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Ozone Technology for Sludge Bulking Control

Bekämpning av slamsvällning med ozonteknologi

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Civilingenjörsprogrammet i miljö- och vattenteknik

Master of Science Program in Aquatic and Environmental Engineering

ABSTRACT

Ozone Technology for Sludge Bulking Control

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Bulking sludge causes major problems in wastewater treatment plants that deal with biological nutrient removal in activated sludge processes. Bulking sludge is caused by filamentous bacteria, which have a negative impact on the sludge settling properties.

Himmerfjärden wastewater treatment plant suffers from this type of problem with bulking sludge which creates a stable layer at the surface that does not settle in the clarifier.

In order to solve this problem, on site generated ozone was used to decrease the amount of filamentous bacteria in the return activated sludge flow. Ozone is a strong oxidant is suitable for non-specific bulking control. It stresses the filamentous bacteria causing inactivation through cell wall disintegration.

The ozone treatment resulted in decreased abundance of filamentous bacteria. Ozone treatment of the recycled activated sludge improves the settling properties of bulking sludge, without interfering with other important microbiological processes e.g. nitrification.

Key words: Wastewater treatment, Activated sludge process, Sludge bulking, Filamentous bacteria, Microthrix Parvicella, Ozone, Non specific bulking control.

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REFERAT

Bekämpning av slamsvällning med ozonteknologi

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Slamsvällning orsakar stora problem i avloppsreningsverk med biologisk rening i aktivt slamprocesser. Slamsvällning orsakas av filamentösa (trådformiga) bakterier, som inverkar negativt på slammets sedimenteringsegenskaper.

Himmerfjärdens vattenreningsverk har drabbats av detta problem som leder till ett stabilt lager av slam på ytan av sedimenteringsbassängen som inte sedimenterar.

För att lösa detta problem behandlades returslammet från sedimenteringsbassängen med ozon för att minska mängden filamentösa bakterier i returslamflödet. Ozon är en starkt oxiderande gas, som är väl användbar för icke-specifik bekämpning av slamsvällning. När ozon kommer i kontakt med den filamentösa bakteriens cellvägg penetreras det in i cellen, varvid cellen lyserar.

Ozonbehandlingen resulterade i en förminskning av antalet filamentösa bakterier. Ozonbehandling av returslam förbättrade sedimenteringsegenskaperna hos svällande slam utan att påverka andra viktiga mikrobiologiska processer t.ex. nitrifikation.

PREFACE

This master thesis work was done for SYVAB, Himmerfjärden wastewater treatment plant and is a part of a M.Sc. Education in Aquatic and Environmental Engineering at Uppsala University. During this master thesis work I had the opportunity to team up with many different professionals; practically minded laboratory personnel, microbiologically educated engineers, inspiring scientists amongst others, without whose help and expertise this report would not have been made.

I would like to give my gratitude to my subject reviewer Associate Professor Sara Hallin, Department of Microbiology at SLU, for your great support and help throughout this work. Thanks to Professor Allan Rodhe, Department of Earth Sciences at Uppsala University for your comments and suggestions on the report.

Thanks to my supervisor Malin Tuvesson, process manager at SYVAB, for your friendship, instructions and all the practical arrangements. I'm glad that you called me that pre-summer day, offering me the job as a process engineer, and at the same time giving me the chance to perform this study.

Thanks to Mats Rydén, Air Liquide for your experience, patience and good friendship. I will also give my gratitude's to Ph.D Krister Eskilsson at Kemira and Caroline Kragelund at Aalborg University, for your microscopic examinations. Thanks to Ph.D Lars Gunnarsson, MD of SYVAB, for recommending me in this business. And of course, thanks to all the staff at SYVAB for your help and friendly efforts. Also thanks to my family, you are the best.

Last and certainly the least, I would like to thank the microorganisms in the activated sludge process for working 24 hours a day, 7 days a week –that's a symbol of true commitment.

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DEFINITIONS AND ABBREVIATIONS

Definitions and Abbreviations for parameters in waste water treatment.

Abbreviation	Parameter	Definition	Unit
$BOD_{5,7}$	Biochemical Oxygen Demand	Amount of oxygen needed for biological oxidation within 5 or 7 days.	mg/L
COD	Chemical Oxygen Demand	Amount of oxygen needed for chemical oxidation.	mg/L
MLSS	Mixed Liquid Suspended Solids	Total amount of sludge in a tank	mg/L
NH_4-N	Ammonium	N concentration in the form of ammonium (NH_4).	mg/L
NO_3-N	Nitrate	N concentration in the form of nitrate (NO_3).	mg/L
NO_2-N	Nitrite	N concentration in the form of Nitrite (NO_2).	mg/L
PO_4-P	Phosphate	P concentration in the form of phosphate (PO_4).	mg/L
P-tot	Total Phosphorus	Organically bound P + polyphosphate + orthophosphate.	mg/L
SS	Suspended Solids	The mass of non-filterable residue in a liquid sample dried at 103-105 ^oC.	mg/L
SV	Sludge Volume	Measure of sludge volume in a cylinder after 30 min settling.	mL/L
SVI	Sludge Volume Index	Measure of the volume of sludge in milliliters occupied by 1 g of a suspension after 30 min settling	mL/g
SQI	Sludge Quality Index	Measure of the volume of diluted sludge in milliliters occupied by 1 g of a suspension after 30 min settling.	mL/g

Glossary for common terms in waste water treatment

Abbreviation	Definition
ASP	Activated Sludge Process
AOB	Ammonia Oxidizing Bacteria
LOX	Liquid Oxygen
NOB	Nitrite Oxidizing Bacteria
O_3	Ozone
RAS	Return Activated Sludge
RNA	A nucleic acid polymer consisting of nucleotide monomers
Settler	Settling tank synonymous with clarifier
Treated	Ozone treated
Untreated	Not treated with ozone
WWTP	Wastewater Treatment Plant
F/M	Food to Microorganisms ratio. High F/M make equal high organic load.
FISH	Fluorescent *In Situ* Hybridization

ACTORS

Actors involved in the experiments performed within this master thesis work.

Actor	Description
Aalborg University	The Aalborg University has cooperation with national and international businesses, organisations and educational institutions. They performed some microscopy studies together with Kemira. See en.aau.dk for more information.
Air Liquide	Air Liquide is the world leader in industrial and medical gases and related services. Their core business is to supply oxygen, nitrogen, hydrogen and many other gases and services to industries and laboratories. See www.airliquide.com for more information.
Kemira	Kemira is a chemicals group that is made up of different business areas; water treatment chemicals among others. Kemira is a global group of leading chemical businesses with unique competitive position and a high degree of mutual synergy. See www.kemira.com for more information.
Swedish University of Agricultural Sciences	Swedish University of Agricultural Sciences is a university with a main focus on sustainable use of biological resources and biological production. See www.slu.se for more information.
SYVAB and Himmerfjärden WWTP	A municipal waste water treatment plant that serves the south-western part of Stockholm area. The average flow is about 110,000 m^3/d; the plant serves a population of 250,000 persons and an industrial load corresponding to an additional 35,000 pe. This thesis was initiated by SYVAB. See www.syvab.se for more information.
Uppsala University	Uppsala University is a university focusing on research and higher education within most fields of science. See www.uu.se for more information.

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1 INTRODUCTION

The activated sludge process (ASP) is the most widespread treatment technology for wastewater, it is a biological process where microorganisms oxidize and mineralize organic matter. All microorganisms enter the system with the influent water and the composition of species depends on influent wastewater, design and operation of the plant. The suspension that is formed during the process is called activated sludge. This active biomass is responsible for the treatment efficiency. The purposes of the ASPs are to oxidize dissolved and particulate biodegradable constituents into suitable end products, capture and aggregate suspended and non-settle colloidal solids into flocs and transform and/or remove nutrients like nitrogen and phosphorus from the wastewater (Tchobanoglous et al. 2003). Recycling of the biomass is a characteristic feature of the ASP in order to keep a high concentration of biomass in the aerated tank (Wanner, 1994).

The ASP consists of a biological step and a separation step (Figure 1). In the biological process, microorganisms convert pollutants into end products such as carbon dioxide. This takes place in the aerated tank where microorganisms are kept suspended by aeration. The oxygen required in the biological process is a result of the carbonaceous biochemical-, nitrogenous biochemical- and inorganic chemical oxygen demand.

Because of the high volumes of treated wastewater at large waste water treatment plants (WWTP) biomass is separated by sedimentation in the clarifier, in order maintain the microbiological population and increase the sludge concentration. Then the activated sludge can be recycled back and distributed to the aerated tank. The clarifier is designed to separate sludge flocs from the biological and chemical treated water, normally the flocs will have higher density than water and thus be separated by gravity. This is the most critical step in the wastewater treatment, and without a good function of the separation step, the entire process is rather pointless (Carlsson and Hallin, 2003). This highlights the significance of good settling properties of the activated sludge. The most important settling properties are fast sludge settling (1 m^3/h or more), that sludge does not occupy an excessive volume after settling, leaves a clear supernatant after sludge settling and sludge does not rise within a 2-3 h period after settling (Eckenfelder, 1992). Problems in the biological oxidation which takes place in the aerated tank will pass on to the clarifier and to the filters, causing the problems in the clarifier to feed back to the aerated tank and so on.

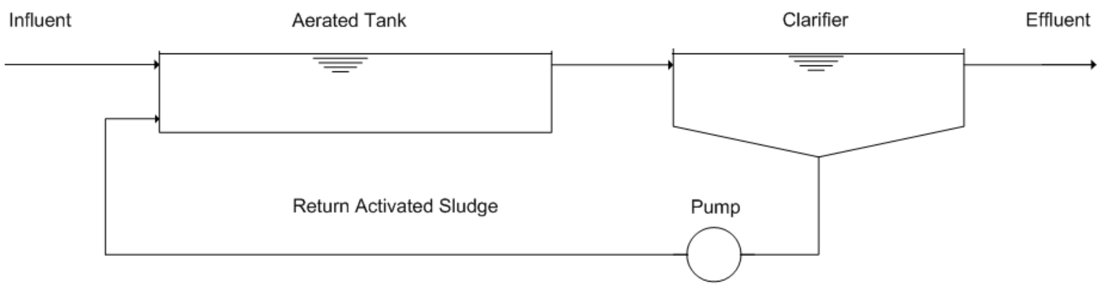

Figure 1. The activated sludge process, the waste water flow in to the aerated tank and out from the clarifier.

The above described bulking of sludge is a major microbial related (solid) separation problem. WWTPs all over the world suffers from bulking and rising sludge due to large amounts of filamentous microorganisms interfering with the sludge settling properties, by building bridges between flocs or creating diffuse flocs (Jenkins et al., 2003). In several international surveys it has been shown that *Microthrix parvicella* is the most common filamentous bacteria to cause sludge separation problems in wastewater treatment plants (Seviour et al., 1999, Rossetti et al., 2004). Much effort and hard work has been put into solving this problem. Nevertheless filamentous bacteria are a normal part of the activated sludge and are needed in small numbers to provide a matrix for floc formation (Blackall, 1999), but if their number and length exceed an acceptable level their presence might lead to sludge bulking (Eckenfelder, 1992).

Large amounts of filaments hinder the thickening, compaction and settling of the suspended solid flocs. Due to bulking sludge floc particles are discharged with the effluent from the settling tanks, which cause problems in the following process steps, and may lead to increased suspended solids concentration in the effluent to the recipient. Potential loss of nitrification due to the increased plant loading may lead to further weakening in the effluent quality.

Blackall (1999) suggest that in extreme cases of the continuous loss of solids a reduction in the oxidation of carbon compound can arise.

Ozone (O_3) is a strong oxidant and very potent disinfectant which can be used in waste water treatment for disinfection and oxidation (Rice, 1996). Several organic substances can be oxidized to a more degradable form by ozonation (Sontheimer et al., 1978). Ozonation of sludge to control sludge bulking and improve sludge treatment has been studied by eg. Caravelli et al. 2006, Boeler et al. 2003, Saayman et al. 1996, Van Leeuwen, 1988a, 1988b, 1989, 1992 and the positive effect from this treatment have been reported to be instantaneous.

My hypothesis was that filamentous bacteria are vulnerable to ozone treatment since they have a high surface area to volume ratio, and for that reason can maintain a higher rate of mass transfer across cell boundaries than other microorganisms. Therefore could ozone penetrate the filamentous bacteria and cause irreversible damage leading to lysis.

The objective of this study was to use ozone dosage in the RAS flow as an application for non-specific activated sludge bulk control. There are two principal ways to control bulking, specific, i.e. the selector reactor approach and non-specific, i.e. the use of selective toxicants (Van Leeuwen, 1988, Stypka, 1998). Specific control of bulking means identification and elimination of conditions that support the production of the specific filaments causing these problems, it targets the cause of the problem. Non-specific bulking control means elimination of filament growth in the sludge, this does not go to the bottom of the symptoms of bulking i.e. it decreases the filaments but does not remove the causes of filament production permanently. The use of strong oxidizing agents is examples of non-specific methods. The experimental part of this project was done at Himmerfjärden WWTP which suffers from filamentous sludge bulking (Figure 2). Previously FISH analyses stated that *M. parvicella* are the dominant filamentous species there.

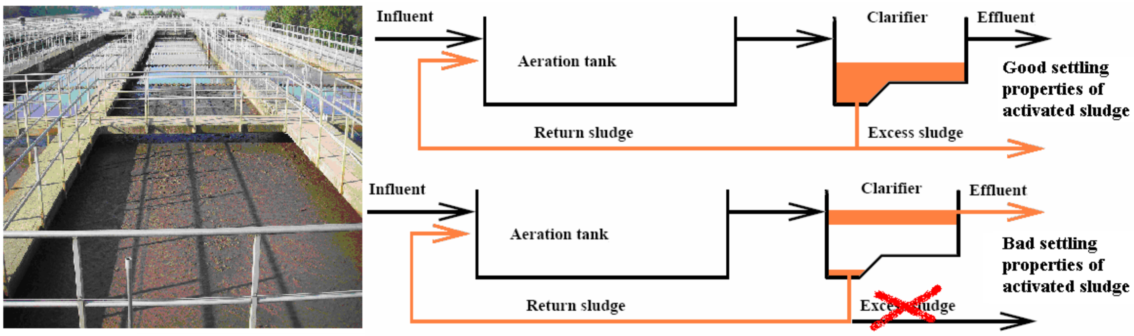

Figure 2. Bulking sludge in the clarifier, Himmerfjärden WWTP 2006.

A literature study and experiment were combined with evaluation of prior process data and continuous microscopic studies of the treated and untreated RAS from the experiment and control line. The experiment performed in this study involved 1/8 of the biological treatment volume at Himmerfjärden WWTP. An identical line (except the ozonation step), was used as a control system. Activated sludge flocs abundance, characteristics and filamentous bacteria content were observed through microscopy in direct light after staining using Gram, Neisser and crystal violet.

To characterize the settling properties Sludge Volume (SV) and Sludge Quality Index (SQI) were measured. Influent and effluent measurements of chemical parameters were also determined to control the treatment capacity. To verify that other important features in the ASP were maintained, such as nitrogen removal, FISH analyses were performed to estimate effects in the nitrifying community composition and abundance.

1.1 Microbial life in Wastewater treatment

Microorganisms play an important role in removing organic compounds, nitrogen and phosphorus in waste water. The most important microorganisms in the water treatment are bacteria (Eckenfelder, 1992, Carlsson and Hallin, 2003). The biological treatment processes can be divided into two principal categories:

1. Suspended growth - The activated sludge process is an example of suspended growth, where the microorganisms are maintained in liquid suspension by the aerated mixing.

2. Attached growth - The fluidized bed, where the microorganisms are attached to grain of sand is an example of attached growth process

The suspended growth principal is the most common treatment process. In this system microorganisms form flocs. The activated sludge flocs contain microorganisms, such as bacteria, fungi, protozoa and metazoan, organic as well as inorganic particulates. Fibres from incoming wastewater and extracellular polymers are also present in the flocs. Extracellular biopolymers work as adhesion different organic and inorganic components together with diverse bacteria to form the flocs. The shapes of the flocs depend on the type of microorganisms that construct it, spherical microorganisms make a roughly spherical shaped floc, and filamentous organisms make an irregular shaped floc (Figure 3). The sludge flocs are categorised by size, and ranges from:

Small ≤ 150 µm ≤ Medium ≤ 500 µm ≤ Large (Jenkins et al., 2003).

Figure 3. Magnified, irregular shaped sludge floc with filamentous organisms.

The effectiveness of a process can be expressed in terms of the decrease of Biological Oxygen Demand (BOD), which determines the amount of dissolved oxygen consumed by microorganisms for the oxidation of organic and inorganic matter, but also the Chemical Oxygen Demand (COD) which determines the oxygen corresponding of the organic material in water that can be oxidized chemically. Other parameters that can be used for the same purpose are DOC and TOC. The microorganisms oxidize dissolved and particulate carbonaceous organic matter into simpler products and supplementary biomass, according to following, simplified equation:

$$\text{organic material + nutrients} \xrightarrow{\text{Microorganisms}} \text{new cells + end products (e.g. } NH_4\text{)}$$

New cells are representing the biomass being produced during this process and end products from this reaction are typically carbon dioxide and water. In nitrogen removal two processes are involved: nitrification and denitrification (Figure 4). During nitrification, bacteria oxidize ammonia to nitrite and nitrate:

$$NH_4^+ + 1{,}5\ O_2 \xrightarrow{\text{nitrosomonas}} NO_2^- + 2\ H^+ + H_2O$$

$$NO_2^- + 0{,}5\ O_2 \xrightarrow{\text{nitrobakter}} NO_3^-$$

Then other bacteria denitrify and nitrate is reduced to gaseous nitrogen, represented by the following simplified equation:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

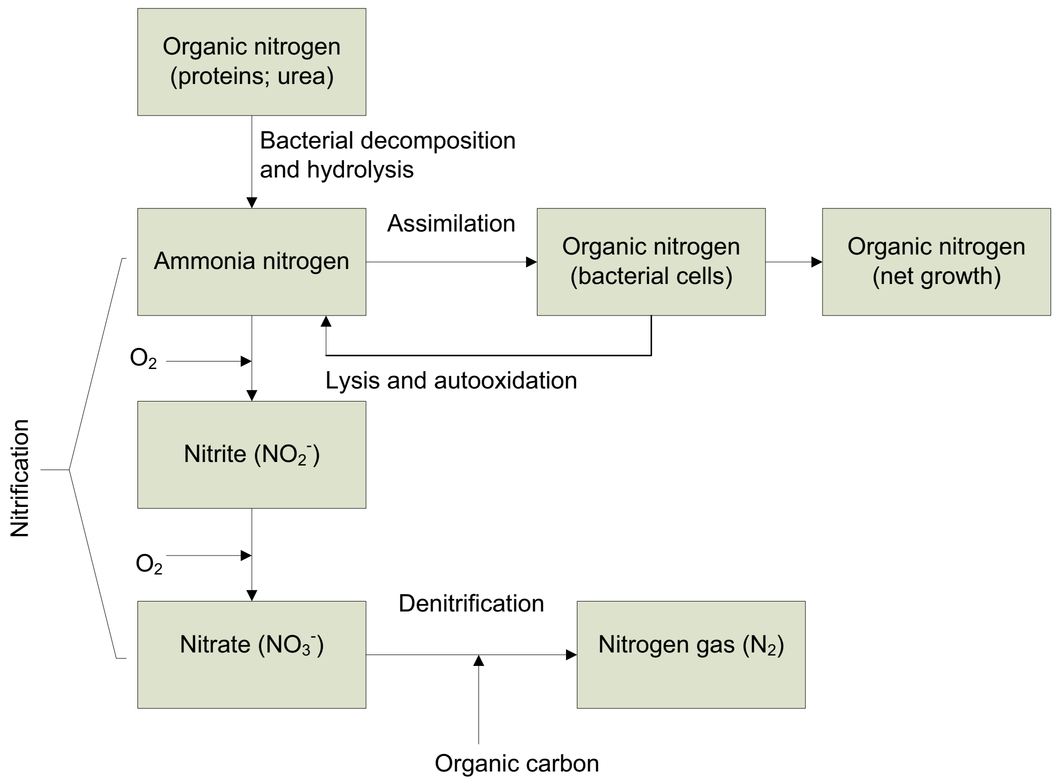

Figure 4. Nitrogen transformations in biological treatment (Tchobanoglous et al. 2003).

2 OZONE

2.1 Physical chemistry

Ozone (O_3) is an allotrope of diatomic oxygen (O_2) with three negatively charged oxygen atoms. The geometry of the ozone molecule is a bent shape, with the bond angle of 117° (figure 5). The ozone molecule is unstable, its heat of formation from oxygen is endothermic, which requires a considerable input of energy. The oxidation potential of 2.07 Volt proves that ozone is a strong oxidizer.

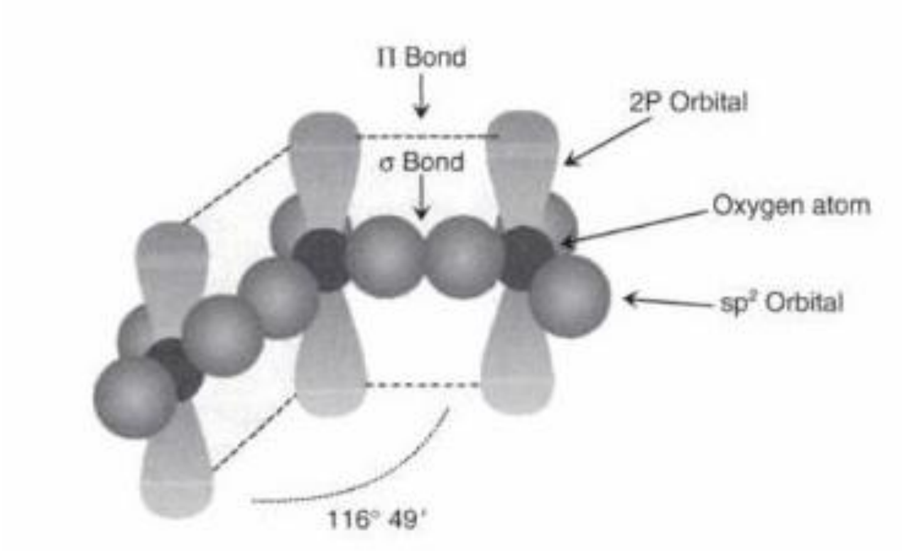

Figure 5. Structure of an ozone molecule. Figure copied from Beltran (2003)

Von Gunten (2003) stated that ozone is the strongest oxidizer available for wastewater treatment. Because ozone is a very reactive and unstable molecule with a short half-life, it has to be generated on site by an ozone generator. When ozone dissolves in water, the molecule can remain as O_3 or it can decompose by several mechanisms (Figure 6), eventually forming the hydroxyl free radical which is a stronger oxidizing agent than is molecular ozone. Several water parameters affect the decomposition e.g. ozone demand, pH, temperature etc. (Rice, 1996). The advantages with ozone are its pure nature and that it does not form any toxic by-products (Von Gunten, 1996). Further information and discussion regarding ozone in water chemistry can be found in Hoigné and Bader (1979).

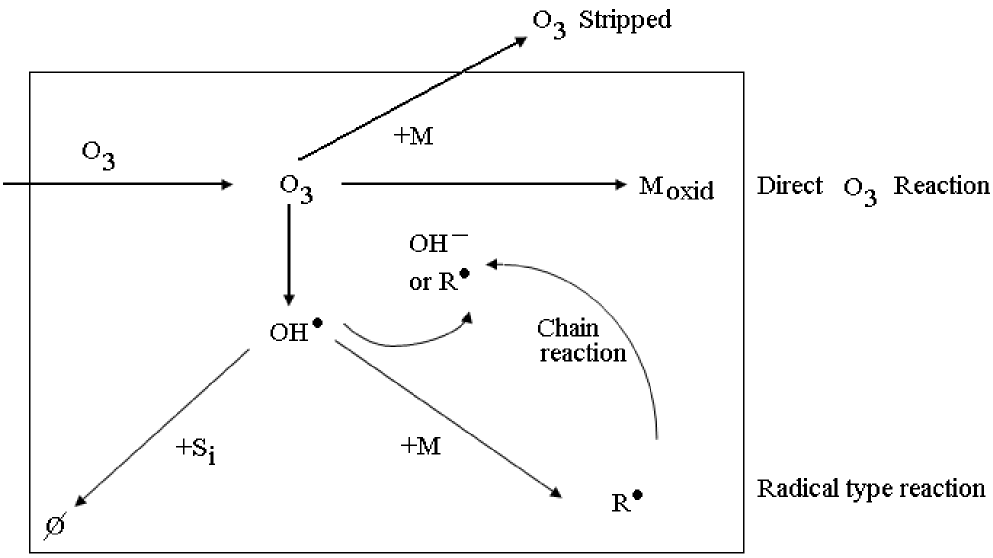

Figure 6. Scheme of reaction of ozone added to an aqueous solution. M=solute, M_{oxid}=oxidized solute, S_i=free radical scavenger i, Ø=products which do not catalyze the ozone decomposition, R=free radicals which catalyze the ozone decomposition (Hoigné and Bader, 1979).

2.2 Ozone generation

The most established way to generate ozone is the corona-discharge. An ozone generator creates ozone artificially with air or pure oxygen gas flowing through an electric field and decomposition of the oxygen molecule through extremely high voltages causes oxygen radical formation. The oxygen radicals bind to oxygen molecules, and forming ozone according to the following reaction mechanisms:

$$1/2\ O_2 \leftrightarrow O \quad \Delta H = +247.4\ kJ$$
$$O + O_2 \leftrightarrow O_3 \quad \Delta H = -103.0\ kJ$$
$$3/2\ O_2 \leftrightarrow O_3 \quad \Delta H = +144.4\ kJ$$

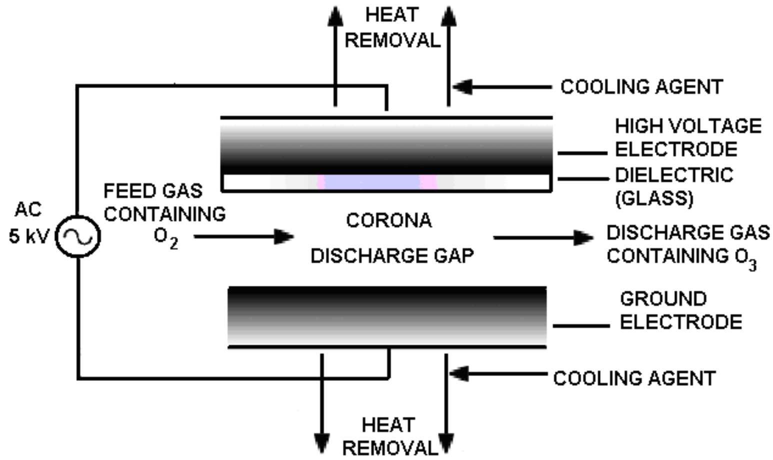

Figure 7. Principle of a basic electrical discharge cell

The yield of ozone formation is basically a function of gas flow and power input. Some main parameters that influence the efficiency is:

- The composition of feed gas i.e. the $N_2 : O_2$ ratio.
- The gas temperature.
- The cooling agent temperature.
- Dew point of the feeding gas.

The humidity of the feed gas also has effects on the discharge properties and on the reaction path. Therefore, the feed gas should normally be dried to a dew point below -60 °C. The efficiency of ozone production also depends on the gap spacing, the pressure, the dielectric, the metal electrode and the electrical circuit (Kogelschatz et al., 1988). The gas flow (V_n) can be calculated using this formula

$$V_n = \frac{100}{\rho} \cdot \frac{M_{O3}}{c} \qquad (1)$$

Where
c = Ozone concentration [wt %]
M_{O3} = Ozone production [kg/h]
ρ = density of the feed gas [kg/m³]

This equation (nr 1) can be rewritten as:

$$M_{O3} = \frac{\rho \cdot V_n \cdot c}{100} \qquad (2)$$

The necessary gas flow and electrical effect can be found using diagrams supplied with the generator. Figure 8 shows the diagrams for the OZAT® OZONGENERATOR TYP CF-6A.

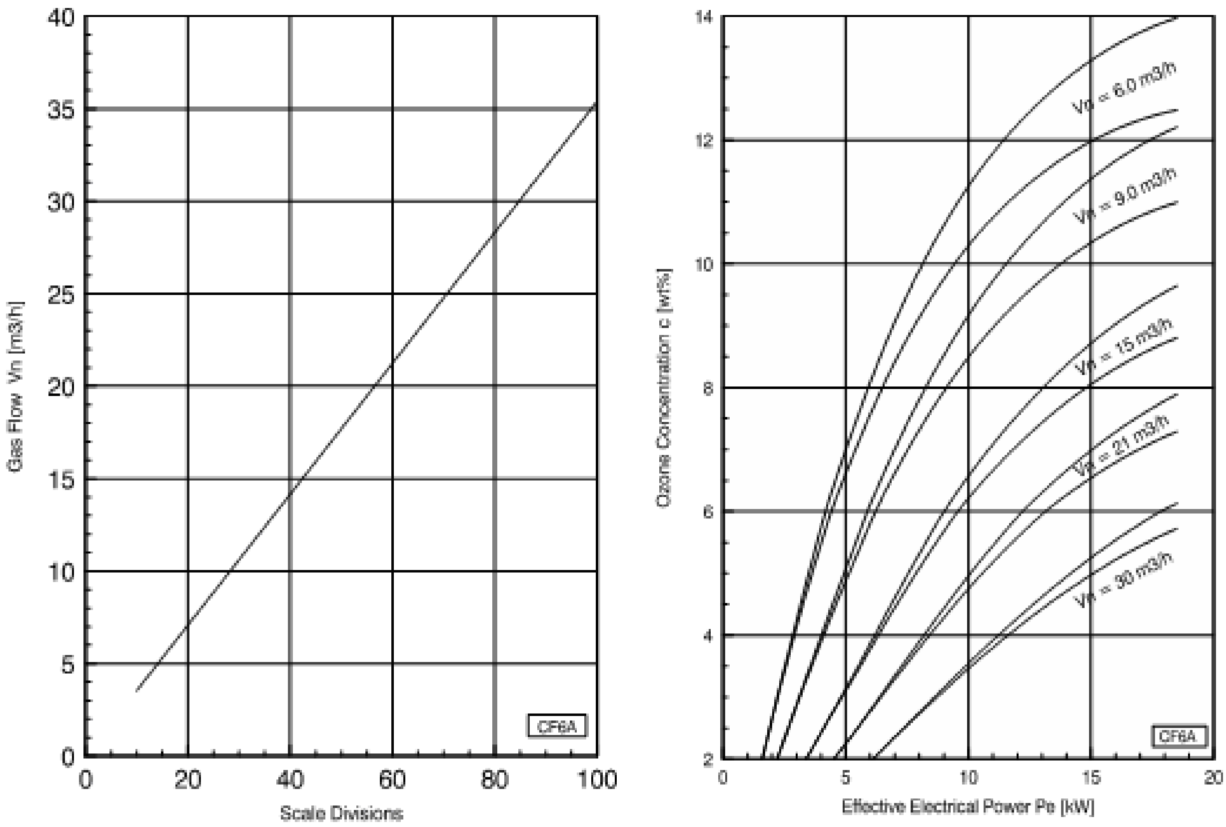

Figure 8 . Gas flow and electrical effect diagram examples for the OZAT® OZONGENERATOR TYP CF-6A.

By using Henrys law, the solubility of ozone in wastewater can be described. Its concentration in the liquid the phase is linearly related to the partial pressure in the gas phase (Lorch, 1987). Ozone is very unstable in water. The decay of ozone in natural water is characterized by a fast initial decrease of ozone, followed by a second phase in which ozone decreases with first order kinetics. Depending on the water quality, the half-life of ozone is in the range of seconds to hours (Von Gunten, 2003).

3 METHODS

3.1 WWTP description

The Himmerfjärden WWTP treats domestic and industrial wastewater using the ASP with a post-denitrification design for nitrogen removal. The effluents enter the recipient bay, Himmerfjärden which empties in the Baltic Sea. An overview is presented in Figure 9.

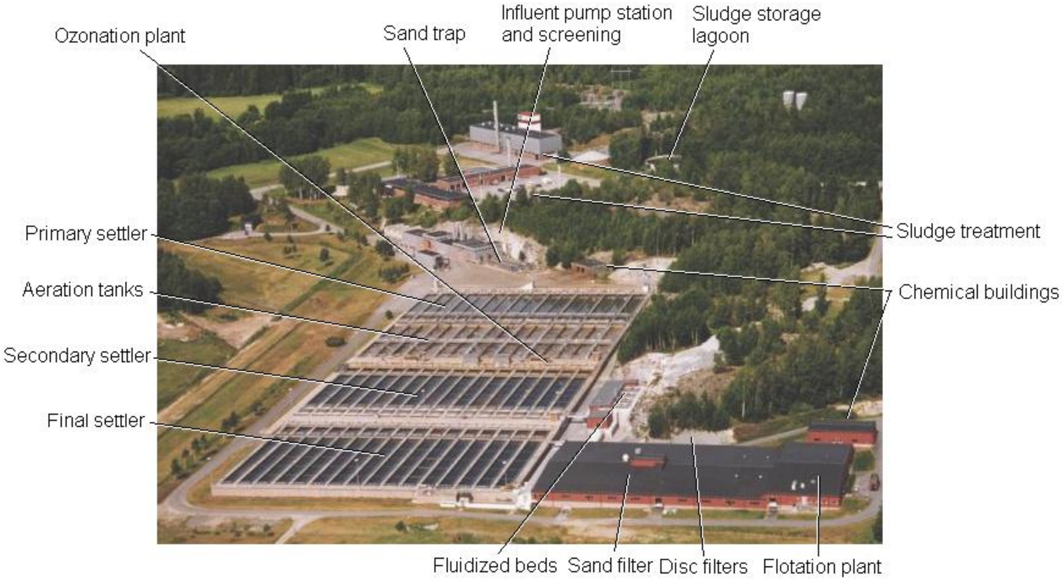

Figure 9. Overview of Himmerfjärden WWTP. The capacity of the biological and chemical unit is 3.9 m^3s^{-1} and towards the mechanical unit 5.2 m^3s^{-1}.

At Himmerfjärden, two basin blocks operate independently of one another, and can be regarded as two separate sub-plants. Prior to the biological treatment, mechanical treatment is applied, and large objects are collected on a grid. The heavy particles are removed in an aerated sand trap. Then lighter particles are removed in a primary settler. At the second stage Iron(II)sulphate is used to precipitate phosphate. No excess sludge is removed from the secondary settlers after the aeration tanks due to sludge bulking problems. The fourth, fifth and sixth stages consists of fluidized beds for denitrification and filters (disc and sand filters) to remove suspended solids. Methanol and ethanol are added to the filters as carbon-source to enhance denitrification. The sludge from the clarifiers is digested to generate biogas. (http://www.syvab.se/)

3.2 Description and operation of experimental plant and reactors

The experimental system used in this study consisted of two of Himmerfjärden WWTPs eight parallel biological treatment lines. The two lines are completely separated as a system. One line had a recycled, ozonated sludge tributary flow (Figure 10), and as a control system a parallel line from the same basin block was chosen. Each line consisted of a 2710 m^3 aeration tank, a 2700 m^3 secondary settler, a 3000 m^3 final settler. In addition, a sludge ozonation unit was connected to the experimental line. The experimental and control line was fed with the same influent and RAS several weeks before the experiment started, then the RAS was separated from the experimental line. The ozone generator was fed with a gas composition of 98 % O_2 and 2 % N_2. The cooling agent consisted of freshwater with a temperature between 20-25 $^{\circ}$C.

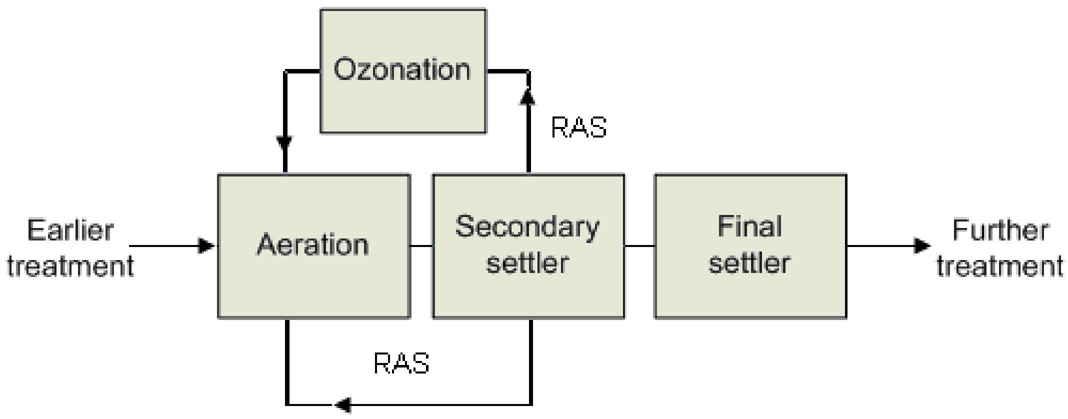

Figure 10. The experiment sequence.

A tributary of the RAS was pumped via a reactor (Figure 11). At the inlet of the reactor an injector consisting of a double venturi system was assembled. The venturi was used to inject ozone into the return activated sludge tributary. The injection of ozone in the return loop optimised the contact with the sludge. This involved dissolution of ozone in the RAS under a vacuum, which is produced by the venturi part along with the high pressure RAS pump. In the reactor, the return activated sludge stream was mixed with ozone and biochemical reactions occurred.

Figure 11. The experimental plant at Himmerfjärden WWTP.

The system was designed with a maximum treatment capacity of 30 m^3/h RAS, which is a tributary corresponding to 10 % of the total, normal RAS flow for one line at Himmerfjärden WWTP. Control runs in the experimental line without ozonation of RAS were also conducted in this study.

With the intention of initially damaging and then controlling the growth of filamentous bacteria, ozone was added into the process continuously during twelve weeks. Ozone dosage in the first 8 weeks was set to a constant value according to table 1. Subsequent the dose was lowered during four weeks, to see if the positive effect remained. After 12 weeks (week 49) the dosing was stopped. This was done in order to evaluate the time taken for the system to reach pre experimental values. The evaluation of the experiment was done by using process data and microscopic analysis (Table 2). By week 52 (after three weeks) several values of the controlled parameters, i.e. the settling properties, reached pre experimental levels.

Table 1. Return activated sludge flow rate and the corresponding suspended solid content, and the dosage for the two periods of operation.

	SS (mg/l) mean values in RAS	RAS flow (m^3/h) mean values	Dose [g O_3/Kg SS]
Period 1 (8 weeks)	4863	18	6.5
Period 2 (4 weeks)	5090	16	4.4

Table 2 Analysed parameters and frequency during the period of operation.

Parameter	*Method*	*Frequency*
Suspended solids after final settler	SS 028112-3	1/week
Suspended solids in return activated sludge	SS 028112-3	1/week
Sludge volume	VAVP24	1/week
Sludge quality index	SS 028113	1/week
COD (influent and effluent to ASP, treated and untreated RAS)	LCK 114	1/week
DOC (influent and effluent to ASP, treated and untreated RAS)	LCK 385 LCK 386	1/week
NO_3-N (Treated and untreated RAS)	LCK 340	1/week
NH_4-N after final settler	LCK 303 LCK 304	1/week
P-tot (Treated and untreated RAS)	SS EN 1189-6	1/week
Microscopic analysis (Total filament growth, extended filament growth and floc form)	Jenkins scale, crystal violet staining	1/week
Microbial screening	FISH, Gram & Neisser staining	Before start and after: 2 weeks, 1 month and 6 months treatment

3.4 Microscopic analysis

The mixed liquid from the aerated tank was continuously monitored by microscopic analysis before, during and after the period of operation. The analyses were performed to identify the abundance of filamentous bacteria, extended growth, total growth, floc shape and size. To evaluate the abundance the Jenkins scale (Jenkins et al., 2003) was used, together with crystal violet staining (Knoop and Kunst, 1998). Double samples were analysed at all times. Samples were also sent to Kemira for FISH analysis of nitrifying bacteria and Gram & Neisser staining of sludge samples to detect filamentous bacteria. The principle of staining is that a sample is stained with different reagents, and one cell component binds the stain more strongly than the other parts of that cell. The Gram staining is performed by first staining the bacteria blue by using a carbol gentian violet, and the rinsing the sample with an alcohol solution. The Neisser performed by first staining the bacteria blue using a methylene solution and a crystal violet solution, after a short contact time a Chrysoidin solution is added, and then the sample is washed with tap water and dried (Eikelboom, 2000). The nitrifying community was analyzed by the FISH gene probes in table 3. FISH is performed by using a probe, which is matching with a unique part of one of the RNA molecules in the ribosomes. The probe is labelled with a fluorescent molecule, which can be visualised when illuminated with ultraviolet light (Jenkins et al. 2003).

Table 3 *Gene probes for FISH analysis.*

Bacteria	Probes	Target	Abundance
Ammonia oxizing bacteria, (AOB)	Nso 1225	*Nitrosomonas* sp	Broadest probe for AOB. Not very good signal
	Cluster 6a	*Nitrosomonas* sp	Targets fewer AOB Gives better signal than Nso 1225
	Nsm 156	*Nitrosomonas* sp	Abundant in some WWTPs
	Nse1472	*N. europea*	Very abundant
	Nmo 218	*N. oligotropha*	Very abundant
	Nsv 443	*Nitrosospira*	Rare but occur in some WWPs
	Nit	*Nitrobacter*	Rare
Nitrite oxizing bacteria, (NOB)	Ntspa 662 + comp	*Nitrospira* sp.	Broadest probe for NOB

3.5 Analysed parameters

Suspended solids, COD and DOC

The objectives with these tests are to get measurements of the amount of suspended matter, chemical oxygen demand and dissolved organic carbon present in the sample. One limitation of the COD test is the incapability to distinguish between biologically inert organic and biologically oxidizable matter (Sawyer, 1994), therefore DOC tests were performed for validation. These tests were performed by standardised laboratory test (Table 2) and the presented values are all mean values of three samples.

Settling properties

The sludge quality index (SQI) is used in routine process control to monitor the settling characteristics of activated sludge.
Fitch and Kos (1976) has suggested the following calculation of the sludge index

$$SQI = \frac{(Settled\ volume\ of\ sludge)}{(Suspended\ solids)}$$

For Sludge Volume < 300 ml/l

And

$$SQI = \frac{\left(200 + \frac{Settled\ volume\ of\ sludge}{3}\right)}{(Suspended\ solids)}$$

for 300 ml/l < Sludge Volume < 800ml/l.

The 30-min settled sludge volume of a biological suspension is used to determine the SQI.

Nitrogen and phosphorus

Nitrate was measured in the treated and untreated RAS to evaluate if the sludge contained unoxidized nitrogen. The appearance of ammonia in the effluent from tertiary treatment were used for the evaluation of the treatment result, since bacteria oxidize ammonia to nitrite during the nitrification, differences between the experimental and control line could indicate a inhibition of the important, slow-growing nitrifiers. Microorganisms contain phosphorus, so it could be used for the determination of cell lysis during the experiment. The determinations of total phosphorus in liquid samples were performed by photometrical methods. These tests were performed by standardised laboratory test (table 2) and the presented values are all mean values of three samples.

4 RESULTS

4.1 Enhancement of sludge quality

Ozone treatment of the return sludge had an immediate effect on sludge bulking, as can be seen in the picture below taken after 4 weeks of treatment (Figure 12).

Figure 12. The experimental and control line. The surface of the control line (right) is covered with sludge, and the experimental line (left) has a clear surface of water.

The sludge settling properties were enhanced and the lower the SQI value the better the sludge settling properties. After one weeks of dosage, there was a 76 ml/g difference between experimental and control line of SQI values, after two weeks the difference was around 100 ml/g (Figure 13). The mean value in the experimental line, for the first period, was 80 ml/l compared to control line which had a mean value of 227 ml/g, i.e. a difference of 147 ml/g. For the second period, with a lower dosage, the difference between both lines was 263 ml/g. One outlier of 500 ml/g the 29/11/2006 is notable (Figure 13), but the reason for this high value of SQI is unknown.

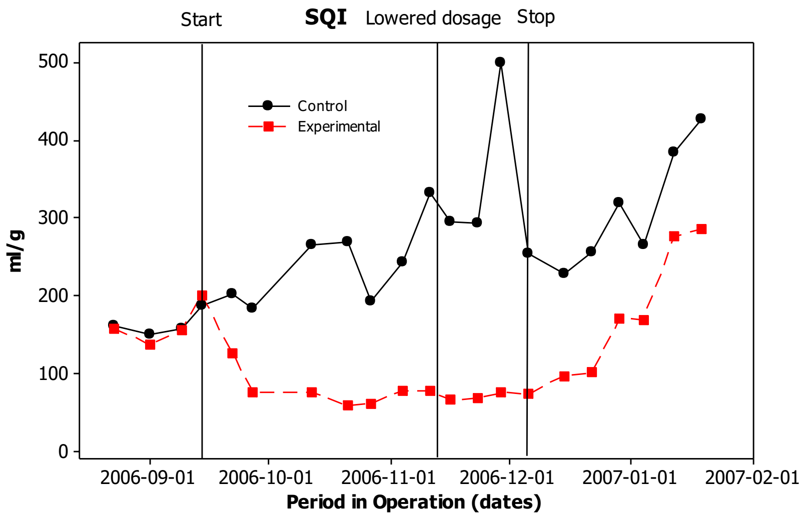

Figure 13. Sludge Quality Index for the control and reference line.

During the period of operation, the suspended solids in effluent of the tertiary treatment were below 10 mg/l, whereas the control line has very fluttering values during this period (Figure 14).

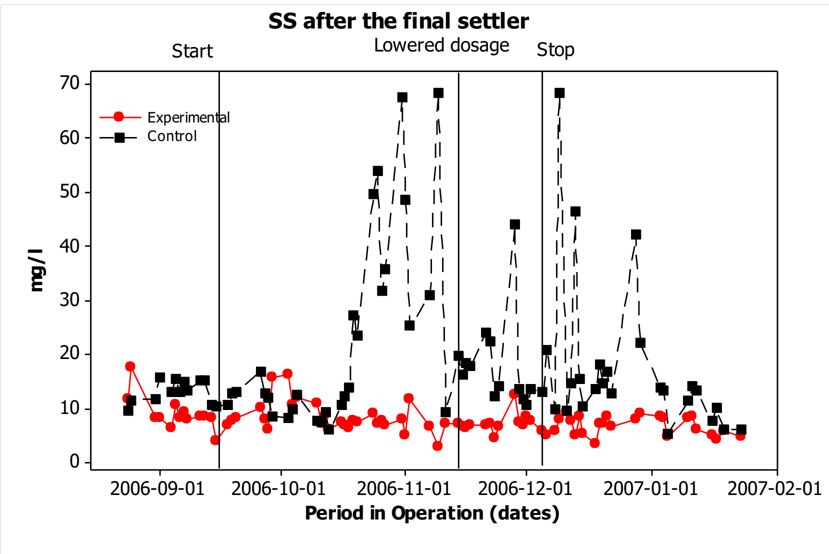

Figure 14. Suspended solids in effluent of the final settler for the control and experimental line.

4.2 Microscopic studies of filamentous microorganisms

The Gram and Neisser staining procedure indicated that the dominant filamentous species in Himmerfjärden ASP is *Microthrix parvicella* (figure 16). The identification of *M. parvicella* was confirmed by gene probes (Mpa 1410) in both the experimental and control line by analysis done by The Kemira company (figure 15).

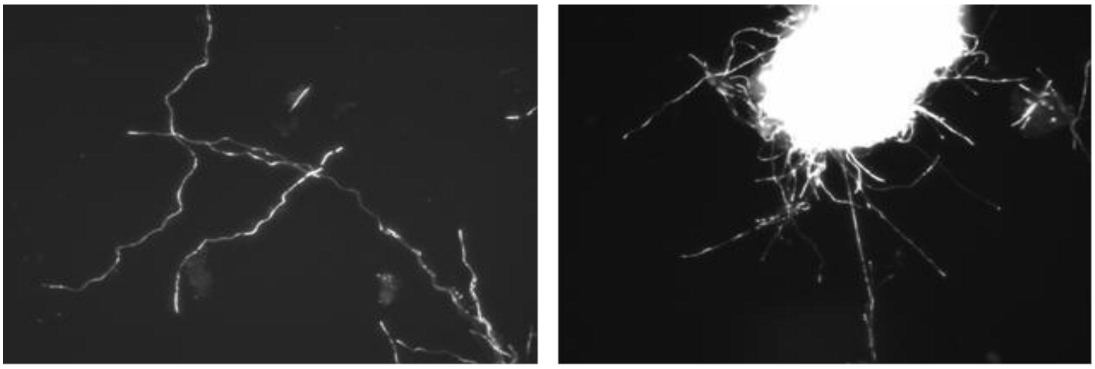

Figure 15. The pictures are from FISH analyse probes performed by Aalborg University/Kemira, and shows several *M. parvicella* in the sludge from Himmerfjärden WWTP.

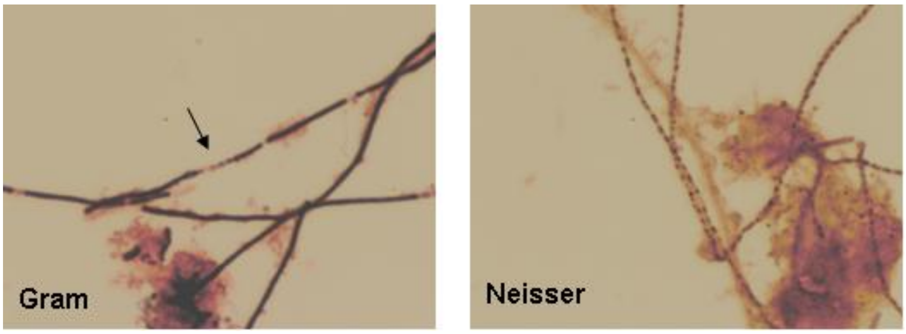

Figure 16. Gram and Neisser staining of the Himmerfjärden WWTPs ASP sludge, showing *M. Parvicella*. These analyses were performed by Aalborg University/Kemira.

Initially there were no differences in abundance of filamentous bacteria observed in the microscope between the samples from the experimental and control line (Figure 17). Filamentous bacteria are twisted inside and capable of forming bridges between sludge flocs which interfere with the settling characteristics of the sludge flocs (Figure 17). *M. parvicella* stain Gram positive, and were full of phosphate granules. After three weeks of dosage the Gram staining showed that *M. parvicella* was either Gram positive with many empty cells or even completely Gram negative. In the control line no changes were observed, *M. parvicella* were present in large numbers.

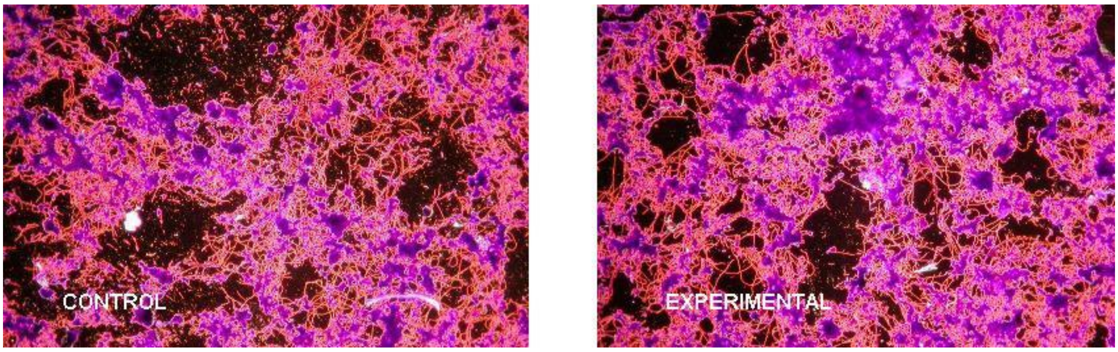

Figure 17. Pictures showing crystal violet staining of sludge samples, showing the total filamentous growth photos are taken three days before the start of ozone treatment.

A change for better shape and floc structure could be seen to begin with directly after start up, and after three weeks the filamentous bacteria in experimental line were damaged permanent (Figure 18). According to Kemiras analysis only a few *M. parvicella* remained inside the sludge flocs. Nematodes and ciliates were not present during any of the analyses and are therefore not taken in for analyse.

Two months after treatment started, the filamentous bacteria were observed merely inside the sludge flocs, only few free filaments were present. The sludge flocs had increased a bit in size (Figure 19). The Gram and Neisser staining showed that *M. parvicella* stained mostly Gram negative and that the phosphorus granules were almost empty. FISH analysis showed that *M. parvicella* was located only inside sludge flocs.

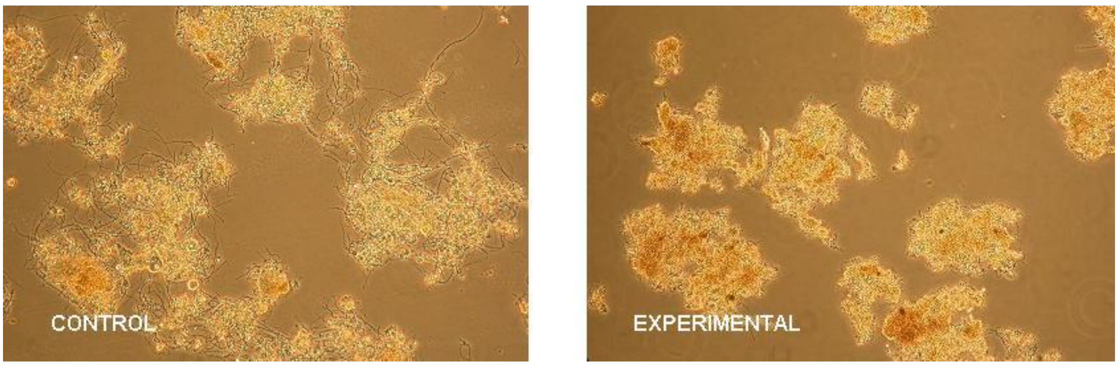

Figure 18. Extended filament growth in the control and experimental line after three weeks of treatment. There is a difference in level, where the control line has abundant extended growth in contrast to the experimental line.

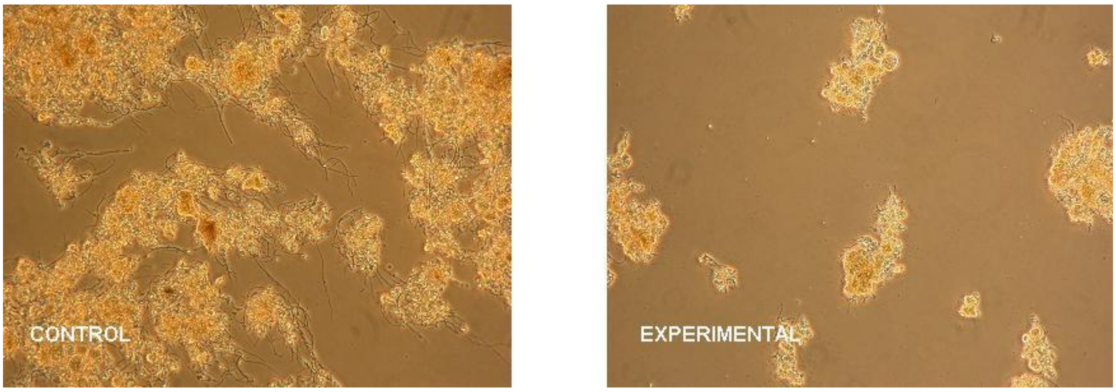

Figure 19. Pictures from the control and experimental line taken two months after treatment started, there were only filamentous bacteria inside the sludge flocs in the experimental line. There is an abundant number in the control sample.

Twelve weeks after the start, three weeks after reduced dosage the sludge flocs were still a bit smaller than in experimental comparative to control line. *M. parvicella* stained Gram positive and contained Neisser positive phosphor granules. The sample from the control line contained numerous *M. parvicella,* which stained Gram positive and contained Neisser positive phosphor granules (Figure 20).

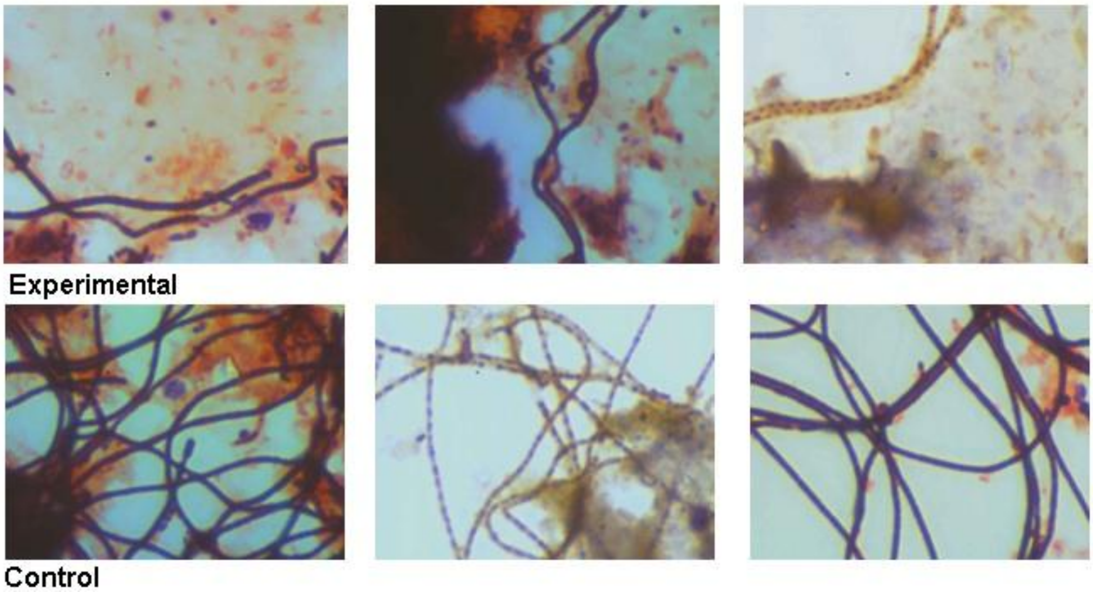

Figure 20. Gram and Neisser stains of sludge samples from experimental and control line.

The result of ozonation gets quite remarkable when looking at the Figures below. The abundance of filamentous bacteria changed rapidly three weeks after the ozone treatment was started, the difference between the mean values for the extended growth for the whole period was 3 units (Figure 21) and the total filament growth (Figure 22) difference between the mean values for the whole period was 2.62 units. A change for better shape and floc structure was observed directly after start up, and after three weeks the filamentous bacteria in experimental line were damaged permanent. The sludge flocs were affected overall, and appeared smaller than before dosage (Figure 23), in addition the ozone treatment resulted in more dense flocs (Figure 24).

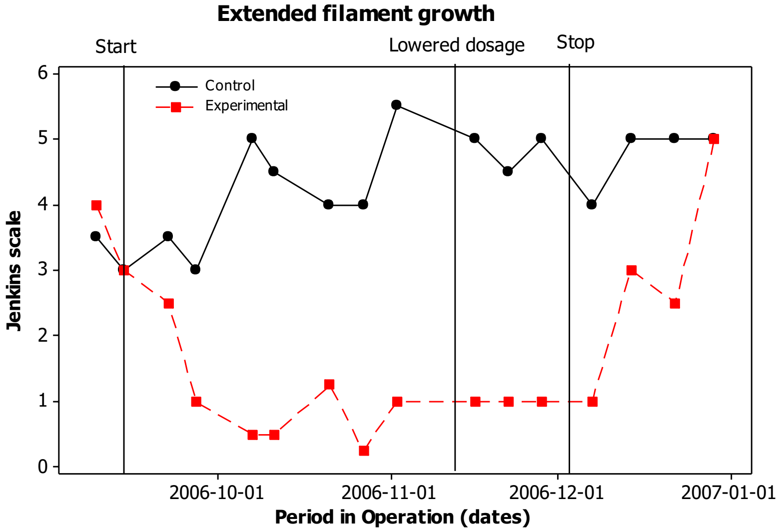

Figure 21. Extended filament growth in experimental and control line according to the Jenkins scale (Jenkins et al. 2003)

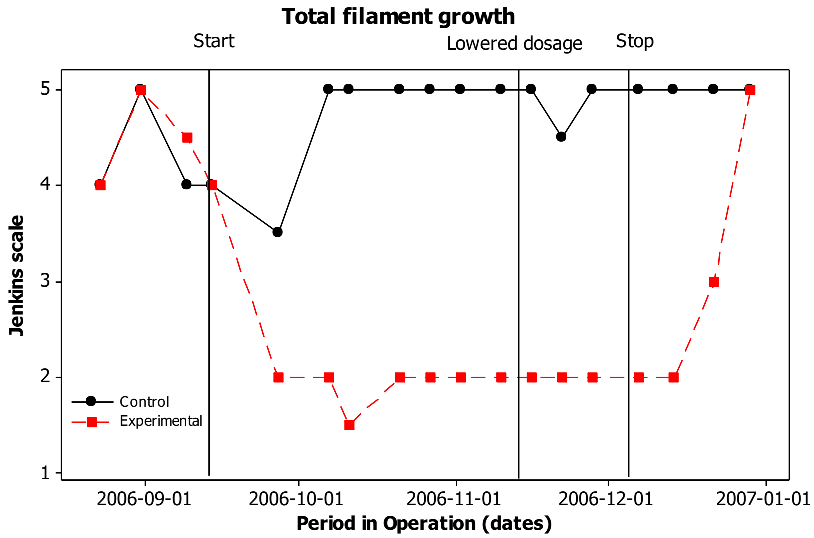

Figure 22. Total filament growth in experimental and control line according to the Jenkins scale (Jenkins et al. 2003)

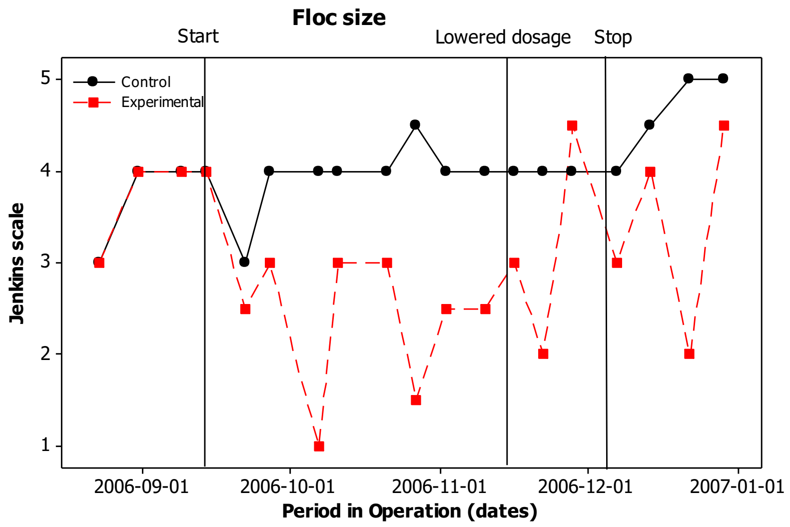

Figure 23. Floc size according to the Jenkins scale (Jenkins et al. 2003)

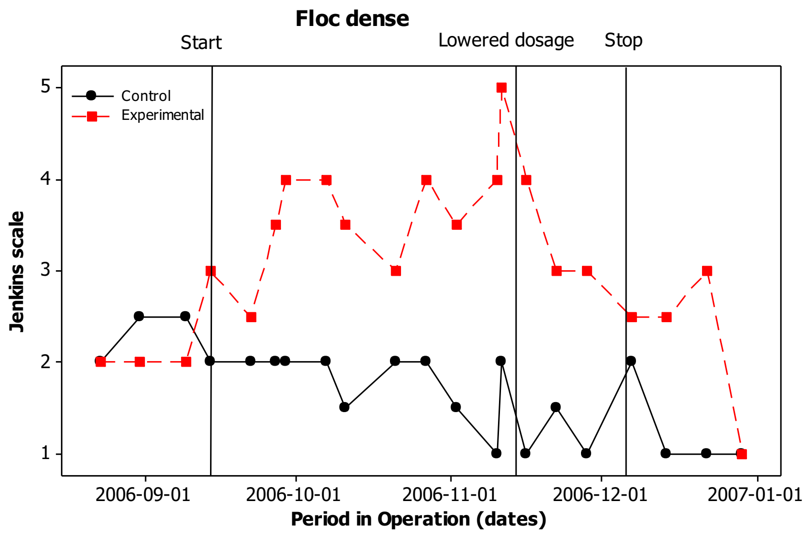

Figure 24. Floc dense according to the Jenkins scale (Jenkins et al. 2003)

4.3 Influence on the return activated sludge

Initially the COD concentration increased in the RAS (Figure 25). After about three-four weeks, the value for the treated and untreated RAS starts to converge. Also the DOC concentration increased (Figure 25) for the treated sludge as expected. After about eight-nine weeks, the value for the treated and untreated starts to converge. A similar trend was observed for total phosphorus (Figure 26).

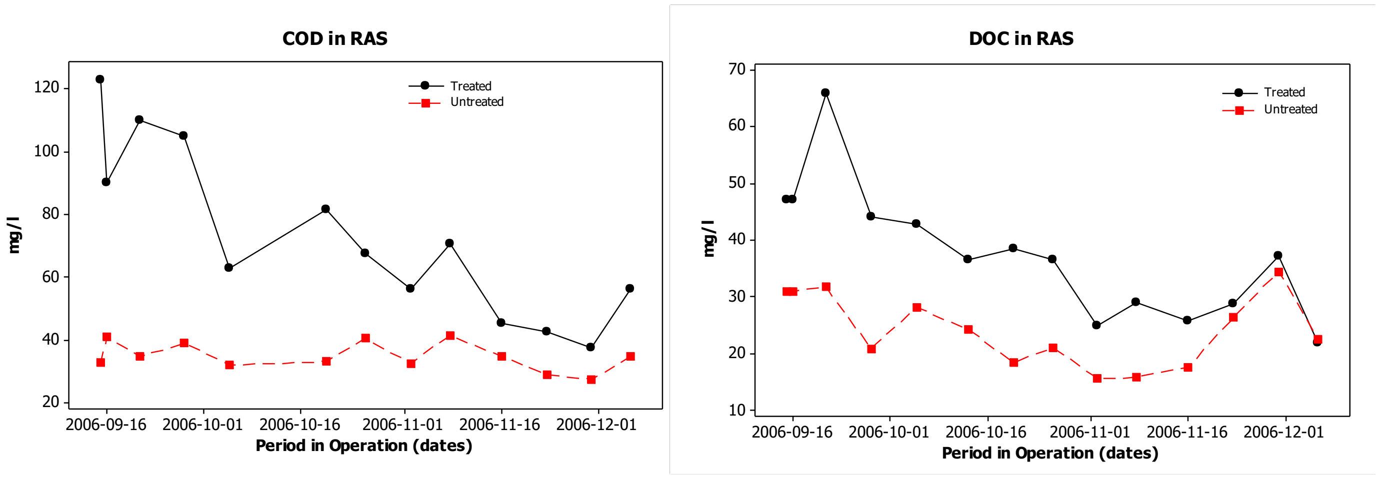

Figure 25. COD and DOC in the treated and untreated RAS in experimental line.

Initially the total phosphorus concentration increased for the treated sludge (Figure 26). After about three-four weeks, the value for the treated and untreated RAS started to converge.

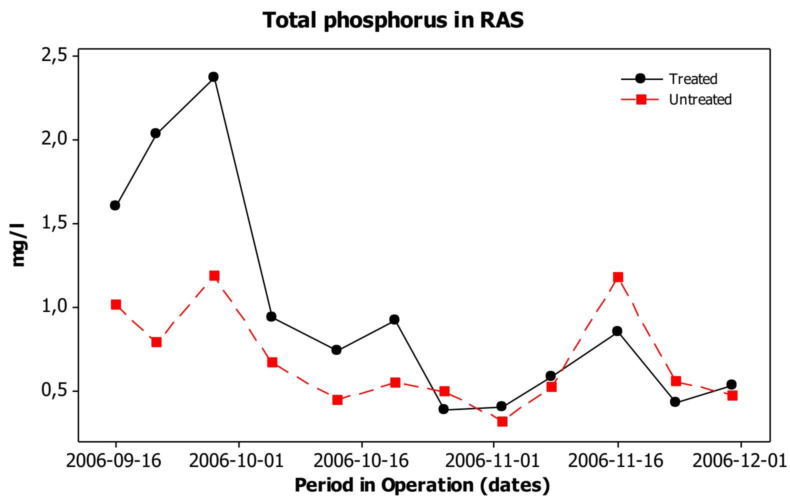

Figure 26. Total phosphorus (P-tot) in the treated and untreated RAS in experimental line.

The mean values of nitrate in the untreated and treated RAS, NO_3-$N_{mean,untreated}$=10.69 mg/l and NO_3-$N_{mean,treated}$=10.98 mg/l for the whole period indicate that there is no vital differences between the experimental and control line (Figure 27).

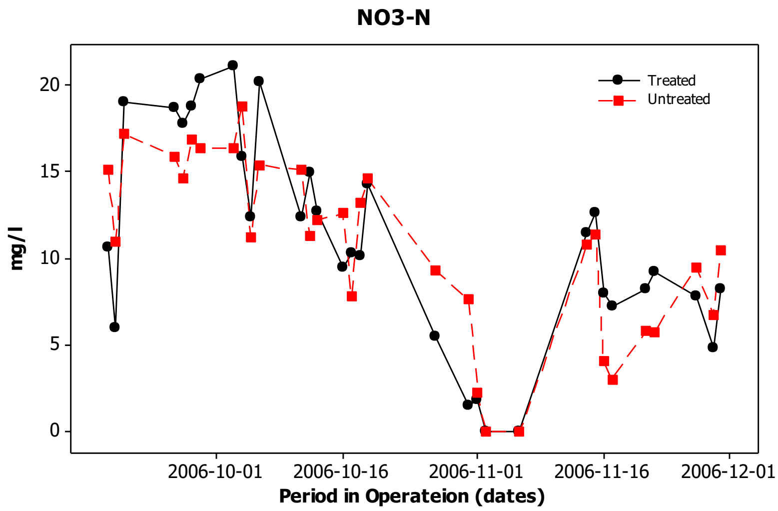

Figure 27. Nitrate (NO_3-N) in the treated and untreated RAS in experimental line.

4.4 Treatment results and effects on RAS

There are no significant differences between influent concentrations and the removal efficiency of COD (Figure 28) or DOC (Figure 29) between the two lines.

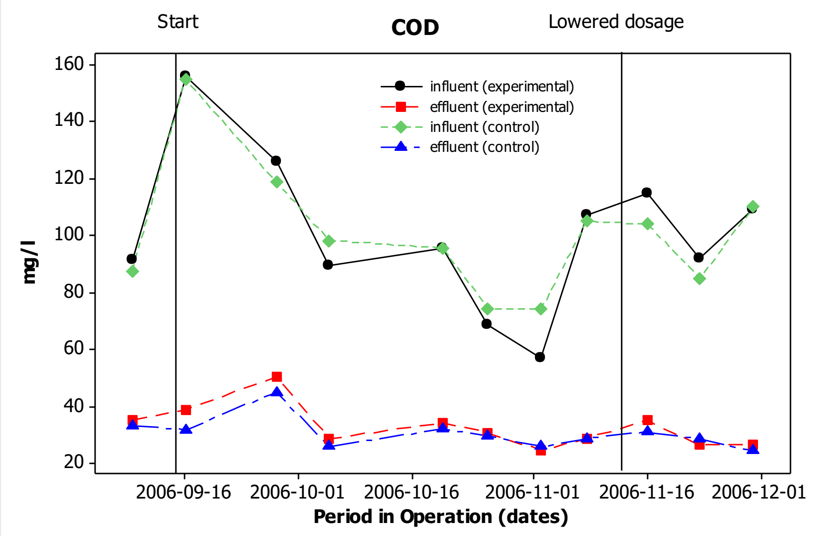

Figure 28 Influent and effluent COD values for the control and experimental lines.

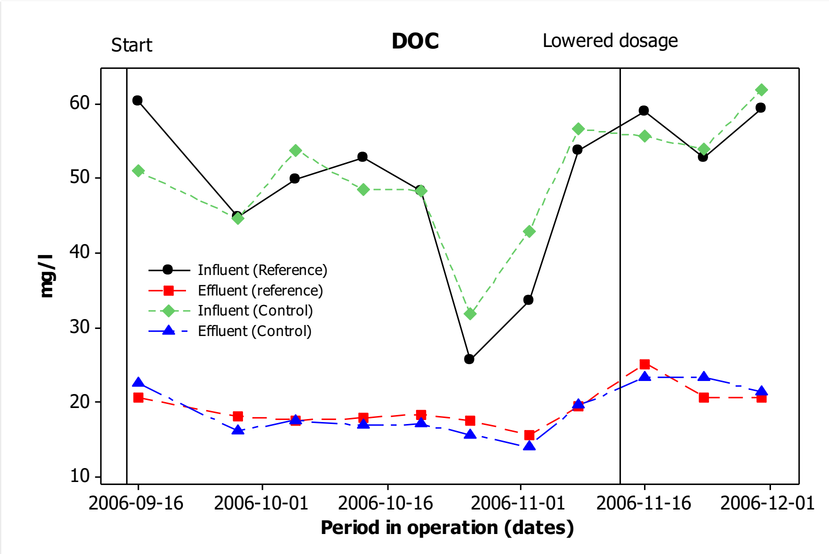

Figure 29 Influent and effluent DOC values for the control and experimental lines.

4.5 The nitrifying community

No significant difference in measurements of ammonia in the effluent from tertiary treatment were observed (Figure 30), $NH4\text{-}N_{mean,\ experimental}$=1.14 mg/l, and $NH4\text{-}N_{mean,\ control}$=1.20 mg/l. This indicates that nitrification was unaffected by the ozone treatment.

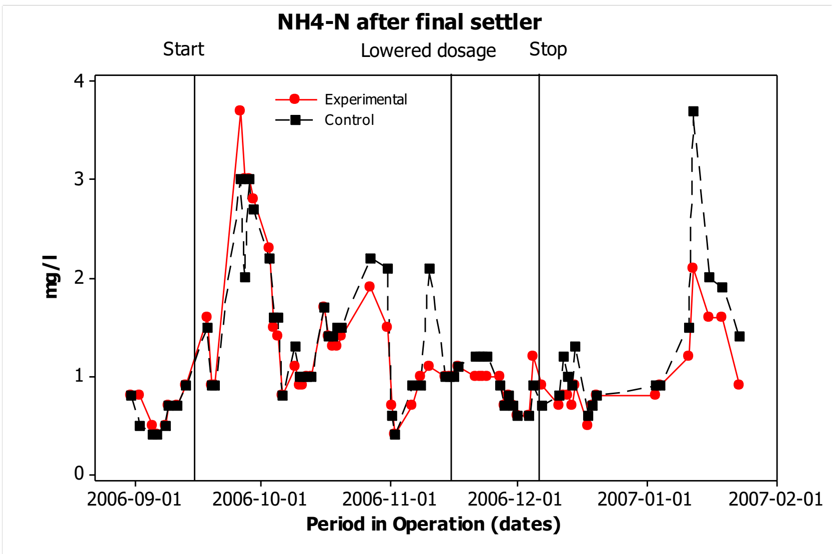

Figure 30. Ammonia in effluent of the tertiary treatment for the control and experimental line.

The AOB and NOB populations were characterized and quantified before and after dosing of ozone. Positive signal for AOB was observed using probes Nso1225, Cluster 6a and Nmo218
No significant difference between the size of AOB population between the control and experimental line was found. After two months a increase in both AOB and NOB populations were observed in the control line as well as the and experimental line. After three months large changes in the NOB population were observed, as hardly any nitrite oxidizing bacteria was found in either control or experimental line. The reason for the changes of the NOB population after three months of operation is unknown.

5 DISCUSSION

5.1 Effects of ozone treatment

The result from the ozone treatment done within this project shows a large enhancement of the sludge settling ability in processes suffering from bulking sludge. The conclusion is supported by the low SQI values obtained after ozone treatment. SQI are an established parameter to characterize sludge settling properties (Henze et al., 1995). High SQI values are typically related to filamentous growth. The control line gave varying values for several parameters during the period of operation, which shows problems with bulking sludge, causing solids to escape the post-treatment.

Ozonation of return activated sludge has in this work been proven to be an effective technique for sludge bulking control at Himmerfjärden WWTP. It has been shown that relatively small ozone doses can be used to demolish the filamentous sludge structures and control the re-growth of filamentous bacteria. The sludge samples from both the control and experimental lines, after that the ozone dosage was lowered, showed that *M. parvicella* stained Gram positive and contained Neisser positive phosphor granules. This result could indicate that the lowered dosage ozone had lower impact on the *M. parvicella* which could influence the rate of re-growth.
Ozone being a non-selective chemical agent affects filamentous and floc forming micro-organisms (Van Leeuwen, 1992). Due to its high redox potential, the impact of ozone on microorganisms is the strongest of all chemicals in use (Rice, 1996, Lorch, 1987). Surveys

indicate that the inactivation of bacteria with ozone is a direct result of cell wall disintegration, and secondary substrates are released into the liquid (White, 1999). Hunt (1999) has performed inactivation of bacteria, and states that the inactivation process is a very complex heterogeneous occurrence which comprise various mass transfer steps and reactions. The oxidizing agent must first diffuse toward the microorganisms' surface, and permeate into the membrane and cytoplasm. The inactivation occurs when vital constituents suffer a certain level of irreparable damage. The spreading advantage of filamentous bacteria likely becomes their disadvantage when it comes to ozone treatment of the RAS.

The ozone technique has also proven effective for excess sludge production management. Salhi et al. (2003) explains the reduction of excess sludge production by ozone and points out the advantages of using this technique. They also confirm that in a continuous process, a 100 % organic excess sludge production reduction can be achieved. Déléris et al. (2003) stated that excess sludge production reduction is one of the most important economic and environmental issues for the next decade.

The increase of the values for COD, DOC and total phosphorus in the treated and untreated RAS is probably an effect of released cell content due to the oxidizing effect of ozone. When the bacteria lysis it leads to an increase of the concentration in the RAS, this effect fades out whereby the filamentous bacteria culture is extinguished and the values for the experimental and control line starts to converge. The results of ammonia concentration in the effluent show that an inhibition of the nitrifying bacteria didn't occur by the treatment of ozone. This was confirmed by abundance and composition of nitrifiers were unaffected by ozone treatment. The FISH analysis of nitrifiers result agrees with the earlier results, that ozone doesn't disturb the nutrient removal. Instead it could affect the effluent quality positively. The sensitive phosphate removing bacteria and the slow growing nitrifying bacteria do not get inhibited by the ozone treatment and the removals of organic and suspended substances can be improved by the treatment (Van Leeuwen, 1988a, 1988b, 1989, 1992).

The results from this study motivate the use of ozone treatment of the return activated sludge from the secondary clarifier in order to defeat problems with bulking sludge. However, large scale experiments will be needed to fully evaluate the efficiency of ozone treatment and in order to optimize related parameters like oxygen flow relative to power input, flow rate of RAS, and to assess the influence of sludge parameters like SS. Work concerning the automatic control of the system should also be considered.

5.2 Practical consideration for ozone generation

For full scale ozone production it is convenient to consider installation of an oxygen generator or purchasing gas from a gas-delivery company. During the course of this project, ozone was generated from liquid oxygen, which was delivered by Air Liquide and stored on site. When considering the alternatives for ozone production it is important to take in to account that ozone generation in air is more complicated than it is in pure oxygen. In air the presence of nitrogen provides additional reaction paths for the formation of ozone, and at very high specific energies, the performance of the discharge changes in a radical way – the ozone formation breaks down completely and all previous generated ozone is destroyed (Kogelschatz et al. 1988).

Two examples of oxygen generation design that could be implemented at a WWTP like Himmerfjärden (Tchobanoglous et al. 2003):

1. Pressure swing adsorption (PSA)
   PSA uses a multibed adsorption process which provides a continuous flow of oxygen gas. It is operated in a cycle of two steps: Adsorption by high pressure separates the oxygen from the feed air, and the adsorbent is regenerated to low pressure.

2. Cryogenic air separation process
   The first step of the process involves liquidisation of air. This is followed by fractional distillation to separate it to its components: nitrogen, oxygen and argon.

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