7,45	2,49	52,6	1,738	24,91	
23/07/2010	100	144	1,15		-532,6	8,945	7,50	2,50	35,8	1,732	24,96	Calibration pH-meter, redox-meter (IN and R). Cleaning conductivity-meter (IN and R).
24/07/2010	100,0	144	1,15		-537,9	9,032	7,48	2,50	37,8	1,681	24,98	
25/07/2010	100,0	144	1,15		-504,2	9,036	7,55	2,51	36,6	1,666	24,97	Calibration redox-meter (IN).
26/07/2010	100,0	144	1,15		-471,5	9,055	7,62	2,51	36,1	1,691	25,00	
27/07/2010	100,0	144	1,15		-398,8	8,971	7,66	2,52	37,1	1,708	24,92	Calibration pH-meter, DO-meter.
28/07/2010	100,0	144	1,15		-510,9	8,787	7,66	2,50	22,3	1,753	24,97	
29/07/2010	106,7	153,6	1,08	8,38	-414,7	8,683	7,67	2,51	23,7	1,733	24,96	Calibration conductivity-meter (R), redox-meter (IN and R). Cleaning pH-meter.
30/07/2010	106,7	153,6	1,08		-356,3	8,454	7,76	2,53	14,1	1,856	24,77	
31/07/2010	113,3	163,2	1,02		-374,0	8,332	7,80		-2,1	1,976		Single data because automatic data capture was off.
01/08/2010	113,3	163,2	1,02									
02/08/2010	113,3	163,2	1,02	8,41	-365,5	8,882	7,83	2,54	-1,3	2,233	24,78	Calibration pH-meter, DO-meter, conductivity-meter (IN). Cleaning redox-meter (IN and R).
03/08/2010	113,3	163,2	1,02		-375,3	8,934	7,84	2,54	1,2	2,287	24,99	

04/08/2010	106,7	153,6	1,08		-401,7	8,960	7,82	2,49	5,9	2,328	24,93	
05/08/2010	106,7	153,6	1,08		-421,5	8,619	7,74	2,52	11,3	2,152	24,86	
06/08/2010	103,0	148,3	1,12	8,46	-387,0	8,775	7,64	2,51	24,9	1,901	24,85	New reject water. Cleaning pH-meter, redox-meter (IN and R), conductivity-meter (IN and R) and DO-meter.
07/08/2010	103,0	148,3	1,12		-331,8	9,443	7,69	2,54	22,1	2,000	24,97	
08/08/2010	103,0	148,3	1,12				7,24	2,57	133,0	1,416	24,68	No inflow rate since 00.20 because the pipe in the tank was not under the reject water level. T started to decrease since 17.41. pH decresed to down to 7,20.
09/08/2010	103,2	148,6	1,12			9,677	7,45	2,53	100,4	1,716	23,05	Progressive re-establishment of prevoius conditions from 11.50. T dropped down to 21,4°C. Calibration pH-meter, DO-meter, redox-meter (IN and R).
10/08/2010	105,3	151,7	1,09		-467,0	9,635	7,71	2,53	6,3	2,155	24,98	
11/08/2010	105,3	151,7	1,09		-492,4	9,631	7,65	2,53	3,7	2,087	24,95	
12/08/2010	105,0	151,2	1,10		-540,5	9,594	7,68	2,51	1,7	2,153	24,93	
13/08/2010	105,0	151,2	1,10		23,2	9,626	7,72	2,51	5,2	2,314	24,91	
14/08/2010	105,0	151,2	1,10		-536,5	9,638	7,71	2,50	1,1	2,318	24,92	
15/08/2010	105,0	151,2	1,10		-546,0	9,612	7,71	2,49	0,2	2,320	24,91	Calibration DO-meter. Cleaning redox-meter (IN and R), pH-meter, conductivity-meter (IN and R).
16/08/2010	105,0	151,2	1,10	8,49	-561,1	9,568	7,69	2,49	1,6	2,245	24,95	
17/08/2010	105,0	151,2	1,10		-525,4	9,465	7,83	2,48	-9,5	2,336	24,92	Calibration pH-meter, DO-meter, redox-meter (IN and R), Problem with calibration DO-meter. pH increased up to 7,85 due to two anoxic period (20 min and 30 min) during DO calibration.
18/08/2010	107,5	154,8	1,07	8,47	-550,2	9,427	7,80	2,50	-2,3	2,248	24,95	Calibration (only air) conductivity-meter (IN and R). Problem to calibrate with standard solution.
19/08/2010	107,5	154,8	1,07		-540,3	9,396	7,79	2,51	-2,6	2,262	24,96	
20/08/2010	107,5	154,8	1,07	8,49	-483,1	9,302	7,77	2,51	4,8	2,193	24,98	
21/08/2010	107,5	154,8	1,07		-505,6	9,219	7,80	2,51	-2,5	2,215	24,97	
22/08/2010	107,5	154,8	1,07		-545,4	9,134	7,76	2,49	-3,9	2,138	24,96	Since 0.00 inflow rate probably started to decrease because the reject water level in the tank was very low and reject water at the bottom had a higher content of suspended solids.

23/08/2010	0,0	0,0	-				7,32	1,61	68,6	1,409	24,89	Reject water was finished. New reject water was not delivered because problems with the truck. pH dropped down to 7,29 and conductivity to 1,395mS/cm. NH4-N was probably depleted because pH did not decreased anymore (ammonia-elbow). Average on data from 5pm. DO set point decreased to 1,6mg/l.
24/08/2010	93,3	134,4	1,24			10,347	7,51	2,04	61,7	1,901	25,00	New reject water since 10 am. Calibration DO-meter, pH-meter, redox-meter (IN and R). Conductivity-meter (IN and R) (only air). Spilled some liquid from inside reactor. DO set point 2,4 mg/l.
25/08/2010	93,3	134,4	1,24		-468,6	10,515	7,31	2,35	41,4	1,861	24,99	Decrease of pH from 7,6 to 7,0.
26/08/2010	94,8	136,6	1,22	8,43	-479,2	10,432	7,14	2,28	37,9	1,794	25,01	DO set point decreased to 2,1 mg/l and before leaving to 2,0 mg/l.
27/08/2010	94,8	136,6	1,22		-526,4	10,407	7,18	2,05	27,1	1,827	25,03	
28/08/2010	94,2	135,6	1,22	8,48	-533,0	10,337	7,27	2,04	21,2	1,870	25,03	DO set point 2,1 mg/l. Increased pump rate; flow was 92 ml/min.
29/08/2010	94,2	135,6	1,22		-501,3	10,269	7,40	2,13	8,4	1,958	25,05	
30/08/2010	94,2	135,6	1,22		-519,7	10,201	7,51	2,26	0,9	2,030	25,02	DO set point 2,3 mg/l
31/08/2010	94,2	135,6	1,22		-535,4	10,168	7,47	2,31	3,9	1,966	25,04	
01/09/2010	94,0	135,4	1,23		-555,6	10,111	7,44	2,29	11,2	1,926	25,03	
02/09/2010	94,0	135,4	1,23		-425,3	10,075	7,40	2,29	10,4	1,962	25,06	Calibration DO-meter, pH-meter. Problem with DOmeter after calibration. Between 14.41 and 16.37 no aeration and pH increased from 7,35 to 7,50.
03/09/2010	94,0	135,4	1,23		-373,7	10,066	7,62	2,19	9,1	2,182	25,12	Probably problems with DO-meter and aeration stopped between 9.52 and 11.27 and pH increased up to 9,65. Calibration pH-meter. Increased mixing 27 ->50 rpm
04/09/2010	94,0	135,4	1,23		-475,9	10,055	7,40	2,31	11,4	2,034	25,06	
05/09/2010	94,0	135,4	1,23		-470,4	10,017	7,35	2,31	15,9	2,002	25,05	
06/09/2010	94,0	135,4	1,23		-430,8	9,978	7,40	2,25	16,8	2,089	25,02	Problem with DO-meter No aeration between 19.44 and 20.23.pH increased from 7,34 to 7,51. Calibration redox-meter (IN and R). Cleaning pH-meter,, conductivity-meter (IN and R) and DO-meter.
07/09/2010	96,0	138,2	1,20		-515,0	9,958	7,58	2,25	-11,8	2,364	25,03	Problem with DO-meter. It seems there was no aeration between 2.29 and 3.39. pH increased from 7,52 to 7,61.
08/09/2010	96,0	138,2	1,20		-521,5	9,935	7,49	2,30	-9,7	2,183	25,04	
09/09/2010	96,0	138,2	1,20		-567,7	9,910	7,47	2,30	-17,5	2,162	25,04	Calibration pH-meter, redox-meter (IN and R).
10/09/2010	91,7	132	1,26	8,39		9,861	7,49	2,3	17,8	2,141	25,05	Average on three data during the day because automatic data capture was off.
11/09/2010	91,7	132	1,26					2,3			25,05	
12/09/2010	91,7	132	1,26					2,3			25,05	

13/09/2010	93,3	134,4	1,24		-362,9	9,689	7,57	2,3	12,2	2,297	25,06	
14/09/2010	99,8	143,8	1,15	8,40	-407,2	9,663	7,47	2,33	7,4	2,009	25,06	
15/09/2010	99,8	143,8	1,15		-503,9	9,646	7,52	2,35	1,7	2,148	25,03	
16/09/2010	99,8	143,8	1,15		-516,6	9,582	7,53	2,39	1,8	2,182	25,04	
17/09/2010	50,0	72	2,31		-420,6	9,466	7,48	2,11	20,0	2,045	25,04	Decreased inflow rate (half) and DO set point to 1,2 mg/l.
18/09/2010	50,0	72	2,31		-500,6	9,368	7,43	1,17	27,1	1,875	25,05	
19/09/2010	50,0	72	2,31		-533,8	9,326	7,46	1,18	46,6	1,878	25,09	
20/09/2010	50,0	72	2,31		-321,4	9,269	7,46	1,23	44,9	1,837	25,07	Calibration pH-meter, redox-meter (IN and R). DO set point to 1,3 mg/l.
21/09/2010	50,0	72			-404,8	9,917	7,50	1,64	22,7	1,774	25,08	New reject water. DO set point 2,3mg/l
22/09/2010	100,0	144			-524,3	10,337	7,55	2,31	21,1	2,026	25,09	
23/09/2010	100,0	158,4	1,05		-434,2	10,386	7,47	2,29	31,0	2,067	25,08	
24/09/2010	89,0	128,2	1,30	8,37	-463,7	10,537	7,51	2,28	24,9	2,231	25,05	DO set point 2,2mg/l
25/09/2010	89,0	128,2	1,30		-551,2	10,631	7,37	2,19	26,1	2,008	25,05	
26/09/2010	88,3	127,1	1,31	8,50	-535,3	10,572	7,32	2,22	29,4	1,995	25,06	
27/09/2010	88,3	127,1	1,31		-528,3	10,510	7,30	2,22	29,9	1,999	25,06	
28/09/2010	88,3	127,1	1,31		-489,3	9,844	7,36	2,17	21,9	1,987		

APPENDIX V –BATCH TESTS ON THE PILOT PLANT-SCALE REACTOR

Table 21A – OUR tests on carriers – data and results from Pilot Plant scale reactor (*)

Date	Initial NH4-N (mg/l)	OUR (Nitrobacters + Nitrosomonas + Heterotrophic)		OUR (Nitrosomonas + Heterotrophic)		OUR (Heterotrophic)		OUR (NOB+AOB+HT)	OUR (AOB+HT)	**OUR (HT)**	**OUR (AOB)**	**OUR (NOB)**
		slope [mg O2/l s]	average [mg O2/l s]	slope [mg O2/l s]	average [mg O2/l s]	slope [mg O2/l s]	average [mg O2/l s]	$g\ O_2 / m^2 / d$	$g\ O_2 / m^2 / d$	**$g\ O_2 / m^2 / d$**	**$g\ O_2 / m^2 / d$**	**$g\ O_2 / m^2 / d$**
02/06/2010	104	-0,003121	-0,002967		-0,002599		-0,001109	7,7255	6,9831	**2,9071**	**4,0760**	**0,7423**
		-0,003069										
		-0,002710		-0,002599		-0,000724						
28/06/2010	104	-0,003623	-0,003097	-0,002946	-0,002666	-0,000654	-0,000862	7,3787	6,5941	**2,2596**	**4,3345**	**0,7846**
		-0,002691		-0,002335		-0,000891						
		-0,002976		-0,002716		-0,001041						
15/07/2010	97,6	-0,003142	-0,003058		-0,002563		-0,001248	7,9642	6,6907	**3,2715**	**3,4192**	**1,2735**
		-0,003192		-0,002510		-0,001041						
		-0,002841		-0,002615		-0,001252						
22/07/2010	103	-0,002494	-0,002399	-0,002244	-0,002151	-0,000835	-0,000919	6,2472	5,6150	**2,4082**	**3,2068**	**0,6323**
		-0,002307				-0,000918						
		-0,002396		-0,002057		-0,001003						
04/08/2010	97,2	-0,002656	-0,002411	-0,002235	-0,002280	-0,000696	-0,000910	6,8579	6,4884	**2,3863**	**4,1020**	**0,3695**
		-0,002611		-0,002735		-0,001004						
		-0,001965		-0,001869		-0,001031						
17/08/2010	101,4	-0,002624	-0,002568	-0,002622	-0,002410	-0,000875	-0,000752	6,6865	6,2934	**1,9721**	**4,3213**	**0,3931**
		-0,002663		-0,002459		-0,000867						
		-0,002416		-0,002150		-0,000515						
26/08/2010	104,4	-0,002785	-0,002759	-0,002708	-0,002588	-0,000973	-0,000739	7,1856	6,7573	**1,9381**	**4,8192**	**0,4283**
		-0,002915		-0,002404		-0,000589						
		-0,002578		-0,002652		-0,000656						
02/09/2010	98,7		-0,002712	-0,002376	-0,002394	-0,000609	-0,000519	7,0610	6,2516	**1,3596**	**4,8920**	**0,8094**
		-0,002710		-0,002487		-0,000589						
		-0,002713		-0,002320		-0,000358						
28/09/2010	98,4		-0,002768		-0,002406	-0,000549	-0,000694	6,7177	6,1524	**1,8201**	**4,3323**	**0,5653**
		-0,002640		-0,002459		-0,000670						
		-0,002896		-0,002352		-0,000781						

(*) in blue are enlighten the measurements whose results are somewhat anomalous. The empty cells represent problems with DO probe, inhibitors or not reliable execution of the test.

Table 22A – OUR tests on activated sludge from Pilot Plant scale reactor

Date	Initial NH4-N (mg/l)	VSS (mg/l)	VSS (mg/l) with 9ml NH4HCO3	VSS (mg/l) with 9ml NH_4HCO_3 and 4 ml $NaClO_3$	VSS (mg/l) with 9ml NH_4HCO_3, 4 ml $NaClO_3$ and 6 ml ATU	OUR (Nitrobacter + Nitrosomonas + Heterotrophic)	OUR (Nitrosomonas + Heterotrophic)	OUR (Heterotrophic)	OUR (Nitrobacter + Nitrosomonas + Heterotrophic)	OUR (Nitrosomonas + Heterotrophic)	OUR (Heterotrophic)
						[mg O_2/l s]	[mg O_2/l s]	[mg O_2/l s]	$gO_2\ gVSS^{-1}\ d^{-1}$	$gO_2\ gVSS^{-1}\ d^{-1}$	$gO_2\ gVSS^{-1}\ d^{-1}$
27/09/2010	106,8	283,8	282,1	281,4	280,4	-0,014109	-0,010367	-0,000941			0,2900
	113,4	286,2	284,5	283,8	282,8	-0,014887	-0,011671	-0,000898	4,5205	3,5531	0,2743
	112,5	284,3	282,6	281,9	280,9	-0,015351	-0,011570	-0,000814	4,6930	3,5462	0,2504
								AVERAGE:	4,6067	3,5496	0,2716

OUR (NOB)	**OUR (AOB)**	**OUR (HT)**
g O_2 $gVSS^{-1}$ d^{-1}	**g O_2 $gVSS^{-1}$ d^{-1}**	**g O_2 $gVSS_1^{-1}$ d^{-}**
1,0571	**3,2781**	**0,2716**

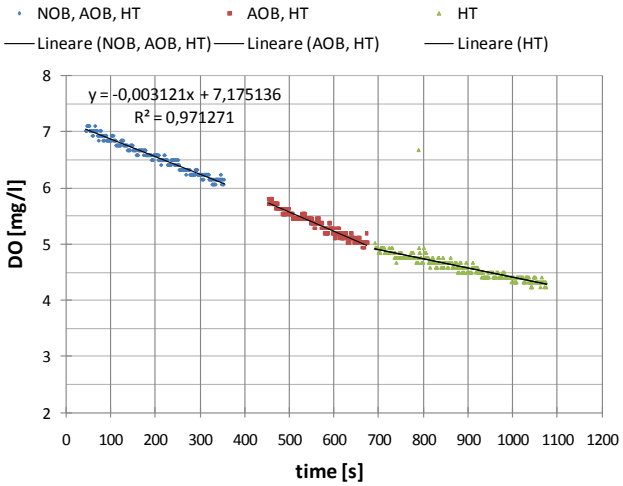

Fig. 18A - 2 June 2010 (I) (25°C)

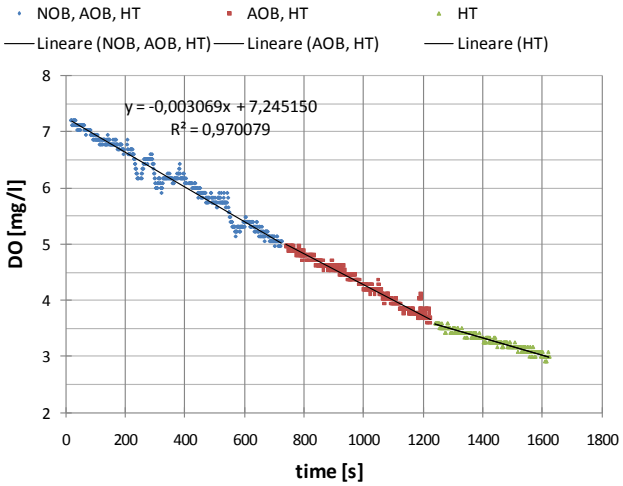

Fig. 19A - 2 June 2010 (II) (25°C)

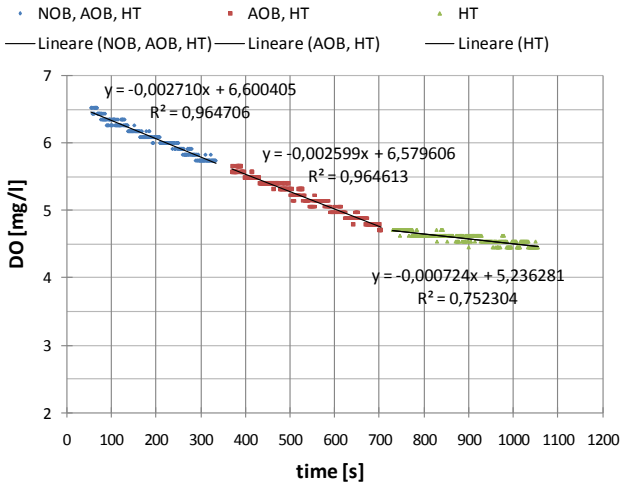

Fig. 20A - 2 June 2010 (III) (25°C)

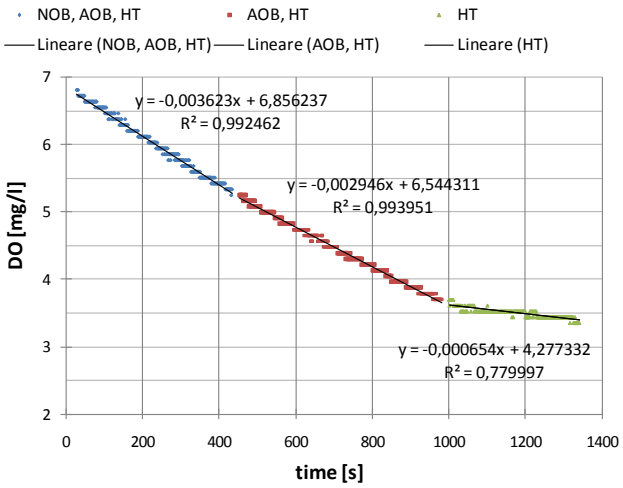

Fig. 21A - 28 June 2010 (I) (25°C)

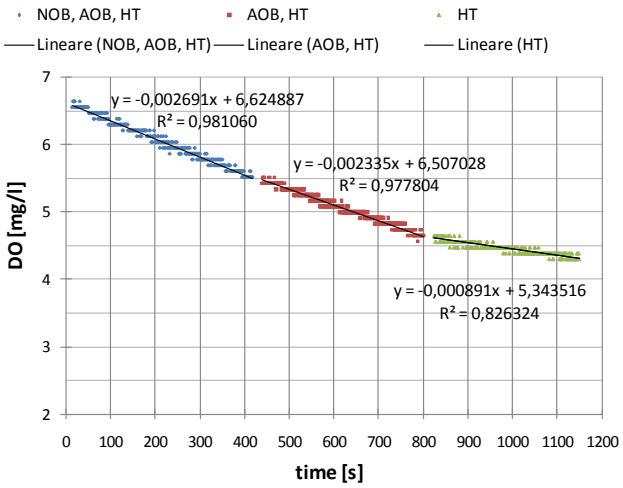

Fig. 22A - 28 June 2010 (II) (25°C)

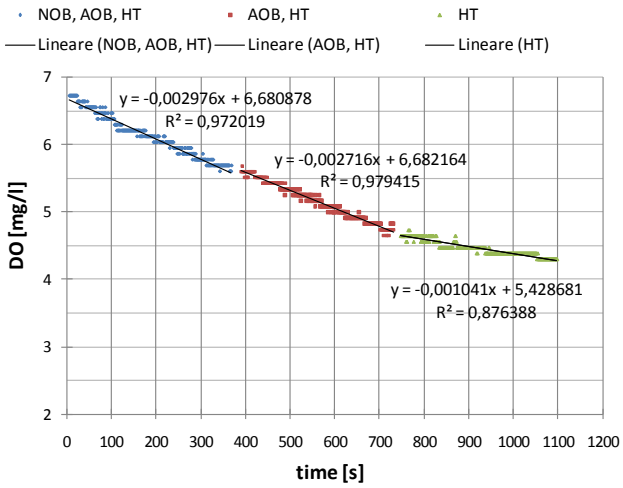

Fig. 23A - 28 June 2010 (III) (25°C)

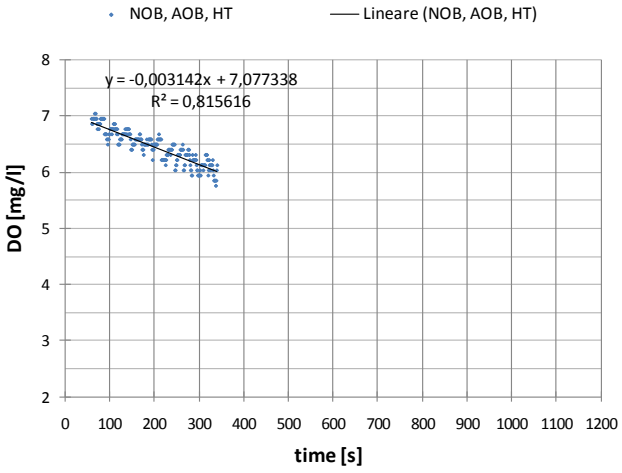

Fig. 24A – 15 July 2010 (I) (25°C)

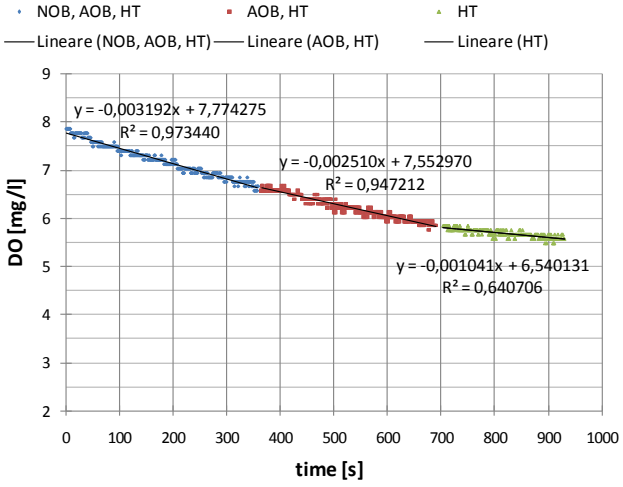

Fig. 25A - 15 July 2010 (II) (25°C)

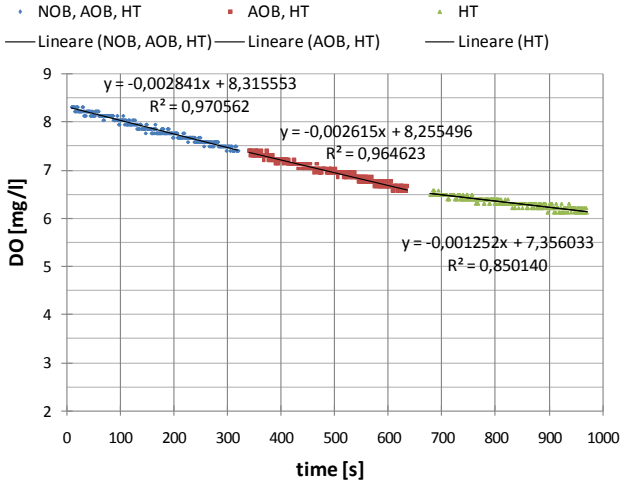

Fig. 26A - 15 July 2010 (III) (25°C)

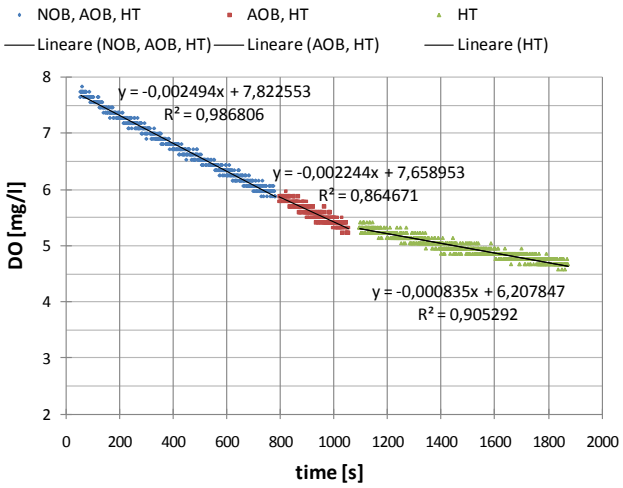

Fig. 27A - 22 July 2010 (I) (25°C)

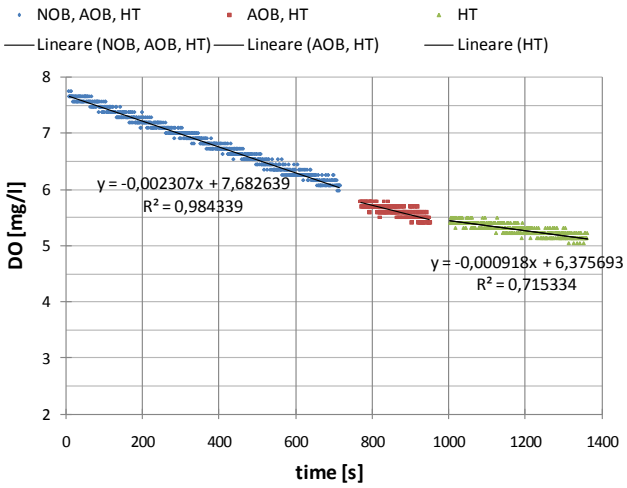

Fig. 28A - 22 July 2010 (II) (25°C)

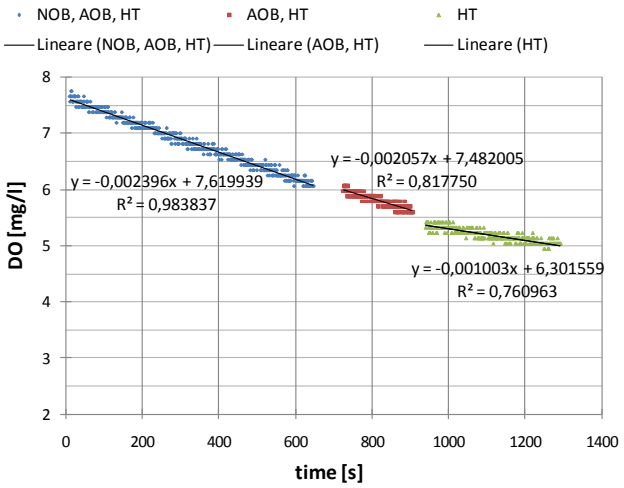

Fig. 29A - 22 July 2010 (III) (25°C)

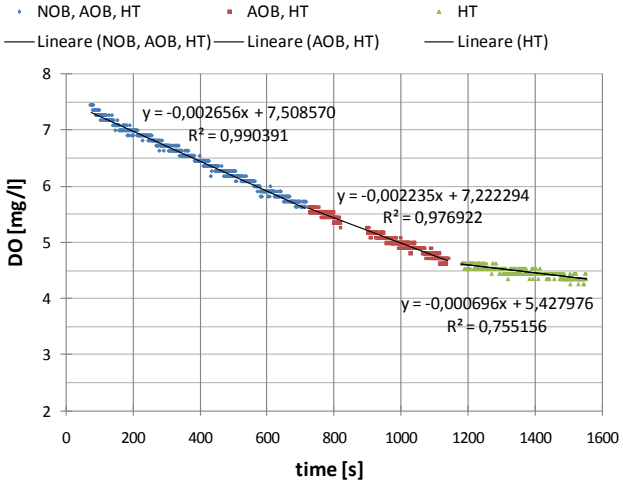

Fig. 30A – 4 August 2010 (I) (25°C)

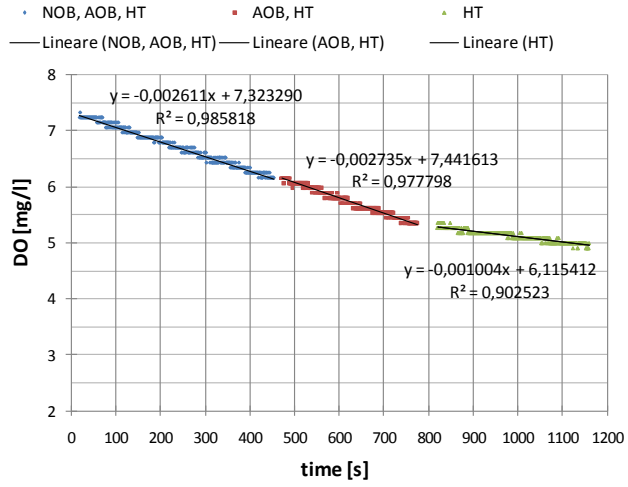

Fig. 31A – 4 August 2010 (II) (25°C)

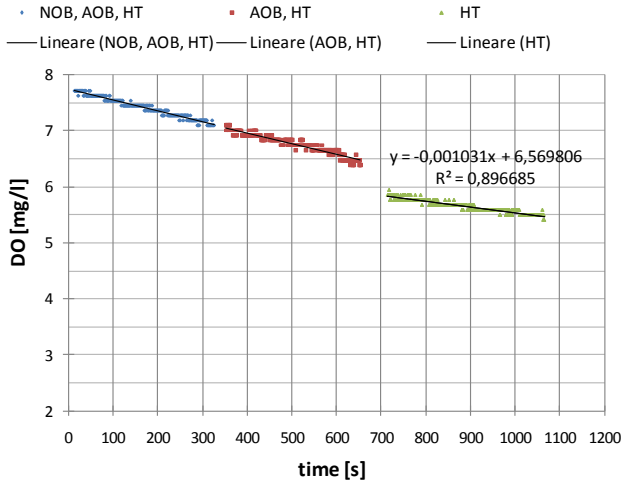

Fig. 32A - 4 August 2010 (III) (25°C)

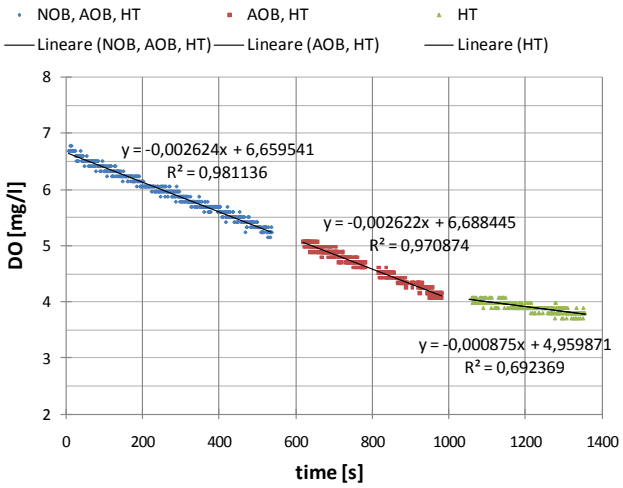

Fig. 33A – 17 August 2010 (I) (25°C)

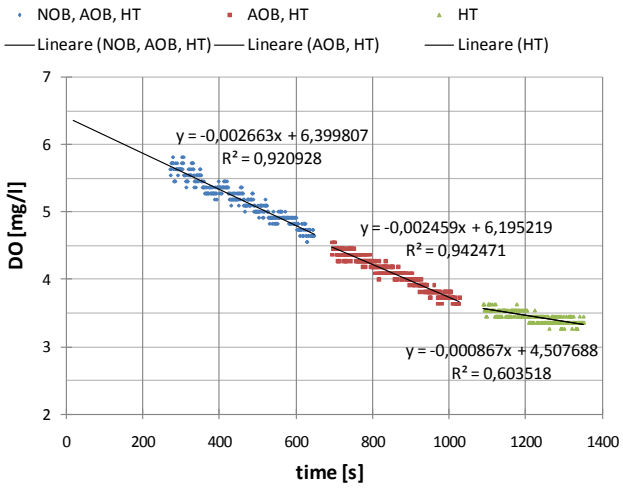

Fig. 34A – 17 August 2010 (II) (25°C)

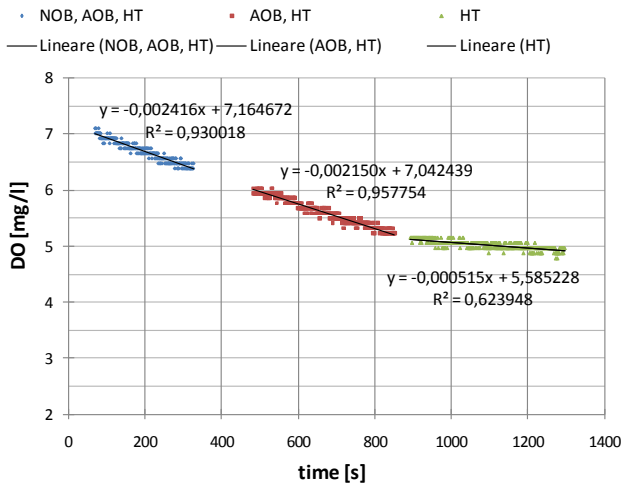

Fig. 35A – 17 August 2010 (III) (25°C)

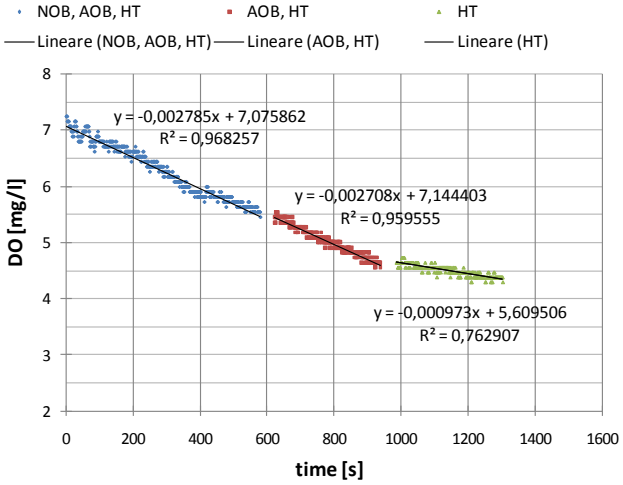

Fig. 36A – 26 August 2010 (I) (25°C)

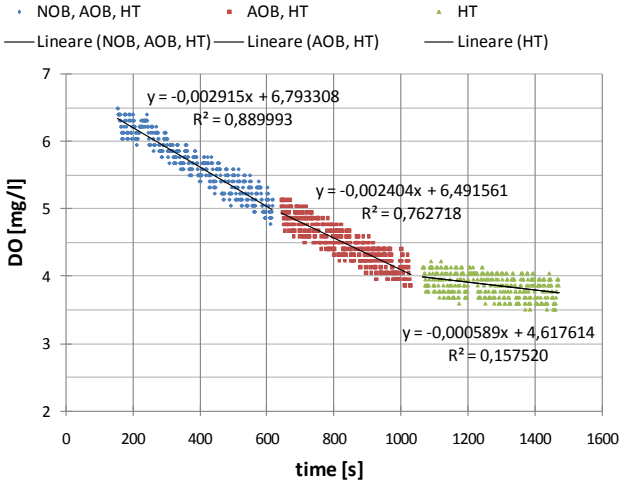

Fig. 37A – 26 August 2010 (II) (25°C)

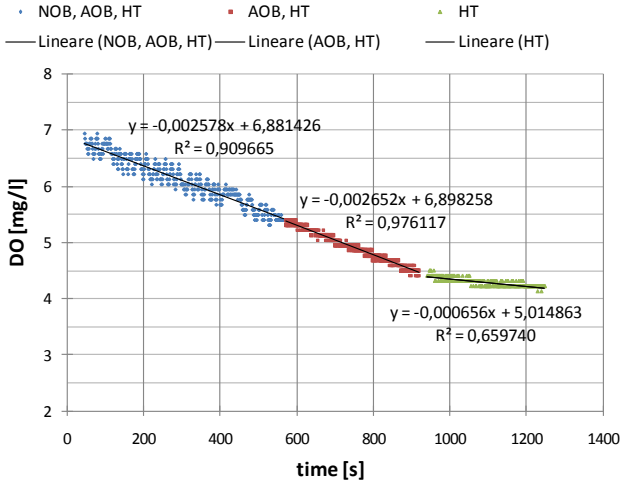

Fig. 38A – 26 August 2010 (III) (25°C)

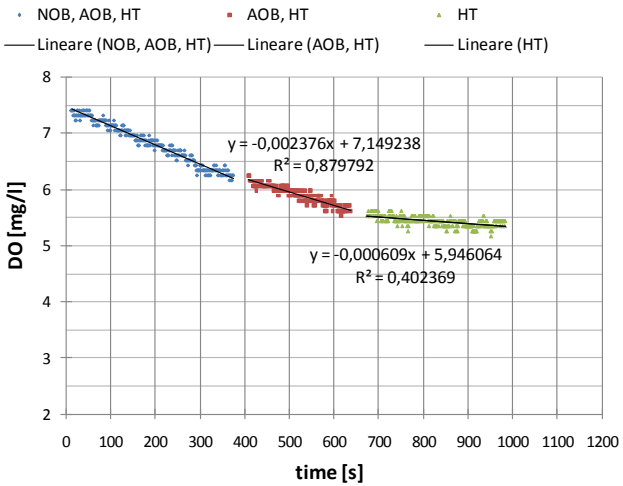

Fig. 39A – 2 September 2010 (III) (25°C)

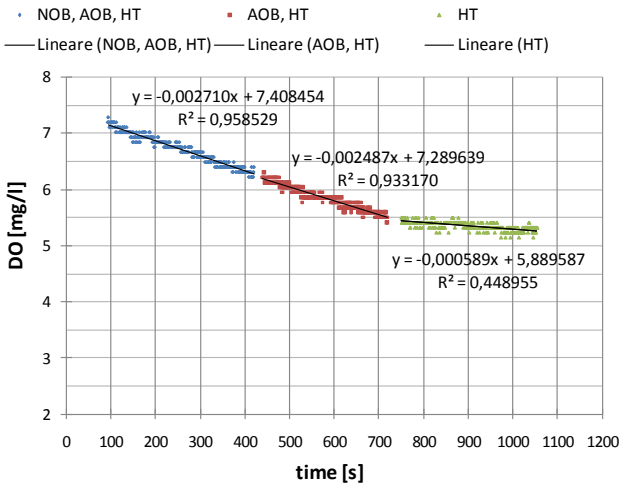

Fig. 40A – 2 September 2010 (III) (25°C)

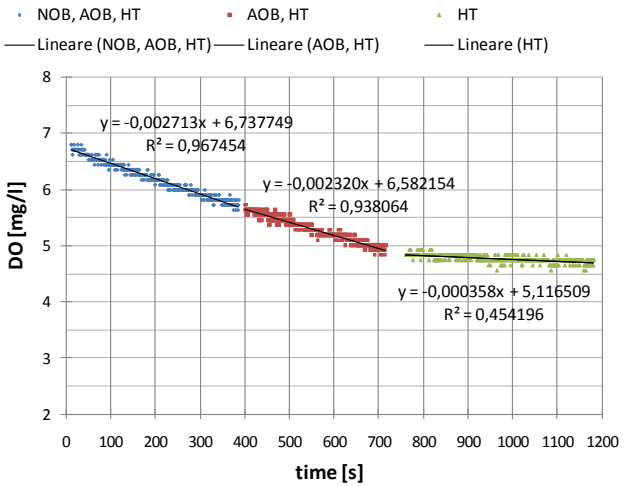

Fig. 41A – 2 September 2010 (III) (25°C)

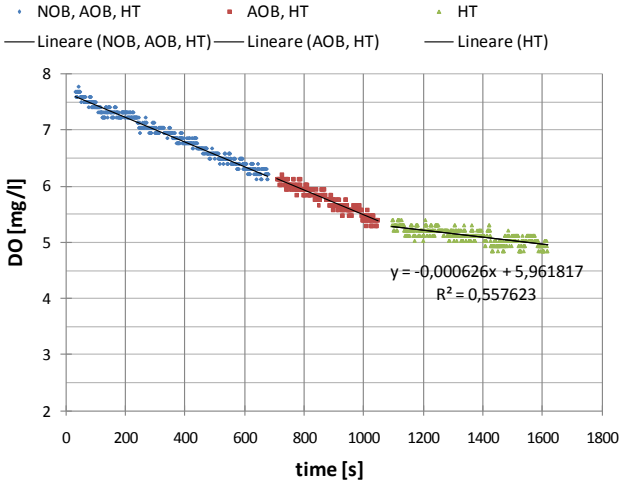

Fig. 42A – 28 September 2010 (I) (25°C)

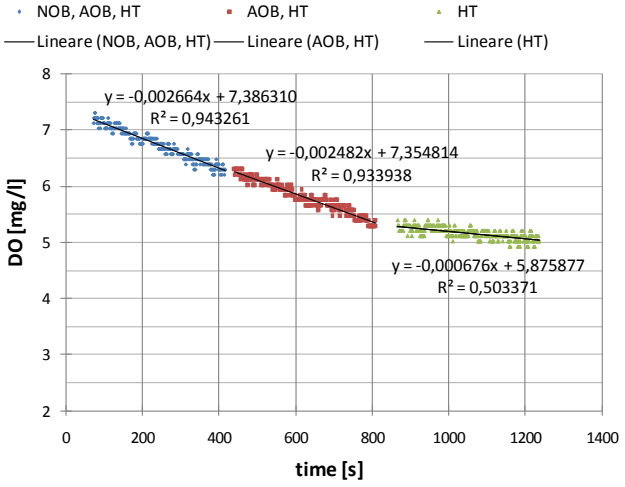

Fig. 43A – 28 September 2010 (I) (25°C)

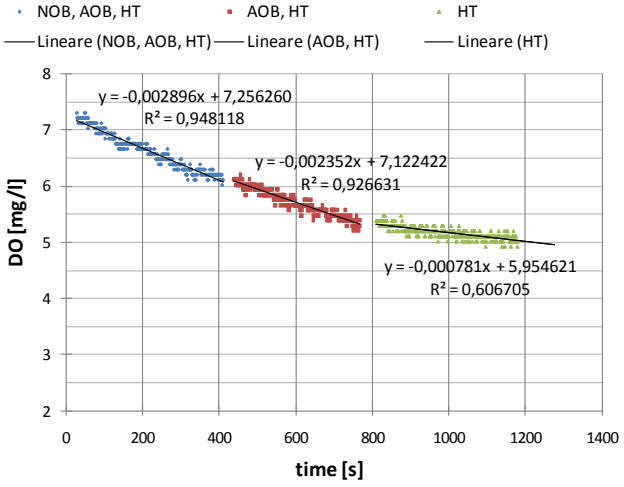

Fig. 44A – 28 September 2010 (I) (25°C)

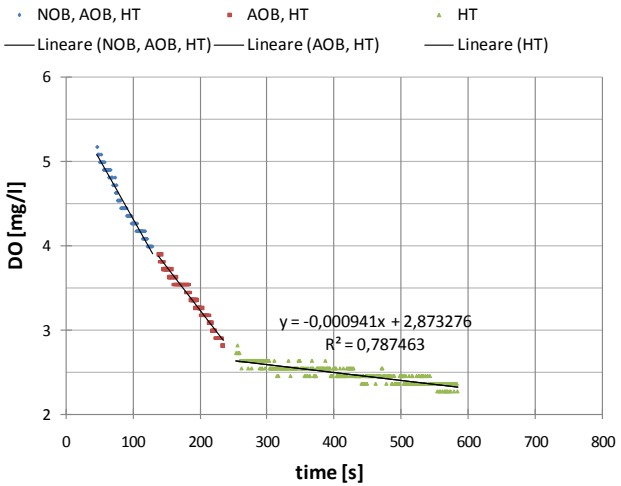

Fig. 45A – 27 Sept. 2010 (I) Act. sludge (25°C)

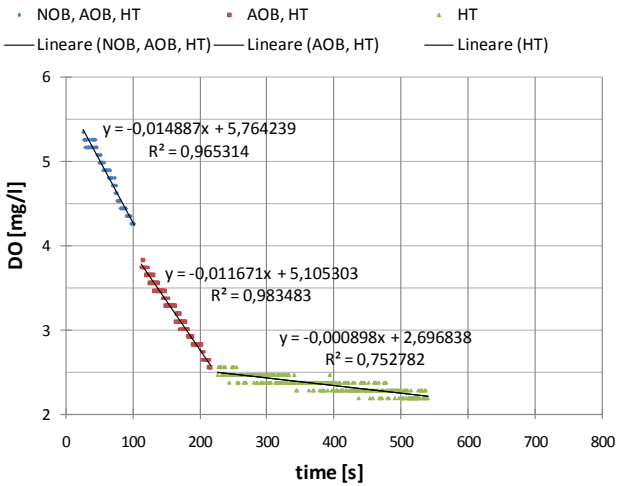

Fig. 46A – 27 Sept. 2010 (II) Act. sludge 25°C)

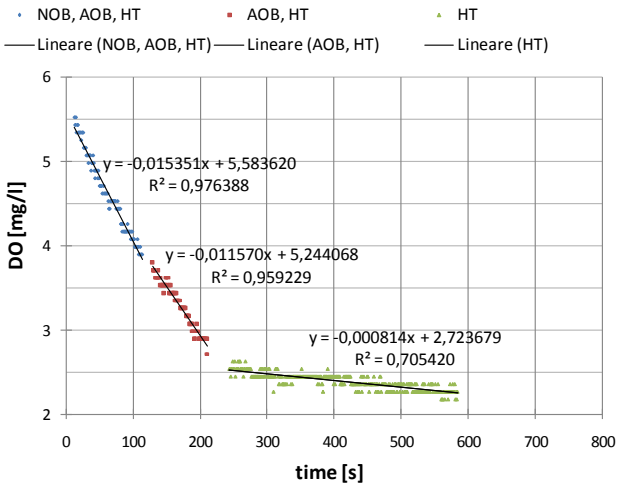

Fig. 47A – 27 Sept. 2010 (II) Act. sludge (25°C)

NUR tests

Table 23A – NUR results

Date	NUR (g N / m^2 / d)
02/06/2010	0,8820
24/06/2010	0,8544
28/06/2010	0,6588
15/07/2010	0,8846
22/07/2010	0,7452
04/08/2010	0,8051
17/08/2010	0,9795
26/08/2010	0,5063
02/09/2010	0,9250
26/09/2010	0,8182

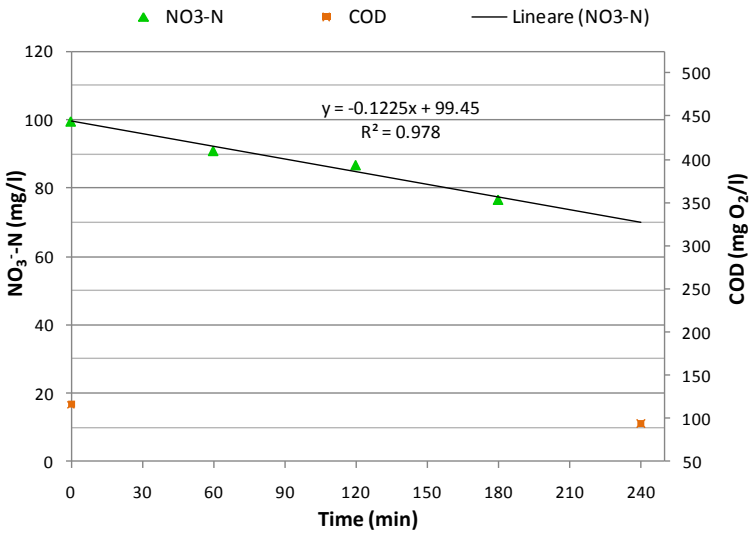

Fig. 48A – 2 June 2010 (25°C)

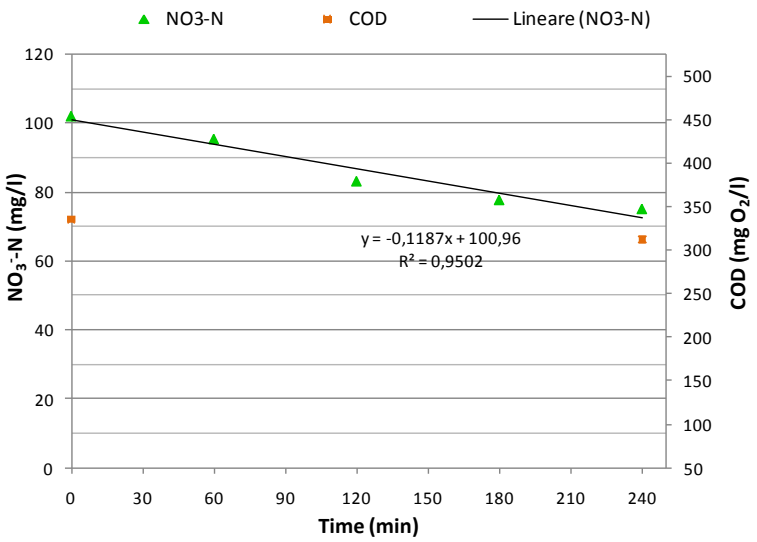

Fig. 49A - 24 June 2010 (25°C)

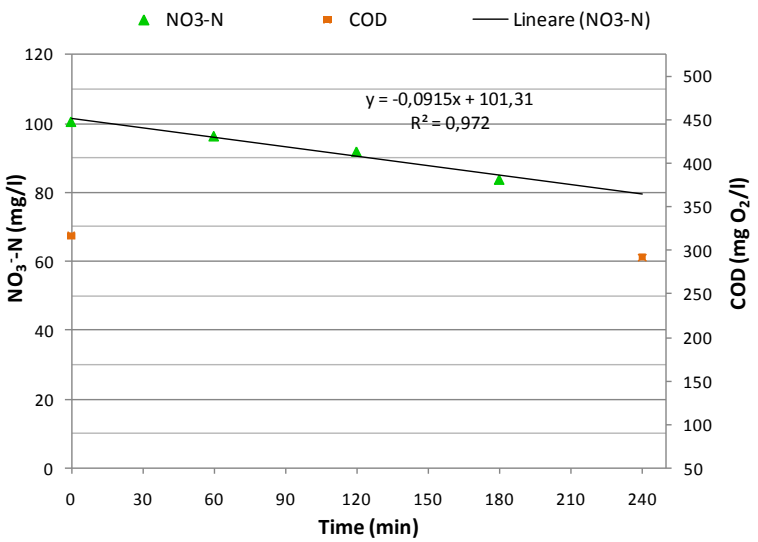

Fig. 50A - 28 June 2010 (25°C)

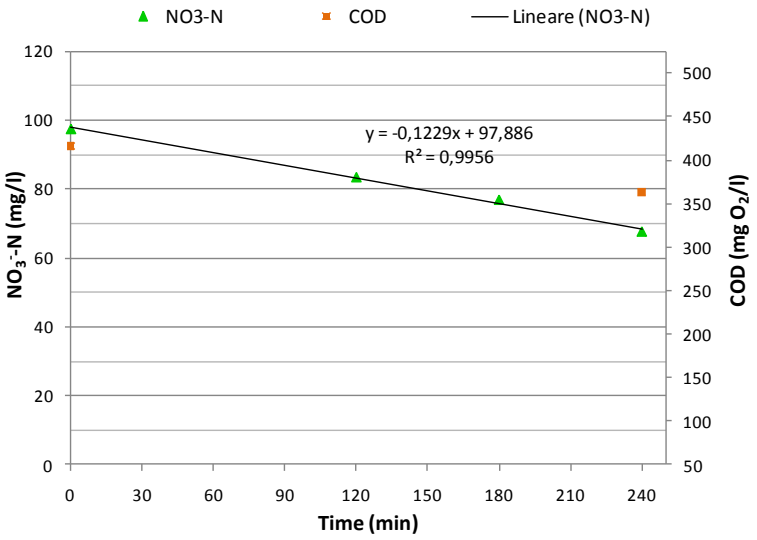

Fig. 51A - 15 July 2010 (25°C)

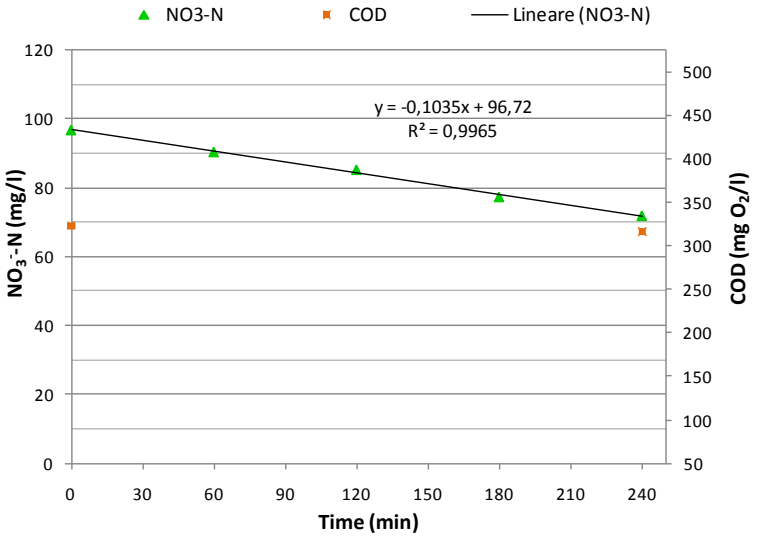

Fig. 52A - 22 July 2010 (25°C)

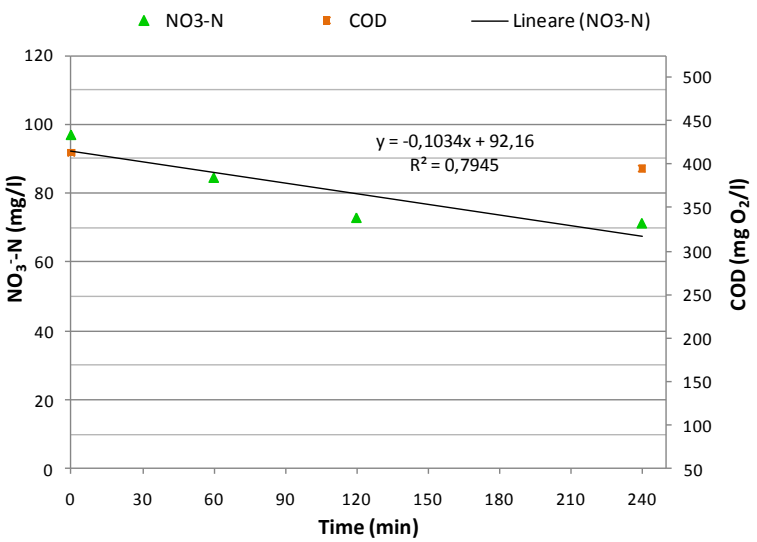

Fig. 53A – 4 August 2010 (25°C)

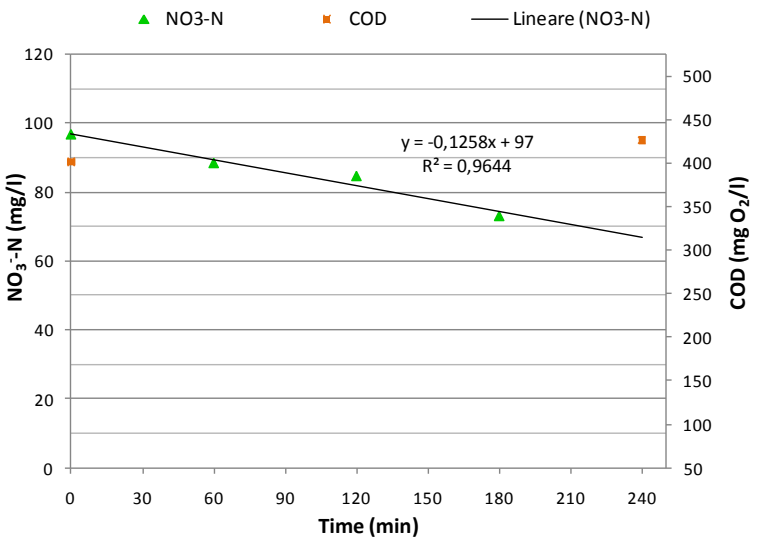

Fig. 54A– 17 August 2010 (25°C)

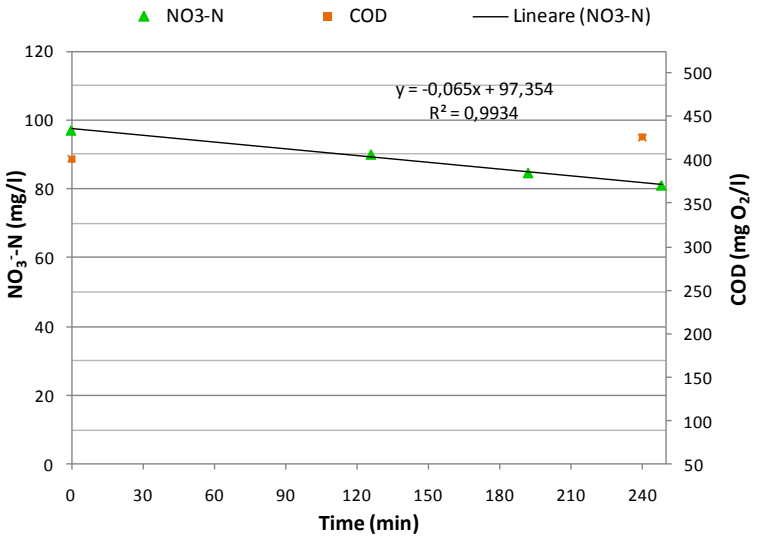

Fig. 55A – 26 August 2010 (25°C)

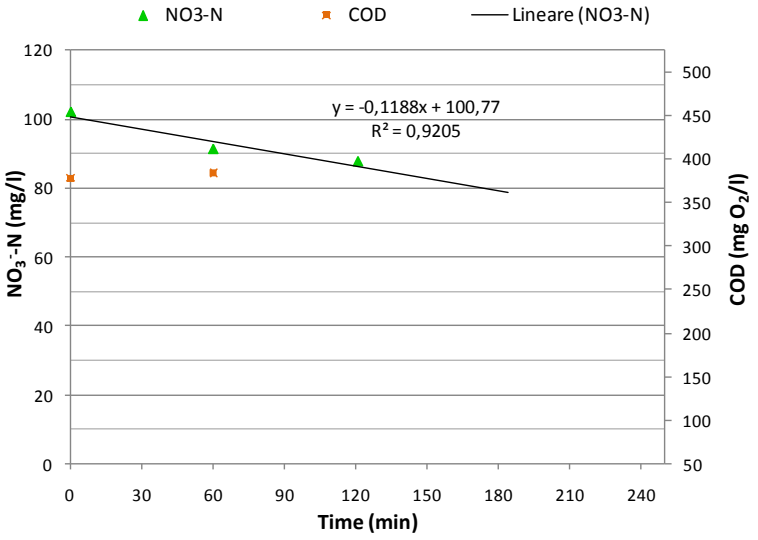

Fig. 56A - 2 September 2010 (25°C)

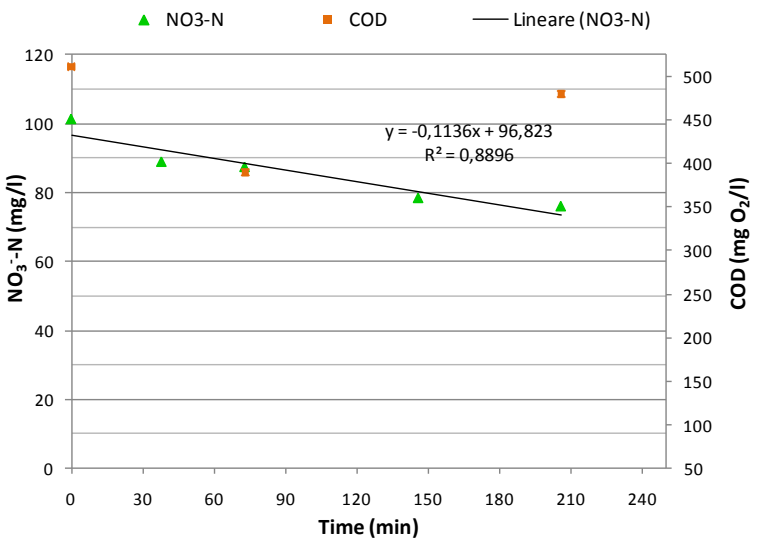

Fig. 57A – 26 September 2010 (25°C)

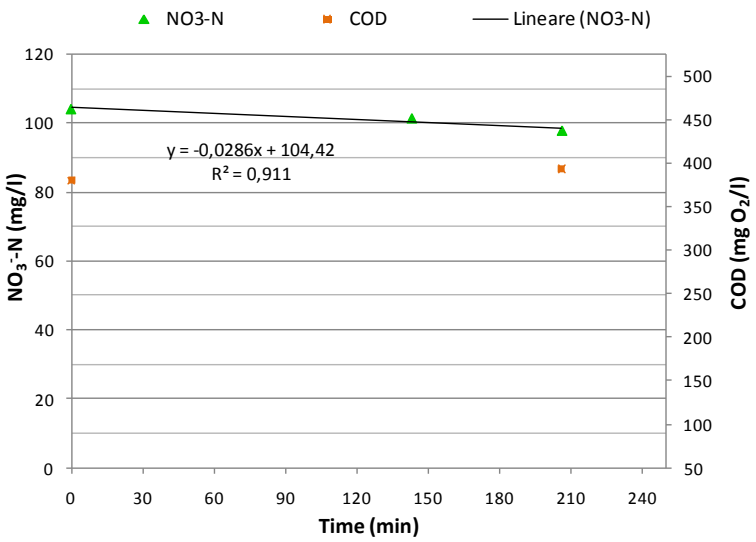

Fig. 58A – 27 September 2010 – activated sludge (25°C)

SAA tests

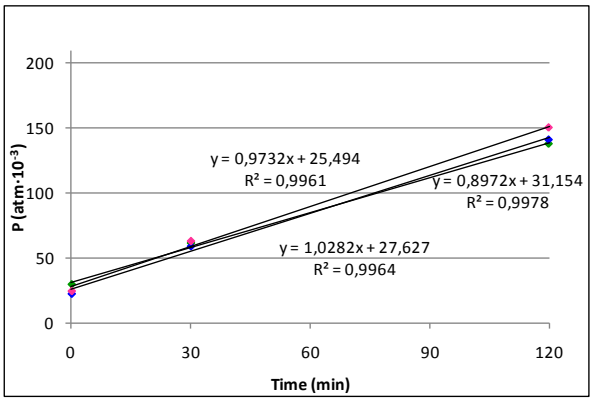

Fig. 59A - 25 May 2010 (25°C)

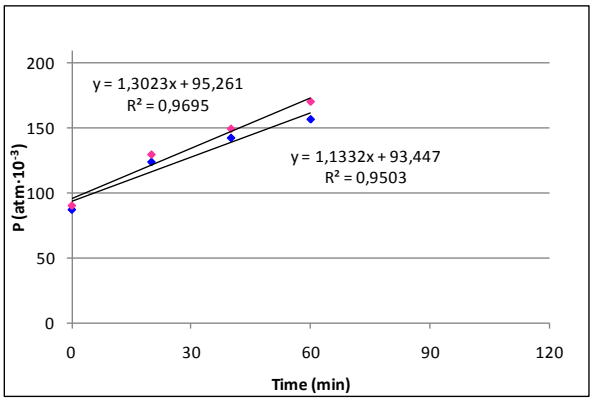

Fig. 60A - 25 May 2010 (35°C)

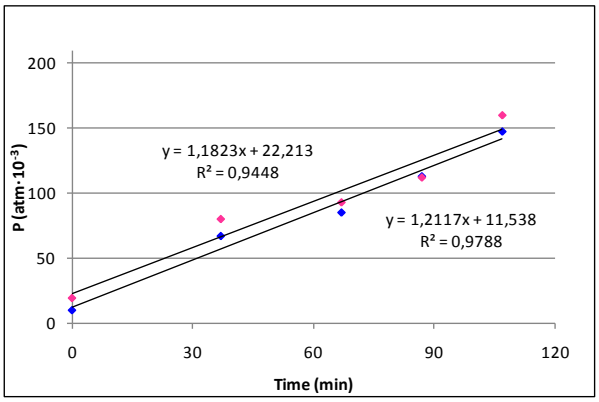

Fig. 61A – 10 June 2010 (25°C)

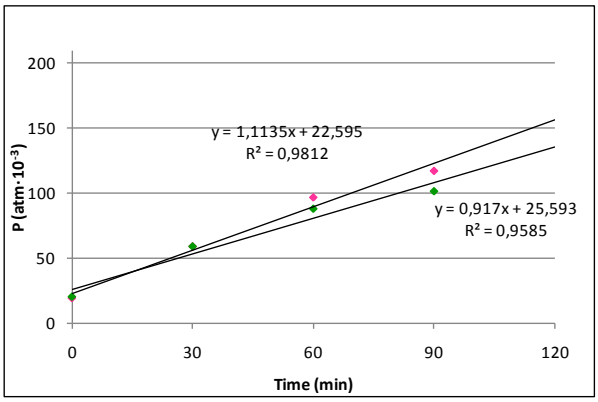

Fig. 62A – 28 June 2010 (25°C)

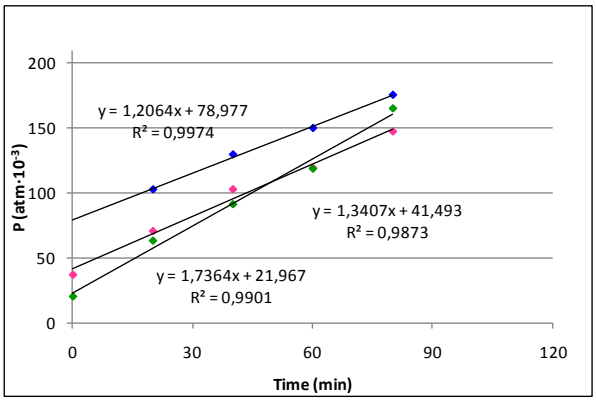

Fig. 63A – 28 June 2010 (35°C)

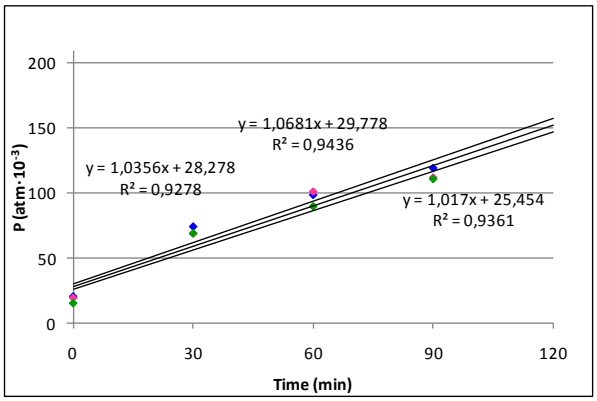

Fig. 64A - 15 July 2010 (25°C)

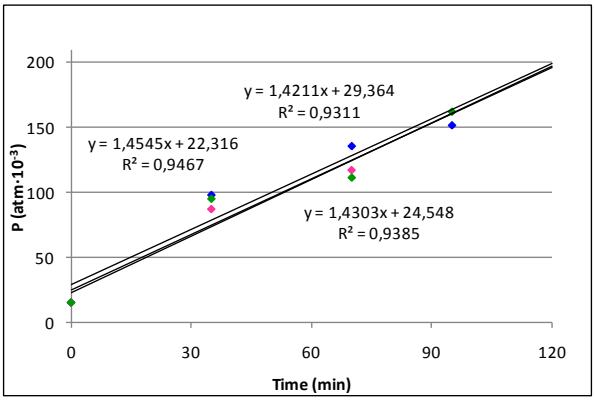

Fig. 65A – 16 July 2010 (35°C)

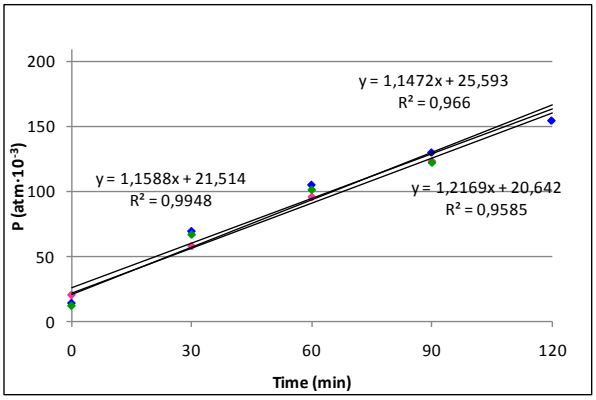

Fig. 66A – 22 July 2010 (25°C)

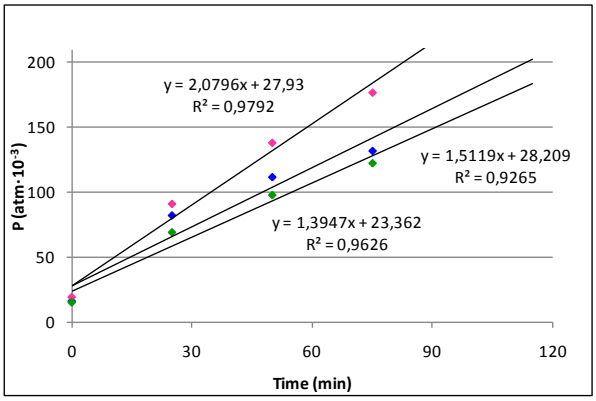

Fig. 67A – 22 July 2010 (35°C)

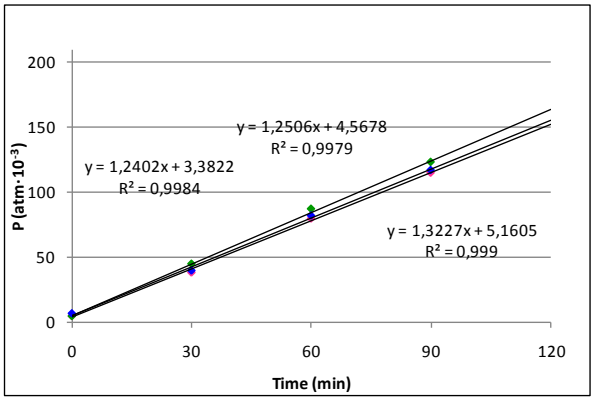

Fig. 68A - 16 August 2010 (25°C)

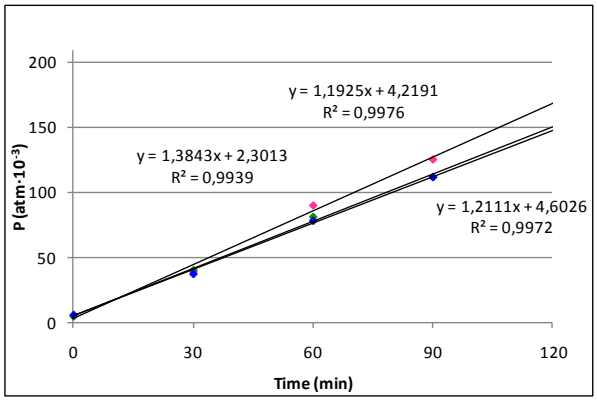

Fig. 69A – 23 August 2010 (25°C)

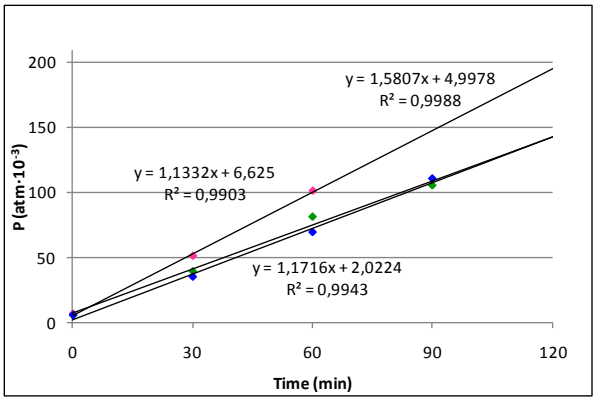

Fig. 70A – 3 September 2010 (25°C)

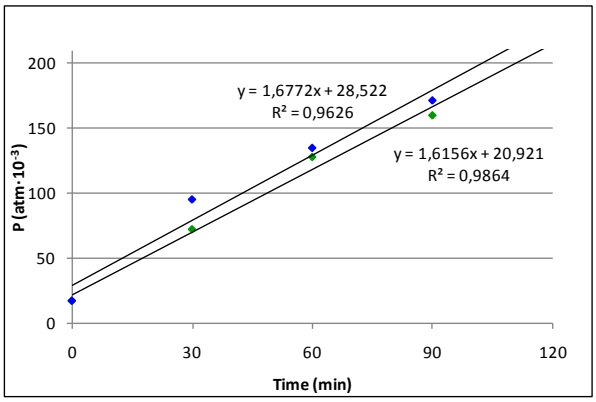

Fig. 71A – 8 September 2010 (25°C)

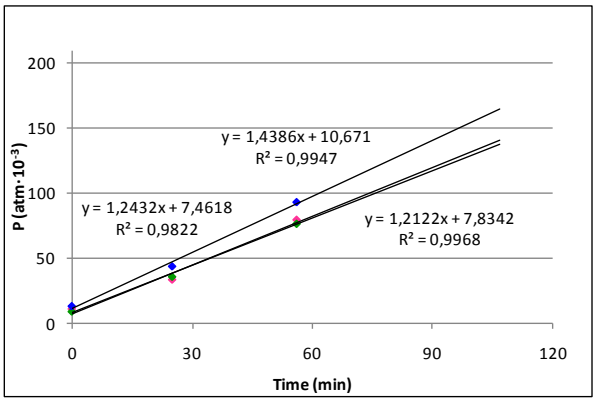

Fig. 72A – 28 September 2010 (25°C)

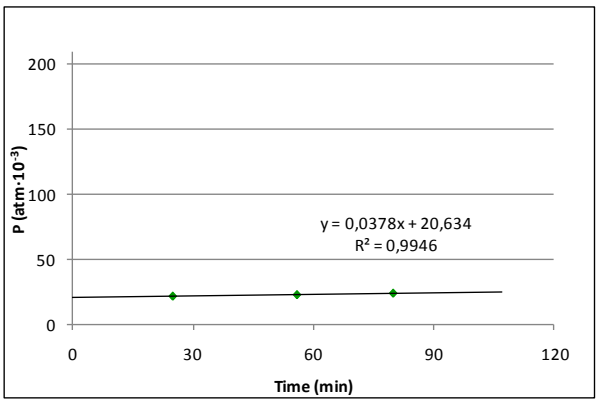

Fig. 73A – 28 September 2010 (25°C) – Activated sludge

Table 24A – SAA results from Pilot Plant scale reactor

Date	Anammox Biomass	
	SAA (25°C) (gN/m^2/d)	SAA (35°C) (gN/m^2/d)
28/05/2010	2,95	3,60
10/06/2010	3,66	
28/06/2010	3,10	4,22
15/07/2010	3,18	
16/07/2010		4,24
22/07/2010	3,59	4,92
16/08/2010	3,89	
23/08/2010	3,86	
03/09/2010	3,96	
08/09/2010	4,12	7,12
23/09/2010	3,21	
28/09/2010	3,97	