USING OZONATION AND ALTERNATING REDOX POTENTIAL TO INCREASE NITROGEN AND ESTROGEN REMOVAL WHILE DECREASING WASTE ACTIVATED SLUDGE PRODUCTION

by

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A Thesis

Submitted to the Faculty of Graduate Studies in Partial Fulfillment

of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

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ACKNOWLEDGEMENTS

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ABSTRACT

The effectiveness of partial ozonation of return activated sludge for enhancing denitrification and waste sludge minimization were examined. A pair of nitrifying sequencing batch reactors was operated in either aerobic or alternating anoxic/aerobic conditions, with one control and one ozonated reactor in each set. The amount of solids decreased with the ozone dose. Biomass in the anoxic/aerobic reactor was easier to destroy than in the aerobic one, generating approximately twice as much soluble chemical oxygen demand (COD) by cell lysis. Increased COD favoured production of extracellular polymers in ozonated reactors, enhancing flocculation and improving settling. Floc stability was also strengthened in prolonged operation in alternating treatment, resulting in declined solids destruction. Dewaterability was better in alternating reactors than in aerobic ones indicating that incorporation of an anoxic zone for biological nutrient removal leads to improvement in sludge dewatering. The negative impact of ozonation on dewaterability was minimal in terms of the long-term operation. Ozone successively destroyed indicator estrogenic compounds, contributing to total estrogen removal from wastewater. Denitrification rate improved up to 60% due to additional carbon released by ozonation. Nitrification rates deteriorated much more in the aerobic than in the alternating reactor, possibly as a result of competition created by growth of heterotrophs receiving the additional COD. Overall, ozonation provided the expected benefits and had less negative impacts on processes in the alternating treatment, although after prolonged operation, benefits could become less significant.

The alternating anoxic/aerobic reactor achieved twice the nitrification rates of its aerobic counterpart. Higher removal rates of estrogens were associated with higher nitrification rates, supporting the contention that the nitrifying biomass was responsible for their removal. The alternating treatment offered the better estrogen biodegradation. Microbial populations in both reactors were examined with fluorescent *in situ* hybridization. Dominance of rapid nitrifiers like *Nitrosomonas* and *Nitrobacter* (79.5%) in the alternating reactor, compared to a dominance of slower nitrifiers like *Nitrosospira* and *Nitrospira* (78.2%) in the aerobic reactor were found. The findings are important to design engineers, as reactors are typically designed based on nitrifiers' growth rate determined in strictly aerobic conditions.

NOMENCLATURE AND ABBREVIATIONS

μ_{max}	maximum growth rate
aE2	17-α-estradiol
ammonification	decomposition of organic nitrogen into ammonia under aerobic or anoxic conditions
AOB	ammonia-oxidizing bacteria
AS	activated sludge, biomass, mixed liquor
ATU	allylthiourea
AUR	ammonia utilization rate
BAT	best available technology
b_{aut}	autotrophic decay rate
b_{max}	maximum decay rate
BNR	biological nutrient removal
BOD	biological oxygen demand
BPT	best practicable technology
C/N	carbon to nitrogen ratio
COD	chemical oxygen demand
CST	capillary suction time
DAPI	4',6-diamidino-2-phenylindole
DGGE	denaturing gradient gel electrophoresis
DO	dissolved oxygen
E1	estrone
E2	17-β-estradiol

EDC	endocrine disrupting compounds
EE2	17-α-ethinylestradiol
ENR	enhanced nutrient removal
EPS	extracellular polymer substances
F/M	food-to-microorganisms ratio (COD/VSS)
FISH	fluorescent in-situ hybridization
GC-MS	gas chromatography - mass spectrometry
HPLC	High performance liquid chromatography
HRT	hydraulic retention time
K_o	half-saturation constants for oxygen
K_{ow}	octanol-water partition coefficient
K_s	half-saturation constants for substrate
K-strategist	microorganism with high substrate affinity and low growth rate
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
NEWPPC	North End Water Pollution Control Center Winnipeg
NO2PR	nitrite production rate
NO2UR	nitrite utilization rate
NOB	nitrite-oxidizing bacteria
NPR	nitrite+nitrate (NO_x^-) production rate
NUR	nitrite + nitrate (NO_x^-) utilization rate
ORP	oxidation-reduction potential (redox)

OSA	oxic-settling-anaerobic activated sludge process
OUR	oxygen uptake rate
PCR	polymerase chain reaction
protonation	addition of a proton to an atom, molecule, or ion
RAS	return activated sludge
RFLP	restriction fragment length polymorphism
r-strategist	microorganism with low substrate affinity and high growth rate
SBR	sequencing batch reactors
sCOD	soluble chemical oxygen demand
SOUR	specific oxygen uptake rate
SRT	solids residence time
SS	suspended solids
SVI	sludge volume index
TCOD	total chemical oxygen demand
TKN	total Kiejdahl nitrogen
TN	total nitrogen
TP	total phosphorus
TS	total solids
TSS	total suspended solids
VSS	volatile suspended solids
WAS	waste activated sludge; excess biomass
WERF	Water Environment Research Federation

WEWPPC	West End Water Pollution Control Center Winnipeg
WWTP	wastewater treatment plant
Y	bacterial yield
YES	yeast estrogenicity screening assay

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1. INTRODUCTION AND OBJECTIVES

1.1. Description of the problem

Organic contaminants are removed in secondary wastewater treatment systems through the metabolic activity of concentrated populations of microorganisms (biomass) in an activated sludge tank. Activated sludge is a suspension of flocs in which living and dead microorganisms, together with inorganic material, are embedded in a polymeric matrix produced and secreted by cells.

Excess biomass produced during the treatment process (called waste activated sludge or WAS) has to be removed in order to prevent accumulation within the system. The process of disposal of WAS and other sludges produced at any wastewater treatment plant may account for 60% of the total plant operating cost. The total cost ranges from $200 to $1000/t dry total solids (TS). Management of the excess sludge production then becomes an important environmental issue. Most municipal wastewater treatment facilities will soon be upgraded to biological nutrient removal (BNR) as government regulators impose nitrogen and phosphorus effluent standards. Typically, the expected best practicable technology effluent (BPT) level permits would require effluent total nitrogen (TN) of 10 mg/L and total phosphorus (TP) concentration of 1 mg/L (Oleszkiewicz and Barnard, 2006).

For the case of fragile receivers such as estuaries, oligotrophic lakes or certain trout streams, the effluent permits may be set as low as TN < 3 mg/L and TP < 0.1 mg/L.

Achieving such low levels requires application of the best available technology (BAT). Application of BAT aggravates the operational problems already faced by BPT plants which include filamentous sludge bulking, biological scum formation, deficiency of easily bio-degradable chemical oxygen demand (COD) for denitrification and enhanced biological phosphorus removal. Upgrades to existing conventional biological treatment systems are done by increasing the solids residence time (SRT). The magnitude of SRT increase is limited by the clarifier's capacity to settle the biomass. This is due to the fact that SRT increase is achieved by decreasing biomass wasting which increases the mixed liquor suspended solids concentration (MLSS). There clearly is a need to decrease the mass and concentration of solids at longer SRT as a substantial portion of the solids is inert and does not contribute to enhanced removal. Simultaneously, the decrease of solids concentration and mass in the reactor should lead to a lesser mass of waste activated sludge and thus lower overall plant operating costs. To engineer the minimization of the biomass production, essential aspects of the treatment process, such as efficiency of removal of organic matter and nutrients from wastewater and maintenance of the reactor and settling properties of the biomass, should be considered.

Several methods for minimizing the amount of biomass produced during wastewater treatment have been identified. They include oxic-settling-anaerobic (OSA) activated sludge process (Saby et al., 2003, Chen et al., 2003), chemical uncoupling metabolism (Liu, 2003, Low and Chase, 1998, Yang et al., 2003, Wei et al., 2003), and predation of bacteria (Lapinski and Tunnacliffe, 2003, Griffiths, 2002). Several strategies based on microorganisms' cell wall breakage have been proposed, including advanced oxidation

processes using O_3, Cl_2, H_2O_2, thermal treatment using temperatures from 40 ^oC to 180 ^oC, chemical treatment using acids and alkali, mechanical disintegration using ultrasound, pulsed power or high-pressure homogenizers (Pincince and Borgatti, 2003, Yoon et al., 2003). Among the advanced oxidation processes, ozonation was selected for examination of excess sludge minimization in this research. It is a technique which besides minimization of WAS production, can help alleviate emerging operational problems with BNR reactors such as growth of poorly settling filamentous microorganisms (Weemaes et al., 2000). The most significant anticipated drawback of this method is possible inhibition of the nitrification process (Boehler and Siegrist, 2004).

1.2.   The proposed technique - ozonation

Ozone (O_3) is widely used in drinking water and wastewater disinfection processes. It is a strong chemical oxidant with one of the highest redox potentials of commonly available compounds. Ozone oxidation destroys the cell walls of microorganisms and releases cytoplasm comprised of water, nutrients such as ammonia, enzymes, ribosomes, and soluble organic compounds to the bulk solution (Beltran, 2003). The free radicals formed during ozone decomposition reactions, HO_2 and HO, are probably the active form in the disinfection process. They react with organics in solution and themselves are lethal for the microorganisms.

Ozone generation systems require a power supply, facilities to prepare feed gas (air, oxygen enriched air or pure oxygen), ozone generator, ozone contactor with the treated liquid, and facilities for the destruction of the off-gas. Ozone generation (the conversion

O_2 to O_3) is the most expensive part of the process, equal to 45 to 60% of operating costs depending whether high-purity oxygen or air is used as feed (Metcalf and Eddy, 2003).

Biodegradability of sludge is increased because ozone hydrolyzes solid organic particles within wastewater treatment systems (Beltran, 2003). For this reason, ozonation has recently been adopted as pretreatment before anaerobic sludge digestion leading to increased generation of biogas (Muller et al., 1998; Weemaes et al., 2000; Goel et al., 2003). Ozonation has also been reported to remove persistent organic compounds such as estrogens with high endocrine disrupting potential from the wastewater (Birkett and Lester, 2003).

To reduce excess biomass production, ozonation has been introduced to the return activated sludge (RAS) line in activated sludge systems (Yasui and Shibata 1994; Sakai et al., 1997; Egeman et al., 2001; Figure 1.1). The process consists of a biomass (sludge) ozonation stage followed by a biodegradation stage. A fraction of the recycled sludge passes through the ozonation unit. Two processes occur here: solubilization of sludge solids to biodegradable organics (due to disintegration of suspended solids (SS)) and, to a lesser extent, mineralization to CO_2 and H_2O (due to oxidation of soluble organic matter). The treated sludge is then recycled to an aeration tank where soluble organic substances generated during sludge disintegration are degraded biologically (Yasui and Shibata, 1994; Ahn et al., 2002; Boehler and Siegrist, 2004).
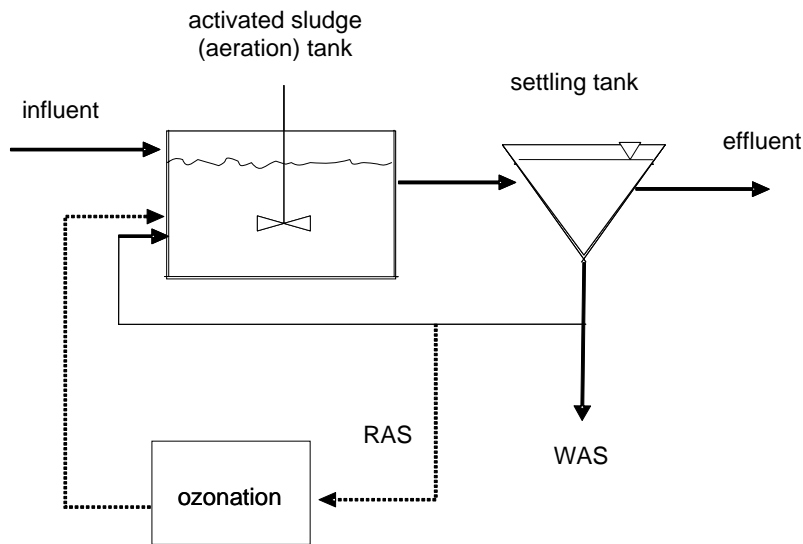
Figure 1.1. Schematic of an ozonation system

A decrease in sludge following ozonation and degradation in an aerobic tank reduces the amount of wasted activated sludge (WAS) from the system (Figure 1.1). The possibility of running a process without excess sludge production (100% reduction efficiency) has been confirmed in full-scale studies, although the process was operated at low food-to-microorganism rate (F/M = 0.045 kg BOD/kg SS per day; Yasui et al. 1996). For their experiments, Yasui et al. applied doses as high as 0.30 g O_3/g SS of initial excess sludge.

1.3.   Impact of partial RAS ozonation on total process performance

Figure 1.2 depicts the numerous impacts ozone oxidation has on sludge and on sludge properties in activated sludge systems. Ozone treatment decreases the size of flocs and increases their density when compared to untreated sludge. Networks of filamentous bacteria significantly disappear following ozone treatment while zoogleal bacteria thrive

inside flocs (Weemaes et al. 2000, Kamiya and Hirotsui, 1998). Sludge settleability is typically improved following ozone treatment (Weemaes et al. 2000, Kamiya and Hirotsui, 1998).
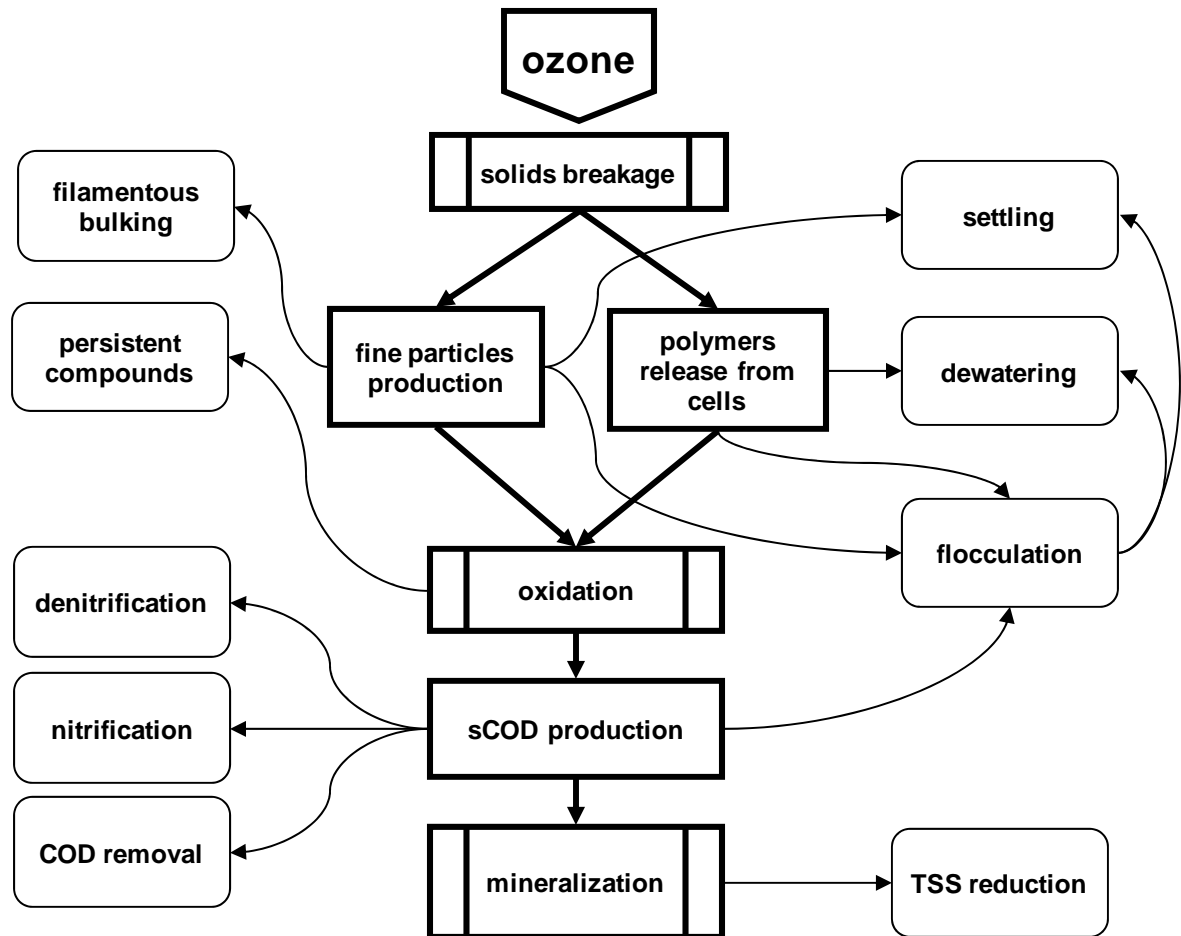
Figure 1.2. Depiction of impacts of sludge ozonation on various aspects of a whole wastewater treatment process

The production of tiny particles increases turbidity and also affects settling and flocculation properties (Keiding and Nielsen, 1997). Through oxidation of organic compounds, simpler compounds are produced and many persistent, potentially non-

biodegradable and often hazardous compounds are destroyed (ex. endocrine disrupters) (Beltran, 2003). The release of polymers from microbial cells enhances flocculation and deteriorates dewaterability (Weemaes et al. 2000). Further oxidation solubilizes polymers generating soluble COD. This additional readily biodegradable carbon has a strong influence on all biological processes that follow: flocculation, heterotrophic COD removal, nitrification and denitrification. The direct killing of the fraction of active biomass is compensated by the generation of additional "easy food" which in turn stimulates microbial activity leading to improved denitrification and biological phosphorus removal (Yasui et al. 1996, Ahn et al., 2002). Wastewater treatment plants in the developed world notoriously suffer from a deficit of biodegradable carbon needed for complete removal of nitrates and phosphorus (Barnard & Oleszkiewicz 2006). Continuous operation of an ozone treatment system may however lead to an accumulation of non-biodegradable compounds. This may result in a decrease in volatile to total mixed liquor solids ratio, MLVSS/MLSS (Yasui and Shibata 1994). Long-term consequences may include a decreased overall activity of biomass nullifying the potential benefits from additional readily biodegradable carbon production (Weemaes et al. 2000).

1.4. Biological nitrogen removal

Heterotrophic denitrification, the biological reduction of nitrate to nitric oxide, nitrous oxide and nitrogen gas, is a process requiring organic carbon as an electron donor for the oxidation–reduction reaction. Carbon deficiency (i.e. high N/COD, P/COD ratio) in typical municipal wastewater is a problem which leads to the high cost of supplying external carbon sources such as methanol and often necessitates the enlargement of the

anoxic zone in the activated sludge tank. When the ozonated sludge is recycled to the anoxic zone, denitrification improves as an additional degradable carbon source is created by ozonation (Ahn et al., 2002, Park et al., 2004). The drawback is that pure oxygen, which is a by-product of ozone breakdown (Metcalf and Eddy 2003), can be introduced into the anoxic zone, creating unfavourable oxidation-reduction potential (ORP) conditions for anaerobic heterotrophs.

The ammonia released from organic compounds and present in wastewater influent is oxidized by nitrifying bacteria in an aerobic, autotrophic process sequentially to nitrite and nitrate. Nitrifiers are the most sensitive species in population of biomass in the activated sludge tank (Metcalf and Eddy, 2003) and require the longest time to reproduce, hence the reactor SRT is determined based on their need (Metcalf and Eddy, 2003). A reduction in nitrification rates after ozonation has been shown to be proportional to the decrease in sludge biomass (Boehler and Siegrist 2004, 2007). Ozonation creates conditions less favourable to nitrifiers. As autotrophs, they obtain their carbon from alkalinity (HCO_3^-) whereas higher COD released from ozonated cells favours heterotrophic growth (Metcalf and Eddy, 2003). Further, during ozonation, additional ammonia is generated. It has also been observed that an increase in ozone dose is followed by decreased sludge pH due to destruction of alkalinity which may drop to as low as 3.0 (Sakai et al., 1997; Weemaes et al., 1999; Park et al., 2004). In full scale plants however, with partial treatment of the return flow with ozone, the pH in the aeration tank was hardly reduced. Other reports are contradictory. Nitrification rates remained constant despite ozonation (Sahli et al. 2003, Sakai et al. 1997). Contrary to

the common contention that nitrifying bacteria reside on the surface of the floc, Boehler and Siegrist (2004) suggest they may be shielded inside the sludge floc due to overgrowth by the faster growing heterotrophs and thus are protected from ozone.

The efficiency of ozonation as a method of excess sludge reduction and its impact on nitrification and denitrification is highly dependent on process operating parameters such as ozone dose, process SRT, or power input for mixing. Therefore research is needed for the optimization of proper ozone dosage and dosing method (Liu and Tay, 2001).

Heterotrophic denitrification can be incorporated into the activated sludge system by converting an initial part of the aerobic tank to anoxic conditions – a process often called preanoxic denitrification. The use of the process has increased recently as regulatory permits call for removal of total nitrogen (ammonia and nitrates) and not just ammonia to protect lakes and coastal waters from eutrophication. A system featuring preanoxic denitrification is generally more cost effective due to savings on aeration and external carbon source. It must be noted however that nitrification in alternating anoxic/aerobic system occurs under significantly different environmental conditions than in a conventional aerated system. A previous report by Lee and Oleszkiewicz (2003) showed that higher ammonia removal rates were achieved in a reactor under alternating anoxic/aerobic conditions than in an identical reactor operated under strictly aerobic conditions. Faster nitrification rates can lead to cost savings in plant design. Thus it is of practical importance to determine the basis for observed faster nitrification in alternating anoxic/aerobic reactors.

1.5.   Objectives

The purpose of the research was to examine the influence of ozonation on sludge minimization and the whole treatment process performance towards final effluent and sludge quality. Nitrification and denitrification performance, bioflocculation processes, as well as settleability and dewaterability of sludge were studied. As an additional goal, the potential of biodegradation of estrogens in secondary treatment and possibilities of their removal through the ozone treatment were investigated.

All experiments were conducted comparing two treatment processes, strictly aerobic and alternating anoxic/aerobic conditions. The impact of ozone on biomass development under nitrifying and denitrifying/nitrifying conditions was evaluated and contrasted. Although a number of earlier lab scale studies employed strictly aerobic and alternating anoxic/aerobic conditions (Boehler and Siegrist, 2004; Deleris et al., 2002), this is the first research aimed at comparing both processes and providing a unique perspective on the effects of ozonation on the two types of treatment.

Additionally, regardless of the ozone application, the employment of the two treatments provided the opportunity to compare the two regimes. The environmental conditions created in reactors were recorded and contrasted, giving the exceptional chance to understand the differences in ammonia removal rates from a microbiological process and diversity approach.

1.6.   Chronology of lab experiments

The objective here is to show the chronology of the research and the progressive age of the biomass which was continually operated. The use of ozone gradually changes the biomass MLVSS/MLSS ratio and the biomass composition. The chronology is important when comparisons are made to literature findings. The experiments reported in literature are often short-term (e.g. few months) and may not reflect the effect of prolonged exposure to ozone treatment and its long-term effects.

1. Activated sludge acclimation period: 22 June 2004 – 19 September 2004 (Chapter 2),

2. WAS reduction, sCOD production, impact on denitrification-nitrification rates and effluent quality: 20 September 2004 – 02 August 2005 (Chapter 3),

3. Flocculation, settling and dewatering properties: 15 March 2005 – 15 August 2005 (Chapter 4),

4. Explanation of higher nitrification rates for alternating anoxic/aerobic treatment: 8 March 2006 – 15 May 2007 (Chapters 5, 6),

5. Transformation of estrogens in activated sludge, destruction of estrogens by ozone: 23 June 2005 – 15 August 2005 (Chapter 7, 8).

2. ACCLIMATION OF ACTIVATED SLUDGE – ACHIEVEMENT OF STEADY STATE

2.1. Objectives

The goal of this thesis chapter is to describe the reactor set up and biomass acclimation and achievement of the steady-state conditions. The reactors were set up to compare the multiple impacts of ozonation under aerobic and alternating anoxic/aerobic conditions. Two reactors were operated for each treatment, one from each set was intended to be ozonated in subsequent thesis chapters and the unozonated reactor served as a control.

2.2. Materials and methods

2.2.1. Assembling of reactors

The laboratory reactors were designed and assembled to simulate a sequencing batch reactor (Figure 2.1). Glass vessels, tubing (Tygon®) and plastic connectors were used; daily operation was supported by peristaltic pumps (Masterflex®) and timers. To ensure homogeneous mixing at all times, each reactor was mixed using a magnetic stirrer No. 3 with a speed of 295 rpm. (Versamix Fisher Scientific). The air was supplied from a laboratory air line with an aeration stone (Fisher Scientific).

Four sequencing batch reactors (SBR) were established with a total liquid volume of 3 L each. The reactors were seeded with biomass from the City of Winnipeg's North End

Water Pollution Control Centre. The Centre operates a non-nitrifying pure oxygen activated sludge BOD removal plant with a design solids residence time (SRT) of 2.5 d.
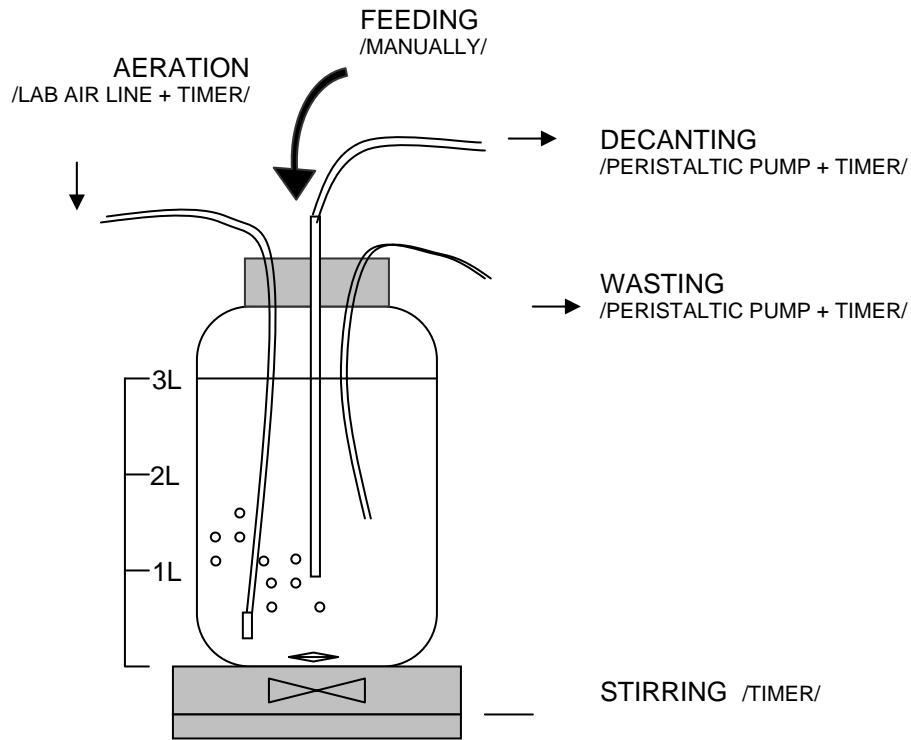
Figure 2.1. Schematic of a sequencing batch reactor.

2.2.2. Operating conditions

The reactors were operated at a temperature of 20 ± 1 ^{o}C achieved by maintaining them in an environmental dark chamber. The design SRT was 12 d while the actual SRT including solids in the effluent was calculated to be 11.2 ± 0.4 d. The actual hydraulic retention time (HRT) was 36 h. The reactors had a sequence of fill, react, waste, settle, and decant and were operated using conditions reported in Table 2.1.

Table 2.1. Operating conditions of aerobic and alternating anoxic/aerobic reactors

Aerobic reactors:

Operation	Time	Air	Mixing
Feeding (2 L)	5 min	-	●
Aerobic reaction	22 h + 10-25 min	●	●
Wasting (0.25 L)	10 min	-	●
Settling	1 h 05 min	-	-
Decanting (1.75 L)	10 min	-	-

Alternating anoxic/aerobic reactors:

Operation	Time	Air	Mixing
Feeding (2 L) + NO_3^- addition	5 min	-	●
Anoxic reaction	3 h + 10-25 min	-	●
Aerobic reaction	7 h	●	●
Anoxic reaction	3 h 30 min	-	●
Aerobic reaction	8 h 30 min	●	●
Wasting (0.25 L)	10 min	-	●
Settling	1 h 05 min	-	-
Decanting (1.75 L)	10 min	-	-

The cycle in the aerobic reactors started when they were filled manually with 2 L of synthetic wastewater consisting of beef and yeast extract as a carbon source and ammonium chloride as an ammonia source (composition in Table 2.2). The feed load was increased twice during the acclimation period by increasing the concentration. Each time 25% higher concentration than the initial one was added (see Table 2.2). After feeding the biomass was stirred and aerated for 22 h 30 min. Wasting of excess biomass was performed by removing 250 mL of reactor content immediately before the end of the aeration phase. The reactor biomass was then allowed to settle for approximately 1 hour

which was followed by decanting of 2 L of supernatant (which is equivalent to effluent), which left 1 L of sludge in the reactors (equivalent to RAS). A new cycle was then started with a new feeding.

The cycle in alternating anoxic/aerobic reactors was similar except the air supply was discontinued for 3.5 h twice during the reaction period, as shown in Table 2.1. Anoxia defined by the measurement of dissolved oxygen and ORP occurred immediately after feeding in the first period of non-aeration. The alternating anoxic/aerobic reactors also received 10 mL of 40 g/L $NaNO_3$ solution at the beginning of the anoxic reaction phase to create a sufficient supply of NO_3^- for denitrification. The ratio between the anoxic period and total time of the cycle in the alternating reactors was 30%.

Dissolved oxygen (DO) in aerobic conditions was set to 5 mg O_2/L and controlled manually using a valve on the pipe connected to the compressed air system. Residual DO was set to 0.1 mg/L for anoxic conditions in the alternating reactor space. The alkalinity remaining in the effluent was high enough to ensure unhindered nitrification. It is generally assumed that alkalinity above 120 mg $CaCO_3$/L is sufficient for unhindered nitrification (Metcalf and Eddy, 2003).

Table 2.2. Synthetic feed composition

Chemical component	Concentration mg/L on days of the experiment		
	1-41	42-55	56- ∞
Beef extract	200.00	250.00	300.00
Yeast extract	200.00	250.00	300.00
$MnSO_4 \cdot 7 H_2O$	5.00	6.25	7.50
$FeSO_4 \cdot 7 H_2O$	2.25	2.81	3.38
KCl	7.00	8.75	10.50
K_2HPO_4	196.25	245.31	294.38
$NaHCO_3$	366.25	457.81	549.38
$CaCl_2$	3.75	4.69	5.63
NH_4Cl	75.00	93.75	112.50
$MgSO_4 \cdot 7H_2O$	50.00	62.50	75.00

2.2.3. Analysis

Total suspended solids (TSS) and volatile suspended solids (VSS) were measured daily for all reactors. Samples were collected during the aeration cycle and analyzed according to Standard Method 2540D and 2540E respectively. Soluble and total chemical oxygen demand (sCOD, TCOD) were measured in quintuplicate using the same sample of the influent and effluent daily by spectrophotometry (Standard Method 5220D, F.S. Spectronic 21, Bausch and Lomb). NH_4^+, NO_3^- and NO_2^- analyses were performed using an automated colorimetric assay (Pulse Instrumentation Ltd and Technicon Autoanalyzer) following Standard Method 4500-NH3-G and 4500-NO3-F (Standard Methods, 1998). The pH was monitored at the end and beginning of the cycle with a pH meter (Accumet Portable AP61, Fisher Scientific,). Alkalinity was determined daily using Standard Method 2320B.5 with potentiometric titration to end-point pH 4.5. The

ORP (redox) was measured with a flow-through and submersion ORP probe (OAKTON, Fischer Scientific,). DO concentration was determined using a DO probe (HQ 10 HACH Portable LDOTM) at the beginning and at the end of the complete cycle. The specific oxygen uptake rate (SOUR) was measured according to Standard Method 2710B.

Samples of mixed liquor were observed regularly using a Leitz Wetzler Germany microscope (at magnifications 400x and 1000x, later using a phase contrast objective). Digital images of the magnified mixed liquor were taken using a Nikon Microscope Eclipse E400 fitted with an Olympus DP70 digital camera and using Image-Pro® Plus software. Approximately 10 slides per sample were evaluated.

2.2.4. Statistics

All data sets, e.g. nitrification and denitrification rate, actual process parameters such as HRT and SRT, biomass concentrations, effluent concentrations, were analysed statistically, and reported as average value with standard deviation over the study period, and analyzed using paired student's t-test with P = 0.05 (Berthouex and Brown 1994). Both replicate reactors and treatments were compared.

2.3. Results and discussion

The measured process parameters are summarized in Table 2.3. As expected, there were differences in some parameters caused by the treatment; yet duplicate reactors were very similar. Each comparison was performed after the system achieved steady-state.

Table 2.3. The main measured process parameters in aerobic and alternating anoxic/aerobic reactors

Parameter	Unit	Aerobic	Alternating anoxic/aerobic	t-test P level
pH (end of the cycle)	-	7.90	8.11	<0.001
Alkalinity (effluent)	mg	140	276	<0.001
VSS/TSS ratio (mixed liquor)	-	0.85	0.79	<0.001
VSS/TSS ratio (final effluent)	-	0.86	0.91	0.108
Total COD (effluent)*	mg/L	39	45	0.001
NO_3^--N (effluent)	mg/L	77.82	52.5	<0.001

* each sample was analyzed five times for COD.

2.3.1. The pH and alkalinity

The feed pH (7.8±0.05) had been set using a buffer (sodium bicarbonate) to provide residual alkalinity in the effluent suitable for the nitrification process to occur. Measured pH and alkalinity in the effluent was higher in alternating reactors than in aerobic ones, likely due to anoxia and denitrification under anaerobic conditions in the alternating reactor. pH during the acclimation phase increased gradually due to the use of more concentrated feed and averaged 7.90±0.17 and 8.11±0.32 in aerobic and alternating reactors respectively .

Alkalinity in the synthetic feed averaged 486±45 mg $CaCO_3$/L. In the effluent it was found to be 140±30 mg $CaCO_3$/L in aerobic and was higher (276±36 mg $CaCO_3$/L) in alternating anoxic/aerobic reactors.

2.3.2. Dissolved oxygen and oxidation-reduction potential

The strategy was to maintain an ample supply of dissolved oxygen (DO) for respiration and nitrification in the aerobic reactors. The rapid oxygen uptake immediately after feeding lowered the DO level in the reactors, however it was always above 3 mg O_2/L. By the end of the cycle the DO was consistent between all reactors at 5.2±0.5 mg O_2/L.

In the alternating reactors, within 10 minutes of commencing anoxic conditions, the DO level was less than 0.2 mg O_2/L. Thereafter the DO level was 0.1 mg O_2/L. By the end of the complete cycle, which was an aeration period, the DO level for all reactors was 5.2±0.5 mg O_2/L. The DO level for the second anoxic period was not measured.

The ORP (oxidation–reduction potential) was analysed occasionally during the acclimation period. It averaged (+)185±45 mV for the aerobic reaction period and (-)237±14 mV for the anoxic reaction period. ORP was in the proper range to assure nitrification and denitrification, in either the aerobic or anoxic period.

2.3.3. Specific oxygen uptake rate (SOUR)

SOUR, defined as oxygen uptake rate per unit mass of volatile suspended solids, is considered a measure of biomass respiration. When oxygen is not limited, SOUR depends primarily on the availability of easily biodegradable carbon and the presence or absence of inhibitory compounds or conditions. SOUR was high (32.5 to 50.4 mg O_2/g VSS h) just after feeding and decreased thereafter being low (5.4 to 13.6 mg O_2/g VSS h)

by the end of the complete cycle (before wasting) SOUR was slightly lower during the anoxic period in alternating reactors (33 ± 8 mg O_2/g VSS h) than during the same reaction period in aerated reactors (41 ± 13 mg O_2/g VSS h) indicating that under anoxic conditions microbial activity could decrease.

2.3.4. Suspended solids

Total and volatile suspended solids in the mixed liquor (MLSS and MLVSS) were measured every day to observe the progress of acclimation of biomass to new conditions. It took three SRT after the implementation of the new feed concentration to achieve steady state MLSS conditions at a level around 1773 ± 223 mg/L in aerobic reactors and 1734 ± 210 mg/L in alternating reactors. There was no difference in TSS levels for the two treatments.

The ratio of VSS/TSS was monitored to determine the fraction of non-biodegradable particles in the sludge. During a period of $1\times$SRT, the content of VSS in TSS increased rapidly due to the addition of soluble biodegradable feed and stabilized at the level of 0.85 ± 0.02 in aerobic reactors and 0.79 ± 0.04 in alternating aerobic/anoxic reactors. This phenomenon can be explained by chemical mechanisms. Higher pH in the alternating than in aerobic reactors could favour precipitation of mineral fraction (Metcalf and Eddy, 2003) resulting in smaller fraction of volatile solids in the mixed liquor. In aerobic reactors, some CO_2 is lost during the aeration period due to air stripping and nitrification reaction. In contrast, anoxic conditions could favour greater CO_2 levels as it is produced in the denitrification process and the aeration period is shorter. Excess alkalinity

(hardness) can react with components of the feed ($MgSO_4$, $CaCl_2$, $FeSO_4$, $NaHCO_3$) and possibly create precipitates of $CaCO_3$ and $Mg(OH)_2$ in the higher pH of the anoxic phase (Benefield and Judkins, 1982; Metcalf and Eddy, 2003, Sawyer et al., 2003).

TSS in the effluent was measured occasionally and averaged 15.5±7.4 mg/L. The VSS/TSS ratio in the effluent was higher however for alternating than for aerobic reactors (0.90±0.09 and 0.86±0.50 respectively). This indicated that the alternating reactors had either greater amounts of volatile solids or that the inorganic solids somehow settled out faster from the sludge. On the contrary, aerobic reactors had a VSS/TSS in the final effluent similar to the VSS/TSS in the mixed liquor (0.85±0.02).

2.3.5. COD removal

The efficiency of COD removal in the reactors was determined daily from the total COD in the influent (feed) and effluent. Average total COD in the feed (composition described in Table 2.2) was 624±28 mg/L and the COD applied to the reactors was 416 mg/L with a relative percent of standard deviation to average level being 4.4%. Total COD in the final effluent averaged 39±11 mg COD/L and 45±15 mg/L for aerobic and alternating reactors respectively.

2.3.6. Nitrogen removal

Ammonium and both nitrate and nitrite were measured in the feed and at the end of the cycle (samples from wasting). The feed contained 36.25±8.55 mg NH_4^+-N/L supplied as

NH$_4$Cl. Additional ammonia was released during degradation of components of the feed. Ammonia was measured at the end of the complete cycle and was found below the detection limit before settling (0.08 mg NH$_4^+$-N/L) except for a few isolated cases when there was a problem with aeration (clogging of diffusers). The feed contained no oxidized forms of nitrogen (NO$_3^-$ and NO$_2^-$). Ammonia in the reactor – during the cycle - was changing due to the ammonification of raw feed which contained TN. This led to some variability in the concentration of NO$_x$.

NO$_2^-$ was not detected in the effluent of aerobic reactors. In alternating reactors, NO$_2^-$ was detected at the beginning of acclimation in the few instances where problems with ammonia removal were observed. The resultant NO$_3^-$ at the end of the cycle was found to be 77.82±6.52 mg NO$_3^-$-N/L in aerobic and 52.5±6.50 mg NO$_3^-$-N/L in alternating reactors. The significantly lower concentration of NO$_3^-$ in alternating reactors likely was due to the occurrence of denitrification in these reactors during the anoxic period.

2.3.7. Biomass structure

Microscopic observation of sludge showed that biomass in the aerobic reactors consisted of abundant protozoa and dense, spherical, compact flocs, while biomass in the alternating reactors consisted mostly of bacteria with fewer filaments, inhabiting weak, thin, more dispersed and elongated flocs. The occurrence of anoxia in the alternating reactors was likely responsible for partial, non-reversible deflocculation of the biomass. Decreased microbial activity and extracellular polymer (EPS) production under anoxic

conditions likely prevented floc formation. Floc formation and floc robustness (i.e. physical resilience to adverse conditions) have been found to be directly related to the rate of microbial activity (Guellil et al. 2001, Jin et al., 2004, Wilen et al. 2004). To quantify the difference in floc structure observed microscopically, the area and perimeter lengths of flocs were compared. The histograms in Figure 2.3 show that aerobic biomass had a greater frequency of smaller flocs with reduced perimeter length
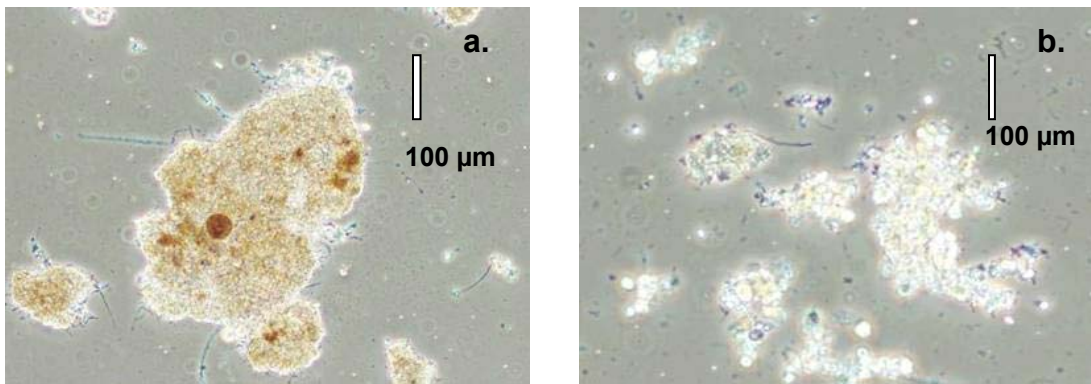
Figure 2.2. Microphotographs of flocs: aerobic reactor (a), alternating reactor (b). Floc size is relative to the 100 μm vertical bar.
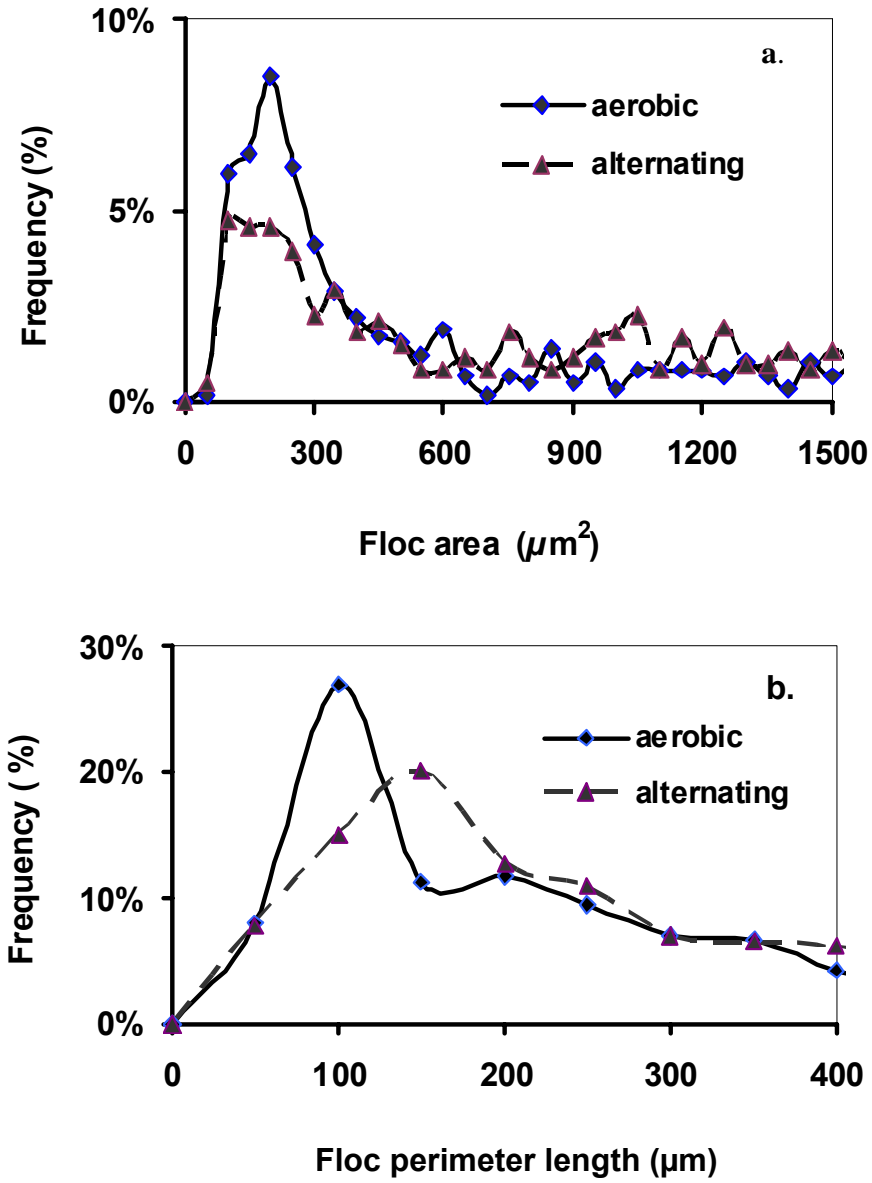
Figure 2.3. The frequency of floc area (a) and floc perimeter length (b) for flocs of aerobic and alternating biomass (samples from the RAS)

2.4.   Conclusions

All reactors were acclimatized for more than five SRT periods before testing commenced. The normal period before steady state is assumed in environmental engineering is three SRT. There was no difference between duplicates of a treatment. Thus one duplicate reactor for a treatment will serve as a control and the other will be subjected to ozonation in subsequent studies presented in this thesis.

3. WASTE ACTIVATED SLUDGE REDUCTION THROUGH OZONATION

3.1.   Introduction

Activated sludge treatment produces excess biomass (waste activated sludge or WAS) which is co-disposed with primary sludge at large costs to municipalities.   Technologies for reducing WAS production are thus gaining attention (Low and Chase, 1999; Liu and Tay, 2001; Wei et al., 2003). One promising technology is ozonation of return activated sludge (RAS) (§1.2). Results of full-scale studies suggest ozonation could even lead to a treatment process with no excess sludge production (Yasui et al., 1996). All of the full scale studies were done on conventional non-nutrient-removal reactors.

The objective of sludge ozonation is to disintegrate sludge biomass, and decrease total TSS and VSS in the ozonated sludge and receiving reactors (Weemaes et al., 2000). Ozonation of sludge has been shown to decrease the ratio of VSS/TSS (Sahli et al., 2003). Further, negligible accumulation of inorganic solids (Sakai et al., 1997) or even a lowering of inorganic solids has been observed (Deleris et al., 2002). However, in some studies, sludge ozonation has been reported to cause a slight increase in total organic carbon (TOC) and TSS in the final effluent (Sakai et al., 1997). More importantly, ozonation could potentially impact biological rates of carbon, nitrogen, or phosphate removal when treated RAS is introduced back into the process. The impact could be due to the destruction or inhibition of the activity of specialized microbial populations. One could anticipate that reduction of ammonia utilization rate (AUR) after ozonation should

be proportional to the sludge reduction. Some researchers have shown that the resulting AUR remained constant – i.e. there was no inhibitory effect of ozonation on nitrification (Sakai et al., 1997; Sahli et al., 2003). It was suggested that nitrifiers overgrown by the faster growing heterotrophs may be protected inside sludge flocs and not be exposed to ozone as much as floc surface-dwelling heterotrophs (Boehler and Siegrist, 2004).

The destruction or solubilisation of organic solids by ozonation of return sludge can improve the overall treatment process by indirectly impacting the SRT. Reduced WAS production leads to an apparently higher SRT if the mixed liquor suspended solids concentration (MLSS) remains constant (Boehler and Siegrist, 2004). Loss of nitrifiers due to ozonation seems to be partly compensated by this increased apparent SRT. Furthermore, there are potential benefits of ozonation in improving the overall biological treatment processes. In a denitrifying plant, the recycle of ozonated RAS to an anoxic reactor improves denitrification as the solubilized degradable organic compounds become an additional carbon source for the reduction of NO_3^- (Ahn et al., 2002; Park et al., 2004, Boehler and Siegrist 2007) (§1.3).

3.2.　Objectives

The purpose of this research was to examine the influence of ozonation on sludge minimization and the whole treatment process performance in regards to final effluent and the activated sludge flocs' structure under strictly aerobic and alternating anoxic/aerobic conditions. The study aimed to compare both processes and provide a perspective on the effects of ozonation on releasing biodegradable carbon.

3.3. Materials and methods

3.3.1. Ozonation

Four sequencing batch reactors were established as pairs under aerobic and alternating anoxic/aerobic conditions as previously described (§ 2.2). In each pair, one reactor served as a control and the other went through the ozone treatment: aerobic (R1 – control, R2 – ozonated) and alternating anoxic/aerobic (R3 – control, R4 – ozonated) (Figure 3.1).

R1	R2	R3	R4
feeding COD	feeding COD	feeding COD + NO_3^-	feeding COD + NO_3^-
aerobic reaction	aerobic reaction	anoxic reaction	anoxic reaction
		aerobic reaction	aerobic reaction
		anoxic reaction	anoxic reaction
		aerobic reaction	aerobic reaction
wasting	wasting	wasting	wasting
decanting	decanting	decanting	decanting
	withdrawing 20% RAS		withdrawing 20% RAS
	ozonation		ozonation
	return to the reactor		return to the reactor

Figure 3.1 Schematic of individual operations in a full cycle in the control and ozonated SBR (R1, R2 – aerobic, R3, R4 – alternating treatment)

For the ozonated reactors, 20% of the return activated sludge (RAS) was withdrawn and treated with ozone. The mass was then returned to the activated sludge reactor immediately before feeding. Ozone was produced from air (compressed laboratory air line) by an ozone generator (OZO 1 VTT Ozomax Ltd.). Ozone was applied to the portion of the RAS in a gas-washing bottle by bubbling the ozone gas (flow 10.5 mg/min) directly into the sludge for different lengths of time to achieve greater ozone doses (maximum retention time in the ozone contactor 16 min). The ozone dose consumed by sludge was analyzed each time by calculating the inlet gas and the off-gas trapped in a potassium iodide solution (Standard Method 2350E). A dose of $7 - 33$ mg O_3 consumed per reactor and day were applied. This corresponded to doses of $0.006 - 0.030$ mg O_3 /mg TSS of ozonated sludge and $0.015-0.080$ mg O_3/mg TSS of initial excess sludge. Expressing ozone dosage per mg of initial excess sludge (which would be wasted if it was not for ozonation) is often used in ozone sludge minimization studies (Sakai et al., 1997; Sahli et al., 2003; Boehler and Siegrist, 2004, 2007). This approach allows comparison between different experiments performed with various settings (volume, recycle ratio, SRT etc.). Doses were applied in increasing amount of 0.005 mg O_3/mg TSS of initial excess sludge for a period of $1\times$SRT. The tests were conducted in two phases (sampling periods): February –April and May-June 2005.

3.3.2. Analysis

TSS and VSS were measured in RAS from ozonated reactors immediately before and after ozonation as well as in RAS from the control reactors. TSS was also measured daily in samples from all reactors before wasting. Wasting was adjusted daily in ozonated

reactors to maintain TSS at around 1800 mg/L at the beginning of the reaction period which was similar to the controls. This resulted in a higher apparent SRT in ozonated reactors (SRT up to 14.9±0.7 d) compared to design SRT of 12 days.

Kinetic studies were performed to calculate substrate utilization and production rates. Samples of 15 mL activated sludge were withdrawn every 30 minutes and analysed for inorganic nitrogen compounds, sCOD, pH, temperature (T, ^{o}C) and DO. Rates were calculated as the concentration of removed or produced compound in time and expressed as mg N/g VSS h where VSS content in the sludge in the beginning of the study was used. This method is used in environmental experiments instead of mg/L h as it allows comparison of studies performed with different volatile matter content. Similarly, AUR, nitrite+nitrate utilization rate (NUR) and nitrite+nitrate production rate (NPR) were calculated based on changes in measured ammonium or nitrite+nitrate concentration.

Soluble COD was measured in quintuplicate (each sample was analyzed five times) in the portion of ozonated RAS directly before and after ozonation as well as in RAS from control reactors using Standard Method 5220D. Starting from February 1st 2005 the use of a F.S. Spectronic 21 Bausch and Lomb spectrophotometer was discontinued and replaced by a HACH DR/2500 ver. 35 with software 2.13. The replacement instrument resulted in obtaining about 20% lower values of COD in high range (150-1500 mg/L) than in the previous phases of the research. The low range (0-150 mg/L) was not affected with the instruments.

To evaluate the production of additional ammonia with ozone treatment, an additional batch test was performed using biomass originated from the aerobic control reactor and created from wastage. The additional reactor was setup and maintained in the same way as the main reactors. After feeding and three hours of aerobic reaction, 20% of sludge was withdrawn and treated with ozone (0.055 mg O_3/mg initial excess sludge) and then the biomass was returned to the tank. Samples of sludge for analysis were taken in 15 minutes intervals.

The analysis of TSS, VSS, sCOD, TCOD, NH_4^+, NO_3^-, NO_2^-, pH, alkalinity, and DO were performed as described in § 2.2.3.

3.3.3. Statistics

All data sets were analysed statistically and reported as average with standard deviation and analyzed using paired student's t-test with P = 0.05 over time (Berthouex and Brown 1994).

3.4. Results and discussion

3.4.1. Final effluent quality

The final effluent quality was not negatively impacted by ozonation in these aerobic and alternating anoxic/aerobic sequencing batch reactors. Total COD in effluent from all reactors remained constant (41±16 mg/L for aerobic and 40±15 mg/L for alternating reactors, respectively), showing no negative effect of ozone treatment on total COD

removal. No statistical difference between the amount of TSS in the effluent from control and ozonated reactors was observed for either type of reactor, as all four reactors were quite variable in effluent TSS (average 16.3 ± 6.3 mg/L). Alkalinity in the final effluent was similar to that during the acclimation period (154 ± 33 and 299 ± 64 mg $CaCO_3$/L for aerobic and alternating reactors, respectively, with no statistical difference between ozonated and control reactors. Ammonia removal was complete in both sets of reactors regardless of ozone dose. Nitrite was never detected in the final effluent.

3.4.2. Biomass structure and microbial activity

Microscopic observation of biomass in the aerobic reactors revealed abundant protozoa, with a backbone of filamentous bacteria in dense, spherical, compact flocs. The biomass in the alternating reactors consisted mostly of bacteria with fewer filaments, inhabiting weak, thin and elongated flocs (chain-type structure). It appeared the aerobic flocs were more resistant to higher doses of ozone than in alternating reactors. (Figure 3.2). Visually the turbidity after the ozone treatment was significantly higher in the alternating reactor. The breakage and dispersion of solids caused by ozone treatment increased slightly the exposed surface area and porosity. Higher surface area may lead to higher surface biosorption capacity.
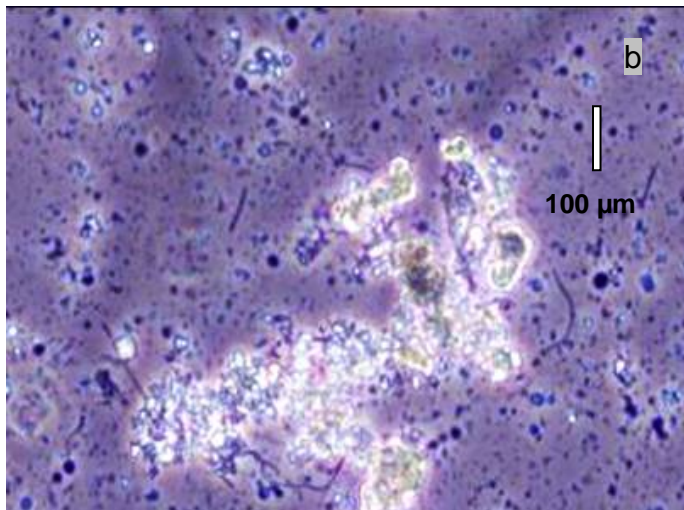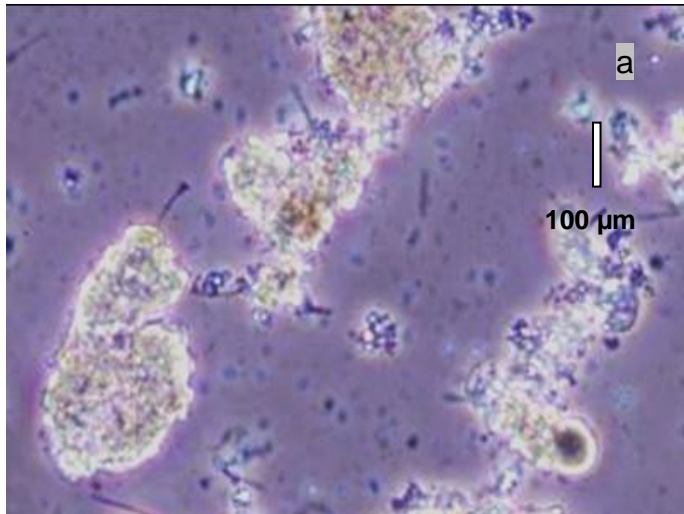
Figure 3.2. Effect of ozone on flocs (dose 0.045 mg O_3 /mg TSS of initial excess sludge); aerobic (a) and alternating (b) reactors. Scale identified by the 100 μm vertical bar

Ozonation decreased pH from 7.59 ± 0.07 to 7.28 ± 0.09 in the ozonated portion of the aerobic RAS and from 7.88 ± 0.11 to 7.64 ± 0.09 for the alternating reactor. However, after returning the ozonated batch to the main reactor together with feed there was a difference in pH between control and ozonated reactors (7.66 ± 0.25 and 7.66 ± 0.23 for aerobic control and ozonated reactor; 7.98 ± 0.23 and 7.96 ± 0.24 for alternating control and ozonated reactor respectively). During seven months of operation of the ozonated

reactors with higher apparent SRT the average VSS/TSS ratio of the mixed liquor dropped slightly from 0.85 ± 0.05 to 0.83 ± 0.06 and from 0.79 ± 0.06 to 0.77 ± 0.05 in the aerobic and alternating reactors, respectively. This decrease in the VSS/TSS ratios indicated an accumulation of inorganic solids in the reactors. During ozone treatment a portion of the particulate matter was solubilized and this soluble matter could be utilized during biological treatment (Yasui and Shibata, 1994; Egemen et al. 2001; Boehler and Siegrist, 2004, 2007).

SOUR measured in the ozonated portion of RAS directly before and after ozone application was not statistically different for aerobic biomass (10 ± 1 and 11 ± 1 mg O_2/g VSS h) showing that microbial activity was not significantly impacted by ozone treatment. However, for the alternating reactor SOUR dropped from 17 ± 2 to 13 ± 1 mg O_2/g VSS h. Surviving microorganisms in the portion of ozonated RAS likely were stimulated by additional carbon released from dead cells and indeed the decrease in SOUR indicated greater destruction of living cells in the alternating than in aerobic reactor. In contrast SOUR measured occasionally in the control and ozonated reactors decreased from 45 mg O_2/g VSS h at the beginning of reaction (cycle) to 4 mg O_2/g VSS h before wasting. The final SOUR level was similar to those obtained during the acclimation period (§2.3.3).

3.4.3. Decrease in waste activated sludge

The RAS that was ozonated experienced a large decrease in biomass that was proportional to ozone dose for the highest doses up to 55 mg and 112 mg for aerobic and

alternating reactors, respectively. Ozonation of 20% of the RAS also consistently reduced the amount of excess solids in the reactors receiving the ozonated RAS compared to the controls. This reduction was somewhat proportional to the ozone dose over the range tested for both reactors and was much greater in the alternating anoxic/aerobic reactor than the aerobic one (Figure 3.3a). This was related to the different structures of the flocs in the alternating and aerobic reactors. Correlation is rather poor in Figure 3.3, what is however beyond doubt is that the magnitude of TSS loss was lower in the aerobic reactor.

For a moderate dose of 0.05 mg O_3/mg TSS of initial excess sludge (24 mg consumed O_3 /reactor) the average decrease in excess sludge was 6.3% and 14.7% of initial excess sludge in the aerobic and alternating reactor compared to their controls, respectively. A decrease in solids resulted in lowering amount of activated sludge that had to be wasted daily. The decrease was up to 25% for alternating biomass. This is comparable to previous research by Boehler and Siegrist (2004) who found an excess sludge reduction of 25-30% up to an optimal dosage of 0.05 g O_3/g TSS and to results of Sievers et al. (2004) where reduction reached 20-35%

Prolonged ozonation actually reduced the magnitude of solids decrease in both reactors. The decline in the benefit of sludge reduction was more pronounced in the alternating reactor (Figure 3.3 b, May-June 2005). The destruction of solids achieved in the alternating reactor was equal or less than 15% for the highest ozone doses used.
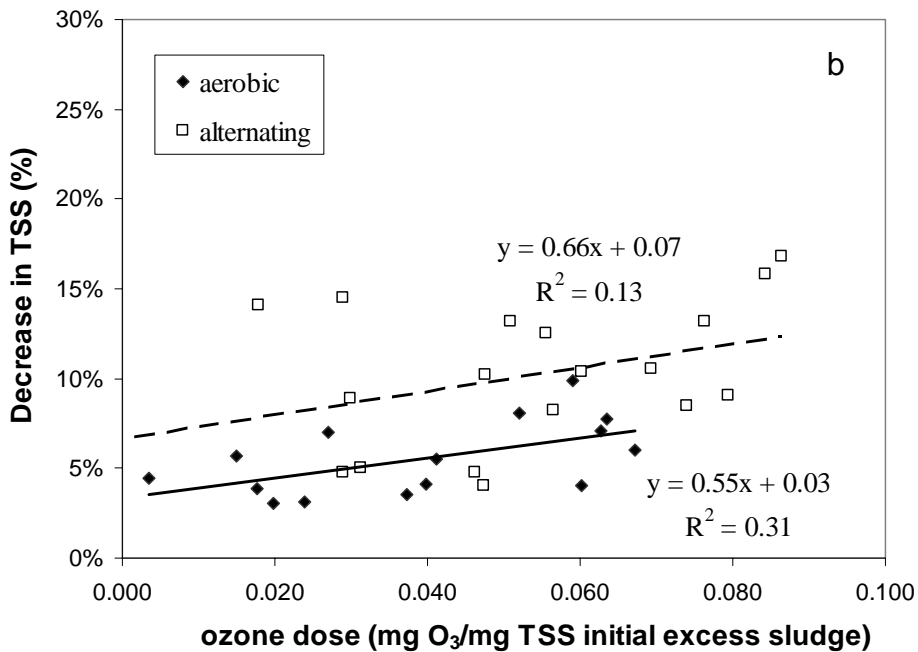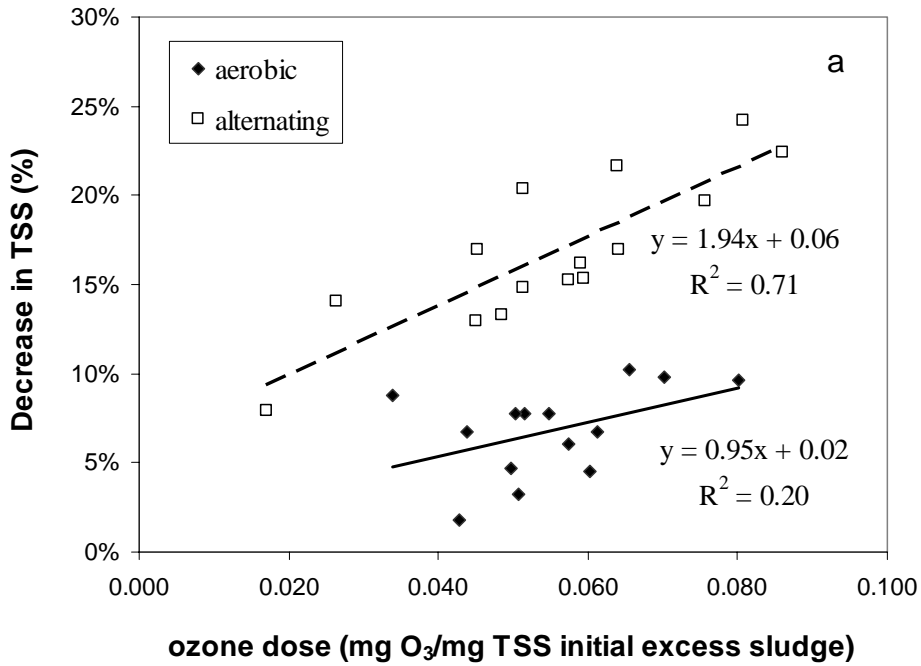
Figure 3.3. Decrease in WAS in the aerobic and alternating reactors after ozone treatment; February-April 2005 (a) , May-June 2005 (b)

3.4.4. The production of additional carbon

Ozonation was expected to generate soluble organic matter by oxidation of complex organic polymers. Production of sCOD during ozone treatment was confirmed, and it correlated with suspended solids destruction. An increase in sCOD was evident with increasing dose of ozone (Figure 3.4a) For the first three months of the experiment (February-April 2005) the effect of ozonation was much greater in the alternating reactor than the aerated one (Figure 3.4a). The sCOD increased with the ozone dose in ozonated reactors, and for the moderate dose of 0.05 mg O_3/mg TSS of initial excess sludge, the increase in sCOD was 8.4% and 15.2% for the aerobic and alternating reactors, respectively. Consequently, the production of sCOD during ozonation in the alternating reactor decreased for the second phase of the tests (Figure 3.4b, May-June 2005). It appeared that prolonged ozonation strengthened the structure of alternating flocs making them more resistant to destruction from ozone.
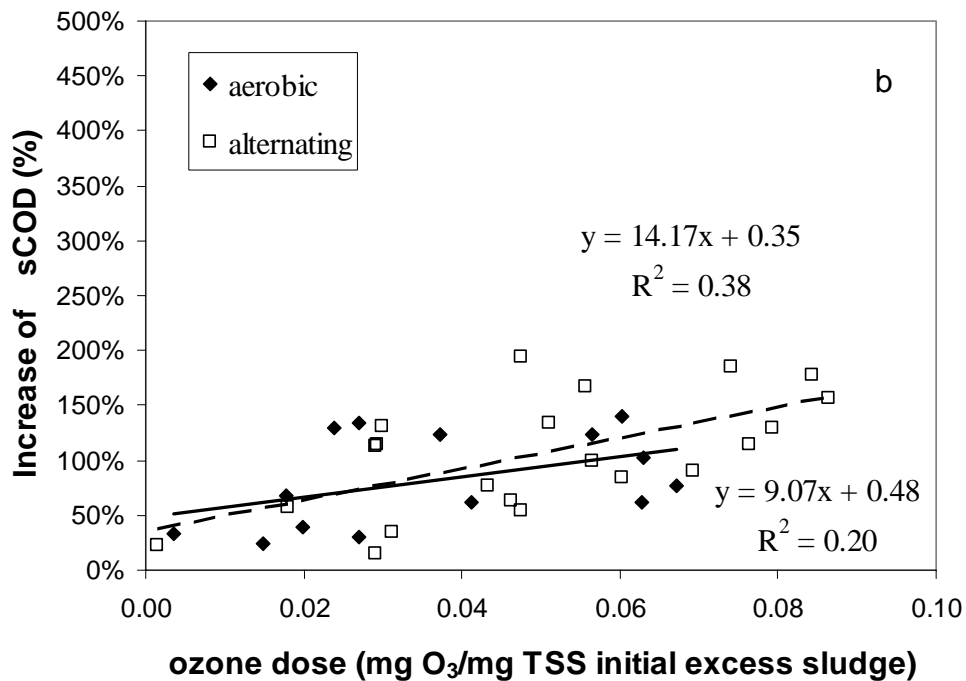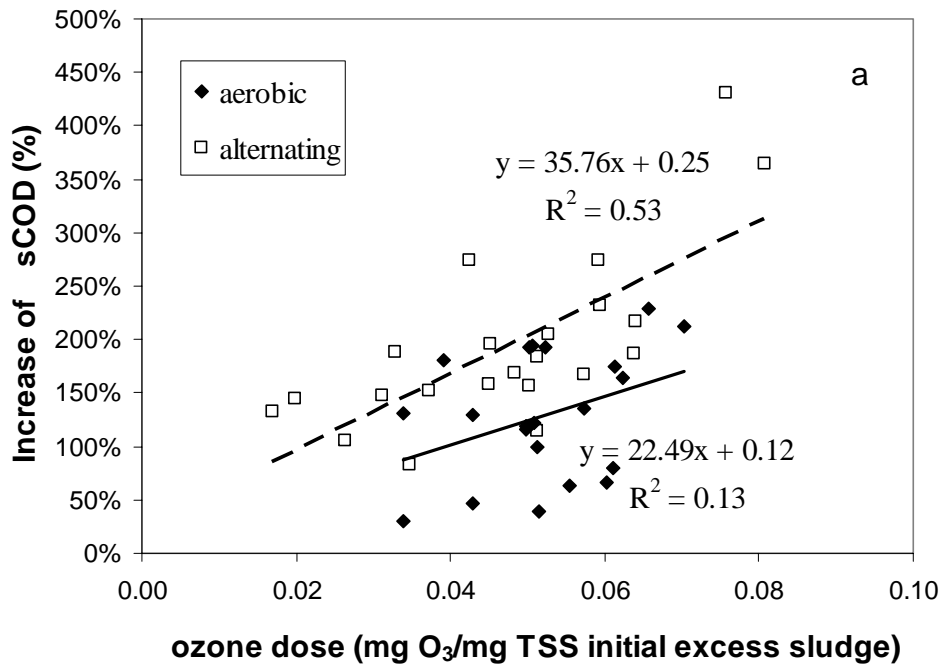
Figure 3.4. Increase in soluble COD in ozonated RAS in the aerobic and alternating reactors after ozone treatment; February-April 2005 (a) , May-June 2005 (b).

The overall COD removal in the reactors did not deteriorate in spite of additional sCOD loads to the ozonated reactors. Amount of TCOD in the effluent did not increase. In the four reactors, the percentage of TCOD removal averaged 90.5% in both control reactors and achieved 91.5% and 92.3% removal in the ozonated aerobic and alternating reactors, respectively. Therefore, ozonation did not negatively impact carbon removal during the treatment process. This carbon removal would consist of both biodegradation and biosorption. Physical-chemical phenomena such as flocculation of particulate and colloidal material released from cells and sorption of dissolved organics mitigate the transfer of organic matter (COD) from the wastewater to the surface of activated sludge flocs. After sorption, the colloids are hydrolyzed by extracellular bacterial enzymes and further COD assimilation occurs (Guellil et al., 2001). Biosorption capacity expressed here as percent of TCOD removed from the bulk solution during the first 15 min of feed (biomass contact) was 45.7% for aerobic and 50.5% and alternating control reactor and was close to the value of 45% reported previously (Guellil et al., 2001). Biosorption was higher in alternating reactors probably due to the larger active surface of the flocs (open structure) compared to the compact flocs in the aerobic reactors. Biosorption capacity increased slightly after ozone treatment (to 53.3 % for aerobic and 53.8% for alternating ozonated reactor) probably due to enlargement of the floc surface and increased porosity during floc destruction. This observation also confirms that biosorption capacity of activated sludge is proportional to COD concentration originating from the influent solids. The latter was first suggested by Fujie et al. (1997).

3.4.5. Nitrogen removal

3.4.5.1. The production of additional ammonia

The concentration of ammonia was also expected to increase in reactors treated with ozone. The study indicated that ozonation resulted in an increase on average of 1.32 mg NH_4-N/L (range 0.06 to 5.72 mg/L). The increase in ammonia is equivalent to 5% of total ammonia supplied with feed to the reactors. For aerobic reactors differences in nitrification rate between pairs of ozonated and control reactors make it impossible to evaluate release rates of ammonia.

An additional batch test was performed using aerobic biomass to evaluate the production of extra ammonia from ozone treatment. After three hours of aerobic reaction 20% of sludge was withdrawn and ozonated. There was a 26% increase in COD that was accompanied by a 3% increase in ammonia during the 30 minutes following treatment.

Figure 3.5 shows the drop in NO_3^- in the final effluent after ozone treatment, in comparison to the control reactor. The level of nitrate in effluent of the ozonated aerobic reactor increased up to 7% for higher doses of ozone. This confirms that the proportional addition of ammonia after ozonation serves as a substrate for the production of NO_3-N during nitrification. This result is approximate only as some NO_3^- can be lost in the ozonated reactor due to ozonation. In spite of these complicating factors it can be concluded that additional ammonia production in both aerobic and alternating treatment

is not significant and will not impact negatively total ammonia removal from the ozonated system.
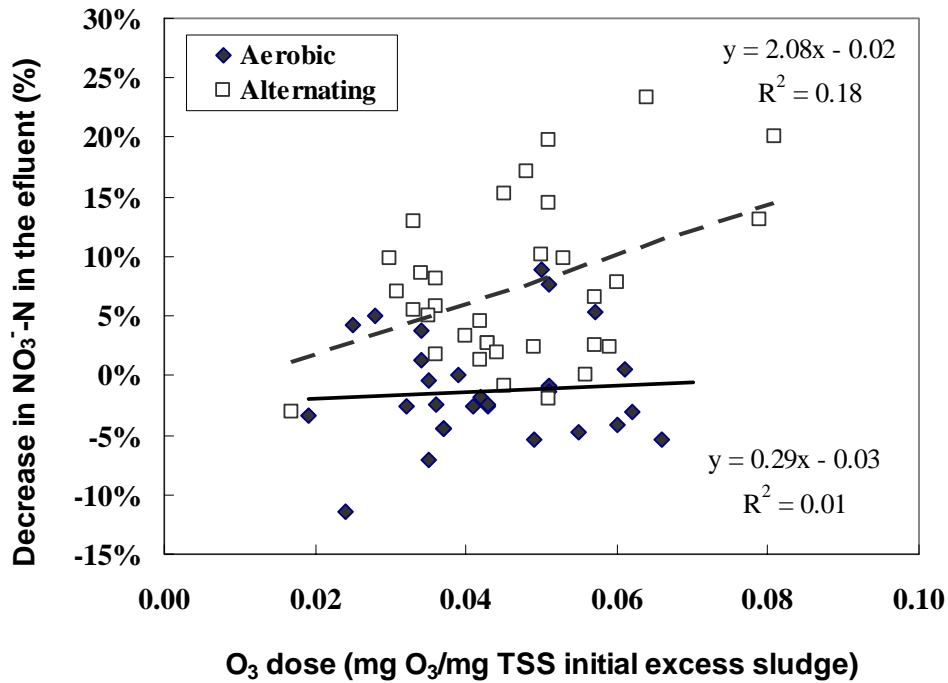
Figure 3.5. Decrease in NO_3^--N in final effluent in ozonated reactors in comparison to the controls.

3.4.5.2. Denitrification

The production of soluble COD from partial ozonation of RAS has the potential to provide a suitable carbon source for denitrification and lead to substantial cost savings in treatment systems having reactions that are carbon-limited. Here, the alternating reactors were exposed to anoxic conditions immediately following ozonation of 20% of the sludge and the subsequent addition of feed containing nitrate (Figure 3.1). Soluble COD generated by ozonation could potentially be used for denitrification. Conversely,

ozonation of the RAS could decrease the population of denitrifiers and could decrease initial rates of denitrification.

Removal of nitrate and nitrite (NO_x utilization rate or NUR) was compared to the control where RAS was not ozonated. Denitrification during the initial anoxic phase in the alternating reactors was enhanced rather than inhibited by ozonation of 20% of the RAS. In the ozonated alternating reactor, the level of nitrate in the final effluent decreased in comparison to the control reactor and in proportion to the ozone dose (Figure 3.5). This drop was linear from 5% for the lowest dose of 0.01 mg O_3 /mg TSS of initial excess sludge up to 20% for the highest dose of 0.08 mg O_3/mg TSS of initial excess sludge, indicating better total denitrification. Kinetic studies were then conducted and the rate of denitrification NUR was significantly improved in the ozonated alternating reactor compared to the control, as indicated by an increase in the specific NUR (Figure 3.6).
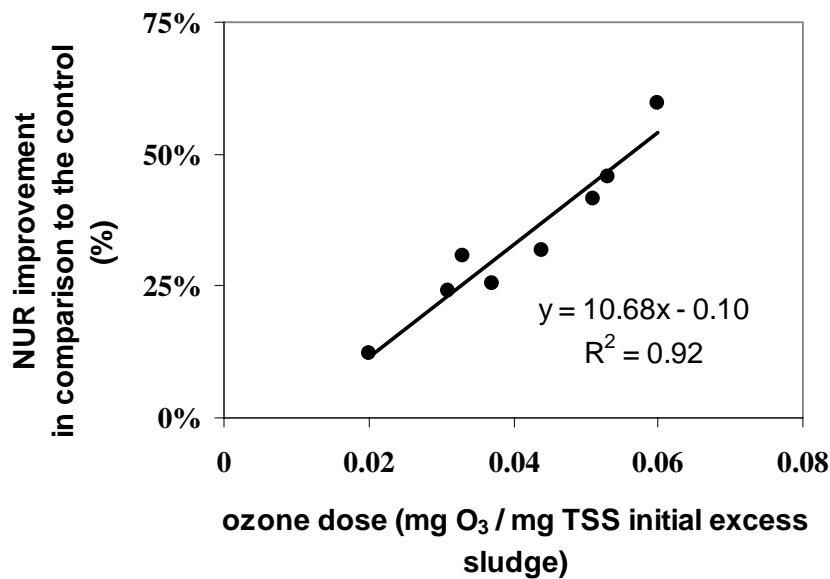
Figure 3.6. Improvement of denitrification in alternating ozonated reactor in comparison to the control (NUR)

3.4.5.3. Nitrification

Complete ammonium oxidation occurred in both aerobic reactors and in alternating reactors during the aerobic phase which commenced immediately after the denitrification phase. Nitrate accumulated in the aerobic reactors and during the aerobic phase in the alternating reactors.

Table 3.1. Ammonia uptake rates (AUR), nitrite+nitrate production (NPR) and utilization (NUR) rates for ozonated and control reactors: results from kinetic studies

	Aerobic			Alternating		
	Control, mg/g VSS h	Ozonated, mg/g VSS h (av±stdev)	Decrease after O_3, %	Control, mg/g VSS h	Ozonated, mg/g VSS h (av±stdev)	Decrease after O_3, %
AUR	4.88±0.43	4.00±0.37	18±7	6.10±0.37	5.75±0.26	7±2
NPR	5.99±0.52	4.67±0.63	22±11	7.75±0.71	7.37±0.76	4±2
NUR	N/A	N/A	N/A	5.78±0.79	7.67±0.84	-34±15

N/A – not applicable

Alternating reactors had higher AUR and NPR than aerobic reactors (Table 3.1) which corroborates earlier findings by Lee and Oleszkiewicz (2003). In both reactors nitrification rates (AUR and NPR) decreased proportionally to the increasing ozone dose. In the alternating reactor, nitrification (AUR, NPR) decreased with ozonation (up to 7%), but was affected far less than the aerobic reactor which showed a 22% decline (Table 3.1, Figure 3.7). It has been suggested previously that nitrifying bacteria may be protected within floc structures. In this study the alternating reactors had flocs that were easier to destroy (based on TSS reduction, sCOD release and microscopy). Thus there was less of an impact on nitrification in spite of the fact that the solids destruction was even greater in the alternating reactors. There must be another mechanism to explain why nitrification

was affected more in the aerobic reactors than in the alternating reactors. The nitrifiers in the remaining 80% of the RAS that was not ozonated were probably responsible for most if not all of the nitrification observed in the reactors. The biomass concentration was always adjusted to 1800 mg TSS/L – similar to the control. The population of nitrifiers could actually be higher in the ozonated reactors than in the control reactors due to the longer SRT maintained to compensate for solids destruction. This additional amount of nitrifier biomass would be even higher in the alternating reactor than in the aerobic reactor. Either way the mass of nitrifiers does not seem to control nitrification rates in these reactors. Further research is needed to quantify the relative impacts of the different SRTs and the ozone doses on nitrifying populations in the reactors.

A more intriguing explanation is that deterioration of AUR and NPR in the aerobic reactor was caused not only by the direct influence of ozone on nitrifiers and floc structure but also by the higher sCOD released during this treatment. Autotrophic nitrifiers could have been suppressed by faster growing heterotrophs. Nitrification rate was hindered more in the ozonated aerobic reactor due to more COD present in the beginning of the cycle (at the same time feed was added) and the time needed for its utilization (Figure 3.7). Both nitrification and heterotrophic COD removal occur simultaneously in the aerobic reactor. Due to their slower metabolism nitrifiers could be easily out-competed by heterotrophs for oxygen and ammonia. Nitrification in alternating reactors started only after the onset of aeration 3.5 h after feeding. By that time only poorly biodegradable COD would be present in the bulk liquid (94 ± 19 mg/L). Such conditions favour nitrification. During the anoxic period readily biodegradable COD is

removed from solution which reduces the competition with faster growing heterotrophs for oxygen but also creates conditions that lead to higher nitrification rates than in purely aerobic reactors. The period of heterotrophic denitrification in alternating reactors also increased the pH (from 7.70 ± 0.11 to 8.20 ± 0.07) and alkalinity in the solution which could further stimulate the rate of nitrification.
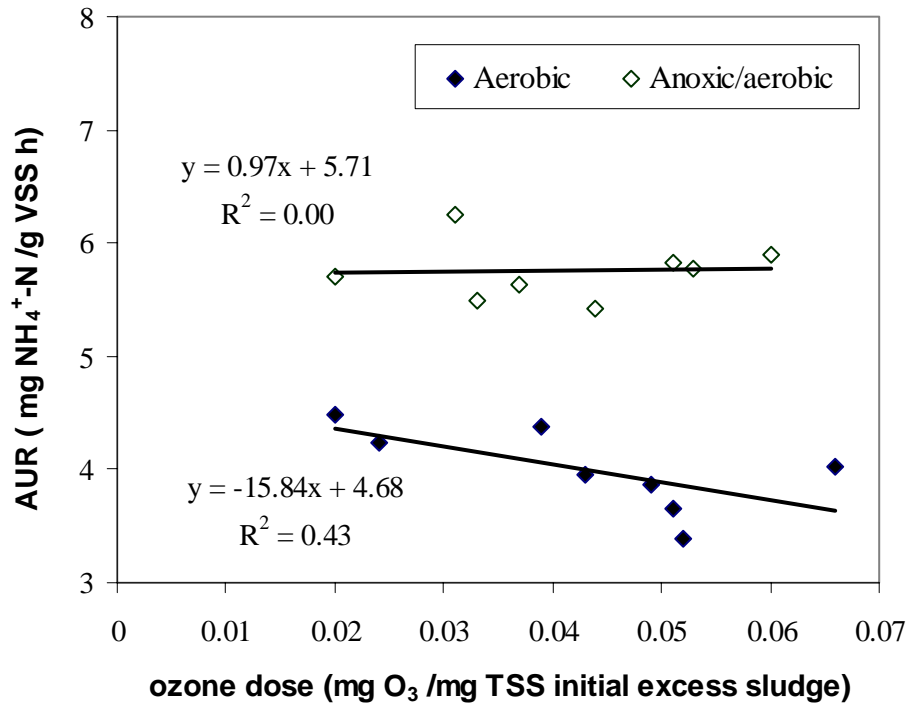
Figure 3.7. Impact of O_3 on nitrification (deterioration in AUR) in ozonated reactors over a range of ozone doses

3.5.   Conclusions

Ozonation of 20% of RAS had no negative impact on the final effluent quality from the SBRs. Total COD and suspended solids in the effluent remained similar to controls and nitrification was complete in both sets of reactors regardless of the ozone dose. After

months of operation there was a slight decrease in the VSS/TSS ratio in the ozonated reactors: 2.3% decrease in aerobic and 2.5% in the alternating reactor.

Ozonation significantly reduced the production of excess sludge in the reactors. The reduction of suspended solids in the bioreactor after ozone treatment was proportional to the ozone dose. After prolonged operation observable decrease in sludge in alternating ozonated reactor became less pronounced. This indicated that flocs became stronger and more difficult to destroy in the alternating reactor. It can be expected that for long-term ozonation greater ozone doses would be required to achieve the same excess solids reduction. This issue has significant engineering implications and will be discussed further in Chapter 4.

Ozonation of sludge increased the soluble COD in the sludge. This additional mass of sCOD correlated with the destruction of solids and greatly increased denitrification rate in the alternating reactor. Ozonation had less of an adverse effect on nitrification rate in the alternating reactor indicating possible negative impact of the increased sCOD on the autotrophic population in the aerobic reactor. Nitrification was however complete in all reactors in spite of additional ammonia released by ozonation.

4. EFFECT OF OZONE TREATMENT ON FLOCCULATION AND SEPARATION CHARACTERISTICS OF SOLIDS

4.1. Introduction

In secondary wastewater treatment systems, the organic contaminants are removed from wastewater through the metabolic activity of concentrated populations of microorganisms (biomass) in the activated sludge tank. The structure and properties of the biological flocs in the activated sludge affect their settling. Efficient separation of these flocs (solids) from treated wastewater in a dedicated final clarifier or within a sequencing batch reactor during the "settle" cycle are essential to the success of the suspended growth biological treatment process. Flocculation and settleability of the solids affect subsequent sludge dewatering processes that strongly influence the cost of sludge management in wastewater treatment plants.

Activated sludge flocs are clusters of living and dead microorganisms, inorganic and organic material coming with the influent wastewater, cellular debris and the products of extracellular hydrolytic activity. The polymeric material, binding them together, is composed of macromolecules produced and secreted by cells. Extracellular polymer substances (EPS) are in the form of a tightly bound capsule (named "bound EPS") or loosely associated colloidal and soluble material ("soluble EPS"). At higher sludge ages (SRT), the cells tend to be surrounded by polymers in more compact aggregates. Polymers which can be found on floc surfaces are attached in relatively weak structures

(Zita and Hermansson, 1994). It was reported that the EPS composition of flocs varies between different types of wastewater treatment systems and depends on wastewater composition: soluble COD content (Sponza, 2003) and COD:N:P ratio (Bura et al., 1998).

EPS originates from biological synthesis and excretion or lysis of microorganisms, and can also contain a variety of substances adsorbed from the incoming wastewater, including micro-pollutants and incorporated particulate material. Chemical composition of EPS and particularly the proteins/carbohydrates ratio can affect the physical properties of the sludge and are also believed to be important in bioflocculation (Morgan et al., 1990). Bioflocculation defines a floc agglomeration process which affects the particle size distribution in the biological sludge (biomass) and its solids separation properties. Several mechanisms are involved in flocculation: binding cells together by polymers (mostly negatively charged); by forming bridges using the presence of bi- and multi-valent cations, especially calcium (Bruus et al., 1992, Keiding and Nielsen, 1997, Morgan et al., 1990, Wilen et al., 2004); DLVO (Derjaguin–Landau–Verwey–Overbeek) cohesion, according to the theory of colloid stability (Zita and Hermansson, 1994) and the hydrophobic effect (Jorand et al., 1998, Liao et al., 2001). In each of these flocculation mechanism models EPS are considered as playing an essential role in bacterial flocculation of activated sludge.

It has been found that samples from anaerobic sludges yielded significantly less EPS than activated sludge (Morgan, 1990). Furthermore, it was reported that the lack of oxygen

during anaerobic phase in full scale biological nutrient removal tank results in deflocculation of sludge (Wilen and Balmer, 1999) because of an inhibition of the microbial activity within the floc-forming aerobic fraction of the biomass(Wilen et al., 2000, Wilen et al., 2004). Alternative metabolism and reduction or oxidations of cations (for example iron) under anoxic or aerobic conditions have been shown to influence bridging and the bioflocculation processes (Wilen et. al. 2000).

The EPS level can influence solids separation in two ways. At low levels of EPS there is a risk that dense layers of bound EPS cannot be sufficiently formed and that loosely attached material more sensitive to shear can be washed out from the system. This may lead to deterioration of the effluent quality particularly during rain and snowmelt periods (Keiding and Nielsen 1997). Settleability and sludge filterability deteriorate due to deflocculation, and increased amounts of small-size particles end up in the effluent (Karr and Keinath, 1978). Increased amounts of EPS can on the other hand form a dense gel that resists the expression of water from the gel pores (Liao et al., 2001, Jin et al., 2004, Neyens et al., 2004). This "bound" water incorporated into the floc matrix cannot be removed through mechanical means such as through thickening or conventional dewatering. It is believed that increased EPS concentration up to a point improves floc formation and settling while excess can deteriorate dewaterability (Houghton et al., 2001). Excessive amounts of EPS have been shown to deteriorate flocculation and settleability as well: the EPS molecules extending out from the cells can prevent the cells from forming close contact (Liao et al., 2001) while bound water can shield the potential binding sites (Neyens et al., 2004). The flocs that are ideal for sedimentation are large,

strong, and have a compact interior. On the other hand, flocs ideal for mechanical dewatering should be of high porosity and have a strong and open interior (Yen 2002). Effective management of sludge characteristics to enhance the settling and subsequent dewatering of sludge requires a moderate level of EPS in the flocs.

Solids destruction by ozonation creates smaller flocs and destroys filamentous networks, improving sludge settleability but decreasing its dewaterability (Weemaes et al., 2000). The ozone also stabilizes the surface charges helping in decreasing sludge volume index (SVI). On the other hand, excessive disintegration and dispersion of flocs can lead to creation of non-settleable solids and deteriorating filterability and dewaterability of sludge (Liu et al., 2001, Boehler and Siegrist, 2004). Further oxidation of polymers causes solubilization and generates soluble COD, increasing the food-to microorganism ratio (F/M) (§3.4.4). This additional, biodegradable carbon generated through ozonation is returned to the activated sludge tank influencing all subsequent biological processes, including EPS formation, biological flocculation, nitrification and denitrification, heterotrophic COD removal. Thus, ozonation has the potential to affect floc structure not only in the ozone-treated sludge but in the entire activated sludge process.

4.2.   Objectives

The following questions were asked in this study:

- What is the difference between the amount of total EPS and bound EPS for biomass developed under strictly aerobic and alternating anoxic/aerobic conditions;

- Is there a relationship between EPS and settling as well as dewaterability;

- What is the impact of ozonation on total and bound EPS;

- Is there a long-term impact of ozone treatment on the amount of total and bound EPS.

4.3.   Materials and methods

Operation of reactors and the ozonation process were performed as described before (§2.2, §3.3.1)

4.3.1.   Analyses

Several methods can be used for extraction and quantification of sludge biopolymers including steaming, centrifugal stripping, alkali stripping and ethanolic extraction. Most methods use centrifugation to separate EPS from cells (Spaeth and Wuertz, 2000). It seems that at present no universal method exists and thus comparison of results coming from different authors must be made with caution. Comparison of treatment within a study can however be useful if the extraction technique is initially standardized (Morgan et al., 1990).

Samples of RAS in the control and ozonated reactors (before and after ozone treatment) were analyzed over a period of one month, to estimate the direct impact of ozonation on EPS as well as to track the long-term influence of ozonation of the sludge. Both total (bound and soluble) EPS, called unwashed EPS, and bound – washed EPS were analyzed

by a method involving thermal treatment at 80°C followed by acetone/ethanol precipitation (Morgan et al., 1990). In the bound EPS analysis each sludge sample was rinsed 3 times with de-ionized water before extraction to remove the soluble EPS fraction. The amount of extracted substance was analysed gravimetrically and then related to the VSS content of the sludge.

A capillary suction time (CST) test was used to analyse the sludge dewaterability. CST was measured daily in the RAS samples by Capillary Suction Timer, Type 30413 (Triton Electronics Ltd.), using 18 mm sludge reservoir.

The settling properties of activated sludge were measured by means of Sludge Volume Index SVI (mL/g) as per Standard Method 2710D. The analyses of TSS, VSS, sCOD, TCOD, NH_4^+, pH, alkalinity and DO were performed as described in § 2.2.3.

4.3.2. Statistics

All data sets were analysed statistically and reported as average with standard deviation and analyzed using paired student's t-test with P = 0.05 (Berthouex and Brown 1994).

4.3.3. Batch tests with the biomass taken from the main reactors

In addition to the daily analyses of sludge in the reactors batch tests were performed outside of the main reactors to evaluate the ability of activated sludge to flocculate and

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settle. The set of batch tests was performed on biomass grown separately (in addition to) from the working main reactors. The biomass was subject to the same anoxic and aerobic regimes the ozonated reactors received the dose of approximately 0.05 mg O_3/mg TSS of initial excess sludge. In the test 5 mL samples of biomass were withdrawn every 30 minutes during the reaction phase. Samples were settled for 5 minutes in the vials and then analyzed for transmittance (wavelength 650 nm) (Spectronic 21, Bausch and Lomb).

One additional batch test of progressively larger ozonation dose was performed three times throughout the study on a 200 mL portion of excess sludge from the main reactors. The whole sludge portion was ozonated continuously for 20 minutes and 20 mL samples were withdrawn after the cessation of ozonation. The dose was then increased and another sample was withdrawn after 20 minutes of ozonation with the new dose. The samples were analyzed for total EPS, bound EPS, CST and sCOD.

4.4. Results and Discussion

4.4.1. Total EPS in reactor sludge

The total EPS of the activated sludge was measured in samples taken prior to feeding (after ozonation where applicable) to examine the effects of the reactor design (aerobic/ alternating) as well as the impact of ozonation on floc characteristics. The aerobic SBR control consistently exhibited a total EPS (397±52 mg/g VSS) that was not different from the alternating control reactor (378±55 mg/g VSS) - Figure 4.1
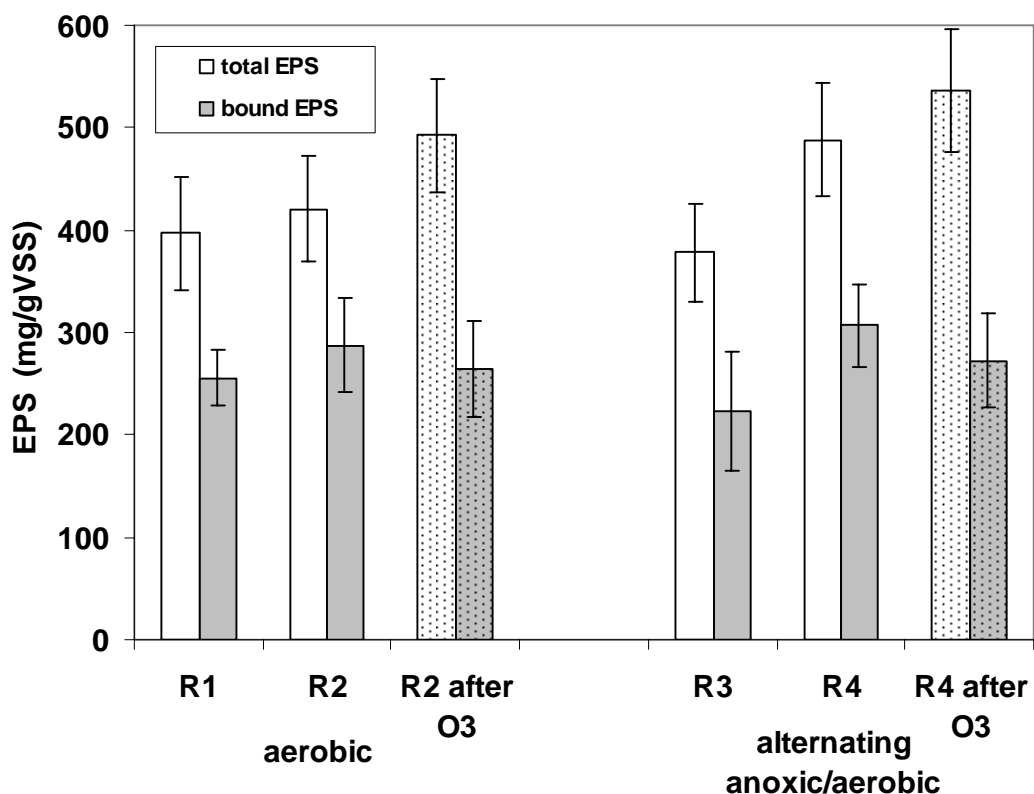
Figure 4.1. The amount of total and bound EPS in RAS for aerobic (R1, R2) and alternating (R3, R4) control and ozonated reactors, respectively. Results "after O_3" mean EPS measured immediately in the ozonated portion of RAS. Values shown are the average and vertical bars represent standard deviation of the mean.

For the control reactors, most of the total EPS was bound rather than soluble, even when standardized to the amount of VSS for each sample (Figure. 4.1). However the ratio of bound-to-total polymers (Figure 4.2), which seems to be a more universal measure for different biomasses, is higher in aerobic reactors ($66\pm5\%$ versus $59\pm12\%$), showing that more polymers are bound in the dense, stronger structure of the floc. It corresponds well with the previous findings of the VSS/TSS ratio and microscopic observation (§2.4.4,

§2.4.7) and may indicate that under limited availability of oxygen fewer biopolymers are produced.
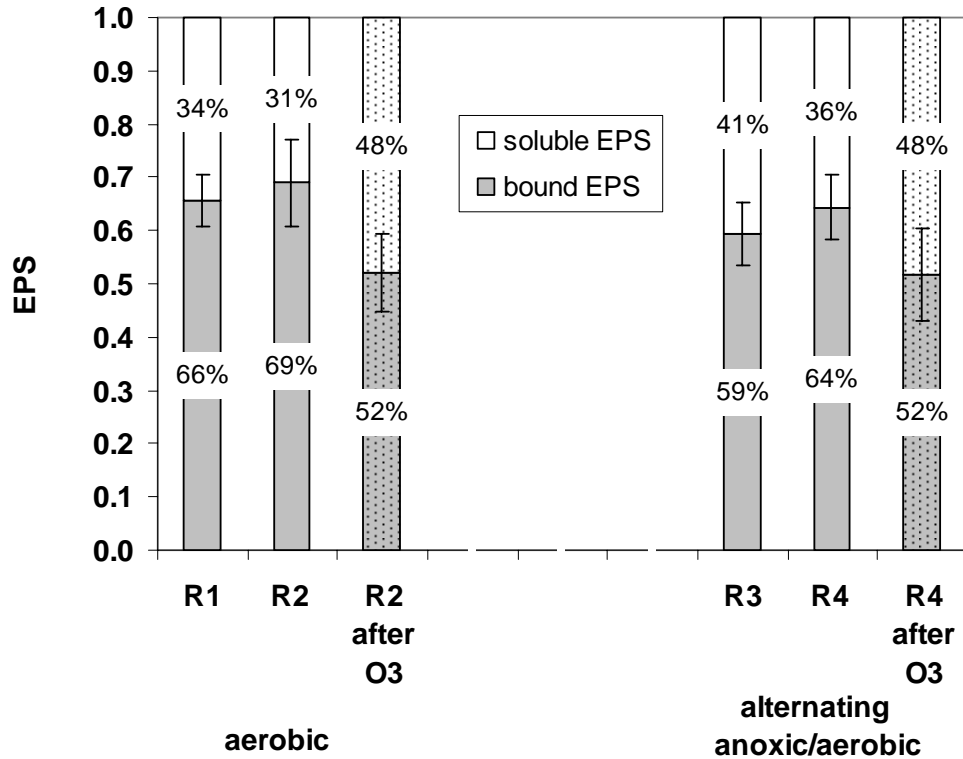
Figure 4.2. Fraction of bound and soluble EPS found in RAS for aerobic and alternating control and ozonated reactors. Soluble EPS calculated as difference between total EPS and bound EPS.

4.4.2. Direct and long-term ozone impact on the EPS content and floc structure

It was anticipated that ozonation would destroy EPS. Comparison of RAS before and immediately after ozone treatment revealed that the amount of measurable total EPS actually increased after oxidation while the amount of bound EPS remained unchanged (Figure 4.1). By destroying part of the flocs and cell walls of microorganisms, ozone

may have caused the release of cytoplasm and membrane components into solution which can become loosely bound to the remaining flocs and thus increase the proportion of measurable EPS as a fraction of the total mass (Figure 4.2). The immediate impact of partial ozonation of the RAS was to increase the amount of soluble EPS in both the aerobic and alternating sludges (Figure 4.2).

The long-term impact of ozonation of 20% of the RAS on EPS level is shown in Figure 4.1 (bars R2, R4). The amount of both total and bound EPS was higher in R2 (aerobic) and R4 (alternating) in comparison to the control reactors. There was a proportional increase in total EPS with the ozone dose when compared to the controls (Figure 4.3).
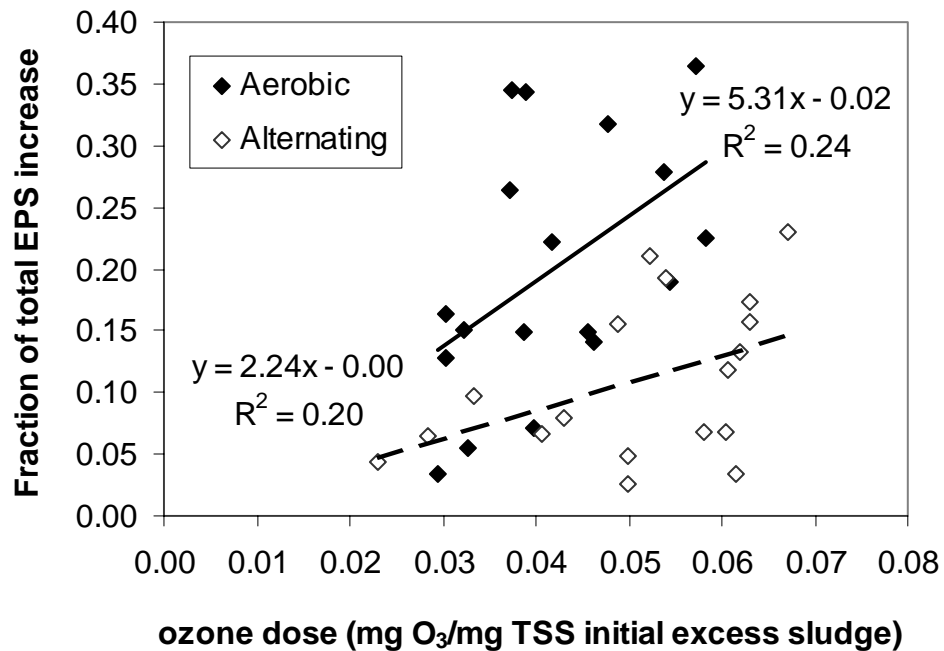
Figure 4.3. Fraction of total EPS increase in ozonated RAS in aerobic and alternating reactors (daily analyses) when compared to control reactors.

Larger ozone dose led to more total EPS produced. The increase was much more pronounced in the aerobic reactor than in the alternating one. A similar trend is reflected in Figure 4.1: "after ozone treatment". Some 17% and 10% of increase in total EPS content for aerobic and alternating ozonated reactor respectively were observed. One possible explanation is the feature of the extraction method, which determines protein, carbohydrates and DNA (Spaeth and Wuertz, 2000). Ozonation of weak, open flocs in an alternating reactor leads to faster biomass destruction (§3.4.2, Figure 3.1) thus quick oxidation of possibly loosely bound EPS and other colloidal and non-settleable material. The produced sCOD consists of volatile fatty acids, alcohols, amino acids which may not be detectable by the acetone/ethanol precipitation (Spaeth and Wuertz, 2000) but are included in fast sCOD production (§3.4.3, Figure 3.2). During ozonation of dense, compact aerobic flocs, with higher content of bound EPS, colloidal and particulate by-products are created - most remain in the liquid phase, not transformed by these ozone doses to products detected as sCOD (0.45µm filtered samples). Both ozone oxidation steps – breakage of flocs to macromolecules and their further solubilization occur in ozonated tank simultaneously. Therefore large errors can be introduced in the interpretation of the data (Figure 4.3).

After long-term ozonation of 20% of RAS, the ratio of bound- to-total polymers (Figure 4.2) was higher in R2 (aerobic) and R4 (alternating) in comparison to the control reactors (bars R1, R3). This phenomenon can be explained by analyzing the food-to microorganisms (F/M) ratio. Each reactor receives the same amount of soluble

wastewater (Food). The destruction of part of the solids (biomass) leads to the corresponding additional sCOD released from cells. This increases the F/M ratio in ozonated reactors which in turn promotes microbial activity and increases the EPS yield.

Liao et al. (2001) suggested that sludge at higher F/M possibly does not consume all the carbon sources available for growth. Excess carbon can be converted to intracellular storage granules and extracellular polymers that accumulate as EPS. If true this can explain why the amount of both total and bound EPS level in ozonated reactors in comparison to initial EPS (control reactor) is higher after prolonged daily day ozonation.

As a result increased amount of polymer helps in flocculation and overcomes the effect of disintegration by ozone while creating stronger flocs. The increase of the fraction of bound EPS in ozonated reactors (Figure 4.2) shows that ozonation followed by the biological step of treatment in a long SRT system favours not only EPS production but also degree of their binding into the floc structure. The histograms in Figure 4.4 show the frequency of floc area and perimeter length in control and ozonated alternating reactor. In comparison to Figure 2.3 it can be noted that after long-term ozonation alternating flocs became similar to the flocs from aerobic control reactor – smaller and round in shape (see §2.4.7.).
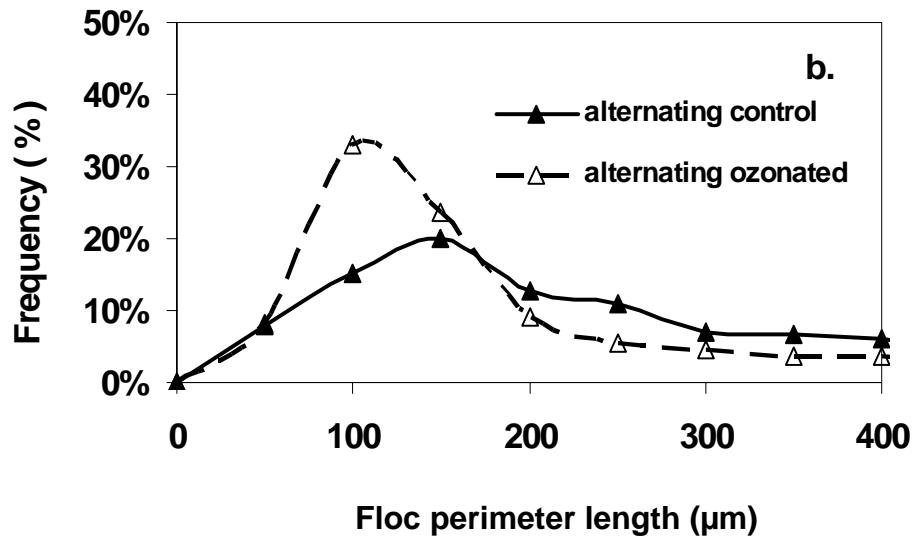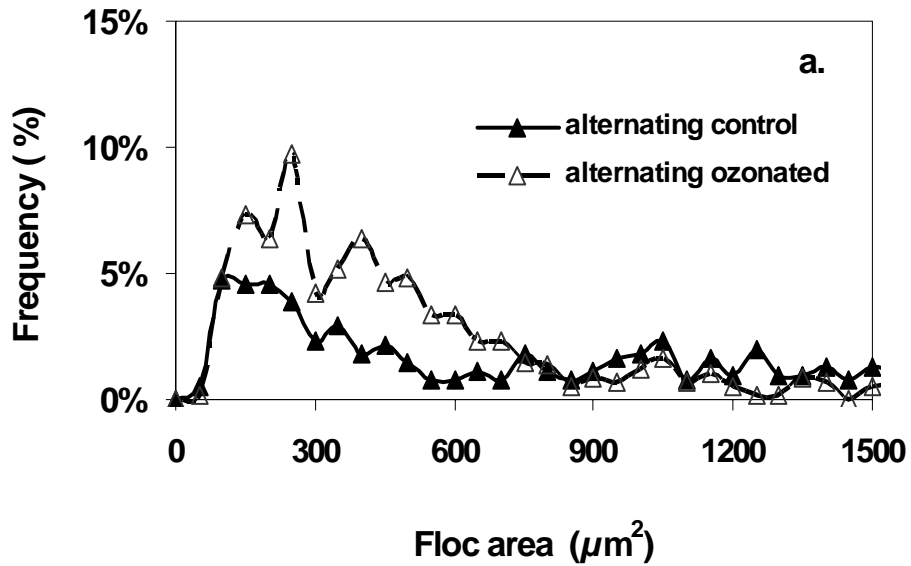
Figure 4.4. The frequency of floc area (a) and perimeter length (b) for flocs for alternating control and ozonated reactors (samples from the RAS). Results from the alternating control reactors were the same as those presented in Figure 2.3.

4.4.3. Settling

The sequencing batch reactors used had typically very low SVI values (<60 mL/g) indicating that settling was rapid in all cases. Control reactors had identical SVI of 59 ± 4

and 59±5 mL/g under aerobic and alternating conditions, respectively. Partial RAS ozonation reduced the SVI to 43±4 in the aerobic reactor and even more in the alternating reactor to 30±3 mL/g. This may be an indication of the improvement in settling properties as has been observed previously (Kamiya and Hirotsuji, 1998, Weemaes et al., 2000). The results in such a range may not be representative of what would happen in a full-scale continuous system. Bilanovic et al. (1999) noted that flocs with lower VSS/TSS have more stable settling properties. Since VSS/TSS decreased after long-term ozone treatment in this research (§3.1, § 3.4.2) the heavier floc could have contributed to the improvement of settleability

To investigate further, batch tests were conducted by measuring transmittance of supernatant in ozonated and control reactors - Figure 4.5 a, b. Ozone treatment and destruction of part of RAS initially produced fine, non-settleable particles which caused turbidity and deterioration of the settling and effluent quality. However after initial ozone-caused deflocculation, biological treatment in the reactors led to re-flocculation which countered that negative effect. Overall transmittance increased over time in all four reactors and settleability in ozonated trains had not deteriorated in comparison to the control reactors. Reflocculation under anoxic conditions was always slower than under aerobic conditions (Figure 4.5b) for both ozonated and control reactors. The flocculation (and possibly EPS production) could have perhaps been inhibited by lack of aerobic microbial activity – which is considered key to efficient floc formation. The latter is in agreement with findings of Wilen et al. (2000)
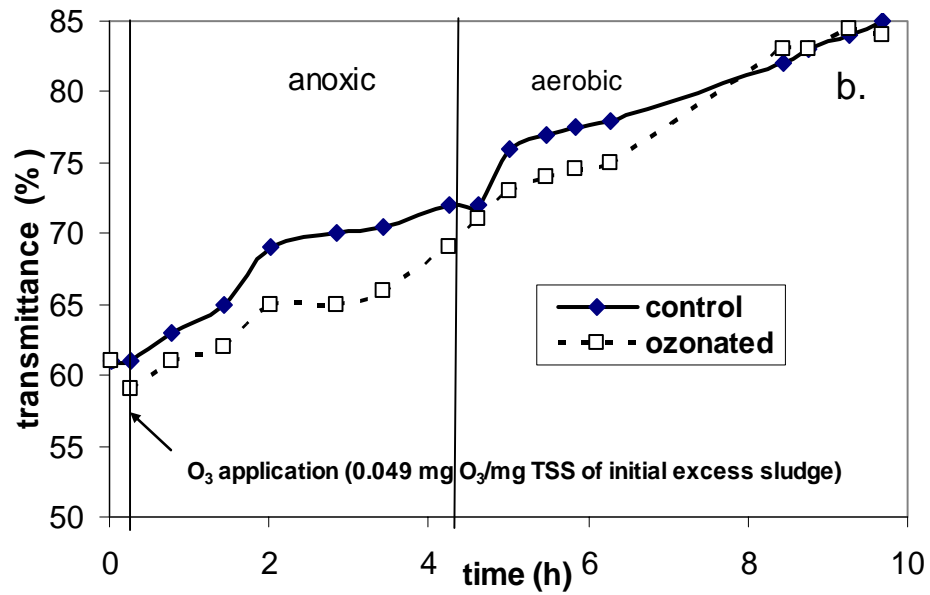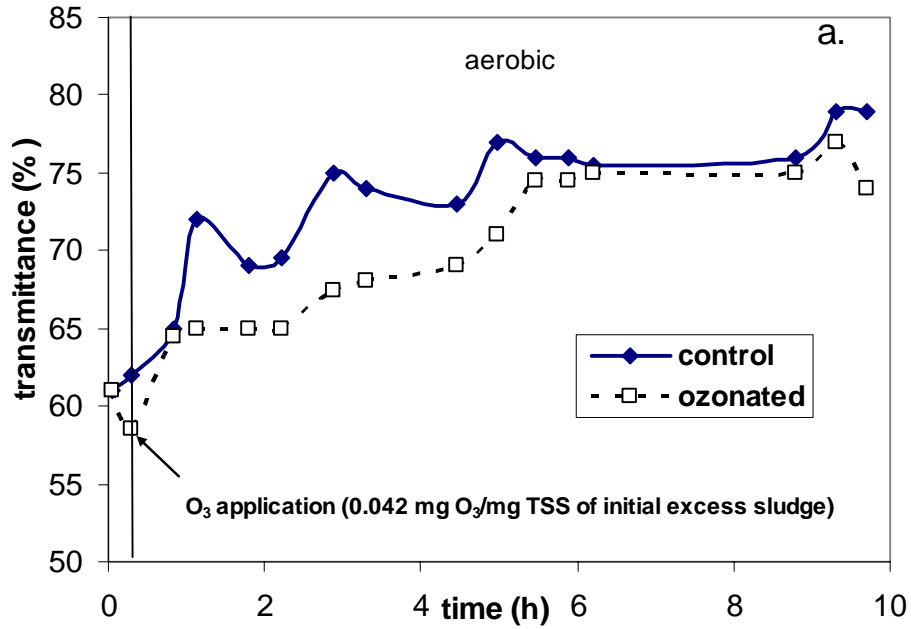
Figure 4.5. Re-flocculation measured as transmittance after 5 min. in ozonated and control reactors, under aerobic (a) and alternating anoxic/aerobic (b) conditions.

After switching to aerobic conditions (Figure 4.5b), additional clarification occurred - in fact the transmittance exceeded the clarity in aerobic reactors and demonstrated possible

improvement of settling in alternating reactors. Overall, ozonation had a positive impact in the final settleability of the sludge.

4.4.4.   Dewatering

The increased amount of bound EPS (Figure 4.2) could adversely affect dewaterability of the sludge. To assess the impact on dewatering, the capillary suction time (CST) test was performed. The CST test is universally   accepted as a method of evaluation of dewaterability of sludge due to its simplicity. The measurements obtained by using this filtration test may not be fully representative of dewaterability by centrifugation dewaterabilities because the latter is a settling phenomenon (Karr and Keinath, 1978). Twelve test periods were chosen for the test. RAS concentration was 5500 to 6000 mg TSS/L. Average results and standard deviations are presented in Figure 4.6. The aerobic reactors had higher CST times and hence poorer dewaterability than the alternating reactors (Figure 4.6). Ozonation had no impact on CST for either reactor.
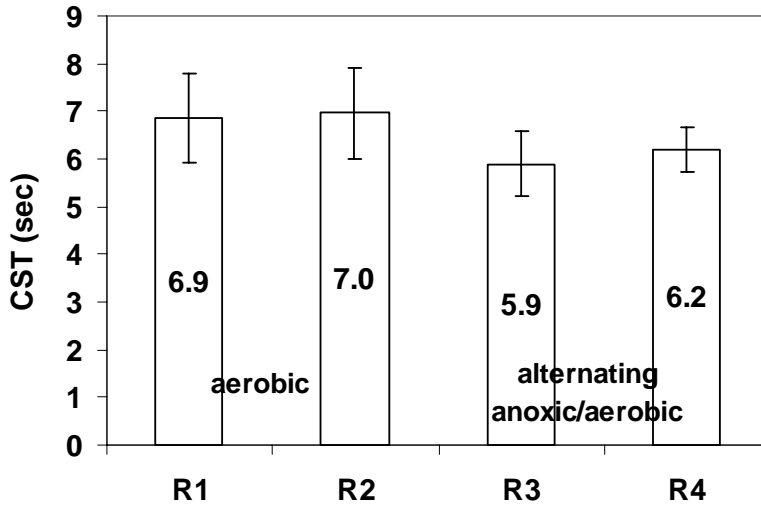
Figure 4.6. Average CST in RAS samples of control (R1, R3) and ozonated (R2, R4) reactors. Errors bars are standard deviations.

4.4.5. Final batch test

A batch study of the impact of ozone dose (Table 4.1) on characteristics of directly treated sludge confirmed that ozonation led to the destruction of flocs, as indicated by the decrease in TSS and increase in sCOD, which was directly proportional to the ozone dose. At the highest doses ozonation decreased the amount of total and bound EPS and adversely impacted the dewatering (CST). Below that threshold of highly destructive doses the oxidation increased soluble COD and the amount of soluble EPS and total EPS. This increase in EPS adversely affected the dewaterability of the sludge (CST). The overall impact was minimized in the long-term operation of the SBR reactors as only 20% of RAS was subjected to ozonation (Figure 4.6).

 Degradation of EPS reduces their water retention properties by releasing bound water (Neyens et al., 2004). In this batch study it was found that increased EPS due to all but

the lowest doses of ozone increased the CST. The latter could be the result of TSS destruction and production of fine particles which clog the filter paper. After returning the ozonated portion of RAS to the reactor the newly produced sCOD was utilized during biological process and re-flocculation occurred under both aerobic and alternating conditions.

The impact of ozonation was greater on the sludge from alternating reactor than from the aerobic one. Ozonation increased the relative and absolute values of the total EPS, soluble/total EPS ratio and sCOD to a greater extent possibly due to the structure of flocs.

Table 4.1. Characteristics of the ozonated fraction of RAS with. increasing dose of ozone

Aerobic reactor				Ozone dose			
Characteristics	mgO_3/mg TSS initial excess sludge	0.000	0.022	0.043	0.058	0.070	0.088
TSS	mg/L	6075	-	-	-	-	5950
sCOD	mg/L	46	62	79	90	107	142
total EPS	mg EPS/g VSS	388	437	440	441	471	199
bound EPS	mg EPS/g VSS	303	314	248	249	279	116
soluble /total EPS	-	0.22	0.28	0.43	0.43	0.41	0.42
CST	sec	6.4	6.6	7.1	7.4	7.7	8.1

Alternating anoxic/aerobic reactor				Ozone dose			
Characteristics	mg O_3/mg TSS initial excess sludge	0.000	0.018	0.034	0.049	0.059	0.078
TSS	mg/L	5575	-	-	-	-	5375
sCOD	mg/L	41	71	97	124	138	175
total EPS	mg EPS/g VSS	542	547	567	558	569	268
bound EPS	mg EPS/g VSS	358	322	261	265	257	88
Soluble /total EPS	-	0.34	0.41	0.54	0.52	0.55	0.67
CST	sec	6.0	6.5	6.5	7.0	8.0	9.0

4.5.   Conclusions

- The ratio of bound to total EPS was higher in aerobic reactors indicating that flocs there had stronger structure than flocs in alternating reactors.

- Ozone destroys flocs and microbial cells thus a fraction of the bound polymeric material was transferred to the solution and led to increased soluble and total EPS.

- Because of additional sCOD produced during ozonation and increased apparent F/M ratio, the production of EPS was favoured in ozonated reactors. The amount of bound EPS increased during biological step in comparison to the control reactors and consequently the floc stability was strengthened.

- The alternating reactors showed the potential to improve settling due to enhanced flocculation, leading to greater clarity of supernatant.

- Ozonation of RAS deteriorated dewaterability as defined by the CST however these impacts were not significant in terms of the long-term operation of the SBR reactors. Dewaterability was measured by CST test and found to be better in alternating anoxic/aerobic reactors than in aerobic ones. This indicated that incorporation of an anoxic zone for biological nutrient removal leads to improvement in sludge dewatering.

5. INVESTIGATION OF REASONS FOR HIGHER NITRIFICATION RATES UNDER ALTERNATING ANOXIC/AEROBIC CONDITIONS.

5.1. Introduction

The previous research showed that higher nitrification rates were achieved in reactors under alternating anoxic/aerobic conditions than in identical reactors operated under strictly aerobic conditions (§3.4.5.3). Faster nitrification rates can lead to cost savings in plant design so the phenomenon is worth closer examination. This study will investigate the relationship between the mode of operation and the nitrification rate and particularly how the environment created in the reactor affects the composition of microbes responsible for nitrification.

Nitrification is the most common method of ammonia removal from wastewater. It is an aerobic autotrophic process where ammonia nitrogen is oxidized to nitrite by ammonia-oxidizing bacteria (AOB) followed by oxidation of nitrite to nitrate by nitrite-oxidizing bacteria (NOB). Most AOB are closely related evolutionarily as genera such as *Nitrosomonas* and *Nitrosospira* form a cluster within the β-subclass of Proteobacteria (Koops et al., 2006). The exceptions include marine *Nitrosococcus spp.* which forms a separate branch within a γ-subclass and ammonium-oxidizing Archaea which are only beginning to be isolated in pure culture. Most of what is known about AOB physiology has been gained from studies of *Nitrosomonas europea* (Costa et al., 2006). Many AOB have been isolated in pure culture although they possibly constitute just a fraction of the

members of AOB community that co-exists in natural environments. AOB are important in many environments including a wide variety of soils and aquatic habitats ranging from marine to freshwater. They also have many practical applications - from agriculture to aquaculture and wastewater treatment. Within the nitrifying wastewater treatment systems such as used in this study the aggregates of suspended nitrifiers are generally dominated by *Nitrosomonas* and *Nitrosospira* spp.. Their composition and abundance depends on the process operating conditions (Geets et al., 2006) (Table 5.1).

Most NOB are distantly related to the AOB belonging to a different subclasses of the Proteobacteria, mostly the alpha group (Abeliovich, 2006). NOB form four phylogenetically distinct groups: *Nitrobacter, Nitrococcus,* and the outlying *Nitrospira* and *Nitrospina* spp. Initial studies of NOB focused on *Nitrobacter*, which are ubiquitous and easily isolated with the higher nitrite concentrations typically used in culturing. More recent studies using molecular techniques have indicated that *Nitrospira* is the dominant NOB in most wastewater treatment systems with *Nitrobacter* playing a minor role, although they often co-exist (Kim and Kim, 2006). *Nitrobacter* spp. are more versatile than *Nitrospira* as they can use a wider variety of organic compounds and can grow under both aerobic and anaerobic conditions (Daims et al., 2001) (Table 5.1). *Nitrospira* were the predominant group of NOB detected in a study of an alternating anoxic/aerobic submerged membrane bioreactor (Sofia et al., 2004).

AOB and NOB interact with each other and are affected by the environmental (physical and chemical) conditions by the supply and concentrations of substrates and products,

and by positive and negative interactions with other populations. Nitrifiers are influenced by factors like pH, alkalinity, salinity, temperature, C/N ratio, as well as concentrations of oxygen, ammonium, nitrite, and organic compounds (Chen et al., 2006; Geets et al., 2006). Many of these factors are interconnected. For example, the abundance of organic compounds affects the competition of autotrophic nitrifiers with various heterotrophs for substrates like oxygen, yet these groups comprise an effective food web (Kindaichi et al., 2004). The alkalinity affects the buffering of the water (nitrification generates acidity) and thus the pH. This in turn affects the ionization of the ammonium which can exist most commonly as NH_4^+ at the pH typical of wastewater or the more bioavailable but toxic free form NH_3 at increasing pH (above 9.4) (Gerardi, 2002). The success of nitrifiers depends not only on their activities during periods of substrate abundance but also their survival during starvation and their ability to quickly flux between these two states (Geets et al., 2006).

Nitrifiers oxidize nitrogen compounds by two-step cooperation between the AOB and NOB yet these organisms also compete for common resources, particularly oxygen. The main kinetic coefficients (half-saturation constants K_s for substrate ammonia/nitrite, half-saturation constants for oxygen K_o, yield Y, growth rate μ_{max} and decay rate b_{max}) for these pairs of AOB (*Nitrosospira, Nitrosomonas*) and NOB (*Nitrospira, Nitrobacter*) are summarized in Table 5.1. It is generally thought that AOB might out-compete NOB for oxygen because of their lower K_s values for oxygen than NOB (Schramm et al., 1999, 2000; Aoi et al., 2004). This greater affinity to oxygen makes them more adaptable to low levels of oxygen.

The availability of oxygen can affect which AOB populations develop. In sequencing batch reactor studies low DO levels (1.0 mg/L) favored *Nitrosospira* (1.2% of the total bacteria) over *Nitrosomonas* (0.7%). Increasing the DO level to 3.5-4.5 mg/L increased the proportions of both types of AOB but especially the *Nitrosomonas* which occupied 3.5% of the total bacterial mass, compared to just 2% for *Nitrosospira* (Li et al., 2006). The length of anoxic periods in alternating reactors also affects the relative composition of *Nitrosomonas* spp. and the proportion of the minor *Nitrosospira* as in one study *Nitrosospira* were enriched with longer anoxic treatment (Mota et al., 2005b). *Nitrosospira* spp. form looser micro-colonies which is probably an adaptation to low oxygen and ammonium conditions (Okabe et al., 2004). Even among the *Nitrosomonas* spp the *N. oligotropha* may be better adapted to low oxygen conditions than *N. europaea*-like microbes (Geets et al., 2006). A survey of activated sludge from different wastewater treatment plants indicated that *N. oligotropha* was ubiquitous whereas *N. europaea* and *N. communis* were found only in sludge from anaerobic/aerobic systems (Limpiyakorn et al., 2005). Availability of DO even affects development of different types of *N. europaea* and their growth kinetics (Park and Noguera, 2004). In general most AOB appear to tolerate periods of starvation and are capable of rapid recovery, although *Nitrosomonas europaea* out-competes other nitrifiers under variable substrate availability (Geets et al., 2006).

The recent detection of *Nitrospira*-like bacteria in various natural soils and the absence of *Nitrobacter* in similar environments might indicate an advantage such as better

adaptation to starvation with their higher substrate affinity (Schramm et al., 1999). *Nitrospira* were the dominant species in wastewater treatment plants with low nitrite and in freshwater (Taylor and Bottomley, 1999). *Nitrosomonas* and *Nitrobacter* on the contrary dominated in fertilized soils, human-impacted water systems and NH_4^+-rich treatment plants (Table 5.1). It was reported (Daims et al., 2001) that organic carbon is able to support the growth of *Nitrobacter* under anoxic conditions, which gives it a competitive advantage for denitrification in soils. Both *Nitrobacter* and *Nitrospira* were detected in wastewater effluent and river sediments downstream of an effluent outlet, yet the *Nitrobacter* dominated closer to the outlet while *Nitrospira* dominated further downstream where nitrogen sources were reduced (Cebron and Garnier, 2005). In alternating anoxic/aerobic reactors treating swine wastes *Nitrospira* were usually the dominant NOB although they were out-competed by *Nitrobacter* in some cases. It appears that the factors selecting for one or the other species remain obscure (Mota et al., 2005b).

Table 5.1. Comparative characteristics of two groups of ammonia oxidizers AOB (*Nitrosospira, Nitrosomonas*) and nitrite oxidizers NOB (*Nitrospira, Nitrobacter*), common in bioreactors.

Bacteria	AOB *Nitrosospira*	AOB *Nitrosomonas*	NOB *Nitrospira*	NOB *Nitrobacter*
Phylogeny	β-Proteobacteria	β-Proteobacteria	δ-Proteobacteria	α-Proteobacteria
Occurrence	Natural soil populations (unfertilized) [3]	Soils with high input of N [3], NH_4^+-rich systems in wastewater treatment plants [2,4,1,9]	Numerically dominant in WWTP NO_2^--weak communities, various soils, freshwater [7, 8, 10]	Soils with high input of N, human-impacted water systems [7, 8]
Organic C substrates [7]	-	Amino acids, fructose, lactose, pyruvate	pyruvate under aerobic conditions	acetate, pyruvate, butyrate, propionate under aerobic and pyruvate under anoxic conditions
Substrate affinity [1, 2, 4, 6]	High; adapted to low substrate and oxygen conditions	Low, can tolerate high substrate concentrations	High; adapted to low substrate and oxygen conditions	Low, can tolerate high substrate concentrations
half-saturation constant K_s mg N/L	Low [2, 3] 1.96 (NH_4^+) [3] 0.01-0.02 (NH_3) [3] 0.08-0.15 (NH_3) [2,11]	High [2, 3] 26.60 (NH_4^+) [3] 5.88-46.20 (NH_4^+) [14] 12.27-27.44 (NH_4^+) [12] 0.17-0.27 (NH_3) [3]	Low [2] 0.14 (NO_2^-) [2] 0.11-0.50 (NO_2^-) [13]	High [2] 7.00 (NO_2^-) [2] 9.88-17.36 (NO_2^-) [12] 1.49 (NO_2^-) [16]
half-saturation constant K_o mg O_2/L	-	0.51 [2] 0.22-0.56 [12]	0.13 [13] 0.47 [13]	1.98 [2] 0.17-4.32 [12]
growth rate μ_{max} [1, 2, 5, 6] **1/h**	Low (K-strategist) -	High (r-strategist) 0.043 [15] 0.039 [17]	Low (K-strategist) -	High (r-strategist) 0.020 [16] 0.032 [18] 0.043 [17]
Yield Y mg VSS /mg N	-	0.120 [15]	-	0.020-0.070 [19] 0.045-0.071 [16]
decay rate b_{max} 1/d	-	0.26 [15]	0.14-0.15 [20]	0.07 [16]

[1] Kim and Kim, 2006; [2] Schramm et al., 2000; [3] Taylor and Bottomley, 1999; [4] Mota et al., 2005; [5] Wagner et al., 2002; [6] Li et al., 2006; [7] Daims et al., 2001; [8] Cebron and Garnier, 2005; [9] Logemann et al., 1998; [10] Abeliovich, 2006, [11] Jiang and Bakken, 1999; [12] Laanbroek et al., 1994; [13] Manser et al., 2005; [14] Stehr et al.,

1995; [15]Vadivelu et al., 2006b (30°C); [16] Vadivelu et al., 2006c; [17]Keen and Prosser, 1987 (30°C); [18]Yoshioka et al., 1982; [19] Philips et al. 2002; [20] Manser et al., 2006.

The operation of sequencing batch reactors in either aerobic or alternating aerobic/anoxic conditions would likely influence the rates of nitrification by affecting both the AOB and NOB. Faster nitrification in the alternating reactor was not expected as these conditions select for denitrifiers at the expense of the conditions (aerobic, autotrophic) preferred by nitrifiers (§2.2.2). Several factors could potentially account for higher nitrification rates under alternating conditions:

1) Competition for oxygen could limit nitrification in the aerobic reactor as the nitrifiers compete with abundant heterotrophs which actively degrade organic compounds in the feed at the same time as nitrification takes place;

2) Nitrification in the alternating reactor could be accelerated by the higher alkalinity and pH created by the preceding denitrification stage (denitrification returns about 50% of the alkalinity lost during nitrification)

3) The higher initial ammonia concentration in the alternating reactor due to ammonification could stimulate nitrifiers' activity;

4) The alternating conditions particularly with the associated effects on oxygen and nitrite concentrations could allow for the development of nitrifier populations that carry out faster nitrification.

5.2.   Materials and methods

Sequencing batch reactors (SBR), established under aerobic or alternating aerobic/anoxic conditions and used as controls in previous research were employed here to investigate

nitrification rates under these two regimes. The reactors were operated as previously described (§ 2.2) with the only difference that air was supplied from electrical air pumps (Elite 801) instead of lab air line. Detailed observations of the environmental and chemical conditions were recorded and batch kinetic studies were performed to deduce which factors could have the most pronounced impact on nitrification rates in the reactors.

To address the potential direct and indirect effects of COD and anoxia batch tests were performed outside of the main lab reactors. The biomass originated from the main reactors and was developed separately under strictly aerobic and alternating conditions. The nitrification process itself was investigated through kinetic studies in an aerated tank but without organic feed (beef and yeast extract; COD). For testing the first step of nitrification, ammonia (ammonium chloride $3.6 - 82.7$ mg NH_4^+-N/L) and alkalinity (sodium bicarbonate 0.5 g/L $NaHCO_3$ per 0.2 g/L NH_4Cl) were supplied in excess; to evaluate the second step of nitrification, nitrite (sodium nitrite 2.0 - 62.7 mg NO_2^--N/L) was used instead of ammonia. In these batch tests the alternating biomass did not experience the anoxic phase; rather the test was performed after 9 h of aeration to exclude the direct impact of anoxic conditions. DO was supplied in excess and was always above 2.5 mg O_2/L. In this way, the nitrifying potential of each biomass was investigated directly as additional environmental factors (COD, denitrification, and anoxia) were excluded.

The impact of increased pH on nitrification was evaluated by dividing the biomass equally into two reactors, and adding additional sodium bicarbonate (0.15-0.20 g/L) to one of them, thereby increasing the pH (7.63 - 7.93) and the alkalinity (from 450 to 612 mg $CaCO_3$/L). Similarly, the impact of the addition of COD was assessed by amending one of two reactors with sodium acetate (0.7-1.10 g/L; food-to-microorganisms ratio F/M 0.23-0.36). Acetate was selected as a simple organic carbon source to avoid the additional ammonia release from complex organic sources such as beef and yeast extract. Each comparison was performed at least twice, and results were compared using student's t-test with P = 0.05.

The nitrite production rate (NO2PR) and nitrite utilization rate (NO2UR) were calculated based on changes in nitrite concentration over time. The rest of analysis (TSS, VSS, sCOD, TCOD, NH_4^+, NO_3^-, NO_2^-, pH, alkalinity, DO) and kinetic studies were performed as described in §2.2.3 and §3.3.2, respectively.

5.3. Results

5.3.1. Nitrification in the main aerobic and alternating reactors

The NH_4^+, NO_2^-, NO_3^- and O_2 profiles from a typical kinetic study were plotted in Figure 5.1. Nitrification was consistently faster during the aerobic phase of the alternating anoxic/aerobic reactor than in the strictly aerated reactor, although both values were in the range reported in similar lab scale systems (3.5-13.1 mg/L h) (You et al., 2003; Lee and Oleszkiewicz 2003, Peng et al., 2004, Hu et al., 2005). During the strictly aerobic

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treatment, ammonia removal and nitrate production occurred simultaneously with organic matter removal. There was occasionally an apparent slight lag in ammonia oxidation in the aerobic reactor at the start of the cycle caused by the high initial soluble COD content and ammonification, but otherwise both the AUR and nitrate production rates were linear throughout the cycle. In the alternating treatment, changes occurring in the initial anoxic phase appeared to impact the subsequent aerobic nitrification. At the time of the switch to aerobic conditions, the environment in the alternating reactor was different from the aerobic one, as the pH, alkalinity, and nitrite concentration were higher, yet the nitrate and COD concentrations were lower.

The alternating reactor had a consistent AUR averaging from 6.10 ± 0.37 mg NH_4^+-N/g VSS h when the reactors were established (§3.4.5.3). Two years later into the study the rate changed little and was 6.21 ± 0.33 mg NH_4^+-N/g VSS h. The AUR for the aerobic reactors was initially 4.88 ± 0.43 mg NH_4^+-N/g VSS h (§3.4.5.3) but gradually decreased over the two years to 3.03 ± 0.31 mg NH_4^+-N/g VSS h. Nitrite transiently accumulated during denitrification in the alternating reactor and remained high after switching to aerobic conditions. On the contrary, it was consistently low in the aerobic reactor (Figure 5.1a), indicating a balance existed between the two phases of nitrification – the production of nitrite by oxidation of ammonia, and the removal of nitrite by its oxidation to nitrate. Nevertheless, nitrate production was faster in the alternating reactor (Figure 5.1b), consistent and proportional to the AUR. The increase in dissolved oxygen was also much faster in the alternating reactor, even though it started in anoxic conditions (Figure 5.1b).
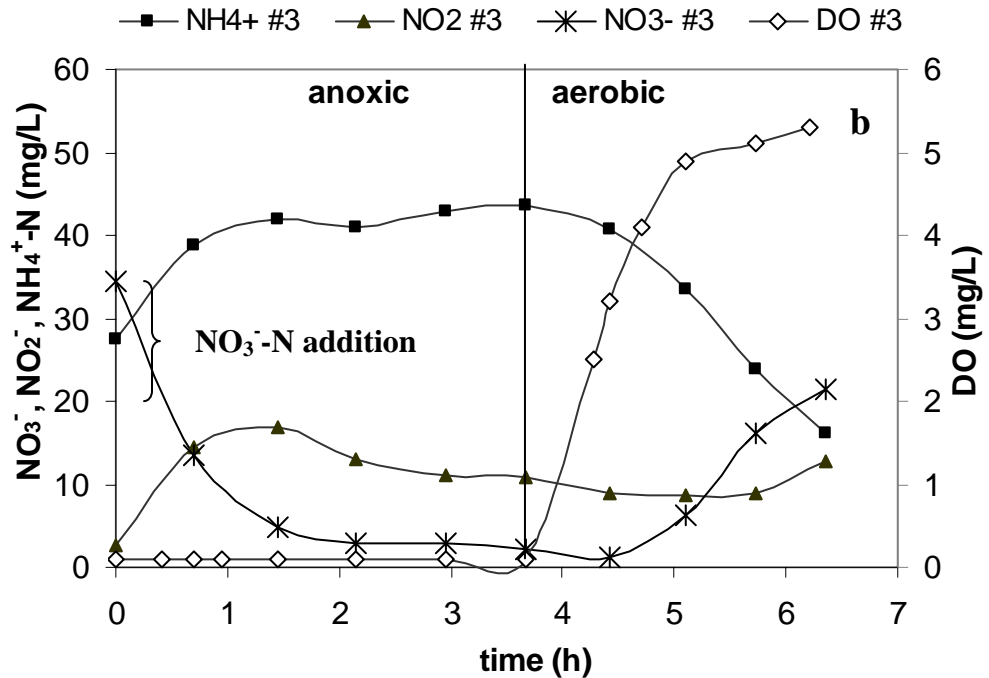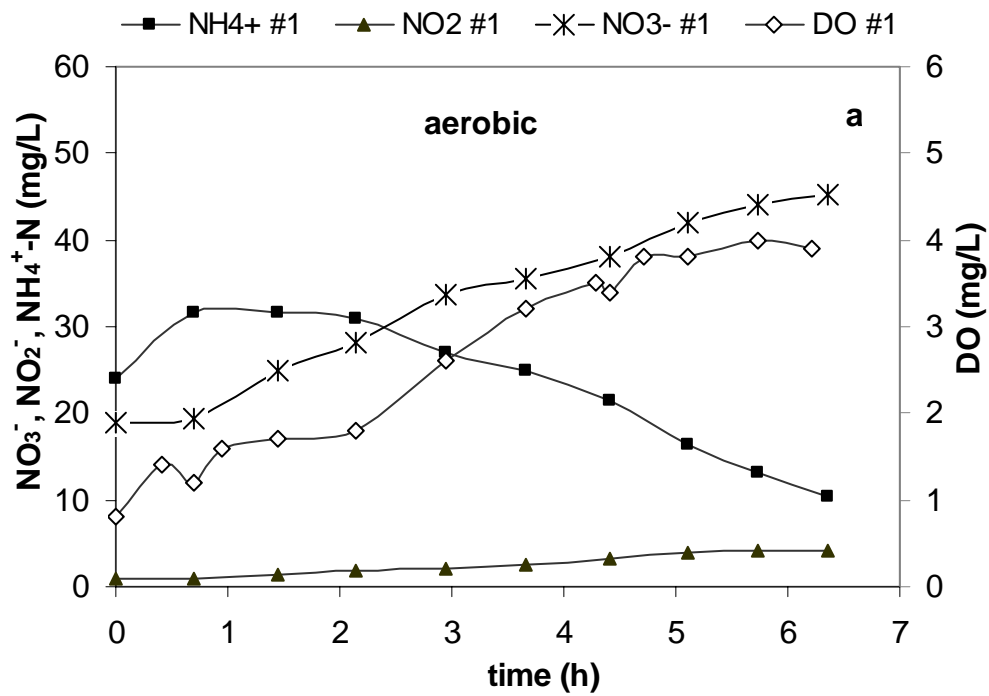
Figure 5.1. Concentration profiles of ammonia, nitrite, nitrate and dissolved oxygen during nitrification stage in the aerobic (a) and alternating reactors (b).

5.3.2. Batch tests: nitrification rate vs. anoxia, alkalinity and COD content

Batch tests were performed to directly compare nitrification rates between biomass from the aerobic and alternating reactors. Figure 5.2 shows the average nitrogen profiles from five separate tests (March-April 2006). Nitrification was monitored as the AUR, NPR, and NO2PR, and normalized to the biomass (VSS). Effects of anoxia and COD on differences between the two types of reactors were removed by conducting the experiments with both types of biomass under the same conditions with no anoxic period and no organic substrate. Nitrification rates in the batch reactors were comparable to those obtained in the main reactors. The biomass from the alternating reactor had higher AUR (6.23 ± 0.96 mg NH_4^+-N /g VSS h for initial ammonia content >15 mg NH_4^+-N/g VSS), and higher nitrite accumulation rate (2.65 ± 0.66 mg NO_2^--N/g VSS h) than the biomass from the aerobic reactor (3.03 ± 0.63 mg NH_4^+-N/g VSS h; 0.46 ± 0.27 mg NO_2^--N/g VSS h, respectively). Operating without the anoxic phase did not significantly impact the nitrification rates for the alternating reactor biomass. Nitrification in terms of ammonia removal, nitrate production, and nitrite accumulation, was inherently faster for "alternating biomass", even if no anoxic stress was applied before the test.
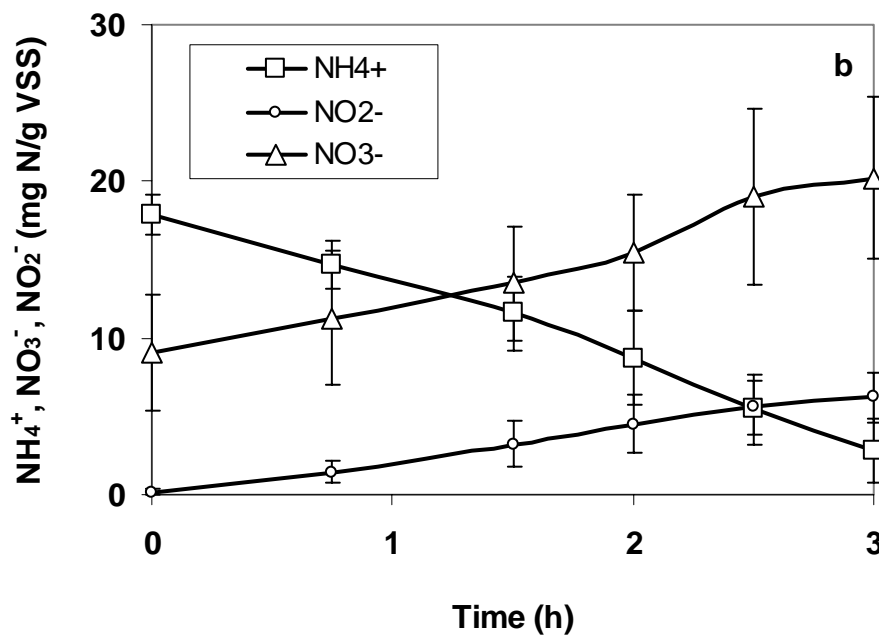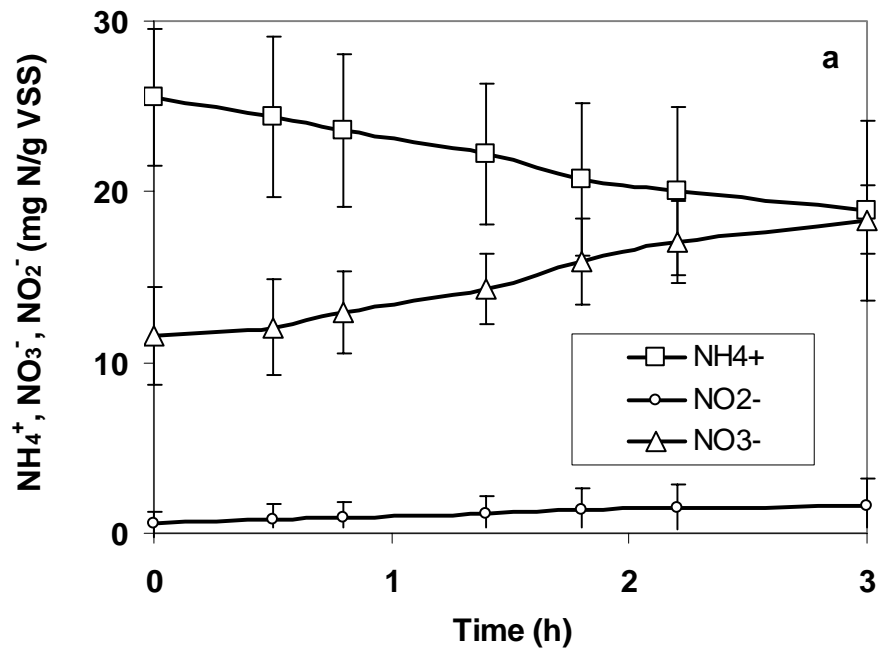
Figure 5.2. Nitrification in typical aerobic batch tests with biomass taken from the aerobic (a) and alternating (b) reactors. Points represent the average of five independent batch tests and error bars show the standard deviation of the mean.

Batch experiments were conducted with both the aerobic and the alternating biomass to test the impact of alkalinity on nitrification rates. Two identical reactors were supplied with excess bicarbonate to mimic the pH existing during nitrification in aerobic vs. alternating conditions. No correlation between the measured pH and the AUR for the aerobic or alternating biomass was found (Figure 5.3).
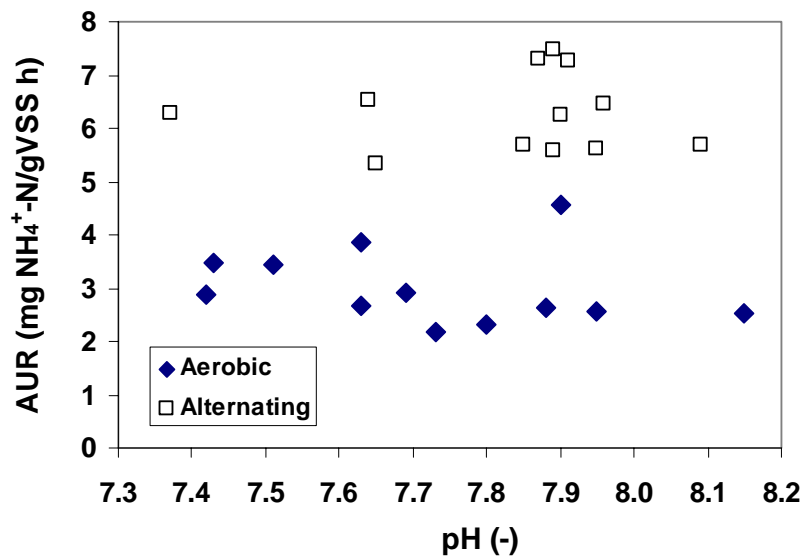
Figure 5.3. Ammonia uptake rate (AUR) vs. average pH during nitrification. Batch aerobic tests conducted on nitrifying communities taken from two different SBR operating conditions.

The elevated rates of nitrification in alternating reactors could be caused by the removal of organics during the anoxic phase thereby reducing the negative impacts on the nitrifiers (§3.4.5.3). The effects of COD were never tested directly. This was remedied here by conducting batch tests in which organic feed was omitted and the effects on nitrification were compared to parallel batch cultures of aerobic and alternating biomass that received the usual synthetic wastewater feed. In tests where COD (acetate) was

added to one portion of equally divided biomass dissolved oxygen plummeted from 5 mg/L to as low as 0.2 mg/L. The AUR decreased by 10% for aerobic biomass (in two experiments) and as much as 24% for a single (due to equipment breakdown) experiment with alternating biomass.

It can be observed from Figure 5.1 that the accumulation of nitrite was fundamentally different in the two SBR reactors.   High concentrations of nitrite accumulated during the denitrification stage in the alternating reactor. This means that the AOB and NOB were exposed to high levels of nitrite even before they would commence nitrification.   The levels of nitrite accumulation were also different in the batch tests in which the anoxic phase and the accompanying nitrite accumulation were excluded (Figure 5.2b).   There was no nitrite initially in those batch tests and thus nitrite production could be measured and directly compared for the two types of biomass. Biomass from the alternating reactor produced more nitrites and at a higher rate which was consistent with the larger nitrification rate (AUR) (Figure 5.4) The results indicated that about 84% of the ammonium accumulated as nitrite prior to further oxidation to nitrate compared to only 38% for the aerobic biomass.   It can be concluded that higher exposure to nitrite is an inherent feature of the nitrifying populations in the alternating reactor and that the exposure extends beyond just nitrite accumulation during the denitrification stage.
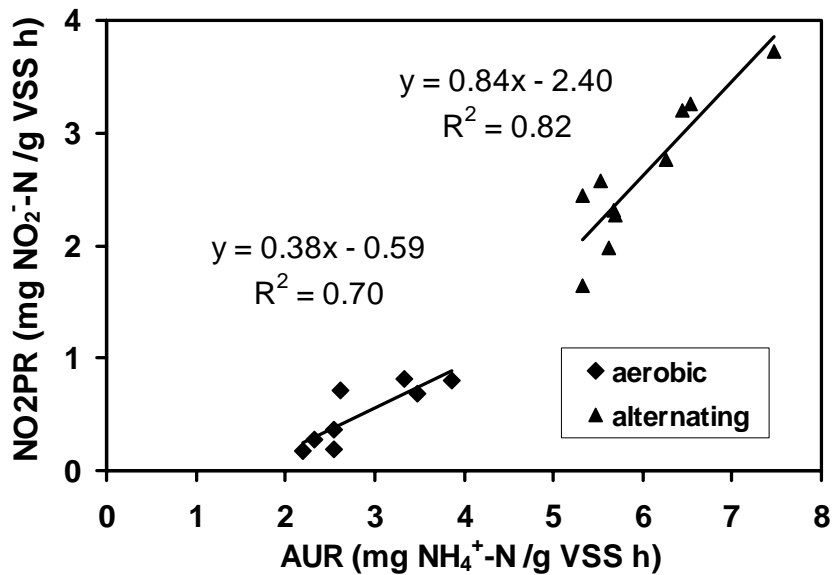
Figure 5.4. Ammonia uptake rate vs. nitrite production (accumulation) rate for two types of biomass in batch tests.

5.3.3. Batch tests: nitrification rate vs. initial substrate concentration

The rate of nitrification can depend on the initial substrate concentration if the nitrifying population is not the limiting factor. In the aerobic reactor linear depletion of NH_4^+ (Figure 5.5a) and production of NO_3^- (Figure 5.5c) suggested zero-order kinetics. The batch experiments with variable initial ammonium content confirmed that the AUR was a consistent 3.03 ± 0.63 mg NH_4^+-N/g VSS h, regardless of the NH_4^+ concentration (Figure 5.5a). The AOB in the alternating reactor gave a fundamentally different response than those in the aerobic biomass. The AUR increased with increasing ammonium at least up to 35 mg NH_4^+-N/g VSS h.
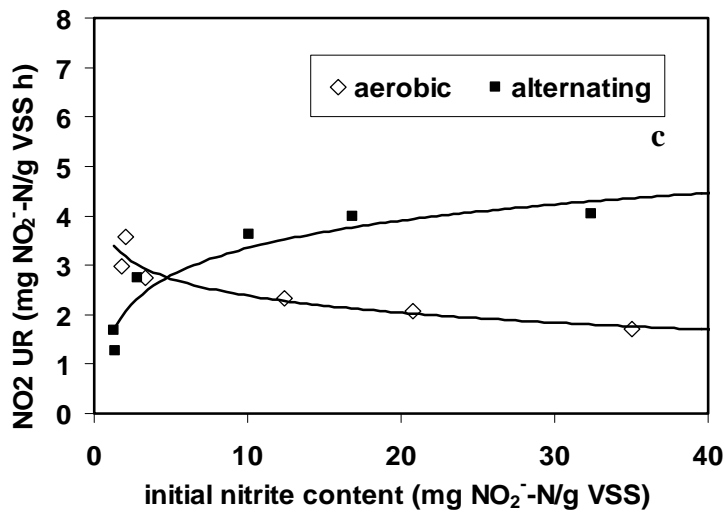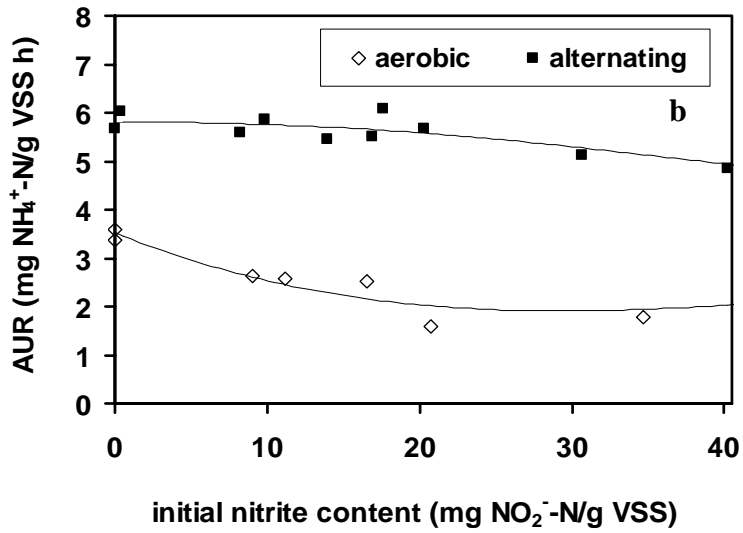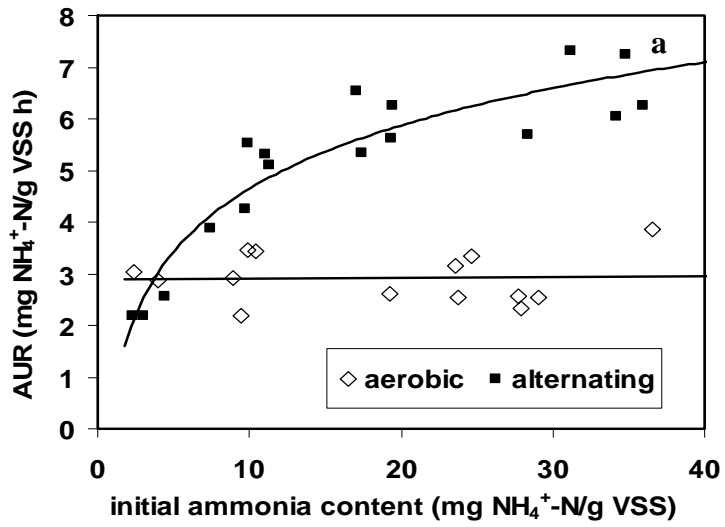
Figure 5.5. Ammonia uptake rate (AUR) vs. initial ammonia (a) and nitrite content (b) and nitrite uptake rate (NO2UR) vs. initial nitrite content (c).   Batch aerobic tests conducted on nitrifying communities from two different SBR operating conditions

Figure 5.5c shows that increased nitrite supply stimulated nitrite oxidation for the community from the alternating reactor but had a slight inhibitory effect on the nitrite utilizing biomass in the strictly aerobic community. Nitrite oxidation was as fast as ammonia oxidation in the batch tests with the strictly aerobic community which was consistent with the fact that nitrite did not accumulate in the main aerobic reactor.   For batch tests under aerobic conditions with the community developed under alternating conditions   nitrite removal was slower than the ammonia oxidation.   This was in agreement with nitrite accumulation in the first hours of the aerobic phase in the main reactor.   It appears that nitrite stimulated the NOB from the alternating reactor however they did not reach the high numerical rates of AUR of the AOB. NOB in the alternating reactor biomass tolerated higher concentrations of nitrite than their aerobic counterparts.

The effect of nitrite on ammonium oxidation was tested by performing   batch experiments analyzing the effect of nitrite amendments on the AUR of each community. Nitrite dose up to 20 mg NO_2^--N/g VSS caused no inhibition of AOB from the alternating reactor (Figure 5.5b). In fact dose as large as 40 mg NO_2^--N/g VSS led to less than 20% decrease in AUR. In contrast the AOB from the aerobic reactor experienced a decreased AUR with increasing nitrite. Some 50% decrease in AUR is shown at a dose of 20 mg NO_2^--N/g VSS - Figure 5.5b.   In summary the AOB in the alternating reactor tolerated higher concentrations of nitrite than their aerobic counterparts.

5.4. Discussion

5.4.1. Environmental conditions

The environments of the two types of treatment were different in spite of the fact they were operated under parallel conditions with the same construction, air flow rates, stirring, temperature, light levels, etc. The anoxic phase had minor impacts on the environmental conditions for the following aerobic treatment. The pH during the nitrification phase of the alternating reactor was higher than the aerobic one (8.17 ± 0.07 and 7.70 ± 0.11 in the beginning of reaction, respectively) as a result of denitrification. This difference in pH is unlikely to have caused such a huge difference in nitrification rate as the pH values measured were within the optimum range for nitrification of wastewater (pH 7.2 to 8.2) and rapid nitrification was demonstrated over a much wider pH range (Tarre and Green, 2004; Gerardi, 2002). The pH could have indirect effects by impacting the protonation of substrates such as ammonia (NH_4^+/NH_3) and alkalinity ($H_2CO_3/HCO_3^-/CO_3^{2-}$) but the magnitude of that is not significant. In the pH range of 7 to 8.5 some 85-99% of the reduced form of nitrogen is present as ammonium ions. The conversion of ammonium ions to free ammonia (NH_3) with increasing pH inhibits *Nitrosomonas* at concentration 10 mg/L and *Nitrobacter* at concentration as low as 0.1 mg/L - leading to the accumulation of nitrite ions (Gerardi 2002). Free ammonia becomes the dominant species at pH >9.4. On the other hand low concentrations of free nitrous acid produced from nitrite in the aeration tank inhibit nitrifying bacteria at low pH (Gerardi, 2002). It appears that higher pH observed in the alternating reactor could slightly inhibit the NOB and contribute to the increase in nitrite during nitrification in

these reactors but could not explain the almost doubling of the AUR in the alternating reactor compared to the aerobic one.

The solid and soluble products of the reactions also accumulate differently in the two reactors and could lead to differences in nitrification. The inorganic content of the sludge in the alternating reactor was higher as a percentage of the total mass (VSS/TSS ratio was lower), but the absolute amount of TSS was relatively constant between the two biomasses (§2.4.4, §3.4.2).

5.4.2. Substrates

The most significant substrates for nitrification are ammonia, oxygen, and alkalinity, although the complex organics in the feed are also an indirect source of nitrogen and biosynthetic building blocks as well as food source for competing organisms.

The nitrogen sources for nitrification were the same for both reactors in that they both received NH_4Cl as well as organic-N in the form of feed (beef and yeast extract). The difference was that ammonification was actively occurring during the nitrification process in the aerobic reactor whereas it was already complete prior to nitrification in the alternating reactor. In the strictly aerobic treatment the ammonia level in the beginning of the nitrification was approximately 16 mg NH_4^+-N/g VSS. In the alternating treatment the ammonia after hydrolysis and ammonification of organic matter (additional NH_4^+ released from feed) was elevated to 26 mg NH_4^+-N/g VSS during the anoxic reaction. These differences in NH_4^+ concentration are unlikely to cause a difference in rates of

nitrification within the overlapping ranges observed. This is concluded as nitrification was independent of ammonium concentration under these conditions. In batch tests there was no correlation between the initial ammonium concentration and AUR (Figure 5.5a) for the aerobic biomass and the acceleration of AUR was not significant for the alternating biomass in this range.

The dissolved oxygen in the bulk solution was maintained slightly higher in the alternating reactors. Consequently oxygen penetration through the flocs to the nitrifiers could be slightly greater due to the looser and more open flocs in the alternating reactor (§2.4.7). Ammonia removal was shown to be negligible in the absence of oxygen (Figure 5.1). Significant nitrification can occur in a range 2.0-2.9 mg O_2/L. Dissolved oxygen of 3 mg O_2/L has been found to be non-limiting for nitrification (Gerardi, 2002). Since the oxygen was maintained >2.5 mg O_2/L it is unlikely that DO was responsible for the differences in nitrification rates - at least directly.

Alkalinity is required as carbon source for biosynthesis and growth of the various nitrifiers but not nitrification itself. Therefore changes in alkalinity are more likely to impact the nitrification rates indirectly by affecting the operation of the enzyme system within nitrifying bacteria (Gerardi, 2002). There was no significant difference in the AUR between reactors supplied with additional alkalinity (Figure 5.3) so elevated pH after denitrification cannot be responsible for higher nitrification rates in the alternating treatment.

The other factor that could impact nitrification rates is the presence of organic matter. The direct effects of organic feed on nitrification are difficult to isolate. Biodegradable organic compounds are a substrate for heterotrophs that would compete with nitrifiers for oxygen, space, nutrients, and more. Organic compounds have also been shown to directly inhibit autotrophs including nitrifiers (Hanaki et al., 1990, Takach et al. 1996, Gieseke et al., 2001, Gerardi, 2002, Chen et al., 2006). Therefore the effects of organic compounds are considered generally to be indirect and related to floc structural changes. In batch tests with acetate addition the AUR decrease was attributed to lack of oxygen (denitrification was even observed) rather than to the direct effect of the addition of rapidly degradable COD. The experiment conducted in such extreme conditions failed to show any significant impact of organic matter on lowering nitrification rates observed in the aerobic reactors relative to the alternating ones. On the contrary, ammonia removal deteriorated less in batch tests with aerobic biomass (10% of AUR decrease) than with alternating biomass (24%) demonstrating that ammonia oxidizers in aerobic biomass can be more resistant to low DO than ammonia oxidizers in the alternating biomass.

5.4.3. Populations

The parallel sequencing batch reactors (SBR) in this study were established from the same "seed" source of activated sludge. They were operated under the same conditions with the same sources of feed except for the anoxic phase and the addition of nitrate at the beginning of that phase. Nevertheless the aerobic reactor exhibited lower nitrification rates consistently over two years of operation. Neither the environmental conditions nor

the substrates had direct significant effect on nitrification rates so the difference is almost certainly due to differences in the nitrifiers. The long acclimation period under different conditions could result in selection for different populations.

The AOB in the biomass from the alternating reactor were fundamentally different from those in the aerobic biomass. They were faster and dependent on ammonium concentration when the anoxic phase was omitted. Biomass from the alternating reactors experienced regular periods of anoxia and started nitrification without any apparent lag. Subjecting the alternating reactor biomass to operation without the anoxic period had no effect on the nitrification rate. There appeared to be no immediate impact of the anoxia on nitrification so regulatory mechanisms like anoxic stress (Lee and Oleszkiewicz, 2003) do not seem to operate here or explain the different rates.

The proportions of different species of nitrifiers in the reactors could be different because of long-term adaptation to the anoxic periods experienced in the alternating reactor. Among the AOB the anoxic periods would select for *Nitrosomonas* spp. This is particularly true because *Nitrosomonas* have even been shown to denitrify (Logemann et al., 1998; Geets et al., 2006) - the activity selected for in these reactors. The *Nitrosomonas* spp. would also benefit from the constant high supply of oxygen during the nitrification stage of the alternating reactor. Conversely the more consistent availability of oxygen but with a decrease in oxygen availability in the aerobic reactor during nitrification (due to the simultaneous heterotrophy) could favour *Nitrosospira* spp. which were reported to have lower K_s and growth rates (Table 5.1). *Nitrosospira* can survive

anoxia and have also been shown to denitrify (Shaw et al., 2006). It was suggested that *Nitrosomonas* can outcompete *Nitrosospira* in environments with high substrate concentrations because of their higher maximum growth rate and low substrate affinity (r vs K-strategists) (Schramm et al., 1999). This would be consistent with the hypothesis that the aerobic biomass had an abundance of *Nitrosospira*-like bacteria with high ammonia affinity and a tolerance of low pH (Pommerening-Roser and Koops, 2005). The biomass from the alternating reactor could be dominated by *Nitrosomonas*-like AOB which are more comfortable with higher ammonia loads and elevated pH. The development of different populations AOB under different operating conditions has been reported for other systems using a variety of molecular techniques (Kuo et al., 2006; Moussa et al., 2006).

The NOB are even more sensitive to low oxygen concentrations than AOB. The oxygen requirement for nitrite oxidation to nitrate is only 1.14 mg O_2 per mg NO_2-N This is much less than the 3.43 mg O_2 per mg NH_3-N required for the oxidation of ammonia yet low oxygen concentrations (< 2 mg/L) inhibit nitrite oxidation even more and cause nitrite accumulation (Chen et al., 2006). NOB are probably more sensitive to low DO because they have higher oxygen saturation coefficients: 1.1 – 1.4 mg O_2/L for NOB compared to 0.3 – 1 mg O_2 /L for AOB (Ciudad et al., 2006).

The kinetics of nitrogen use may be a consequence of development of different populations. The kinetics of substrate utilization are often described by Monod-type equations where the rate is dependent on the concentration of a limiting substrate and the

half saturation constant K_s for that substrate. At sufficiently high ammonium concentrations the reaction becomes zero-order and becomes independent of substrate concentration. At sufficiently low concentrations the reaction becomes first-order. The nitrification rate of the biomass developed in the aerobic reactor was independent of ammonium concentration which is typical of systems with high ammonium levels as used in these SBR reactors. The biomass from the alternating reactor was dependent on the initial ammonium concentration in the batch tests just like *Nitrosomonas* spp. in pure culture studies (Taylor and Bottomley, 2006). Examples of rates from culture studies of effects of ammonium concentration on *Nitrosomonas* kinetics are summarized in Table 5.1. In addition AOB in soils are also affected by ammonium concentrations. Additional ammonium-stimulated nitrite production was three- to six-fold faster with *Nitrosomonas europea* than with *Nitrosospira* sp. (Taylor and Bottomley, 2006). The dependence of ammonium oxidation on ammonium concentration in the biomass from the alternating reactor would suggest higher proportion of *Nitrosomonas* spp. in that biomass relative to the biomass from the aerobic reactor although the populations were not quantified directly.

The kinetics of the second step of nitrification would be affected by the very different levels of nitrite that are experienced in the two reactors. Ammonium oxidation is usually the rate limiting step for the entire nitrification process (Gerardi, 2002). In most stable full scale nitrification processes nitrites do not accumulate. In the aerobic reactor in this study nitrite did not accumulate suggesting that the potential nitrite oxidation by NOB was at least as fast as ammonium oxidation. In the alternating reactor incomplete

denitrification during the anoxic phase led to a transient increase in nitrite. The nitrite was removed by the NOB once aerobic conditions were restored. However, the high concentration of nitrite would select for different types of NOB. Higher concentrations of nitrite in the alternating reactor could favour the development of *Nitrobacter* spp. due to their higher tolerance of nitrite and faster nitrite oxidation kinetics (Kim and Kim 2006). *Nitrobacter* was reported to outcompete *Nitrospira* in environments with high nitrite concentrations whereas *Nitrospira* are better competitors in environments with low substrates concentration as a result of their higher affinity for nitrite (Mota et al., 2005).

 The observed nitrite accumulation in the alternating reactor could be explained by initial *Nitrobacter* inhibition after switching to the aerobic conditions. It was reported that after substrate starvation *Nitrobacter* needs time to recover before it can use the nitrite (Tappe et al., 1999) although other evidence suggests low decay rates for NOB and no delays to reach the maximum growth rates (Manser et al., 2006). This could also explain the nitrite accumulation in batch tests without the anoxic phase and denitrification (Figure 5.2b). Low nitrite concentrations in the aerobic reactor could favour *Nitrospira* spp., which prefer low nitrite level and cannot tolerate elevated concentration of this substrate (> 0.01 mg NO_2-N/L) (Kim and Kim, 2006).

The relative proportions of different species of NOB have been attributed to nitrite concentrations in several other systems. Kim and Kim (2006) examined NOB populations in a low-nitrite continuous biofilm airlift reactor where slow but efficient *Nitrospira* occupied 59% of the total bacteria while fast and tolerant *Nitrobacter* made up

only 5%. The results were very different for an SBR with relatively high nitrite concentration where the populations were reversed and where the faster *Nitrobacter* occupied 64%, and only 3% of total bacteria belonged to the slower *Nitrospira*. Specific nitrite oxidation activity, determined by estimating the nitrifying populations from the FISH images were 10.5 mg N/g NOB h for *Nitrospira* and 93.8 mg N/g NOB h for *Nitrobacter*. Nitrite concentration was concluded to be the most critical factor affecting *Nitrospira* and *Nitrobacter* distribution. In another study (Wagner et al., 2002) two chemostats were inoculated with the nitrifying biofilm containing *Nitrospira* and operated under identical conditions with low nitrite levels. A *Nitrobacter* culture was then added to both chemostats and nitrite content in the influent was elevated in one of them. In the control chemostat *Nitrospira* dominated (~10% *Nitrobacter* vs. ~75% *Nitrospira*). In the chemostat with high nitrite levels, growth of *Nitrobacter* occurred and this population overtook *Nitrospira* after approx. 30 days (~40% *Nitrobacter* vs. ~7% *Nitrospira*). Interestingly the dominance of *Nitrobacter* over the *Nitrospira* caused by elevated nitrite concentration could not be reverted by lowering the available nitrite concentration to the original level.

5.5. Conclusions

The significant difference between nitrification rates in aerobic and alternating anoxic/aerobic treatment seems to be caused by different populations of AOB and NOB rather than by direct effects of environmental conditions such as pH, alkalinity, oxygen, or organic carbon availability. Reduced oxygen and low nitrite level in the strictly

aerobic treatment could favour *Nitrosospira* and *Nitrospira* species which would explain lower nitrification rates. Periods of anoxia and high nitrite levels in the alternating reactor could favour *Nitrosomonas* and *Nitrobacter* species which would account for the higher nitrification rates particularly the higher specific ammonium oxidation rate. As the next step molecular tools will have to be used to investigate the actual population dynamics in the studied SBR reactors.

6. EXPLANATION OF HIGHER NITRIFICATION RATES UNDER ALTERNATING ANOXIC/AEROBIC CONDITIONS: MOLECULAR ANALYSIS

6.1. Introduction

The development of different AOB and NOB populations under different operating conditions for wastewater treatment has been studied using a variety of molecular techniques. Commonly used molecular methods such as denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphism (RFLP), cloning and sequencing (Logemann et al., 1998, Regan et al., 2003, You et al., 2003, Cébron and Garnier, 2005, Mota et al., 2005, Mertoglu et al., 2006) are based on DNA extraction and polymerase chain reaction (PCR). They are fundamental tools in microbial analysis particularly for identifying novel species but have quantitative limitations due to DNA extraction and purification biases. The other approach is *in situ* detection with molecular probes and fluorescent microscopy. The method called Fluorescent In-Situ Hybridization (FISH) is commonly used in environmental studies for identifying the community structure with strain specific probes and quantifying selected groups of bacteria with image software (Wagner et al., 1995, 2002, Logemann et al., 1998, Okabe et al., 1999, Morgenroth et al., 2000, You et al., 2003, Wilen et al., 2004, Kindaichi et al., 2004, Mota et al., 2005, Kim and Kim 2006, Li et al., 2006a, Li et al., 2006b). FISH however may be limited due to low cellular rRNA content or limited accessibility of probe target sites. The method does not provide information about the distribution of microbial community.

The purpose of this phase of the research was to use FISH in order to compare and contrast the pair of AOB (*Nitrosospira, Nitrosomonas*) and NOB (*Nitrospira, Nitrobacter*) selected as representatives of K and r-strategists in strictly aerobic and in alternating anoxic/aerobic reactors.

6.2. Materials and methods

Two groups of AOB (*Nitrosospira, Nitrosomonas*) and NOB (*Nitrospira, Nitrobacter*) were selected for FISH analysis. Oligonucleotide probes targeted for 16SrRNA sequences (Nsv 443, Nsm 156, Ntspa 662, NIT 3: Table 6.1) were used for quantifying the nitrifiers. Details on the oligonucleotide probes are available at probeBase (Loy et al., 2003). The hybridization and washing procedures were carried out according to Amann et al. (1995). The microscopy was performed using Nikon Microscope Eclipse E400 with camera Olympus DP70. Image-Pro® Plus software was used for counting target populations in relation to the total microbial population in the sample.

Table 6.1. 16S rRNA-targeted oligonucleotide probes used (Loy et al., 2003).

Probe	Specificity	Sequence (5' -3')	FA %[1]	label
Nsv 443	*Nitrosospira spp.*	CCGTGACCGTTTGTTCCG	30	FITC
Nsm 156	*Nitrosomonas spp.*	TATTAGCACATCTTTCGAT	5	Cy3
Ntspa 662	*Genus Nitrospira*	GGAATTCCGCGCTCCTCT	35	Cy3
NIT 3	*Nitrobacter spp.*	CCTGTGCTCCATGCTCCG	40	FITC

[1] FA - formamide concentration in the hybridization buffer.

Activated sludge samples were collected from the two types of reactors and stained with 4',6-diamidino-2-phenylindole (DAPI) and one of four probes given in Table 6.1. For

each combination of reactor and probe four independent samples were analyzed. Each sample was taken in one-week intervals. In total at least 70 microscopic fields were examined for each reactor and probe combination. In each field the average area of cells stained with DAPI was 0.072 mm^2 with no statistically significant difference between the aerobic and alternating biomass samples (Student's t-test with P=0.05).

Positive controls were performed for probe Nsm 156 (labelled with Cy3) and NIT 3 (labelled with FITC) using cultures of *Nitrosomonas europea* and *Nitrobacter winogradskyi* (American Type Culture Collection). For negative controls a culture of *Escherichia coli* was used. Additionally sludge samples stained only with DAPI were examined using Cy3 and FITC filters to confirm a lack of background fluorescence.

6.3. Results

Different responses to increasing substrate concentrations from the two communities in batch tests confirmed that their nitrification kinetics were fundamentally different (Figure 5.5). Analyzing the most common populations of AOB and NOB by FISH showed a clear difference in relative populations between the two types of reactors (Table 6.2)

The AOB in the aerobic reactor were dominated by *Nitrosospira* whereas the alternating reactor was dominated by *Nitrosomonas*. The total proportion of AOB of all cells stained with DAPI was greater in the alternating reactor. The biggest difference was in the proportions of the AOB populations between the reactors. *Nitrosomonas* made up 81% of the total AOB detected in the alternating reactors while it amounted to only 19% in the

Table 6.2. Percentage of AOB and NOB populations relative to DAPI stain, in two types

of treatment.

	AEROBIC		ALTERNATING	
AOB				
Nitrosospira (Nsv 443)	16.1	± 3.4 %	4.8	± 1.2 %
Nitrosomonas (Nsm 156)	3.8	± 2.1 %	21.1	± 1.2 %
NOB				
Nitrospira (Ntspa 662)	7.9	± 2.2 %	3.3	± 1.9 %
Nitrobacter (NIT 3)	2.9	± 1.3 %	10.4	± 1.7 %
Ratio AOB/NOB	1.85		1.90	

aerobic ones.   The NOB in the aerobic reactor were dominated by *Nitrospira* whereas the

alternating reactor had a greater proportion of *Nitrobacter*. The total proportion of NOB

was greater in the alternating reactor. *Nitrobacter* population made up 76% of the NOB

detected in the alternating reactor and only 27% in the aerobic reactor.   Conversely

Nitrospira made up 73% of the total NOB in the aerobic reactor and only 2.4% in the

alternating one. Both the AOB and NOB were more abundant in the alternating reactor.

6.4.   Discussion

Kinetics studies presented in the previous chapter (Figure 5.4) and FISH analysis

demonstrated that the alternating reactor had a larger community of AOB. The total

fractions of AOB relative to the total microbial population in this study averaged 25.9%

for the alternating reactor and 19.9% for the aerobic one (Table 6.2).   Using the equation

suggested by Mota et al. (2005b) for calculation of the ratio of active AOB to active

biomass the fraction of AOB was computed as 9.7% and 15.9% for the aerobic and

alternating reactors, respectively. Although the FISH results were higher than those from the mathematical modelling it is known that the FISH method does not always correlate with targeted bacteria activity. Wagner et al (1995) have shown that AOB have a mechanism to maintain their ribosome content even under relatively low activities. Given that nitrifying population sizes less than 10% are supposed to be sufficient for nitrification in WWTP (Gerardi 2002) the FISH results appear to overestimate nitrifier populations. On the other hand a huge variation of nitrifying populations in bioreactors has been reported in literature. The AOB were reported to range from 6% of the total microbial population for lower ammonia content in influent (Li et al., 2006a) through 18-30% for 65-70 mg NH_4^+-N/L (Okabe et al., 1999, Morgenroth et al., 2000, Kindaichi et al., 2004) up to 35-76% for 500-700 mg NH_4^+-N/L ammonia initial concentrations (Wagner et al.,1995, Daims et al., 2001, Li et al., 2006b). In this study the nitrifiers may be overabundant compared to full scale WWTP due to reactors' design (SBR mode, high SRT, HRT), use of synthetic feed promoting nitrifiers' growth, and long-term system operation (> 2.5 years) selecting for nitrifiers. The elevated temperature during this particular phase of the experiment ($23.9 \pm 0.8\ ^{o}C$) could also contribute to their higher relative participation in the biomass.

NOB usually are present in lower numbers than AOB in the total nitrifying population. This is due to differences in generation times between AOB and NOB (Gerardi 2002). In this research total NOB fractions were estimated as 10.8% for aerobic and 13.7% for alternating treatment (Table 6.2). The percentage of NOB populations in reported batch

studies varied widely: from 2.5% (Li et al 2006a) through 14% (Morgenroth et al., 2000) to 22-39% (Mota et al., 2005b, Kindaichi et al., 2004).

The ratio of AOB to NOB (Table 6.2) averaged 1.9 for both reactors and was lower than the range of 2.0 to 3.5 reported as sufficient for complete nitrification (You et al., 2003, Li et al., 2006a) The latter may explain why neither ammonia nor nitrite were ever detected in the final effluent. As the ratio is similar in both types of reactors apparently the transient nitrite accumulation in the alternating reactor is not due to a relative lack of the NOB population but rather inherent lower NO2UR in comparison to AUR rates for alternating biomass (Figure 5.5 a, c).

Using the four FISH probes in this study 29% more nitrifiers were detected in the alternating reactor (Table 6.2). Due to the presence of the anoxic zone more nitrifiers in alternating reactor could be expected due to more favourable conditions for autotrophs in the aerobic phase (higher pH and alkalinity after denitrification) and 40-60% lower autotrophic decay (Siegrist et al. 1999; Van Loosdrecht and Henze, 1999; Martinage and Paul, 2000; Lee and Oleszkiewicz, 2003; Salem et al., 2006). As the total number of AOB and NOB was not investigated it can be concluded that the clear shift in population is the main reason for different nitrification rates for two types of treatment.

The environmental conditions in the reactors described in §5.4.1 selected for different nitrifying populations (Table 6.2) between the two types of treatment regimes. The reasons for the shift in populations can be deduced from detailed analysis of oxygen and

substrates changes in the main reactors as well as profiles from batch tests performed separately. In the aerobic reactor ammonia removal and nitrate production occurred simultaneously with organic matter removal without nitrite accumulation. Heterotrophic degradation consumed oxygen decreasing its availability for nitrifiers. Previous research showed that the aerobic biomass consists of dense, spherical, compact flocs, that were stronger due to a high content of bound extracellular polymer substances (EPS) (§4.4.1). Such structure provides a higher mass transfer resistance and can decrease the penetration of oxygen and substrates to the internal layers (Wilen et al., 2004). The access to substrates might be even more limited for this biomass in such conditions. The slower K-strategists *Nitrosospira* and *Nitrospira* which are adapted to low oxygen and nitrite conditions (Schramm et al., 1999) were detected by FISH in aerobic biomass in 4.2- and 2.7-times larger amounts than the r-strategists *Nitrosomonas* and *Nitrobacter*, respectively.

The faster r-strategists *Nitrosomonas* and *Nitrobacter* are better competitors in environments with high oxygen and substrate concentrations (Schramm et al., 1999). They have proliferated in the biomass developed under alternating anoxic/aerobic conditions with 4.3- and 3.2-fold improvement over the K-strategists *Nitrosospira* and *Nitrospira*. The period of the anoxic reaction in alternating treatment during which the readily biodegradable COD is removed from solution reduces the competition with faster growing heterotrophs for oxygen, and makes oxygen more available during the nitrification phase (§3.4.5.3). The alternating biomass consists mostly of bacteria with fewer filaments inhabiting weak thin and elongated flocs (chain-type structure) (§2.4.7)

with less bound EPS (§4.4.1). The transfer of oxygen and substrates is easier into such open aggregates (Wilen et al., 2004). Similar changes in community structure due to oxygen and nitrite concentration were also observed in aerated biofilms (Schramm et al., 2000, Gieseke et al., 2001, Okabe et al., 1999, 2004).

The availability of dissolved oxygen is most likely the main reason for the shift in the nitrifying population for the ammonia oxidation step. The initial level of ammonia in the main reactors could also have an influence. This factor as discussed before (§5.4.2) does not seem to be significant when both initial ammonia values are placed on the graph from batch tests (Figure 5.5a). The competition with heterotrophs, floc structure and thus the access to oxygen are critical factors here. This is consistent with findings of Li et al. (2006a).

It was suggested that *Nitrosomonas* spp. may be more resistant to nitrite or free nitrous acid than *Nitrosospira* spp (Vadivelu et al., 2007d) which might also be important when high nitrite build-up is desirable for a nitritation (ammonia oxidation that stops at nitrite production) process in a wastewater treatment plant. The dominance of a particular species of AOB could occur as a result of the nitrite presence regardless of the differences in oxygen concentrations. The batch tests showed that *Nitrosomonas* from alternating biomass turned out to be resistant to nitrite inhibition in the range typically occurring in the main alternating reactor (0 to 20 mg NO_2^--N/g VSS). Aerobic biomass tested for the same range of nitrites showed some decrease in AUR although total inhibition was never observed. It is possible that the minor fraction of nitrite-resistant

Nitrosomonas could partly take over the ammonia oxidation process during the time when *Nitrosospira* is inhibited with increasing doses of nitrite in the aerobic reactor.

The kinetics of the second step of nitrification were very likely controlled mainly by access to oxygen as the NOB are more sensitive to low oxygen concentrations than AOB (Okabe et al. 1999, Schramm et al., 2000, Mertoglu et al. 2006). The very different levels of nitrite present in the two reactors could also influence the nitrifying genera. In the aerobic reactor nitrite did not accumulate (Figure 5.1a), showing that nitrite oxidation by NOB was at least as fast as the ammonium oxidation (Figure 5.5a, 5.5c). Incomplete denitrification during the anoxic phase in the alternating reactor caused a transient increase in nitrite which remained high during the beginning of the aerobic stage (Figure 5.1b; §5.3.1). The nitrite was removed by the NOB in the following aerobic treatment phase although NO2UR was lower than AUR. The temporary elevated concentration of nitrite could favour the development of *Nitrobacter* spp. due to their higher tolerance of nitrite and faster nitrite oxidation kinetics (Mota et al., 2005, Kim and Kim 2006, Vadivelu et al., 2006a). It is clear from the batch tests (Figure 5.5c) that for low nitrite content (<5 mg NO_2^--N/g VSS) the aerobic nitrifiers are better competitors which is consistent with conditions suitable for *Nitrospira* spp. In the range of higher nitrite levels the alternating reactor bacteria identified as dominantly *Nitrobacter* proliferate (Table 6.2). The relative proportions of different species of NOB have been attributed to nitrite concentrations in other systems (Wagner et al., 2002, Kim and Kim, 2006, Li et al., 2006a).   Okabe et al. (1999) speculated that *Nitrobacter* spp. compete well only when both the oxygen and nitrite concentrations are elevated. It should be noted that in spite of

a clear dominance of *Nitrospira* in the aerobic system *Nitrobacter* still contribute to almost one third of the NOB population. This fact can explain fast nitrite removal thanks to high resistance of this species to inhibitory effects of nitrite, free ammonia and free nitrous acid (Vadivelu et al., 2006a, 2007).

Although it is difficult to clearly delineate whether oxygen or nitrite is mainly responsible for the nitrifying population composition shift it is evident that conditions in the aerobic phase following denitrification result in development of "faster" nitrifiers. Improved nitrification is obviously better for the whole treatment process.

The full scale reactor design starts with definition of nitrifiers' growth rate which is converted to the design SRT and then reactor volume. The growth rate in the actual wastewater is determined using a fully aerobic batch test regardless if the plant will involve a pre-anoxic stage or not.   Knowledge of nitrifier speciation and actual ammonia utilization differences when anoxia is involved could save significant capital costs of the reactor volume. The knowledge should also be useful in convincing the municipalities to use the alternating mode of operation and pre-denitrification. Beside faster nitrification rates the use of pre-denitrification saves oxygen and restores alkalinity while using raw wastewater carbon. The biodegradable carbon would have to be added in a post-denitrification situation. The knowledge of microbial environmental preferences would also be important when designing nitrification bioaugmentation strategies (Head and Oleszkiewicz, 2004) to prevent potential washout of organisms not adapted to actual conditions in the bioreactor.

6.5. Conclusions

Different populations of ammonia oxidizing bacteria and nitrite oxidizing bacteria developed in aerobic and alternating anoxic/aerobic treatments led to very significant differences between the respective overall nitrification rates. Reduced oxygen availability in the strictly aerobic reactor caused by heterotrophic competition and dense floc structure favoured the K-strategist *Nitrosospira* and *Nitrospira* species which explains the lower nitrification rates. Periods of anoxia, easier access to oxygen during the nitrification phase for nitrifiers in the more open flocs in the alternating reactor and possibly elevated nitrite concentration after denitrification selected for r-strategist *Nitrosomonas* and *Nitrobacter* species. The r-strategists account for the higher nitrification rates. Besides the benefits of denitrification in the anoxic phase it appears that using alternating treatment creates the environment for nitrifying population with higher nitrification rates with the potential for reducing the costs of nitrogen removal. It should be added that current full scale design protocols call for determination of nitrification rates under strictly aerobic conditions which may lead to over-design of the nitrifying tanks.

7. BIOTRANSFORMATION OF ESTROGENS IN NITRIFYING ACTIVATED SLUDGE UNDER AEROBIC AND ALTERNATING ANOXIC/AEROBIC CONDITIONS

7.1. Introduction

Natural and synthetic estrogens are present in low concentrations (1-115 ng/L) in municipal wastewater treatment plant effluents. Although at these levels they may not pose a direct threat to human health, effects have been observed in aquatic organisms exposed to effluent discharges (Routledge et al, 1998). Particularly common and potent are the two natural estrogens: estrone (E1) and 17-β-estradiol (E2) excreted physiologically as well as the synthetic analogue of E2 the 17-α-ethinylestradiol (EE2), used in pharmaceuticals (Birkett and Lester, 2003). Since the sources of natural estrogens in wastewater cannot be eliminated and synthetic estrogen amounts cannot be decreased significantly recent efforts have concentrated on ways to improve and optimize their removal through the treatment system.

Biological degradation of estrogens can occur during aerobic wastewater treatment (Johnson and Sumpter 2001). Investigations of removal of estrogens in wastewater treatment plants as well as batch experiments with activated sludge have demonstrated the potential for conversion of E2 to E1 and subsequent removal of E1 and to a lesser extent EE2 under aerobic conditions (Tanghe et al. 1998, Ternes at al. 1999, Vader et al.

2000, Lee and Liu 2002, Andersen et al. 2003, Joss et al. 2004). High rates of elimination of estrogenic compounds were achieved under nitrifying conditions at long solids retention time (SRT) with elevated temperatures accelerating the growth rate of microbes responsible for degradation. According to Vader et al. (2000) the nitrifying biomass has the potential to oxidize EE2 because of its capability to produce ammonium monoxygenase enzymes responsible for metabolizing organic compounds. Shi et al. (2004) have shown that nitrifying activated sludge and the ammonia-oxidizing bacterium *Nitrosomonas europaea* could degrade E1, E2 and EE2 and that the rate of degradation of estrogens was correlated to rates of nitrification.

The fate of estrogens under low redox conditions is still unclear although the few batch studies that have been done suggest that conversion of E2 to E1 is possible although subsequent removal of E1 or biodegradation of EE2 is very limited (Lee and Liu 2002, Andersen et al. 2002, Joss et al. 2004, Czajka and Londry, 2006). Mechanisms of estrogen oxidation are more limited under anoxic conditions as oxygen-dependent reactions such as those of ammonium monooxygenase are not possible. Modern treatment plants include anaerobic and anoxic zones for biological phosphorus and total nitrogen removal and there is a need to understand the effects of anoxic treatments on estrogens and other pharmaceuticals.

7.2. Objectives

The objective of this study was to determine relationship between availability of oxygen, nitrification rate and estrogen removal. The goal was to perform batch tests to assess the

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extent of transformation of E2 and EE2 by nitrifying activated sludge under aerobic, anoxic and alternating conditions. The extents and rates of loss of estrogens were compared to biological parameters such as nitrification rates in three separate experiments.

7.3.   Materials and methods

7.3.1. Basic experimental protocols

The nitrifying biomass originated from laboratory sequencing batch reactors operated for 12 months as described in §2.2. Three batch experiments (Table 7.1) were performed at $20\pm1^{o}C$ to assess the biodegradation of estrogens by the biomass from the two types of bioreactors. For each experiment two reactors were set up: R1 (aerated) and R2 (alternating aerobic/anoxic) containing biomass that originated from the main sequencing batch reactors and was acclimated for one SRT as duplicates. New biomass never before exposed to estrogens was used each time to eliminate acclimation and related growth-linked biodegradation. Each batch reactor was then amended with 5 mg/L (0.1 mL) each of E2 and EE2 from methanolic stock solutions at the same time as the addition of synthetic wastewater feed. Unrealistically high estrogen concentration were used in this study for analytical ease and to enhance the detection of metabolites. The high levels of estrogens helped to determine the kinetics of degradation under conditions where sorption would not contribute as significantly to the overall loss of the estrogens.

Table 7.1. The parameters of activated sludge and related estrogen removal for experiments 1-3.

Experiment	#1		#2			#3		
Reactors	R1	R2	R1	R2		R1	R2	
Biomass	Mixed		Aerobic	Alternating		Aerobic	Alternating	
Conditions	Aerobic	Anoxic	Aerobic	Anoxic	Aerobic	Aerobic	Anoxic	Aerobic
F/M, g/g d	0.14	0.14	0.26		0.24	0.66		0.70
VSS, g/L	3.52	3.53	1.89		2.09	1.83		1.71
VSS/TSS	0.79	0.78	0.86		0.79	0.82		0.84
Time of reaction, hours	7	7	8	3	5	7	3	4
E2 influent, mg/L	4.74	4.11	5.2		4.2	4.26		4.84
E2 effluent, mg/L	0.14	0.42	0.40	0.80		0.34	0.18	
E1 influent, mg/L	1.22	0.42	1.10		0.00	0.00		0.00
E1 effluent, mg/L	3.44	3.56	2.60	3.60		3.52	3.56	
EE2 influent, mg/L	5.18	4.14	4.30		4.40	4.76		5.18
EE2 effluent, mg/L	4.04	3.92	3.40		3.40	4.16		4.04
E2 removal %	97	90	92	76	5	92	90	6
					81			96
Constant rate E2 removal, log(mg/L)/h	0.409	0.332	0.387	-	0.184	0.236	-	0.212
E1+E2, removal %	40	12	50	38	N/A	9	9	14
					N/A			23
EE2 removal %	22	5	21	-	23	13	-	22
EE2 removal rate, mg EE2/g VSS h	0.276	-	0.227	-	0.334	0.039	-	0.088
E1+E2 removal rate, mg (E1+E2)/g VSS h	0.105	-	0.109	-	N/A	0.041	-	0.093
AUR, mg NH_4^+/g VSS h	5.21	-	5.04	-	6.46	2.33	-	2.97

N/A – not applicable

Estrogens (E2 and EE2) were added in similar fashion to an equivalent volume of deionized water aerated through the course of a test as a negative control.

The transformation of the estrogens was followed over time (15 minute intervals) by collecting mixed liquor samples (0.25 mL; added to 0.25 mL methanol) that were centrifuged and the centrate was analyzed for estrogens by an HPLC (as per Czajka and Londry, 2006). The rest of analyses (pH, DO, TSS, VSS, COD, NH_4^+) were performed as per §2.2.

7.3.2. Detailed experimental design

Experiment 1 was performed using biomass created by mixing wastage from the main reactors (aerobic and alternating) that was cultivated under strictly aerobic conditions for one SRT. Then, the biomass was split equally into two reactors: R1 (aerated) and R2 (anoxic) which were subsequently spiked with E2 and EE2. Each reactor was analyzed for 7 h. The anoxic reactor was kept anoxic for the full time of the treatment. (Table 7.1)

Experiment 2 differed in that two types of biomass were used: aerobic reactor R1 received biomass developed under strictly aerobic conditions (created from wastage from main aerobic reactors) whereas R2 received biomass developed under alternating anoxic/aerobic conditions (created from alternating reactors wastage). The reactors were maintained for one SRT prior to the experiment. Estrogens were added and analyzed for 8 hours. R1 was continuously aerated while R2 was anoxic during first 3 h and later the air was supplied during next 5 hours (Table 7.1).

Experiment 3 was identical to experiment 2 except that during the acclimation period before the experiment the biomass in each reactor was starved during 1×SRT (no feed supplied) to decrease its biological activity. The F/M ratio was therefore significantly higher in this experiment (Table 7.1).

7.4. Results and discussion

The results of the three experiments are summarized in Table 7.1. No transformation was observed in the negative controls as levels of E2 and EE2 remained constant (E2 = 3.83±0.31 mg/L, EE2 = 8.94±0.47 mg/L). No E1 was observed in the negative controls (detection limit 0.01 mg/L).

7.4.1. E2 → E1 transformation

The rapid transformation of E2 to E1 was observed in all three experiments and examples are shown in Figure 7.1 and 7.2. The loss of E2 occurred during the first one or two hours of incubation and then a steady state concentration of E2 was observed (0.14-0.80 mg/L). In some cases samples were taken again 24 h after exposure and still no loss of estrogens took place compared to the final samples (7-8 hrs) suggesting a steady state had been reached.
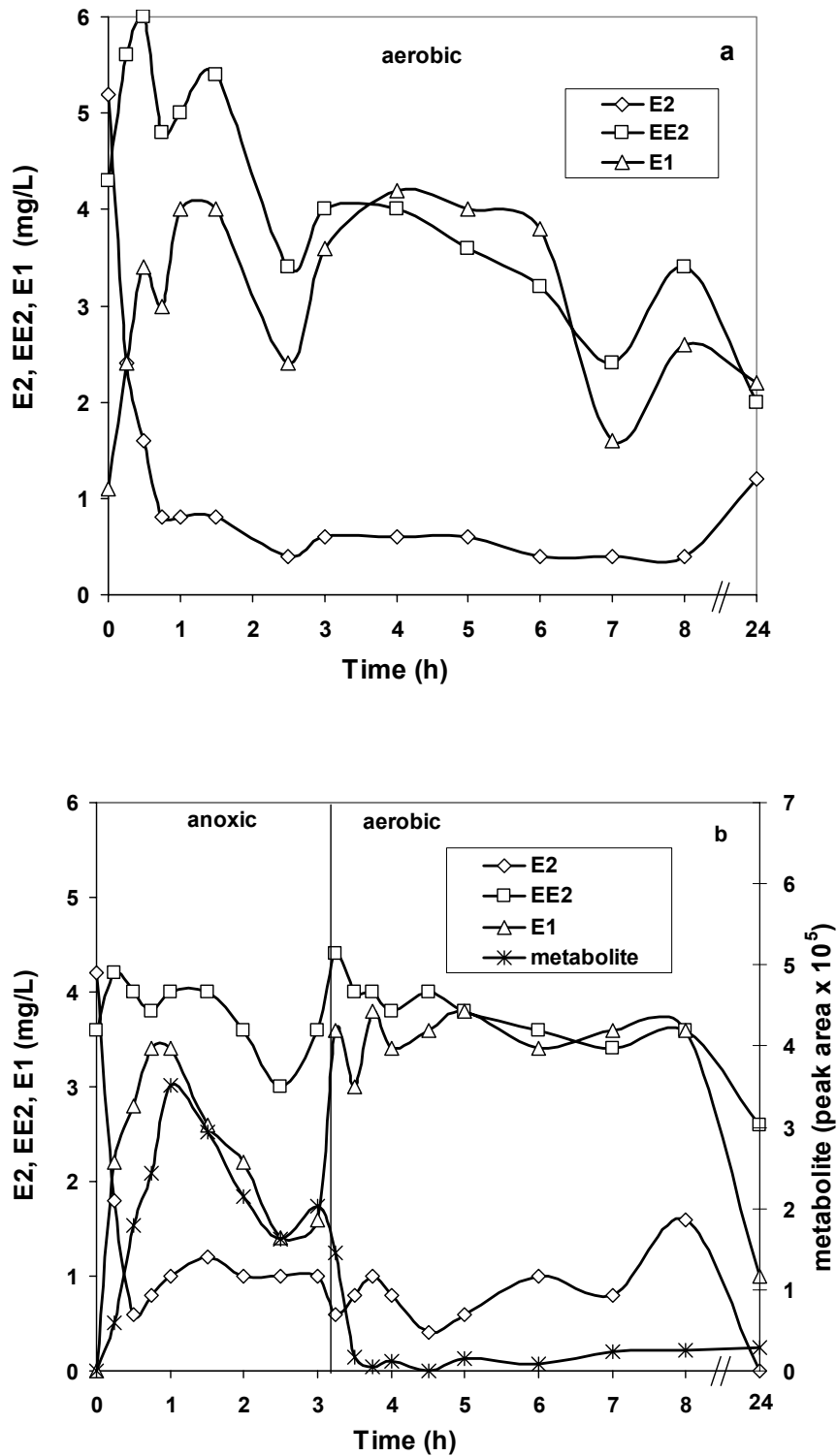
Figure 7.1. Concentration of estrogens EE2, E2 and E1 in activated sludge batch experiments under aerobic (a) and alternating anoxic/aerobic (b) conditions (Experiment 2). The vertical bar in (b) indicates the time when the reactor was switched from the initial anoxic incubation to aerated aerobic conditions.

The final concentration of E2 in the experiments was calculated from the average of the last three time points and is summarized in Table 7.1. The E2 removal averaged 94% over 7-8 hours in the aerobic reactors. The E2 removal under anoxic denitrifying conditions was only 85% over the 3-7 hours (Table 7.1). In experiments 2 and 3 further transformation of E2 occurred after switching from anoxic to the aerobic conditions (Figure 7.1b, 7.2b) with final E2 removal in alternating reactors averaging 89% for the final two experiments (Table 7.1).

The rate of E2 depletion calculated as the semi-log constant averaged 0.344 log(mg/L)/h for aerobic treatments compared to only 0.243 log(mg/L)/h for anoxic treatments (Table 7.1) indicating faster transformation under aerobic conditions. In fact, if the biomass developed partly from aerobic sludge in the first experiment is excluded the average with fully developed denitrifying biomass was only 0.198 log(mg/L)/h. For biomass with lowered bacterial activity (experiment 3 – starvation conditions) the conversion rate was slower than in Experiment 1 and 2 under aerobic conditions. Taking into account that E2 was not altered in the negative control the results are very consistent with the hypothesis that the loss of E2 was due primarily to biological activities. It is possible that some of the loss of estrogens could be due to sorption as well, particularly during the first 15 min when the estrogens were being mixed into the sludge.
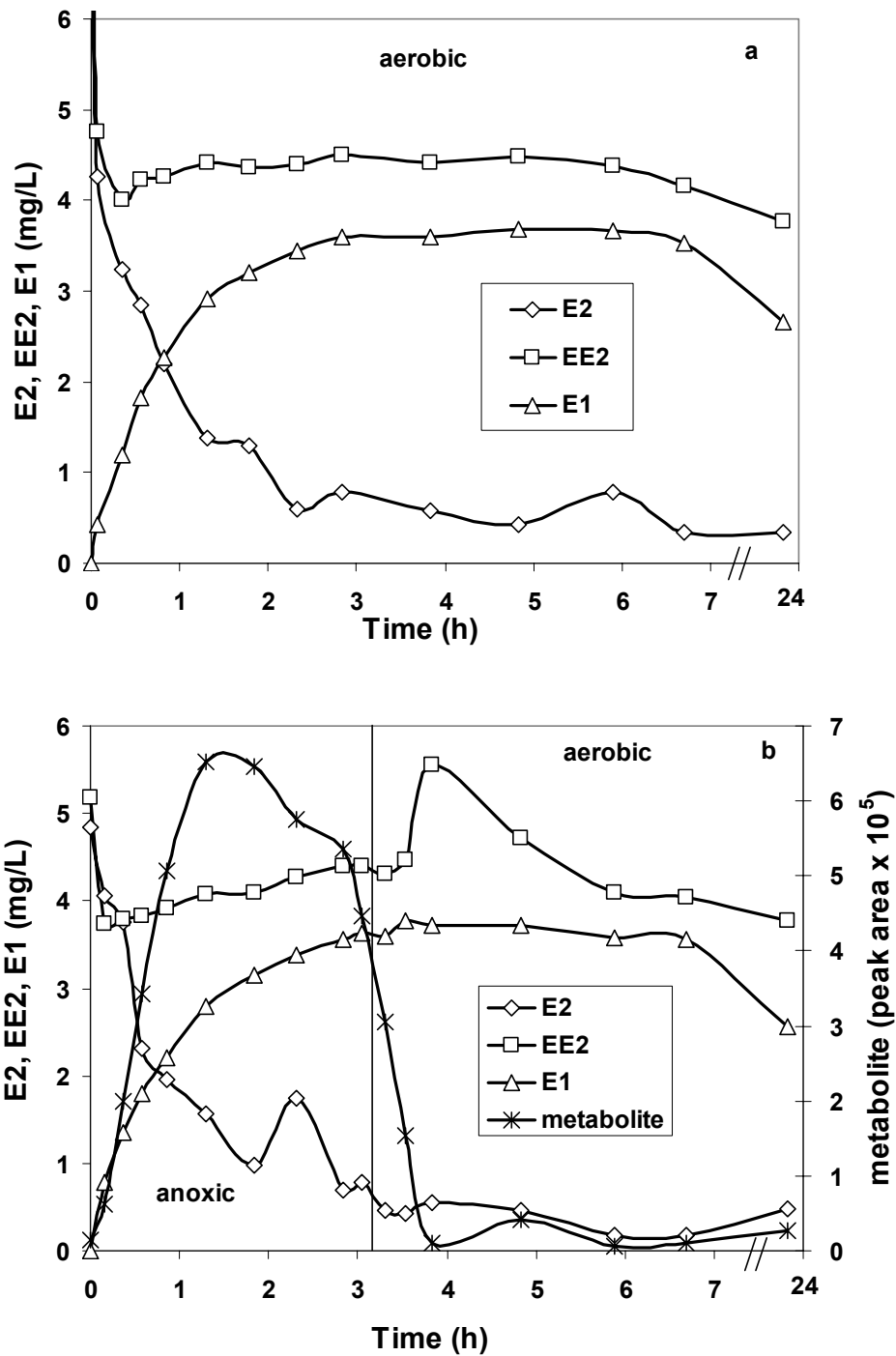
Figure 7.2. Concentration of estrogens EE2, E2, and E1 in activated sludge batch experiments under aerobic (a) and alternating anoxic/aerobic (a) conditions (Experiment 3, starved biomass). The vertical bar in (b) indicates the time when the reactor was switched from the initial anoxic incubation to aerated aerobic conditions.

In all cases the loss of E2 was accompanied by accumulation of E1 (Figure 7.1 and 7.2). The accumulation of E1 corresponded with the loss of E2 and was close to stoichiometric especially under aerobic conditions. The conversion of E2 to E1 is frequently observed and is probably due to microbial enzymatic activity of an NADH-dependent hydroxysteroid dehydrogenase (Czajka and Londry, 2006). This conversion would cause a decrease in the estrogenicity of the final effluent as E1 is less estrogenic than E2 , but this removal could be short-lived as E1 could easily be transformed back to E2 under other conditions (Routledge et al. 1998).

These experiments demonstrated the potential of activated sludge to oxidize E2 to E1 if either oxygen or nitrate was used as an electron acceptor. This conversion is likely to occur quickly in activated sludge processes regardless of whether they are adapted for nitrification or have been previously exposed to periods of anoxia for denitrification.

7.4.2. E1 removal and formation of a metabolite

E1 turned out to be relatively persistent in the activated sludge treatment. After the initial formation of E1 reached its maximum within three hours (Figure 7.1 and 7.2) further aerobic incubation resulted in only a small reduction of the amount of E1. The concentration of E1 at the end of the experiments ranged from 2.60 to 3.60 mg/L (Table 7.1). In reality, it is more useful to compare the total amount of E1+E2 (Table 7.1) which also decreased over time particularly in the second experiment (Figure 7.1a). Under aerobic conditions the loss of E1+E2 ranged from 50% in experiment 2 (Figure 7.1a) and

40% in experiment 1 to only 9% in experiment 3 where biomass with lowered bacterial activity was used (Figure 7.2 a) (Table 7.1). This decrease in the total E2+E1 represented the true loss of estrogenicity in the wastewater during the aerobic treatment of the activated sludge in these batch reactors. The rate of removal of E1+E2 $((E1_{inf} + E2_{inf}) - (E1_{eff} + E2_{eff}))$ under aerobic conditions was greater in the first two experiments than in the third experiment with starved biomass (Table 7.1)

These batch reactors were developed to enhance autotrophic nitrification rather than heterotrophic activities that would facilitate biodegradation of organic compounds like estrogens, and the organisms in the sludge had never been exposed to estrogens, yet some removal of estrogens still occurred. Rates of estrogen removal could be even faster in conventional activated sludge, particularly as it is regularly exposed and acclimated to estrogens, but this study demonstrates that some removal should take place even if the activated sludge process is operated for biological nutrient removal plants.

The transformation of E2 to E1 and the loss of the E2+E1 pair happened during the most active phase of nitrification in the reactors. In order to test a possible impact of nitrification on estrogen transformations the rate of E2+E1 decrease during ammonia removal was calculated for both the aerobic and alternating reactors. There was a correlation between the rates of E2+E1 removal and nitrification (Table 7.1). The relationship between nitrification rate (as AUR) and estrogens removal rate was plotted in Figure 7.3. Higher nitrification rates were associated with higher rates of E2+E1 removal which is consistent with previous observations (Vader et al. 2000, Shi et al. 2004).

Under anoxic conditions removal of E1 (or E1+E2) was not observed over the 3-7 hour

incubation times. However, when the biomass used was from initial reactors adapted to

the alternating anoxic/aerobic treatment (Experiments 2 and 3 but not 1) an additional

metabolite was observed in the HPLC chromatograms (Figure 7.4). The metabolite was

not observed under aerobic conditions (or in the negative control) and is probably related

to the denitrifying activity of the sludge. The additional metabolite accumulated as the

amount of E1 increased (Figure 7.1b, 7.2b) suggesting that it formed as a metabolite from

the E2 although this was not independently tested.
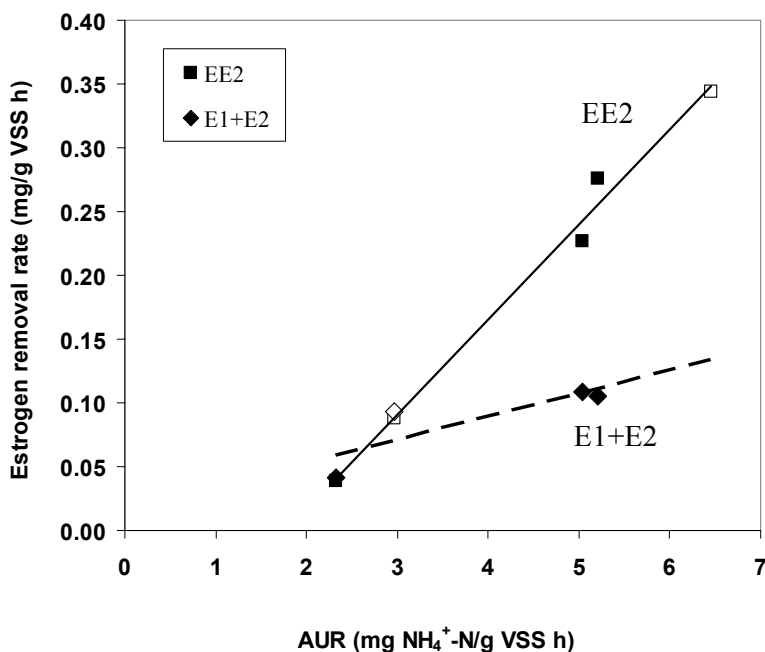
Figure 7.3. Relationship between nitrification rate (as ammonia uptake AUR) and removal rate for EE2 (squares) and E1+E2 (diamonds) for both aerobic reactors (R1 ■ ◆) and the aerobic phase of the alternating anoxic/aerobic reactors (R2 □ ◇).

The retention time of the metabolite just before E2 was subsequently discovered to be the same as 17-α-estradiol. Further research is needed to confirm the identity of the metabolite (by GC-MS) but the results were consistent with other cultures under anoxic conditions that were shown conclusively to form 17-α-estradiol (Czajka and Londry, 2006). Exact concentrations could not be calculated because a standard curve for 17-α-estradiol was not performed on the day that the samples were analyzed but the amount was roughly estimated by comparison to the peak area response for 17-β-estradiol (Figure 7.1 , 7.2b). Interestingly, the metabolite decreased in concentration and E1 increased in concentration once the reactors were aerated (Figure 7.1b and 7.2b).
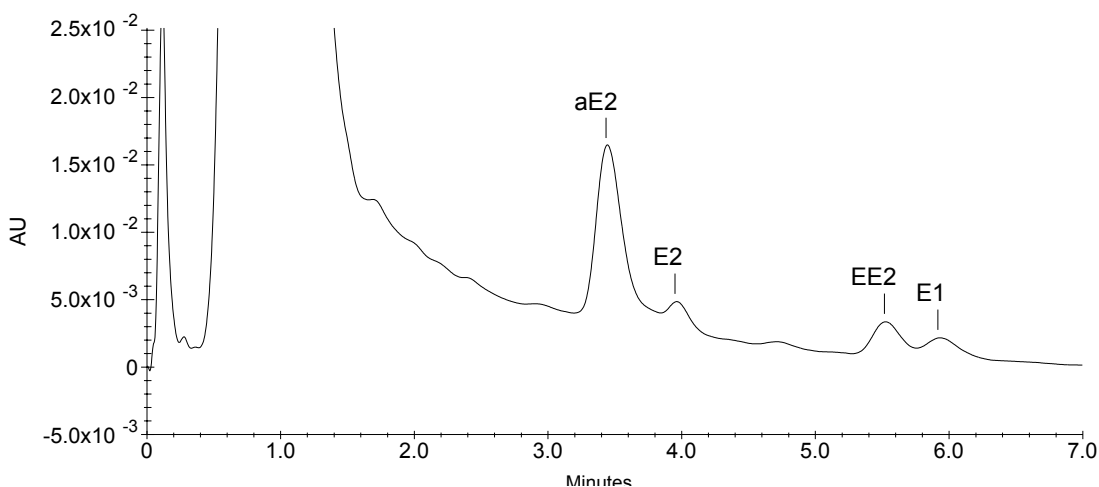
Figure 7.4. HPLC chromatogram of a sample of activated sludge during anoxic treatment in the third experiment, showing the relative sizes of the peaks for E1, EE2, E2, and the putative metabolite 17-α-estradiol (aE2).

Formation of the metabolite represents a reversible oxidation/reduction reaction called racemization in which the 17-β-estradiol could become converted to the 17-α-estradiol through the E1 ketone (Czajka and Londry, 2006). This conversion has been observed previously under sulfate-reducing, iron-reducing, and methanogenic conditions (Czajka

and Londry, 2006) but this is the first report of the observation during denitrification. The 17-α-estradiol is much less estrogenic than E2 but since it could be converted back to E1 (and potentially back to E2) a permanent loss of estrogenicity is doubtful. However, this interconversion could explain fluctuations of estrogenicity as measured by general assays such as the yeast estrogenicity assay during wastewater treatment processes that include anoxic conditions.

7.4.3. EE2 removal

The synthetic estrogen EE2 is considered to be the most difficult to degrade in activated sludge. No removal was observed under anoxic conditions in these experiments but removal was observed of up to 22% for aerobically-developed biomass and to 23% for biomass when aerobic treatment was preceded by anoxia. For the starved aerobic sludge in the third experiment the removal of EE2 decreased to 13%. The removal in the alternating conditions was not affected and remained at 22% (Table 7.1).

The rates of EE2 removal were higher in the aerobic phase of the first two experiments (0.276 and 0.227 mg/g VSS h) than in the starved sludge (0.039 mg/g VSS h) (Table 7.1). Rates of removal were greater in the aerobic phase following the anoxic phase in the two experiments with alternating reactors where this condition was tested (0.334 and 0.088 mg/g VSS h, respectively) (Table 7.1) as observed previously (§3.4.5.3, §5.1.3). There was a good correspondence of high nitrification rate with the higher EE2 removal rate under alternating conditions (Figure 7.3). Close inspection of Figures 7.1 and 7.2 shows that EE2 removal occurred during the active phase of nitrification and was hindered after

the end of nitrification when the ammonia was depleted and the DO level and pH rapidly increased (detailed data for NH_4^+, pH and DO in Appendix 7.1, 7.2, 7.3). It supports the hypothesis that the nitrifying biomass is responsible for EE2 removal.

For each experiment the rate of EE2 degradation turned out to be faster than the rate of E1+E2 degradation (Table 7.1) although the final residue of EE2 was slightly higher than E1 (Table 7.1). There was an overall similarity in the biodegradability of the natural estrogen compared to the synthetic one under the aerobic nitrifying conditions.

7.5.   Conclusions

- E2 was readily converted to E1 in the activated sludge. The conversion was incomplete as some E2 remained even after extended incubation. The conversion of E2 to E1 was faster under aerobic (nitrifying) than anoxic (denitrifying) conditions and faster in sludge that was active than in sludge that had been starved.

- The combined E2+E1 removal achieved 50% under aerobic conditions. Under anoxic conditions for denitrifying biomass the E2+E1 concentration initially decreased due to formation of a metabolite. After aeration the metabolite disappeared restoring the former amount of E2+E1. Subsequent loss of E2+E1 occurred under nitrifying conditions.

- The metabolite formed under denitrifying anoxic conditions was consistent with 17-α-estradiol and appeared to be formed from the E1 intermediate.

- EE2 was persistent under anoxic conditions whereas under aerobic nitrifying conditions as much as 23% could be removed during the active nitrification phase of treatment.

- The EE2 and E1 removal rates increased with increasing nitrification rates. The increase was higher for EE2 than E1.

- The total removal of estrogens was similar in aerobic and alternating reactors.

- Biodegradation of E1 and EE2 was hindered under conditions of relatively lower bacterial activity.

- Further research is needed to determine:

  - the rates of estrogen loss through biodegradation and sorption with lower estrogen concentrations

  - the identity and extent of accumulation of the metabolite under anoxic conditions

  - the removal of estrogens using fresh activated sludge from diverse biological nutrient removal plants.

8. IMPACT OF OZONATION ON TOTAL ESTROGEN REMOVAL

8.1. Introduction

Aerobic/anaerobic biodegradation and adsorption of endocrine disrupting compounds (EDC) onto suspended solids during treatment is not sufficient in estrogenicity removal. The researchers started looking at additional advanced treatment processes. Advanced oxidation techniques employ a combination of O_3 with H_2O_2 or UV irradiation. These typical advanced water treatment technologies may also act as tertiary or post-treatment for wastewater (Gehr et al., 2003; Beltran, 2003, Ternes et al., 2003). Ozonation has been shown to have a high potential for oxidation of pharmaceuticals in drinking water and wastewater (Ternes et al., 2002, 2003; Huber et al. 2003, 2005; Chu and Lau 2007; Kim et al. 2007; Zhang et al. 2007). The average removal of seven groups of pharmaceuticals with doses 5 to 15 mg O_3/L exceeded 78% in municipal final effluent (Ternes et al., 2003). Studies on EE2 showed that ozonation was sufficient to reduce EE2 activity (Huber 2004). The combined ozone + H_2O_2 methods seemed to be even the more promising. During drinking water treatment more than 90% of EDC was destroyed (Birkett and Lester, 2003). In another research (Huber et al., 2003) the measured oxidation for slow-reacting pharmaceuticals averaged 57% with a dose of 2 mg O_3/L and increased to 89% with combined O_3/H_2O_2 process. The effectiveness of the oxidation method depends not only on technology design but also on the quality of treated effluent. A dose of 20 mg O_3/L is considered economically accepted for secondary effluent disinfection (Gehr et al., 2003, Ternes et al., 2003). In this research on waste activated sludge (WAS) minimization the portion of RAS was treated with around 15 times

stronger doses of ozone (§.3.3.1). Beside WAS reduction and denitrification/settling improvement this technology could also have additional positive impact on estrogen removal from the system. This is significant as there is a mounting concern about persistence of these compounds in the environment.

8.2. Objectives

The purpose of this study was to determine the direct impact of ozonation in the range of doses used in previous research on estrogen removal in the ozonated portion of the sludge. The other objective was to evaluate the potential benefits of partial ozonation of RAS on estrogens removal from the wastewater in the whole system.

8.3. Material and methods

The portion of activated sludge adequate in volume and concentration to ozonated fraction of RAS (V=0.25 L, TSS=5400 mg/L) was amended with 5 mg/L (0.1 mL) each of E2 and EE2 from methanolic stock solutions, (§7.1.2.1.) This sludge was then ozonated as described before (§3.3.1) and 0.25 mL portion of sludge was withdrawn every 0.5 min. The estrogens have been analysed as per 7.1.2.1. An identical test with a batch of deionized water instead of activated sludge was performed as a control run. No statistical analysis were performed, as these were single experiments due to the lab limitations.

The impact of partial ozone treatment on the estrogen removal in the system was not evaluated in the laboratory but calculated using the developed mathematical model.

Settings used in the control and ozonated batch reactors served as a base for mathematical modeling of the process. For the simplicity of the calculations two cases have been considered:

Case 1: no sorption of the estrogens to the sludge particles (all estrogen in liquid phase or effluent),

Case 2: the complete sorption of the estrogens to the sludge particles (all estrogen in sludge or solid phase).

8.4. Results and discussion

8.4.1. Direct destruction of estrogens by ozone

There was no difference in estrogen destruction when ozonating sludge or deionized water tests indicating that impact of sludge particles shielding against the ozone was not significant in this range of doses. The drop of estrogen indicators spiked to the portion of the ozonated sludge with increasing ozone dose is shown in Figure 8.1. The dose as low as 0.01 mg O_3/mg TSS initial excess sludge greatly reduced the concentration of the indicators E2 and EE2 and the E1 formation was quickly suppressed. At a dose of 0.025 mg O_3/mg TSS initial excess sludge each compound's level dropped below detection limit regardless of its initial concentration. The dose of 0.05 mg O_3/mg TSS initial excess sludge (equal to 270 mg/L) was optimal for sludge reduction and denitrification improvement in anoxic/aerobic treatment. It can be concluded that complete oxidation of estrogenic compounds in the portion of sludge during the ozone treatment is feasible.
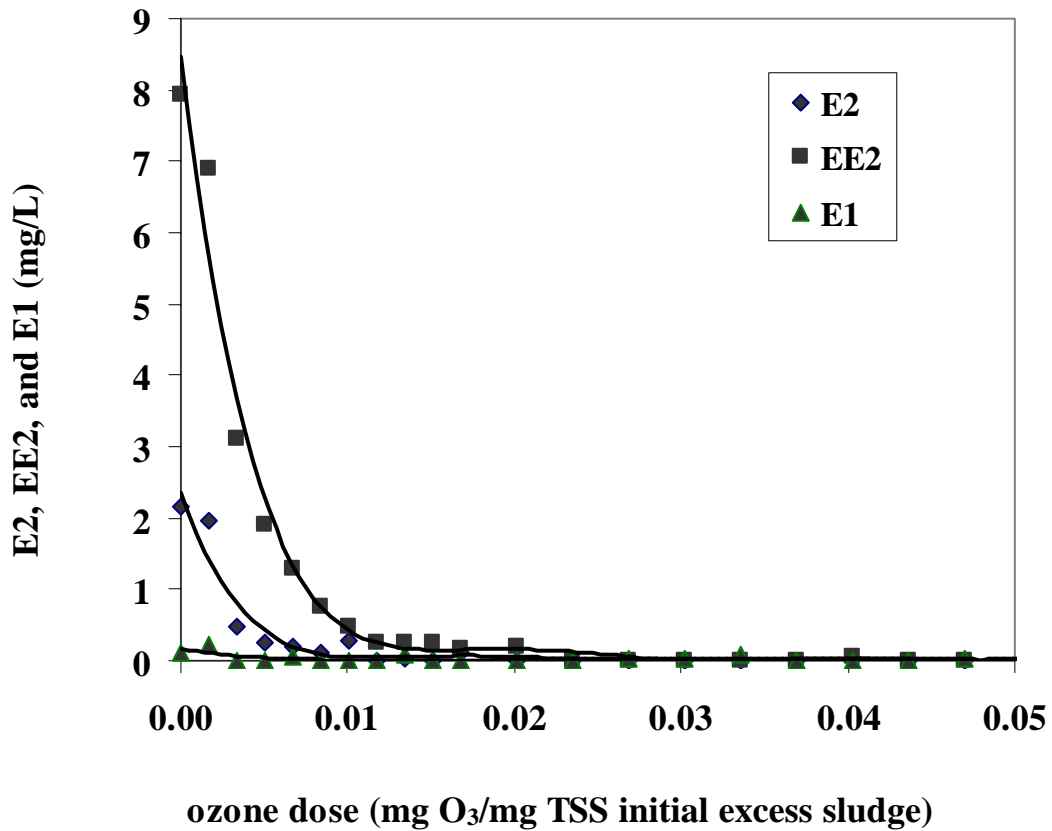
Figure 8.1. Estrogen (E2, EE2, E1) removal from ozonated portion of the sludge with increasing ozone doses

8.4.2. Impact of partial ozonation on overall estrogens removal

The behaviour of estrogen and other endocrine disrupting compounds (EDC) in wastewater treatment plants is dependent upon the physicochemical properties and structure of the sludge. EDC preferentially adsorb onto sludge particles because of their hydrophobic properties (Layton et al., 2000; Ying et al., 2003, Andersen et al., 2005, Suzuki and Maruyama, 2006). EE2 has the highest octanol-water partition coefficient (log K_{ow} 4.15) among tested estrogens. It was reported to have the highest tendency to

adsorb to solids (Birkett and Lester 2003). In some batch tests however sorption effects do not appear to be a factor (Ternes 1999).

During everyday ozone treatment as practiced in this study (§3.3.1) estrogens present in the ozonated RAS fraction (both in solid or liquid phase) are assumed to be destroyed. This daily destruction of EDC in ozonated portion of RAS will impact the whole activated sludge system in terms of estrogenicity reduction. Knowing that estrogens are partly adsorbed to the solids and partly remain in the solution it can be concluded that the estrogen concentration will be reduced after ozone treatment both in the effluent and wasted activated sludge. The percent and distribution of that decrease depends on amount of estrogens remaining in the solids (due to adsorption) or liquid phase. The ideal case would be to concentrate estrogens in the solid phase This would result in estrogen-free liquid phase (effluent) and more estrogen would be destroyed by ozone as they would stay in concentrated RAS. In such a situation only WAS would contain estrogens. In the other non-realistic case assuming no sorption to the particles it would be expected to have all the estrogens in the effluent as well as liquid portion of WAS, but sludge particles would be estrogen-free.

8.4.3. Mathematical model assumptions

The potential benefits of the partial ozonation technique were evaluated using the mathematical model developed**.**

Assumption 1: The same daily concentration of selected estrogen enters the system with the influent.

Assumption 2: In ozonated sludge (ozone dose 0.05 mg O_3/mg TSS initial excess sludge) estrogens are decreased to non-detectable limit (Figure 8.1); portion of return activated sludge after ozone treatment is estrogen-free.

Assumption 3: The fraction of WAS being returned to the system for compensation of solids destruction is neglected as not significant (10 %*1/12=0.8%)

Assumption 4: Neither biodegradation nor additional oxidation occurs during activated sludge treatment.

8.4.4. Symbols

Figure 8.2 shows the schematic of operation of the ozonated reactor with flow distribution. Corresponding with the schematic flow diagram of SBR operation in the Figure 8.3 explains the calculation of flows and indicator compound concentration during the first cycle when estrogen is applied. The following symbols were used in the model (Figure 8.2, 8.3):

V – volume of the reactor, L;
SRT – solids residence time, d;
R – recycle ratio ($R=Q_R/Q$);
c – concentration of selected estrogen EE2, mg/L;
f – fraction of ozonated sludge.
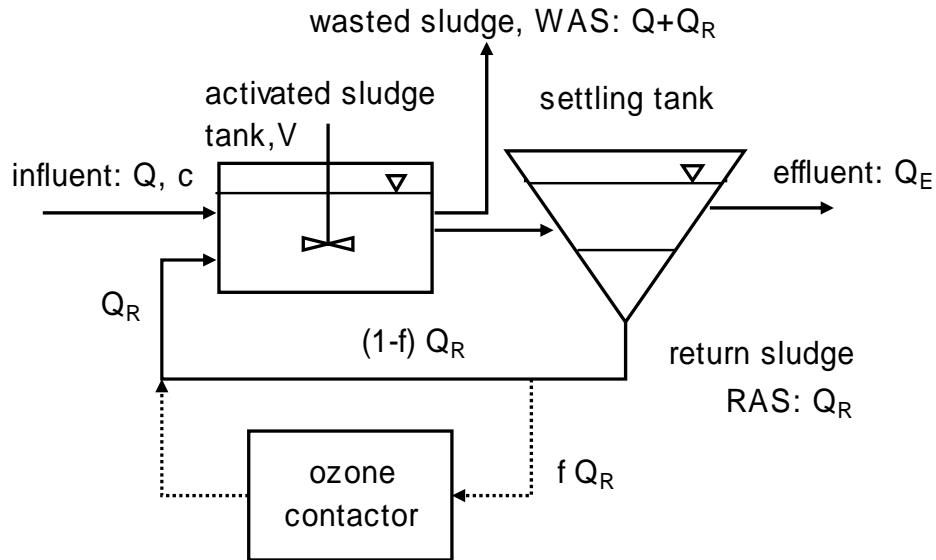
Figure 8.2. Schematic of the reactor's operation mode
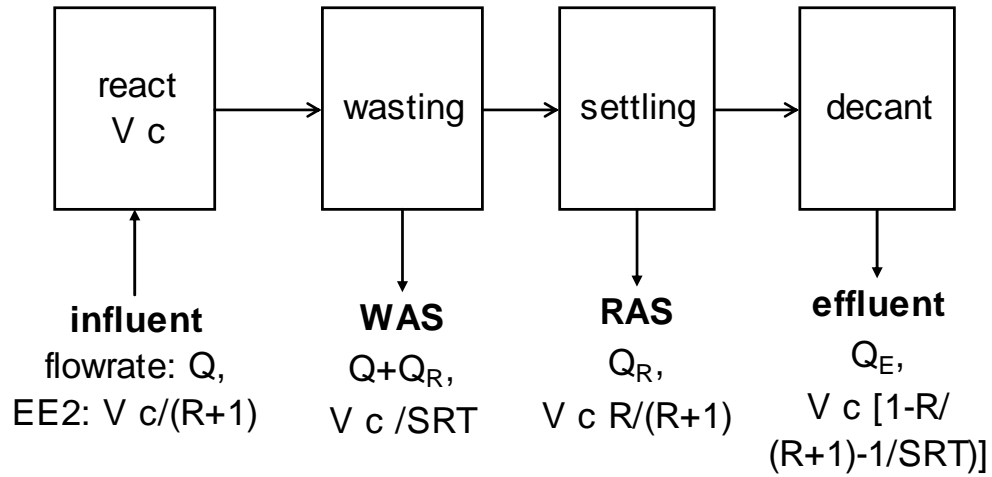
Figure 8.3. Flowrates and amount of EE2 in the first cycle in SBR

8.4.5. Case 1. The estrogens remain entirely in the liquid phase

The effluent intercepts the estrogens (no sorption)

S – the amount of EE2 in the effluent, mg/d (Figure 8.3) calculated as a limit of geometrical progression:

$$S = \lim S_n = \frac{a_n}{1-q}$$

$$a_n = V \cdot c \cdot (1 - \frac{R}{R+1} - \frac{1}{SRT});$$

a) control train

$$q_{contr} = \frac{R}{R+1}$$

$$S_{contr} = \frac{V \cdot c \cdot (1 - \frac{R}{R+1} - \frac{1}{SRT})}{1 - \frac{R}{R+1}} = V \cdot c \cdot (1 - \frac{R}{R+1} - \frac{1}{SRT}) \cdot (R+1) \qquad 8.1$$

b) ozonated train

$$q_{ozon} = (1-f)\frac{R}{R+1}$$

$$S_{ozon} = \frac{V \cdot c \cdot (1 - \frac{R}{R+1} - \frac{1}{SRT})}{1 - (1-f)\frac{R}{R+1}} = V \cdot c \cdot (1 - \frac{R}{R+1} - \frac{1}{SRT}) \cdot \frac{(R+1)}{1+Rf} \qquad 8.2$$

c) Benefit of the treatment in case 1, η 1:

$$\eta_1 = \frac{S_{contr} - S_{ozon}}{S_{contr}} = \frac{R+1 - \frac{R+1}{1+Rf}}{R+1} = \frac{Rf}{Rf+1} \qquad 8.3$$

Conclusion 1:

When the estrogens remain in the liquid phase the benefit of partial RAS ozone treatment is dependent on R (recycle RAS ratio) and f (the fraction of ozonated RAS). For settings in the experiment (R=50%, f=20%) the benefit of ozone treatment for estrogens in the

final effluent is $\eta_1 = 9.1\%$. The same benefit will be achieved for estrogen remaining in the liquid phase of WAS. For higher possible fraction of ozonated sludge (30%) η_1 may increase to 13%. Including the solids decrease in the ozonated train a higher benefit for liquid phase of WAS would be achieved. Conversely a lower benefit in the final effluent would be expected due to higher estrogens accumulation in the RAS.

8.4.6. Case 2. Estrogens are adsorbed entirely to solids

The WAS accumulates estrogens (0 mg EE2 in the effluent; Figure 8.2).

S – the amount of EE2 in the WAS, mg/d

$$S = \lim S_n = \frac{a_n}{1-q}$$

$$a_n = V \cdot c \cdot \frac{1}{SRT};$$

a) control train

$$q_{contr} = 1 - \frac{1}{SRT}$$

$$S_{contr} = \frac{V \cdot c \cdot \dfrac{1}{SRT}}{1 - 1 + \dfrac{1}{SRT}} = V \cdot c \qquad 8.4$$

b) ozonated train

$$q_{ozon} = (1-f) \cdot (1 - \frac{1}{SRT}) = 1 - f - \frac{1}{SRT} + \frac{f}{SRT}$$

$$S_{ozon} = \frac{V \cdot c \cdot \dfrac{1}{SRT}}{1 - 1 + f + \dfrac{1}{SRT} - \dfrac{f}{SRT}} = \frac{V \cdot c}{f(SRT-1)+1} \qquad 8.5$$

c) Benefit of the treatment in case 2, η_2:

$$\eta_2 = \frac{S_{contr} - S_{ozon}}{S_{contr}} = 1 - \frac{1}{f \cdot SRT + 1 - f} = \frac{(SRT-1)f}{(SRT-1)f + 1}$$ 8.6

Conclusion 2:

When the estrogens remain in the sludge the benefit of partial RAS ozone treatment is dependent on SRT and the fraction of ozonated RAS. For settings in the experiment (SRT=12, f=20%) the benefit for estrogens leaving the system with WAS is $\eta_2 = 68.8\%$. Including lower wasting for solids destruction compensation in ozonated reactor we would get higher benefit of the method in WAS, however estrogens accumulation in RAS would increase slightly (1.1%). For the long SRT (=15 days) and ozonation of 30% of RAS (above which the process may not be economical) η_2 can increase up to 80%. In this scenario the final effluent would be always estrogen-free.

8.4.7. Validation of the model

The model was validated by cycle-to-cycle calculation of accumulated EE2 concentration. The initial amount of 3 mg/d of EE2 was assumed. For case 1, 12 iterations were performed which corresponded with one SRT period (12 days). For case 2, due to much faster progress of accumulation 54 iterations equal to 4.5×SRT were employed for the checking. Figures 8.4 and 8.5 show the graphical interpretation of the model for case 1 and 2 respectively during 1×SRT period. For settings used in the experiment, the percentage benefits η_1 and η_2 obtained from the graphs and excel data spreadsheets are consistent with the values calculated according to equations 8.3 and 8.6.

Similar validation was performed for situation when WAS decrease is included in the model and again geometrical progression calculations were in agreement with graphical interpretation and cycle-to cycle data.
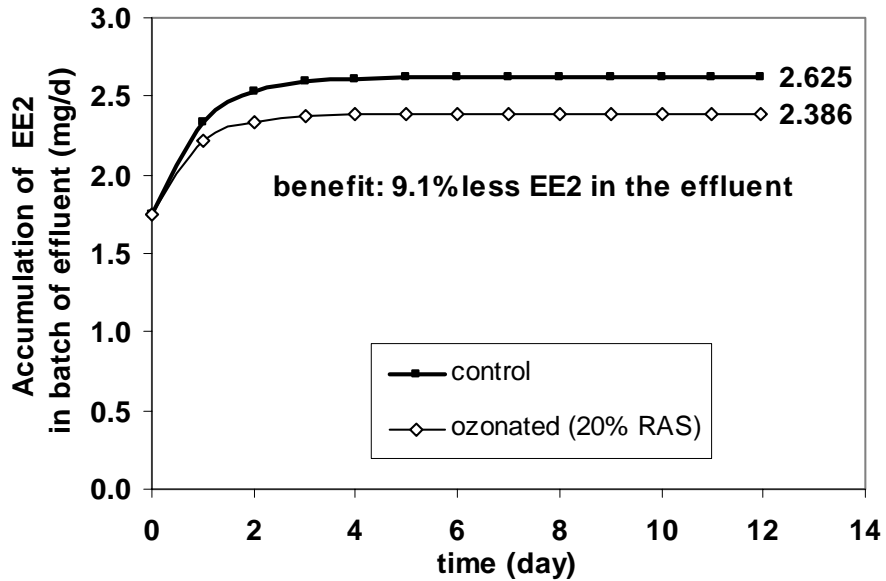
Figure 8.4. The graphical interpretation of the model (case 1) for the settings in the experiment; initial load of the estrogens 3 mg/Ld
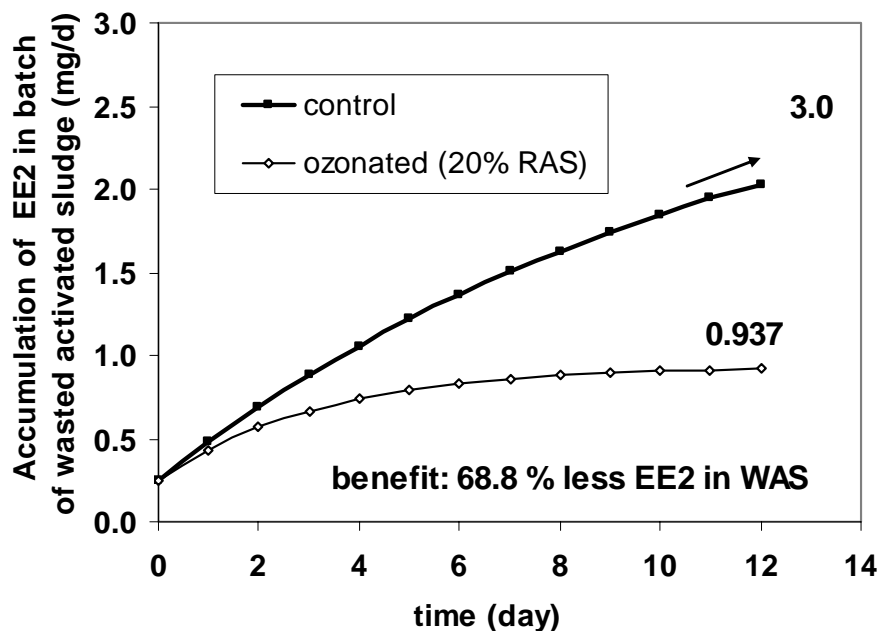
Figure 8.5. The graphical interpretation of the model (case 2) for the settings in the experiment; initial load of the estrogens 3 mg/Ld

8.5. Conclusions

The mathematical simulation was performed with neglecting any estrogen biodegradation during activated sludge treatment. Calculations show that partial ozonation of RAS might provide for concomitant endocrine disrupters removal.   This additional benefit is achieved due to the high ozone doses applied and repeatability of ozone treatment. Everyday destruction of EDC in ozonated fraction of RAS slows down their accumulation in the sludge resulting in decreased estrogen concentration both in the final effluent and WAS. The scale of this benefit depends on how big a fraction of EDC is intercepted by sludge particles (by sorption). In the best case scenario when all EDC are adsorbed to solids the method can provide up to 68% of reduction of EDC in WAS. On the other hand in the worst case situation with no sorption to sludge particles only 9.1%

of EDC reduction could be expected in liquid fraction of RAS and the effluent. Additional calculations performed factoring in the WAS decrease during each cycle (solids destruction compensations) showed that accumulation of estrogens in RAS does not increase significantly. Further benefit in terms of lowered WAS estrogenicity is achieved with slight deterioration of the effluent quality.

The real-life case is closer to the hypothetical case 1 as estrogens are reported to be hydrophobic and thus having high affinity to solids. The estimation of what fraction of estrogens entering the system is adsorbed onto sludge particles requires separate investigation.

9. ENGINEERING SIGNIFICANCE

The research was aimed at resolving several technical issues in nitrogen removal processes as used in full scale biological nutrient removal plants. These included: the use of ozone to reduce the generation of waste activated sludge; use of the new ozone-induced solubilised COD to augment denitrification; improvement of settleability; impact of ozonation on nitrification; impact of ozonation on removal of estrogenic compounds; explanation of differences between nitrification rates in a strictly aerobic reactor and in one with an anoxic pre-denitrification phase. This chapter contains the engineering and economic significance of the key findings.

9.1. Cost of ozonation technology

Decrease in the costs related to sludge processing due to WAS minimization can compensate the costs of ozone production (Ahn et al., 2002). Denitrification improvement after ozone treatment should be taken into consideration.   Full scale plants that treat carbon-deficient wastewater purchase methanol as the source of soluble COD (sCOD). Cities across America (e.g. Washington DC, Tampa FL, Arlington VA and in the near future Winnipeg MB) spend millions of dollars on methanol for denitrification. It was found (Park et al., 2004) that ozone lysates are actually better source of sCOD than methanol (denitrification rate as high as 3.66 mg NO_3-N/g VSS h as sole carbon source was achieved).

For areas with high cost of sludge disposal even the highest expenses for ozone treatment resulting from expensive electrical energy are compensated by decrease in excess sludge reduction. For moderate disposal costs the largest expenses for ozone production would be not economically justified. For the low price level the use of ozonation to reduce the costs of sludge disposal is cost effective. For low expenses of sludge disposal only low costs ozone generation is acceptable. This is particularly feasible as high costs of sludge management are usually associated with high energy costs. In Winnipeg where pure oxygen is generated commercially for activated sludge aeration and energy costs are the cheapest on the continent the costs of ozone could well become the lowest in America.

In pilot studies with real wastewater much higher sludge reduction was achieved (Yasui et al. 1996) than in this research however higher ozone doses were used. None of the studies have reported (Yasui and Shibata 1994; Yasui et al. 1996; Sakai et al., 1997; Egeman et al., 2001; Ahn et al., 2002) the phenomenon of floc strengthening which was observed in this study after long-term ozonation. This warrants further research as floc strengthening has significant bearing on the costs-effectiveness of the ozone addition. If the large scale findings are confirmed there would be no need for successive increases in applied ozone doses.

Intensive sludge disintegration accompanied by soluble COD production has been observed only up to a certain ozone dose. Above that threshold dose the generated soluble COD will be oxidized nullifying the additional benefits expected from generation of additional carbon in denitrification. This threshold was not reached in these studies

and it would not be advisable to operate the process above that threshold even for the benefit of deeper sludge reduction. The exceptions could be areas with extreme problems with sludge management. In such cases higher ozone doses could be applied accepting the fact that the costs will increase significantly.

The improvement in sludge settling after ozone treatment (§4.4.3) and endocrine disrupters' removal are other previously un-quantified benefit of the technology which actually could tip the balance for the use of technology in higher-costs scenarios. Winnipeg's West End Water Pollution Control Center (WEWPPC) has for a number of years used RAS chlorination to improve settleability of its bulking filamentous sludge. In such cases the switch to ozone would allow the operators to achieve multi-tiered operational improvements. When one adds all of the environmental effects such as settling improvement, destruction of EDC and control of filamentous growth to the economic benefits of lower sludge production costs and lower denitrification costs then partial RAS ozonation becomes a very promising and competitive technology in any economic scenario.

9.2. Volume savings as a result of higher nitrification rates

The upgrade of conventional carbonaceous BOD removing wastewater treatment plants to biological nutrient removal (BNR) requires an increase in SRT for nitrification. Such is the case in Winnipeg where three plants are looking at tripling of the tank size capacity and other improvements at a cost close to a 1 billion CAD (Szoke 2008). The plant

conversion to nitrogen removal involves an increase in the solids inventory in the tank which requires an increase in SRT. Any decrease in the required biomass (sludge) SRT is translated to volume savings because solids inventory increases linearly with increasing SRT (Yuan et al., 2000). As SRT is inversely proportional to the nitrification rate the benefits of increased rates due to bacterial speciation become clear.

The promotion of faster nitrifiers may allow the maintenance of full nitrification at a lower solids inventory than in plants designed conventionally with the kinetic parameters obtained through a fully aerobic protocol such as the currently "binding" WERF (2003) manual. The values for nitrifiers maximum specific growth rate μ_{aut} max can be calculated according to that manual, chapter 26.4 "Low F/M Bioassay". The procedure specifies that equations 9.2 could be used for SBR without denitrification only. Thus adapting it to the alternating system becomes questionable in light of the findings reported in this thesis nevertheless the modeling for the two systems was performed.

$$\mu_{max} = \frac{AUR}{c} \qquad\qquad 9.1$$

$$c = \frac{V_w \cdot (S - S_o) \cdot SRT}{(1 + b_{aut} \cdot SRT) \cdot V_p} \qquad\qquad 9.2$$

where:

AUR - ammonia uptake rate, mg/L d

V_w – influent wastewater rate, 2L/d

S – influent ammonia, 37 mg/L,

S_o – effluent ammonia, 0 mg/L

V_p –   reactor operating volume, 3 L

b_{aut} –   autotrophic decay rate, 1/d

Two different values of b_{aut} [1/d] were used: 0.153 for aerobic and 0.058 for alternating conditions as reported earlier (Lee and Oleszkiewicz, 2003). For alternating biomass, the aerobic SRT was calculated as (7+8.5)/24 · 12 SRT = 7.75, which was used in the growth rate equation. ΔNH_4^+ was used as N instead of ΔTKN due to lab analytical limitations. AUR rates from four kinetic studies were used for calculations. The results obtained at 20^oC for $\mu_{aut\ max}$ were 1.14±0.09 for the aerobic system and 1.96±0.13 for the alternating system. The difference was consistent with the faster rates and the hypothesis put forward in this research. Increase in the alternating growth rate was 72%. The nitrification rates in the alternating treatment at 6.21±0.33 mg NH_4^+-N/g VSS h were twice as high as in the aerobic reactors at 3.03±0.31 mg NH_4^+-N/g VSS h (§5.3.1). Some 30% higher population of ammonia oxidizers was determined using FISH (§6.4) which could explain doubling in ammonia removal rates (1.72x1.30=2.24). Assuming reduction in reactor volume proportional to SRT decrease (SRT= $1/\mu_{max}$) the aerobic reactor volume could be decreased by (1.72-1)/1.72=42% with the development of faster nitrifiers.

The resulting 42% aeration volume decrease is theoretical and should be tested in pilot and in full scale side-by-side tests. As the price of installed concrete soars at well over CAD1300/m^3 and the construction costs double every three or four years the savings in reactor volume become quite significant and worth further studies.

10. OVERVIEW

10.1. The "big picture" introduction

Biological nutrient removal (BNR) is being put into practice as new regulations impose nitrogen and phosphorus effluent standards on municipalities in Canada and in the developed world. A typical effluent permit in Western Canada and in European Union (EU) is 10-15 mg TN/L and 1 mg TP/L and calls for application of what is called best practicable technology (BPT) of biological nutrient removal. The three City of Winnipeg plants are expected to meet TN <15 mg/L and TP<1 mg/L. In sensitive areas such as the newly established US ecological zones (US EPA, 2001) the best available technology (BAT) of enhanced nutrient removal (ENR) is expected to bring the effluent quality to levels of 3 mg TN/L and 0.1 mg TP/L. BNR processes beside requiring higher capital and operating costs introduce new operational problems such as filamentous bulking, biological scum formation, alkalinity requirements, temperature dependency of nitrification and deficiency of organic carbon for denitrification and biological phosphorus removal.

At the same time regulations place restrictions on sludge (biosolids) disposal which became increasingly difficult due to high costs of processing (from $300 to $1200/t dry solids) and the increasing public opposition to land application, thermal oxidation (incineration) and landfilling. The difficulty in finding a solution where final disposal is unreasonably limited by public lack of trust in engineering solutions is best evidenced in the City of Winnipeg. With the most successful WinGro land application program in the

country a biosolids (sludge) management study for the City of Winnipeg has been on-going for 5 years. In Winnipeg's case raw sludge from the South and West plants has to be trucked to the North End facility further accentuating the risks and difficulties of sludge management. One option to improve the situation could be to decrease the sludge mass by reduction of the waste activated sludge (WAS). Research has shown that the ozonation of a fraction of the return activated sludge (RAS) prior to return to the aeration tank may achieve that goal. The ozonation technology may also alleviate some of the other problems of the BNR processes such as elimination of filamentous bulking, improvement of settleability; improved denitrification and potentially partial destruction of endocrine disrupting compounds (EDC).

Ozonation may affect the fragile nitrifier population hence it is necessary to assess that impact. The aeration tank where nitrification takes place is the largest and most expensive liquid stream structure at the treatment plant. Any decrease in nitrification activity would translate to a large increase of capital costs. It was hypothesized that nitrification can proceed faster after an anoxic period of pre-denitrification. There is a need to prove that hypothesis and also establish the magnitude of the negative ozonation impact on both aerobic and alternating reactor biomass.

10.2. Objectives

The specific objectives included defining the impact of RAS ozonation on: sludge (biomass) settleability; sludge dewaterability; quality of the final effluent; reduction of waste activated sludge (WAS); improvement of denitrification; nitrification; destruction

of natural and man-made estrogens. The impacts were to be defined on fully aerobic nitrifying biomass and the biomass in a pre-denitrifying alternating bioreactor. Another key objective was to test the hypothesis that biomass grown in an alternating anoxic-aerobic bioreactor generates faster nitrifying population than one found in a strictly aerobic reactor.

10.3. Research summary

The effectiveness of ozonation in enhancing denitrification and waste sludge minimization were examined in sequencing batch reactors with two sets of aerobic and alternating anoxic/aerobic conditions. The reactors were acclimated before commencing sampling and analyses for a period of time longer than $5 \times SRT$. In each set one reactor served as a control and the other was subject to ozone treatment (doses in the range of 0.016-0.080 mg O_3/mg TSS of initial excess sludge). The level of total suspended solids (TSS) in each reactor was controlled at 1800 mg/L.

Ozonation of 20% of RAS had no adverse impact on the final effluent quality from the lab reactors. The total COD and suspended solids in the effluent remained similar in test reactors and controls. An insignificant accumulation of inorganic solids in the ozonated reactors was observed after prolonged operation. Nitrification was complete in both sets of reactors regardless of the ozone dose.

The amount of WAS decreased with the ozone dose. Biomass in the anoxic/aerobic reactor was easier to destroy (up to 25% of the initial excess sludge was destroyed) than

in the aerobic (10% destroyed) one and generated approximately twice as much soluble COD by cell lysis. The difference was caused by different floc structure developed under alternating anoxic/aerobic conditions. The presence of the anoxic period made the flocs from alternating reactors weaker and deflocculated in comparison to dense, strong and compact aerobic flocs. In long-term ozonation however the alternating flocs became stronger due to significant amount of additional sCOD delivered to the system. With time the effect of reduction of WAS solids was less pronounced. This indicates that for prolonged operation of partial sludge ozonation an increase in ozone doses may be required to continuously maintain the same solids destruction effect.

Denitrification rate improved up to 60% due to the additional carbon released by ozonation. Nitrification rates deteriorated much more in the aerobic than in the alternating reactor. This effect was possibly not due to direct destruction of nitrifying autotrophs but rather due to competition created by growth of heterotrophs receiving the additional sCOD. Overall, ozonation provided the expected benefits in denitrification at the doses that could still be tolerated by the nitrifying population. Ozonation exerted lesser negative impact on nitrification in the alternating reactors.

The influence of partial ozonation of return activated sludge on settling properties and dewaterability of sludge was investigated using settling kinetic studies, extracellular polymeric substances (EPS), sludge volume index (SVI) and capillary suction time test (CST). For extraction and quantification of sludge biopolymers thermal-ethanolic extraction was employed. The ratio of bound-to-total EPS was higher for strictly aerobic reactor than for alternating anoxic/aerobic one indicating stronger structure of aerobic

flocs. After ozone treatment the fraction of bound EPS was released and solubilized, increasing soluble EPS. Increased apparent food-to-microorganism ratio caused by release of sCOD from destroyed cells favoured the production of EPS in ozonated reactors, enhancing flocculation that contributed to improved settling. Dewaterability measured by CST test was better in alternating anoxic/aerobic reactors than in aerobic ones indicating that incorporation of an anoxic zone for biological nutrient removal leads to improvement in sludge dewatering. The negative impact of ozonation on dewaterability was minimal in terms of the long-term operation.

Excess sludge (WAS) minimization and denitrification rate improvement decrease the costs related to sludge processing and additional source of carbon. These savings have potential to compensate the costs of ozone production. The final benefits depend on local energy prices and whether ozone is generated from air or pure oxygen as could be the case in Winnipeg.

Higher nitrification rates in alternating reactor (6.16 ± 0.34 mg NH_4^+-N/gVSS h) in comparison to strictly aerobic one (3.03 ± 0.31 mg NH_4^+-N/gVSS h) were observed consistently over a period of operation in excess of one year. The phenomenon was investigated by comparing environmental conditions. In the alternating reactor, pH, alkalinity, oxygen, and nitrite were higher at the onset of aerobic nitrification. On the other hand the COD was already consumed at the onset of nitrification following heterotrophic denitrification. Kinetic studies and batch tests with biomass developed under aerobic and alternating conditions revealed that factors like pH and alkalinity,

COD level and presence of the anoxic zone were insufficient to explain the divergent nitrification rates. Nitrifying genera vary in nitrification kinetics and sensitivity to environmental conditions. K-strategists *Nitrosospira* and *Nitrospira spp.* could dominate in aerobic reactors as they are adapted to low nitrite and oxygen conditions. *Nitrosomonas* and *Nitrobacter* spp. as r-strategists are better competitors with abundant substrates and have higher nitrite tolerance so they could excel under alternating conditions. Microbial populations in both reactors were examined with fluorescent in situ hybridization (FISH) and kinetic batch studies to determine the effects of ammonium, nitrite, and oxygen. FISH revealed a dominance of rapid nitrifiers like *Nitrosomonas* and *Nitrobacter* (79.5%) in the alternating reactor compared to a dominance of slower nitrifiers like *Nitrosospira* and *Nitrospira* (78.2%) in the strictly aerobic reactor. Nitrifiers in the aerobic reactor operated at maximum rates and were unaffected by ammonium or nitrite whereas nitrifying rates in the alternating reactor were proportional to ammonium or nitrite concentrations. The alternating conditions were better because they selected for faster nitrifiers due to their kinetics, growth, and decay rates. The findings are of major importance to the design engineers as the reactors are typically designed based on nitrifiers' growth rate determined in strictly aerobic conditions. Selection for faster nitrifiers by use of pre-denitrification has significant impact on the process selection and cost-effectiveness analysis in process comparison. Smaller aeration zones in modern BNR and ENR treatment plants translate to large capital costs savings.

Natural and synthetic estrogens present in municipal wastewater can be biodegraded during treatment in activated sludge. The objective was to assess the extent of transformation of 17-β-estradiol (E2) and 17-α-ethinylestradiol (EE2) by a nitrifying activated sludge and to evaluate potential relationships between availability of oxygen, nitrification rate and estrogen removal. For each batch experiment the biomass was amended with E2 and EE2 from methanolic stock solutions. EE2 was persistent under anoxic conditions; under aerobic conditions the observed level of its removal was 22%. E2 was readily converted to estrone (E1). The conversion was faster under aerobic (nitrifying) than under anoxic (denitrifying) conditions. During initial anoxic conditions a metabolite consistent with 17-α-estradiol transiently accumulated and was subsequently removed when the reactor was aerated. Higher estrogen removal rates were associated with higher nitrification rates. This supports the contention that the nitrifying biomass was responsible for their removal. The alternating treatment offered better estrogen biodegradation due to higher nitrification rates.

The direct impact of ozonation in the range of doses used in the previous research on estrogen removal was investigated to evaluate the extent of decomposition. Immediately below the moderate dose 0.05 mg O_3/mg TSS initial excess sludge the concentration of the indicators E2 and EE2 dropped below detection limit. That dose is optimal for sludge reduction and denitrification improvement without negative effects on nitrification. Assuming complete oxidation of estrogenic compounds in the portion of sludge during ozone treatment a mathematical simulation was performed. The simulation neglected any estrogen biodegradation during activated sludge treatment. Calculations show that partial

ozonation of RAS has a potential for removal of endocrine disrupters at the ozone doses applied and due to repeatability of ozone treatment.

10.4.   Recommendation for future work

The observed floc structure strengthening after prolonged every-day ozonation may lead to the necessity of application of higher ozone doses and may increase the operational costs of the treatment. This negative consequence of process adaptation could however be less pronounced in a full scale facility where variations in influent composition and temperature cause flocs to be more vulnerable for de-flocculation. The next step should be pilot- or full-scale testing of this method of sludge reduction with emphasis on the potential long-term changes of the floc structure.

Further research is needed also to determine the rates of estrogen loss through biodegradation and sorption with lower estrogen concentrations. Potentially YES (yeast estrogenicity screening) assay cold be used to analyse all estrogens in low-range (ng/L). To investigate further the potential of nitrifying sludge in estrogen removal in the batch studies a method of nitrification inhibition should be developed. Allylthiourea (ATU) as an inhibitor for ammonia oxidizers and $NaClO_3$ for nitrite oxidizers could be used (Surmacz-Gorska et.al., 1996). Further studies on accumulation of the metabolite 17-α-estradiol under anoxic conditions should be performed. The 17-α-estradiol compound was detected so far only in alternating biomass and never in the aerobic biomass even after many hours of anoxic reaction. The issue of nitrification impact on estrogens biotransformation is even more interesting in the light of findings regarding

microbiological structure of the nitrifying population as potentially different species could show different EDC removal activity due to their inherent substrate affinity.

Everyday destruction of EDC in ozonated fraction of RAS slows down their accumulation in the sludge resulting in decrease estrogen concentration both in final effluent and WAS. It is recommended to perform further studies to evaluate the benefit of partial ozonation on persistent compounds in pilot or full scale.

A long term side-by-side full-scale or large pilot scale studies of the continuous flow anoxic $\rightarrow$ aerobic reactors and aerobic nitrifying reactors are necessary. The process should be controlled and data interpreted through the use of commercial simulators (such as BioWin or GPS-X) such that a full picture of savings offered by the presence of long-term pre-anoxic conditions can be assessed. The research should be accompanied by studies to determine autotrophic decay rates and temperature correction factors for decay and growth rate. Full microbiological analysis using FISH and other biomass speciation and quantifying methods should be used to assess conditions that lead to the generation of "faster" nitrifying biomass. One of the outcomes of this work should be introduction of changes to the currently "binding" protocols for determination of autotrophic growth rates as pre-requisite to design of nitrification bioreactors.

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12. APPENDICES

Appendix 2.1 Total and volatile suspended solids in the final effluent

TSS day	R1	R3	R3	R4		VSS	R1	R3	R3	R4
8/19/2004	8	22	6	15			8	21	6	14
8/21/2004	8	6	5	12			4	5	4	10
8/29/2004	12	8	6	18			12	8	6	18
9/2/2004	17	14	26	22			16	13	21	18
9/8/2004	20	23	21	29			20	23	20	29
9/12/2004	21	21	17	17			21	21	17	17
9/14/2004	9	14	16	9			9	13	16	8
9/17/2004	12	14	13	8			7	8	12	8
9/19/2004	15	28	13	37			11	13	12	28
av	13.5	16.4	13.6	18.4		av	11.9	13.7	12.6	16.6
stdev	5.0	7.1	7.2	9.6		stdev	5.9	6.3	6.3	7.9

VSS/TSS day	R1	R3	R3	R4
8/19/2004	1.00	0.96	1.00	0.93
8/21/2004	0.50	0.83	0.80	0.83
8/29/2004	1.00	1.00	1.00	1.00
9/2/2004	0.94	0.93	0.81	0.82
9/8/2004	1.00	1.00	0.95	1.02
9/12/2004	1.00	1.00	1.00	1.00
9/14/2004	1.00	0.96	1.03	0.88
9/17/2004	0.58	0.59	0.88	1.07
9/19/2004	0.72	0.45	0.92	0.76
av	0.86	0.86	0.93	0.92
stdev	0.20	0.20	0.09	0.11

t-test **0.108**

Appendix 2.2 Calculations of the real SRT (d) in the reactors

	av	stdev	min	max
Xw	450		450	450
Xe	31	14.8	45.8	16.2
Xw+Xe	481		495.8	466.2
1/SRT=Xw*3/X	0.0891		0.0918	0.0863
SRT	**11.23**		**10.89**	**11.58**
			0.34	0.36

Xw TSS in WAS, mg
Xe TSS in effl., mg
Xw+Xe TSS total wasted from the system, mg

SRT calculated 11.23±0.36 d

Appendix 2.3   pH end of the cycle, 10:15 am

	# reactor	R1	R3	R3	R4
phase 1					
2004.06.23	1	6.97	6.99	7.15	7.22
2004.06.24	2	6.40	6.19	7.32	7.35
2004.06.25	3	8.20	8.26	7.92	8.70
2004.06.26	4	7.76	8.01	8.27	8.68
2004.06.27	5	7.14	7.52	8.02	8.35
2004.06.28	6	6.95	6.99	7.49	7.50
2004.06.28	6	7.85	7.80	7.50	7.59
2004.06.29	7	6.85	6.80	7.46	7.95
2004.06.30	8	7.34	7.60	8.17	8.06
2004.07.01	9	7.38	7.55	7.91	8.07
2004.07.02	10	7.51	7.60	8.17	8.36
2004.07.03	11	7.70	7.29	8.06	8.29
2004.07.04	12	7.67	7.36	8.09	8.29
2004.07.05	13	7.68	7.47	8.33	8.41
2004.07.06	14	7.72	7.42	8.35	8.41
2004.07.07	15	7.65	7.30	8.32	8.37
2004.07.08	16	7.64	7.19	8.25	8.29
2004.07.09	17	7.48	6.91	8.08	8.36
2004.07.10	18	7.37	7.25	7.26	8.01
2004.07.11	19	7.40	7.21	7.53	8.13
2004.07.12	20	7.56	7.34	7.80	8.07
2004.07.13	21	7.29	7.67	8.03	8.23
2004.07.14	22	7.30	7.70	8.06	8.19
2004.07.15	23	7.41	7.69	7.99	7.99
2004.07.16	24	7.42	7.70	7.94	8.00
2004.07.17	25	7.20	7.39	7.67	7.74
2004.07.18	26	7.37	7.47	7.91	7.94
2004.07.19	27	7.47	7.65	8.01	8.05
2004.07.20	28	7.52	7.69	8.05	8.12
2004.07.21	29	7.51	7.67	8.10	8.14
2004.07.22	30	7.51	7.68	8.00	8.15
2004.07.23	31	7.30	7.42	7.77	8.02
2004.07.24	32	7.44	7.56	7.83	8.02
2004.07.25	33	7.32	7.48	7.82	7.96
2004.07.26	34	7.50	7.67	7.95	8.14
2004.07.27	35	7.42	7.64	7.78	8.08
2004.07.28	36	7.45	7.65	7.34	8.00
2004.07.29	37	7.44	7.61	7.64	7.96
2004.07.30	38	7.48	7.67	7.59	8.03
2004.08.01	40	7.69	7.70	8.25	8.33
2004.08.02	41	7.36	7.58	7.89	8.15
phase 2					
2004.08.03	42	7.74	7.71	7.91	8.18
2004.08.04	43	7.77	7.79	7.88	8.04
2004.08.05	44	7.72	7.75	7.84	8.02
2004.08.06	45	7.73	7.75	8.58	8.47
2004.08.07	46	7.65	7.69	8.42	8.32
2004.08.08	47	7.61	7.64	8.02	8.01
2004.08.09	48	7.64	7.66	8.03	8.00
2004.08.10	49	7.61	7.68	7.98	8.11
2004.08.11	50	7.60	7.61	7.81	7.75
2004.08.13	52	7.33	7.40	7.98	7.99
2004.08.14	53	7.88	7.83	8.04	7.95
2004.08.15	54	7.88	7.93	7.98	7.93
2004.08.16	55	7.91	7.98	7.83	7.81

	# reactor	R1	R3	R3	R4
phase 3					
2004.08.17	56	7.92	8.03	7.92	7.24
2004.08.18	57	8.07	8.15	7.92	7.59
2004.08.19	58	7.97	8.12	7.93	7.34
2004.08.20	59	8.11	7.87	7.54	8.70
2004.08.21	60	8.17	7.54	7.57	8.41
2004.08.22	61	8.06	7.63	7.69	8.14
2004.08.23	62	8.09	7.60	7.62	8.09
2004.08.24	63	8.06	8.15	8.08	7.95
2004.08.25	64	8.03	8.10	7.83	8.33
2004.08.26	65	8.03	8.11	7.55	8.26
2004.08.27	66	8.30	8.24	8.60	8.45
2004.08.28	67	7.99	8.00	8.09	8.05
2004.08.29	68	8.03	8.18	8.50	8.16
2004.08.30	69	7.94	7.99	8.39	8.11
2004.08.31	70	7.80	7.87	8.25	7.95
2004.09.01	71	7.77	7.83	8.23	7.92
2004.09.02	72	7.74	7.79	8.52	8.63
2004.09.03	73	7.71	7.83	8.06	8.22
2004.09.05	75	7.87	7.88	7.95	7.93
2004.09.06	76	8.00	8.05	8.01	8.07
2004.09.07	77	7.67	7.82	7.91	8.05
2004.09.08	78	7.79	7.97	7.68	8.08
2004.09.09	79	7.62	7.77	7.96	7.92
2004.09.10	80	7.84	7.94	8.26	8.18
2004.09.11	81	7.60	7.74	7.99	8.04
2004.09.12	82	7.70	7.86	8.60	8.41
2004.09.13	83	7.74	7.87	8.50	8.35
2004.09.14	84	7.76	7.86	8.44	8.38
2004.09.15	85	7.68	7.81	8.42	8.23
2004.09.16	86	7.87	7.90	8.42	8.22
2004.09.17	87	7.82	7.87	8.36	8.17
2004.09.18	88	7.81	7.86	8.30	8.01
average		**7.89**	**7.91**	**8.10**	**8.11**
stdev		**0.17**	**0.17**	**0.33**	**0.31**
t-test		**0.546**		**0.832**	
t-test aerobic vs. alternating		**0.000**			

Appendix 2.4   Alkalinity in the effluent and the feed, mg CaCO$_3$/L

	# reactor	R1	R3	R3	R4	feed
phase 1						
7/9/2004	17	80	120	220	240	
7/27/2004	35	90	119	236	248	
phase 2						
8/5/2004	44	115	120	278	275	
8/11/2004	50	118	115	275	255	
8/14/2004	53	135	125	255	250	
8/16/2004	55	145	141	258	250	
phase 3						
8/18/2004	57	190	184		253	
8/20/2004	59	121	112			
8/22/2004	61	120	135	381	267	449
8/23/2004	62	110	200	338	275	498
8/24/2004	63	125	138	250	272	583
8/25/2004	64	125	114	258	260	454
8/26/2004	65	130	115	245	270	507
8/27/2004	66	121	113	232	252	477
8/29/2004	68	164	123	289	285	458
8/30/2004	69	185	173	289	276	459
average		**139**	**141**	**285**	**268**	**486**
stdev		**29**	**33**	**51**	**11**	**45**
t-test		**0.893**		**0.402**		
t-test aerobic vs. alternating		**0.000**				

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Appendix 2.5   Total and volatile suspended solids in mixed liquor, mg/L, and volatile to total suspended solids ratio; acclimation period

TSS						VSS						VSS/TSS				
	day	R1	R2	R3	R4		day	R1	R2	R3	R4		day	R1	R2	R3	R4
phase 1																	
2004.06.23	1	3840	3710	3960	3890	2004.06.23	1	2910	2610	2790	2940	2004.06.23	1	0.76	0.70	0.70	0.76
2004.06.24	2	3740	3280	3460	3550	2004.06.24	2	2550	2410	2660	2960	2004.06.24	2	0.68	0.73	0.77	0.83
2004.06.24	2	2800	2839	2717	2812	2004.06.24	2	1930	2002	1957	2161	2004.06.24	2	0.69	0.71	0.72	0.77
2004.06.25	3	2340	2250	2180	2270	2004.06.25	3	1770	1530	1650	1720	2004.06.25	3	0.76	0.68	0.76	0.76
2004.06.26	4	2410	2140	1750	2430	2004.06.26	4	1370	1310	1020	1530	2004.06.26	4	0.57	0.61	0.58	0.63
2004.06.27	5	1900	1800	1880	2430	2004.06.27	5	1490	1260	1320	1250	2004.06.27	5	0.78	0.70	0.70	0.66
phase 2																	
2004.06.28	6	2000	1740	1704	1650	2004.06.28	6	1310	1260	1070	1250	2004.06.28	6	0.66	0.72	0.63	0.76
2004.06.29	7	1490	1680	1510	1590	2004.06.29	7	940	1010	870	1130	2004.06.29	7	0.63	0.60	0.62	0.71
2004.06.30	8	1580	1630	1660	1530	2004.06.30	8	1140	1210	1180	1430	2004.06.30	8	0.72	0.74	0.71	0.78
2004.07.01	9	1660	1720	1590	1570	2004.07.01	9	1220	1190	1190	1050	2004.07.01	9	0.73	0.69	0.75	0.67
2004.07.02	10	1700	1610	1790	1770	2004.07.02	10	1220	1160	1240	1310	2004.07.02	10	0.72	0.72	0.69	0.74
2004.07.03	11	1590	1370	1512	1490	2004.07.03	11	1160	1050	1067	1150	2004.07.03	11	0.73	0.77	0.71	0.77
2004.07.04	12	1370	1320	1399	1320	2004.07.04	12	980	1020	1053	1060	2004.07.04	12	0.72	0.77	0.75	0.80
2004.07.05	13	1200	1400	1537	1570	2004.07.05	13	1020	1080	1173	990	2004.07.05	13	0.85	0.77	0.76	0.63
2004.07.06	14	1440	1430	1427	1540	2004.07.06	14	1160	1090	1163	1150	2004.07.06	14	0.81	0.76	0.82	0.75
2004.07.07	15	1370	1410	1391	1420	2004.07.07	15	1060	1140	982	1070	2004.07.07	15	0.77	0.81	0.71	0.75
2004.07.08	16	1290	1210	1398	1410	2004.07.08	16	960	920	1089	1040	2004.07.08	16	0.74	0.76	0.78	0.74
2004.07.09	17	1350	1330	1381	1240	2004.07.09	17	970	1120	1072	1020	2004.07.09	17	0.72	0.84	0.78	0.82
2004.07.12	20	1390	1440	1574	1480	2004.07.12	20	920	1070	1134	1040	2004.07.12	20	0.66	0.74	0.72	0.70
2004.07.15	23	1380	1410	1226	1270	2004.07.15	23	900	880	1022	1010	2004.07.15	23	0.65	0.62	0.83	0.80
2004.07.18	26	1310	1310	1232	1200	2004.07.18	26	1220	1120	974	1020	2004.07.18	26	0.93	0.85	0.79	0.85
2004.07.21	29	1180	1110	932	1080	2004.07.21	29	850	1045	822	740	2004.07.21	29	0.72	0.94	0.88	0.69
2004.07.26	34	1200	1170	990	1180	2004.07.26	34	1000	970	840	800	2004.07.26	34	0.83	0.83	0.85	0.68
2004.07.29	37	1410	1370	1210	1290	2004.07.29	37	1120	960	1100	1250	2004.07.29	37	0.79	0.70	0.91	0.97
2004.08.03	42	1220	1384	1123	1020	2004.08.03	42	1100	1040	1040	890	2004.08.03	42	0.90	0.75	0.93	0.87
2004.08.05	44	1320	1210	1100	1110	2004.08.05	44	1110	1070	980	960	2004.08.05	44	0.84	0.88	0.89	0.86
2004.08.09	48	1420	1280	1140	1220	2004.08.09	48	1240	1120	1000	1020	2004.08.09	48	0.87	0.88	0.88	0.84
phase 3																	
2004.08.18	57	1460	1220	1300	1204	2004.08.18	57	1200	1020	1100	990	2004.08.18	57	0.82	0.84	0.85	0.82
2004.08.19	58	1560	1320	1320	1408	2004.08.19	58	1300	1160	1100	1140	2004.08.19	58	0.83	0.88	0.83	0.81
2004.08.21	60	1380	1380	1300	1394	2004.08.21	60	1200	1190	1140	1100	2004.08.21	60	0.87	0.86	0.88	0.79
2004.08.29	68	1730	1830	1860	1890	2004.08.29	68	1490	1560	1450	1500	2004.08.29	68	0.86	0.85	0.78	0.79
2004.09.02	72	1680	1700	1750	1760	2004.09.02	72	1460	1490	1360	1390	2004.09.02	72	0.87	0.88	0.78	0.79
2004.09.03	73	1890	1940	1980	2000	2004.09.03	73	1610	1700	1530	1560	2004.09.03	73	0.85	0.88	0.77	0.78
2004.09.08	78	1700	1780	1840	1837	2004.09.08	78	1460	1480	1320	1460	2004.09.08	78	0.86	0.83	0.72	0.79
2004.09.09	79	1660	1840	1800	1800	2004.09.09	79	1460	1620	1360	1320	2004.09.09	79	0.88	0.88	0.76	0.73
2004.09.10	80	1680	1740	1900	1950	2004.09.10	80	1400	1440	1320	1560	2004.09.10	80	0.83	0.83	0.69	0.80
2004.09.11	81	1820	1720	1700	1720	2004.09.11	81	1530	1430	1330	1360	2004.09.11	81	0.84	0.83	0.78	0.79
2004.09.12	82	1790	1800	1620	1650	2004.09.12	82	1500	1540	1290	1350	2004.09.12	82	0.84	0.86	0.80	0.82
2004.09.13	83	1650	1730	1650	1680	2004.09.13	83	1430	1420	1240	1230	2004.09.13	83	0.87	0.82	0.75	0.73
2004.09.14	84	2040	1970	1910	1949	2004.09.14	84	1720	1680	1570	1560	2004.09.14	84	0.84	0.85	0.82	0.80
2004.09.15	85	1970	2030	1800	1840	2004.09.15	85	1670	1700	1400	1420	2004.09.15	85	0.85	0.84	0.78	0.77
2004.09.16	86	1940	2110	1790	1810	2004.09.16	86	1650	1750	1580	1530	2004.09.16	86	0.85	0.83	0.88	0.85

TSS

	day	R1	R2	R3	R4
2004.09.17	87	1790	1800	1760	1760
2004.09.18	88	2070	1980	1920	1860
2004.09.19	89	2000	2120	1900	1810
average		1767	1778	1728	1740
stdev		195	254	216	211
t-test		**0.699**		**0.320**	

VSS

	day	R1	R2	R3	R4
2004.09.17	87	1600	1560	1440	1440
2004.09.18	88	1750	1670	1500	1520
2004.09.19	89	1680	1760	1500	1430
average		1506	1509	1363	1381
stdev		164	210	150	169
t-test		**0.874**		**0.337**	

VSS/TSS

	day	R1	R2	R3	R4
2004.09.17	87	0.89	0.87	0.82	0.82
2004.09.18	88	0.85	0.84	0.78	0.82
2004.09.19	89	0.84	0.83	0.79	0.79
average		0.85	0.85	0.79	0.79
stdev		0.02	0.02	0.05	0.03
t-test		**0.549**		**0.822**	

Appendix 2.6   SOUR mg O2/g VSS h, samples from the reactors

	day	time	R1	R3	R3	R4
7/27/2004	35	12:30 PM	39.6	42.5	34.1	32.5
8/2/2004	41	12:30 PM	52.4	52.0	40.9	42.0
9/4/2004	74	12:30 PM	50.4	49.1	38.9	42.3
8/10/2004	49	2:00 PM	32.7	32.9	31.7	21.5
7/27/2004	35	2:30 PM	28.3	29.1	23.6	23.5
8/11/2004	50	4:00 PM	19.7	15.4	23.1	35.8
8/5/2004	44	4:30 PM	12.9	14	36.0	46.0
8/6/2004	45	4:30 PM	18.5	17.9	19.3	22.6
7/4/2004	12	9:00 AM	12.3	8.3	7.3	5.9
8/3/2004	42	9:00 AM	13.6	9	5.6	-
8/13/2004	52	9:00 AM	11.8	10.5	5.4	7.9
7/2/2004	10	9:30 AM	5.7	9.5	6.7	8.0
9/5/2004	75	10:00 AM	8.3	8.9	6.9	5.8
7/20/2004	28	11:00 AM	8.7	5.4	7.0	5.6

Appendix 2.7   Redox potential (ORP) in the reactors for selected time, mV

	day	time	R1	R3	R3	R4
8/8/2004	47	12:00 PM	-43	-46	-67	-67
9/3/2004	73	12:00 PM	-36	-41	-28	-35
9/5/2007	75	2:00 PM	-5	12	-224	-219
9/6/2007	76	2:30 PM	8	28	-238	-258
7/20/2004	28	3:00 PM	185	183	-239	-246
7/25/2004	33	4:00 PM	148	167	16	9
9/1/2004	71	5:00 PM	150	100	217	83
7/5/2004	13	6:40 PM	177	236	164	171
8/14/2004	53	8:00 AM	206	230	227	220
9/3/2004	73	8:30 AM	240	217	183	233
7/22/2004	30	8:30 AM	245	256	242	236
8/23/2004	62	10:00 AM	170	169	162	164
8/25/2004	64	10:00 AM	135	140	130	135

Appendix 2.8   Total COD in the effluent and feed, mg/L

	reactors						Aerobic		Alternating		
day	R1	R3	R3	R4	feed		R1	R3	R3	R4	feed
phase 1											
2004.07.19	27	95.5	96.0	93.3	93.0		41	36	63	66	
2004.07.20	28	96.5	95.0	93.5	91.5	65.0	31	45	61	82	474
2004.07.21	29	94.5	96.0	94.5	91.8		51	36	51	79	
2004.07.22	30	92.0	93.2	92.0	89.5		50	39	50	76	
2004.07.24	32	92.5	94.0	93.8	90.8	64.0	46	33	34	62	478
2004.07.26	34	93.5	89.5	93.8	92.1		37	76	34	49	
2004.07.27	35	95.5	96.5	96.0	94.0		46	36	41	62	
2004.07.28	36	97.0	95.5	93.0	92.7	63.5	25	39	62	65	479
2004.07.29	37	97.0	96.0	97.0	94.5		30	40	30	55	
2004.07.30	38	96.0	95.5	96.0	94.5	66.5	40	45	40	55	425
average							**40**	**43**	**47**	**65**	**464**
stdev							**9**	**13**	**12**	**11**	**26**
phase 2											
2004.08.03	42	94.0	92.0	92.5	93.0	56.5	55	76	70	65	606
2004.08.04	43	94.5	93.5	94.5	94.0	61.3	43	52	43	47	589
2004.08.05	44	95.8	93.8	94.9	94.8	61.3	36	50	42	43	582
2004.08.06	45	95.5	94.5	93.0	93.9	62.0	38	46	58	51	568
2004.08.07	46	97.0	96.5	96.0	96.5	58.0	28	31	35	31	600
2004.08.09	48	96.0	95.0	95.5	95.5	59.9	30	39	34	34	551
2004.08.10	49	96.5	95.9	96.0	96.2	63.5	38	44	43	41	508
2004.08.11	50	96.0	95.5	94.0	97.0	64.0	38	42	56	29	517
2004.08.13	52	94.0	94.0	95.5	94.0	59.5	42	42	26	42	548
2004.08.14	53					64.2					501
2004.08.15	54					62.4					510
2004.08.16	55	98.0	98.0	97.5	97.5	66.0	20	20	25	25	467
average							**37**	**44**	**43**	**41**	**545**
stdev							**10**	**14**	**15**	**12**	**45**
phase 3											
2004.08.17	56					57.5					623
2004.08.18	57					58.0					614
2004.08.19	58					58.5					605
2004.08.20	59	94.0	97.5	96.0	96.5	57.0	68	30	46	41	632
2004.08.21	60	94.5	94.5	94.5	96.0	51.5	32	32	32	17	672
2004.08.22	61					51.9					665
2004.08.23	62					51.9					665
2004.08.24	63	92.0	93.5	91.5	94.0	53.5	36	20	42	15	634
2004.08.25	64					56.0					627
2004.08.26	65					56.5					618
2004.08.27	66					56.5					618
2004.08.29	68	93.8	96.5	95.0	96.0	54.0	62	35	50	40	662
2004.08.30	69	96.0	95.9	95.5	94.5	54.5	35	36	39	48	640
2004.08.31	70	95.2	96.0	95.0	95.0	55.9	46	38	48	48	622
2004.08.31	70					55.0					638
2004.09.01	71	94.0	97.0	96.0	95.0	56.5	55	27	37	46	603
2004.09.02	72	96.0	96.0	95.5	96.0	54.5	37	37	41	37	641
2004.09.03	73	95.5	97.0	96.5	95.2	55.0	41	27	32	44	632
2004.09.04	74					62.1					571
2004.09.05	75					56.0					613
2004.09.07	77	96.5	95.0	97.5	97.0	59.0	32	46	23	27	624
2004.09.08	78	96.0	95.8	96.0		55.0	43	45	43		692
2004.09.09	79	97.0	97.5	97.0	97.0	58.0	31	26	31	31	641
2004.09.10	80	97.0	97.5	96.0	96.2	57.5	31	26	43	40	650
2004.09.11	81					57.5					602
2004.09.12	82					60.5					569
2004.09.13	83					56.5					635
2004.09.14	84					57.8					585
2004.09.14	84					56.0					628
2004.09.15	85	95.5	96.0	94.5	96.0	57.5	44	39	55	39	599
2004.09.16	86	97.0	97.0	95.5	97.0	57.3	29	29	44	29	611
2004.09.17	87	95.5	96.0	91.0	95.5	57.2	44	38	90	44	597
2004.09.18	88	95.5	96.5	94.5	96.5	57.3	44	33	54	33	595
2004.09.19	89	96.5	96.0	94.0	96.0	57.9	32	38	59	38	586
average							**41**	**33**	**45**	**36**	**624**
stdev							**11**	**7**	**15**	**10**	**28**
average							**40**	**39**	**45**	**45**	
stdev							**10**	**12**	**14**	**16**	
t-test							**0.676**		**0.906**		

Appendix 2.9   NO$_3^-$, NO$_2^-$ and NH$_4^+$ at end of the cycle (samples from the reactors before wasting)
N- mg/L

	day	NO$_3^-$				NO$_2^-$				NH4$^+$				
		R1	R3	R3	R4	R1	R3	R3	R4	R1	R3	R3	R4	feed
phase 1														
13-Jul-04	21	74.23	84.32	22.89	3.92			31.17	45.55			14.98		
14-Jul-04	22	94.41	97.16	24.74	1.83			33.91	39.39			13.17		
15-Jul-04	23	62.99	64.11	36.30	24.61			41.22	42.85			21.49		17.48
17-Jul-04	25	64.11	67.46	35.23	28.82			24.40	34.17			6.02		23.78
19-Jul-04	27	54.49	55.16	33.98	23.33			20.06	37.42					18.05
20-Jul-04	28	72.85	69.78	37.28	23.00			22.28	36.56			32.95		19.20
21-Jul-04	29	72.85	74.90	37.48	26.38			21.06	39.11			7.17		16.91
22-Jul-04	30	97.39	74.90	42.88	26.07			13.61	37.58					
24-Jul-04	32	74.90	77.35	49.11	30.58			12.08	40.64					
26-Jul-04	34	67.74	68.76	43.06	24.01			3.00	30.44					17.36
27-Jul-04	35	69.44	69.44	46.64	47.80			2.08	28.36					
28-Jul-04	36	72.64	76.16	50.39	54.53			4.66	30.42					
29-Jul-04	37	70.88	69.82	46.03	55.86			0.23	27.33					16.78
30-Jul-04	38	41.66	54.08	41.66	26.76									
31-Jul-04	39	39.18	59.05	49.12	29.25									17.36
phase 2														
3-Aug-04	42	46.63	61.53	49.12	29.25									20.85
4-Aug-04	43	44.15	66.50	59.05	56.57									
5-Aug-04	44	68.99	81.40	68.99	49.12									
6-Aug-04	45	66.50	78.92	61.53	51.60									
7-Aug-04	46	66.50	86.37	71.47	46.63									20.27
9-Aug-04	48	61.53	86.37	61.53	24.28									
10-Aug-04	49	54.08	86.37	73.95	51.60									
11-Aug-04	50	78.92	111.21	96.31	73.95									
13-Aug-04	52	33.40	31.36	11.62	17.06									
14-Aug-04	53	17.06	20.47	16.04	12.98									21.54
14-Aug-04	53	65.40	74.25	49.74	47.02									
15-Aug-04	54	77.65	85.82	55.87	52.46									
16-Aug-04	55	83.78	84.46	55.19	51.78									25.82
phase 3														
17-Aug-04	56	81.17	89.63	63.35	58.34									25.82
19-Aug-04	58	85.10	93.25	61.54	57.92									26.18
20-Aug-04	59	72.63	74.96	36.80	44.88			4.66						25.46
21-Aug-04	60	72.94	64.05	43.79	41.46									25.46
23-Aug-04	62	78.86	62.03	45.82	51.12									30.08
24-Aug-04	63	81.20	67.64	55.79	50.03									30.31
25-Aug-04	64	81.98	80.42	57.04	52.37									36.38
26-Aug-04	65	79.64	81.98	30.55	51.59									42.68
27-Aug-04	66	80.42	77.30	60.16	53.14									24.92
29-Aug-04	68	67.95	78.08	57.04	47.69									30.08
30-Aug-04	69	76.66	70.98	50.34	52.60									44.05
31-Aug-04	70	73.82	74.39	49.43	51.02									41.27
1-Sep-04	71	76.66	76.66	52.60	53.74									39.61
2-Sep-04	72	77.22	76.09	50.56	52.38									37.39
3-Sep-04	73	76.66	74.39	49.99	53.40									38.50
7-Sep-04	77	76.66	63.59	48.25	48.91									32.95
8-Sep-04	78	78.93	76.26	46.91	46.91									28.51
9-Sep-04	79	78.93	73.59	48.91	50.92									41.37
10-Sep-04	80	78.26	74.26	48.91	48.91									55.27
11-Sep-04	81	85.57	84.56	62.76	61.75									33.17
12-Sep-04	82	89.43	85.07	62.25	55.15									43.57
13-Sep-04	83	84.05	91.46	57.59	56.68									46.95
14-Sep-04	84	83.55	82.63	64.28	54.14									32.52
15-Sep-04	85	83.04	81.52	61.14	61.75									55.58
16-Sep-04	86	67.78	77.12	55.09	50.11									31.51
17-Sep-04	87	76.92	78.95	59.66	51.02									38.39
18-Sep-04	88	70.32	79.97	49.50	50.01									40.68
19-Sep-04	89	73.47	77.12	50.01	52.04									
average		**78.21**	**77.43**	**52.86**	**52.14**									**36.25**
stdev		**5.25**	**7.66**	**8.09**	**4.51**									**8.55**
t-test		**0.561**		**0.546**										
t-test aerobic vs. alternating		**0.000**												

Appendix 2.10 The frequency of floc diameter length; number and percentage
(samples from the RAS)

Floc diameter, µm	Number of flocs in the range				Percentage of flocs in the range (%)			
	R1	R3	R3	R4	R1	R3	R3	R4
0	0	0	0	0	0%	0%	0%	0%
10	15	8	15	11	3%	1%	2%	2%
20	164	269	110	184	28%	46%	18%	31%
30	74	134	72	186	13%	23%	12%	31%
40	50	58	87	62	9%	10%	14%	10%
50	71	39	84	29	12%	7%	14%	5%
60	44	21	52	15	7%	4%	8%	3%
70	46	22	57	31	8%	4%	9%	5%
80	26	15	46	22	4%	3%	8%	4%
90	24	10	31	18	4%	2%	5%	3%
100	17	4	14	2	3%	1%	2%	0%
110	12	4	13	15	2%	1%	2%	3%
120	14	1	7	5	2%	0%	1%	1%
130	10	2	8	7	2%	0%	1%	1%
140	4	0	2	2	1%	0%	0%	0%
150	4	0	1	4	1%	0%	0%	1%
160	2	0	4	2	0%	0%	1%	0%
170	1	0	1	1	0%	0%	0%	0%
180	2	0	2	0	0%	0%	0%	0%
190	3	0	0	0	1%	0%	0%	0%
200	1	0	0	0	0%	0%	0%	0%
210	1	0	2	0	0%	0%	0%	0%
220	0	0	2	0	0%	0%	0%	0%
230	0	0	1	0	0%	0%	0%	0%
240	0	0	0	0	0%	0%	0%	0%
250	1	0	0	0	0%	0%	0%	0%
260	1	0	1	0	0%	0%	0%	0%
Total	587	587	612	596	100%	100%	100%	100%

Appendix 2.11 The frequency of floc perimeter length; number and percentage
(samples from the RAS)

Floc perimeter, µm	Number of flocs in the range				Percentage of flocs in the range (%)			
	R1	R3	R3	R4	R1	R3	R3	R4
0	0	0	0	0	0.00%	0.00%	0.00%	0.00%
50	47	79	48	48	8.02%	13.41%	7.84%	8.07%
100	158	249	92	197	26.96%	42.28%	15.03%	33.11%
150	66	109	123	140	11.26%	18.51%	20.10%	23.53%
200	69	51	78	54	11.77%	8.66%	12.75%	9.08%
250	55	30	67	33	9.39%	5.09%	10.95%	5.55%
300	41	27	43	27	7.00%	4.58%	7.03%	4.54%
350	39	13	40	22	6.66%	2.21%	6.54%	3.70%
400	25	8	38	21	4.27%	1.36%	6.21%	3.53%
450	18	12	26	18	3.07%	2.04%	4.25%	3.03%
500	13	7	12	10	2.22%	1.19%	1.96%	1.68%
550	17	2	10	9	2.90%	0.34%	1.63%	1.51%
600	6	1	7	0	1.02%	0.17%	1.14%	0.00%
650	6	1	5	5	1.02%	0.17%	0.82%	0.84%
700	3	0	5	2	0.51%	0.00%	0.82%	0.34%
750	4	0	2	1	0.68%	0.00%	0.33%	0.17%
800	7	0	3	2	1.19%	0.00%	0.49%	0.34%
850	3	0	2	3	0.51%	0.00%	0.33%	0.50%
900	1	0	3	2	0.17%	0.00%	0.49%	0.34%
950	2	0	2	0	0.34%	0.00%	0.33%	0.00%
1000	3	0	0	0	0.51%	0.00%	0.00%	0.00%
1050	1	0	0	0	0.17%	0.00%	0.00%	0.00%
1100	1	0	0	0	0.17%	0.00%	0.00%	0.00%

Floc perimeter, µm	Number of flocs in the range				Percentage of flocs in the range (%)			
	R1	R3	R3	R4	R1	R3	R3	R4
1150	0	0	1	0	0.00%	0.00%	0.16%	0.00%
1200	0	0	1	1	0.00%	0.00%	0.16%	0.17%
1250	0	0	1	0	0.00%	0.00%	0.16%	0.00%
1300	0	0	1	0	0.00%	0.00%	0.16%	0.00%
1350	1	0	0	0	0.17%	0.00%	0.00%	0.00%
1400	0	0	0	0	0.00%	0.00%	0.00%	0.00%
1450	0	0	1	0	0.00%	0.00%	0.16%	0.00%
1500	0	0	1	0	0.00%	0.00%	0.16%	0.00%
Total	586	589	612	595	100.00%	100.00%	100.00%	100.00%

Appendix 2.12   The frequency of floc area, number and percentage (samples from the RAS)

area, µm²	Number of flocs in the range				Percentage of flocs in the range (%)			
	R1	R3	R3	R4	R1	R3	R3	R4
0	0	0	0	0	0%	0%	0.0%	0.0%
50	1	3	3	1	0%	1%	0.5%	0.2%
100	35	39	29	29	6%	7%	4.7%	4.9%
150	38	73	28	44	6%	12%	4.6%	7.4%
200	50	58	28	38	9%	10%	4.6%	6.4%
250	36	58	24	58	6%	10%	3.9%	9.7%
300	24	51	14	25	4%	9%	2.3%	4.2%
350	17	30	18	31	3%	5%	2.9%	5.2%
400	13	24	11	38	2%	4%	1.8%	6.4%
450	10	15	13	28	2%	3%	2.1%	4.7%
500	9	20	9	29	2%	3%	1.5%	4.9%
550	7	16	5	20	1%	3%	0.8%	3.4%
600	11	13	5	20	2%	2%	0.8%	3.4%
650	4	13	7	14	1%	2%	1.1%	2.3%
700	1	6	5	14	0%	1%	0.8%	2.3%
750	4	5	11	9	1%	1%	1.8%	1.5%
800	3	4	7	8	1%	1%	1.1%	1.3%
850	8	6	5	3	1%	1%	0.8%	0.5%
900	3	8	7	5	1%	1%	1.1%	0.8%
950	6	6	10	4	1%	1%	1.6%	0.7%
1000	2	1	11	7	0%	0%	1.8%	1.2%
1050	5	5	14	10	1%	1%	2.3%	1.7%
1100	5	4	5	4	1%	1%	0.8%	0.7%
1150	5	4	10	6	1%	1%	1.6%	1.0%
1200	5	2	6	3	1%	0%	1.0%	0.5%
1250	4	4	12	1	1%	1%	2.0%	0.2%
1300	6	4	6	1	1%	1%	1.0%	0.2%
1350	4	11	6	5	1%	2%	1.0%	0.8%
1400	2	0	8	4	0%	0%	1.3%	0.7%
1450	6	4	5	0	1%	1%	0.8%	0.0%
1500	4	6	8	3	1%	1%	1.3%	0.5%
1550	6	4	6	3	1%	1%	1.0%	0.5%
1600	10	1	3	1	2%	0%	0.5%	0.2%
1650	5	1	6	1	1%	0%	1.0%	0.2%
1700	6	2	3	1	1%	0%	0.5%	0.2%
1750	7	1	4	2	1%	0%	0.7%	0.3%
1800	2	3	9	1	0%	1%	1.5%	0.2%
1850	6	2	3	1	1%	0%	0.5%	0.2%
1900	1	3	6	1	0%	1%	1.0%	0.2%
1950	6	1	5	1	1%	0%	0.8%	0.2%
2000	9	0	3	0	2%	0%	0.5%	0.0%
2050	5	2	1	1	1%	0%	0.2%	0.2%
2100	4	2	4	1	1%	0%	0.7%	0.2%
2150	1	1	4	1	0%	0%	0.7%	0.2%
2200	2	2	3	1	0%	0%	0.5%	0.2%
2250	2	1	5	2	0%	0%	0.8%	0.3%
2300	3	3	5	0	1%	1%	0.8%	0.0%

area, µm²	Number of flocs in the range				Percentage of flocs in the range (%)			
	R1	R3	R3	R4	R1	R3	R3	R4
2350	3	0	7	1	1%	0%	1.1%	0.2%
2400	5	4	3	1	1%	1%	0.5%	0.2%
2450	2	1	3	2	0%	0%	0.5%	0.3%
2500	0	4	2	0	0%	1%	0.3%	0.0%
2550	3	0	4	0	1%	0%	0.7%	0.0%
2600	5	0	5	1	1%	0%	0.8%	0.2%
2650	2	0	1	2	0%	0%	0.2%	0.3%
2700	2	1	4	0	0%	0%	0.7%	0.0%
2750	4	1	3	2	1%	0%	0.5%	0.3%
2800	0	2	1	1	0%	0%	0.2%	0.2%
2850	3	2	2	0	1%	0%	0.3%	0.0%
2900	2	0	2	2	0%	0%	0.3%	0.3%
2950	1	2	3	2	0%	0%	0.5%	0.3%
3000	2	2	1	3	0%	0%	0.2%	0.5%
3050	5	1	4	2	1%	0%	0.7%	0.3%
3100	0	0	5	2	0%	0%	0.8%	0.3%
3150	0	0	5	6	0%	0%	0.8%	1.0%
3200	0	1	3	1	0%	0%	0.5%	0.2%
3250	5	0	4	1	1%	0%	0.7%	0.2%
3300	1	2	3	2	0%	0%	0.5%	0.3%
3350	3	1	6	0	1%	0%	1.0%	0.0%
3400	2	1	3	1	0%	0%	0.5%	0.2%
3450	2	0	3	2	0%	0%	0.5%	0.3%
3500	2	0	1	2	0%	0%	0.2%	0.3%
3550	2	1	3	1	0%	0%	0.5%	0.2%
3600	0	0	4	0	0%	0%	0.7%	0.0%
3650	0	0	2	0	0%	0%	0.3%	0.0%
3700	1	0	0	0	0%	0%	0.0%	0.0%
3750	1	0	0	1	0%	0%	0.0%	0.2%
3800	4	3	3	3	1%	1%	0.5%	0.5%
3850	1	1	3	1	0%	0%	0.5%	0.2%
3900	2	0	2	1	0%	0%	0.3%	0.2%
3950	3	2	6	0	1%	0%	1.0%	0.0%
4000	0	2	2	1	0%	0%	0.3%	0.2%
Total	471	556	503	524	80%	95%	82.2%	87.9%

Appendix 3.1   Ozone doses applied - summary

	Q ozone flow, mg O$_3$/min		V titrate reading, mL		y$_R$ residue of ozone, mg		t time of ozonation, min		y$_A$ ozone applied, mg		y$_C$ ozone consumed, mg		η efficiency, %	
	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4
					y$_R$= V*N*24				y$_A$=Q*t		y$_C$ = y$_A$-y$_R$		y$_C$/y$_A$	
9/20/2004	9.8	9.8	5.50	11.00	3.3	6.6	1.1	1.1	10.78	10.78	7.48	4.18	69.4%	38.8%
9/21/2004	9.8	9.8	10.80	14.00	6.5	8.4	1.1	1.1	10.78	10.78	4.30	2.38	39.9%	22.1%
9/22/2004	9.8	9.8	8.25	10.10	5.0	6.1	1.1	1.1	10.78	10.78	5.83	4.72	54.1%	43.8%
9/23/2004	9.8	9.8	9.50	10.10	5.7	6.1	1.1	1.1	10.78	10.78	5.08	4.72	47.1%	43.8%
9/24/2004	9.8	9.8	10.80	11.20	6.5	6.7	1.1	1.1	10.78	10.78	4.30	4.06	39.9%	37.7%
10/31/2004	10	10	17.20	17.10	10.3	10.3	1.5	1.5	15.00	15.00	4.68	4.74	31.2%	31.6%
11/1/2004	10	10	32.50	29.60	19.5	17.8	2.5	2.5	25.00	25.00	5.50	7.24	22.0%	29.0%
11/2/2004	10	10	37.80	39.50	22.7	23.7	2.5	2.5	25.00	25.00	2.32	1.30	9.3%	5.2%
11/3/2004	10	10	35.80	30.50	21.5	18.3	2.5	2.5	25.00	25.00	3.52	6.70	14.1%	26.8%
11/4/2004	10	10	40.20	35.00	24.1	21.0	2.5	2.5	25.00	25.00	0.88	4.00	3.5%	16.0%
11/5/2004	10	10	24.50	17.60	14.7	10.6	2.5	2.5	25.00	25.00	10.30	14.44	41.2%	57.8%
11/6/2004	10	10	23.50	18.20	14.1	10.9	2.5	2.5	25.00	25.00	10.90	14.08	43.6%	56.3%
11/7/2004	10	10	21.30	19.50	12.8	11.7	2.5	2.5	25.00	25.00	12.22	13.30	48.9%	53.2%
11/8/2004	10	10	34.50	22.80	20.7	13.7	2.5	2.5	25.00	25.00	4.30	11.32	17.2%	45.3%
11/9/2004	10.8	10.8	50.00	48.50	30.0	29.1	5.0	5.0	54.00	54.00	24.00	24.90	44.4%	46.1%
11/10/2004	11	11	46.00	37.00	27.6	22.2	4.0	4.0	44.00	44.00	16.40	21.80	37.3%	49.5%
11/11/2004	11	11	27.00	34.00	16.2	20.4	4.0	4.0	44.00	44.00	27.80	23.60	63.2%	53.6%
11/12/2004	11	11	60.00	59.60	36.0	35.8	5.0	5.0	55.00	55.00	19.00	19.24	34.5%	35.0%
11/13/2004	11	11	95.00	95.00	57.0	57.0	8.0	8.0	88.00	88.00	31.00	31.00	35.2%	35.2%
11/14/2004	10.6	10.6	94.40	95.20	56.6	57.1	8.0	8.0	84.80	84.80	28.16	27.68	33.2%	32.6%
11/15/2004	10.6	10.6	93.00	93.50	55.8	56.1	8.0	8.0	84.80	84.80	29.00	28.70	34.2%	33.8%
11/19/2004	10.6	10.6	100.50	96.50	60.3	57.9	8.0	8.0	84.80	84.80	24.50	26.90	28.9%	31.7%
11/20/2004	10.6	10.6	102.60	97.20	61.6	58.3	8.0	8.0	84.80	84.80	23.24	26.48	27.4%	31.2%
11/21/2004	10.6	10.6	107.60	106.00	64.6	63.6	8.0	8.0	84.80	84.80	20.24	21.20	23.9%	25.0%
11/22/2004	10.7	10.7	109.00	111.00	65.4	66.6	8.0	8.0	85.60	85.60	20.20	19.00	23.6%	22.2%
11/24/2004	10.7	10.7	111.80	108.80	67.1	65.3	8.0	8.0	85.60	85.60	18.52	20.32	21.6%	23.7%
11/25/2004	10.7	10.7	114.20		68.5		8.0	8.0	85.60	85.60	17.08		20.0%	

	Q ozone flow, mg O_3/min		V titrate reading, mL		y_R residue of ozone, mg		t time of ozonation, min		y_A ozone applied, mg		y_C ozone consumed, mg		η efficiency, %	
	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4
					$y_R = V*N*24$				$y_A = Q*t$		$y_C = y_A - y_R$		y_C/y_A	
11/26/2004	10.7	10.7	107.30	107.00	64.4	64.2	8.0	8.0	85.60	85.60	21.22	21.40	24.8%	25.0%
11/27/2004	10.7	10.7	103.10	103.00	61.9	61.8	8.0	8.0	85.60	85.60	23.74	23.80	27.7%	27.8%
11/28/2004	10.7	10.7	27.30	26.00	65.5	62.4	8.0	8.0	85.60	85.60	20.08	23.20	23.5%	27.1%
11/29/2004	10.38	10.38	25.80	25.60	61.9	61.4	8.0	8.0	83.04	83.04	21.12	21.60	25.4%	26.0%
11/30/2004	11.23	11.23	24.50	27.60	58.8	66.2	8.0	6.5	89.84	73.00	31.04	6.76	34.6%	9.3%
12/1/2004	11.16	11.16	26.00	27.00	62.4	64.8	8.0	8.0	89.28	89.28	26.88	24.48	30.1%	27.4%
12/2/2004	11.16	11.16	28.50	28.40	68.4	68.2	8.0	8.0	89.28	89.28	20.88	21.12	23.4%	23.7%
12/3/2004	11.24	11.24	27.90	26.70	67.0	64.1	8.0	8.0	89.92	89.92	22.96	25.84	25.5%	28.7%
12/6/2004	11.16	11.16	26.30	27.50	63.1	66.0	8.0	8.0	89.28	89.28	26.16	23.28	29.3%	26.1%
12/7/2004	11.16	11.16	28.05	27.25	67.3	65.4	8.0	8.0	89.28	89.28	21.96	23.88	24.6%	26.7%
12/8/2004	11.16	11.16	29.00	29.10	69.6	69.8	8.0	8.0	89.28	89.28	19.68	19.44	22.0%	21.8%
12/9/2004	11.16	11.16	29.80	28.60	71.5	68.6	8.0	8.0	89.28	89.28	17.76	20.64	19.9%	23.1%
12/10/2004	11.16	11.16	27.90	27.30	67.0	65.5	8.0	8.0	89.28	89.28	22.32	23.76	25.0%	26.6%
12/11/2004	11.16	11.16	31.40	28.80	75.4	69.1	8.0	8.0	89.28	89.28	13.92	20.16	15.6%	22.6%
12/12/2004	11.16	11.16	30.06	30.50	72.1	73.2	8.0	8.0	89.28	89.28	17.14	16.08	19.2%	18.0%
12/13/2004	11.16	11.16	30.00	31.50	72.0	75.6	8.0	8.0	89.28	89.28	17.28	13.68	19.4%	15.3%
12/14/2004	11.16	11.16	30.20	29.90	72.5	71.8	8.0	8.0	89.28	89.28	16.80	17.52	18.8%	19.6%
12/15/2004	11.16	11.16	29.80	31.70	71.5	76.1	8.0	8.0	89.28	89.28	17.76	13.20	19.9%	14.8%
1/16/2005	10.50	10.27	39.00	38.50	93.6	92.4	11.0	11.0	115.50	112.97	21.90	20.57	19.0%	18.2%
1/17/2005	10.50	10.50	42.00	41.50	100.8	99.6	11.0	11.0	115.50	115.50	14.70	15.90	12.7%	13.8%
1/18/2005	11.00	10.50	39.10	36.50	93.8	87.6	10.0	9.9	110.00	104.13	16.16	16.53	14.7%	15.9%
1/19/2005	10.60	10.27	38.50	35.50	92.4	85.2	10.0	9.9	106.00	101.84	13.60	16.64	12.8%	16.3%
1/20/2005	10.50	10.40	36.80	35.50	88.3	85.2	10.0	9.9	105.00	103.13	16.68	17.93	15.9%	17.4%
1/21/2005	10.50	10.50	45.70	38.20	109.7	91.7	12.0	10.5	126.00	110.25	16.32	18.57	13.0%	16.8%
1/23/2005	10.50	10.50	51.80	36.50	124.3	87.6	13.5	10.0	141.75	105.00	17.43	17.40	12.3%	16.6%
1/24/2005	10.50	10.50	51.80	37.50	124.3	90.0	13.5	10.5	141.75	110.25	17.43	20.25	12.3%	18.4%
1/25/2005	10.50	10.50	31.30	29.00	75.1	69.6	8.0	8.0	84.00	84.00	8.88	14.40	10.6%	17.1%
1/26/2005	10.32	10.32	29.50	27.80	70.8	66.7	8.0	8.0	82.56	82.56	11.76	15.84	14.2%	19.2%
1/27/2005	10.32	10.32	36.80	32.80	88.3	78.7	10.0	9.0	103.20	92.88	14.88	14.16	14.4%	15.2%
1/28/2005	10.32	10.32	44.50	33.50	106.8	80.4	12.0	9.0	123.84	92.88	17.04	12.48	13.8%	13.4%

	Q ozone flow, mg O_3/min		V titrate reading, mL		y_R residue of ozone, mg		t time of ozonation, min		y_A ozone applied, mg		y_C ozone consumed, mg		η efficiency, %	
	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4
					$y_R = V*N*24$				$y_A = Q*t$		$y_C = y_A - y_R$		y_C/y_A	
1/29/2005	10.32	10.32	36.20	35.50	86.9	85.2	10.0	10.0	103.20	103.20	16.32	18.00	15.8%	17.4%
2/3/2005	9.20	9.20	48.00	51.00	115.2	122.4	15.0	15.0	138.00	138.00	22.80	15.60	16.5%	11.3%
2/4/2005	10.27	10.27	58.80	56.50	141.1	135.6	16.0	15.0	164.32	154.05	23.20	18.45	14.1%	12.0%
2/6/2005	10.27	10.27	44.10	45.50	105.8	109.2	12.0	12.0	123.24	123.24	17.40	14.04	14.1%	11.4%
2/7/2005		10.27		34.2		82.1		10.0		102.70		20.62		20.1%
2/10/2005	10.27	10.27	40.20	39.10	96.5	93.8	10.8	11.0	110.92	112.97	14.44	19.13	13.0%	16.9%
2/11/2005	10.27	10.27	42.50	41.60	102.0	99.8	12.0	12.0	123.24	123.24	21.24	23.40	17.2%	19.0%
2/13/2005	10.27	10.27	47.50	41.20	114.0	98.9	13.0	12.0	133.51	123.24	19.51	24.36	14.6%	19.8%
2/16/2005	10.27	10.27	40.20	42.00	96.5	100.8	12.0	12.0	123.24	123.24	26.76	22.44	21.7%	18.2%
2/17/2005	10.27	10.27	53.90	55.20	129.4	132.5	15.0	16.0	154.05	164.32	24.69	31.84	16.0%	19.4%
2/18/2005	10.27	10.27	53.80	52.50	129.1	126.0	15.0	15.0	154.05	154.05	24.93	28.05	16.2%	18.2%
2/20/2005	10.27	10.27	55.30	53.70	132.7	128.9	15.0	15.0	154.05	154.05	21.33	25.17	13.8%	16.3%
2/21/2005	10.27	10.27	57.60	54.60	138.2	131.0	16.0	16.0	164.32	164.32	26.08	33.28	15.9%	20.3%
2/22/2005	10.32	10.32	58.10	53.50	139.4	128.4	15.0	15.0	154.80	154.80	15.36	26.40	9.9%	17.1%
2/23/2005	10.32	10.32	75.30	34.00	180.7	81.6	19.0	9.0	196.08	92.88	15.36	11.28	7.8%	12.1%
2/25/2005	10.27	10.27	58.70	35.40	140.9	85.0	15.0	9.0	154.05	92.43	13.17	7.47	8.5%	8.1%
2/27/2005	10.32	10.32	54.80	46.50	131.5	111.6	15.0	13.0	154.80	134.16	23.28	22.56	15.0%	16.8%
3/3/2005	10.32	10.32	59.00	36.30	141.6	87.1	16.5	10.3	170.28	105.78	28.68	18.66	16.8%	17.6%
3/4/2005	10.32	10.32	59.00	36.50	141.6	87.6	16.0	10.3	165.12	105.78	23.52	18.18	14.2%	17.2%
3/8/2005	10.32	10.32	53.05	35.00	127.3	84.0	15.0	10.3	154.80	105.78	27.48	21.78	17.8%	20.6%
3/9/2005	10.32	10.32	56.50	37.60	135.6	90.2	16.0	10.3	165.12	105.78	29.52	15.54	17.9%	14.7%
3/14/2005	10.32	10.32	54.50	34.10	130.8	81.8	15.0	10.3	154.80	105.78	24.00	23.94	15.5%	22.6%
3/16/2005	10.27	10.27	53.20	36.00	127.7	86.4	15.0	10.3	154.05	105.29	26.37	18.89	17.1%	17.9%
4/11/2005	10.60	10.03	57.10	39.20	137.0	94.1	15.0	10.3	159.00	102.83	21.96	8.75	13.8%	8.5%
4/12/2005	10.60	10.60	55.00	26.90	132.0	64.6	16.0	10.3	169.60	108.65	37.60	44.09	22.2%	40.6%
4/15/2005		10.60		37.5		90.0		10.5		111.30		21.30		19.1%
4/18/2005		10.60		38.2		91.7		11.0		116.60		24.92		21.4%
5/7/2005	10.27	10.27	33.00	57.20	79.2	137.3	11.0	18.0	112.99	184.90	33.79	47.62	29.9%	25.8%
5/8/2005	10.27	10.27	24.50	37.50	58.8	90.0	10.0	11.0	102.72	112.99	43.92	22.99	42.8%	20.3%
5/9/2005	10.27	10.27	29.20	36.50	70.1	87.6	10.0	10.0	102.72	102.72	32.64	15.12	31.8%	14.7%

	Q ozone flow, mg O_3/min		V titrate reading, mL		y_R residue of ozone, mg		t time of ozonation, min		y_A ozone applied, mg		y_C ozone consumed, mg		η efficiency, %	
	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4
					$y_R = V*N*24$				$y_A = Q*t$		$y_C = y_A - y_R$		y_C/y_A	
5/10/2005	10.27	10.27	39.00	39.10	93.6	93.8	10.0	10.0	102.72	102.72	9.12	8.88	8.9%	8.6%
5/11/2005	10.27	10.27	39.00	40.20	93.6	96.5	10.0	10.0	102.72	102.72	9.12	6.24	8.9%	6.1%
5/12/2005	10.80	10.80	40.20	78.60	96.5	188.6	10.0	20.0	108.00	216.00	11.52	27.36	10.7%	12.7%
5/13/2005	10.80	10.80	40.20	48.00	96.5	115.2	10.0	12.0	108.00	129.60	11.52	14.40	10.7%	11.1%
5/16/2005		10.80		38.90		93.4		12.0		129.60		36.24		28.0%
5/17/2005		10.80		52.50		126.0		15.0		162.00		36.00		22.2%
5/18/2005		10.80		42.50		102.0		11.5		124.20		22.20		17.9%
5/19/2005		10.80		48.50		116.4		12.5		135.00		18.60		13.8%
5/20/2005		10.80		39.50		94.8		12.0		129.60		34.80		26.9%
5/24/2005	13.60	13.60	44.50	57.20	106.8	137.3	10.0	13.0	136.00	176.80	29.20	39.52	21.5%	22.4%
5/25/2005	12.00	12.00	40.60	43.90	97.4	105.4	10.0	11.0	120.00	132.00	22.56	26.64	18.8%	20.2%
5/26/2005	13.60	13.60	45.00	48.60	108.0	116.6	10.0	10.5	136.00	142.80	28.00	26.16	20.6%	18.3%
5/27/2005	13.60	13.60	50.20	51.00	120.5	122.4	11.0	11.0	149.60	149.60	29.12	27.20	19.5%	18.2%
5/30/2005	10.80	11.30	40.60	47.00	97.4	112.8	11.1	11.5	119.70	129.95	22.26	17.15	18.6%	13.2%
5/31/2005	10.80	11.30	58.10	56.20	139.4	134.9	15.0	15.0	162.00	169.50	22.56	34.62	13.9%	20.4%
6/1/2005	10.80	11.30	61.10	57.90	146.6	139.0	15.0	15.0	162.00	169.50	15.36	30.54	9.5%	18.0%
6/15/2005	7.84	7.84	31.90	35.60	76.6	85.4	10.0	11.0	78.40	86.24	1.84	0.80	2.3%	0.9%
6/16/2005	7.84	7.84	29.60	26.20	71.0	62.9	10.0	10.0	78.40	78.40	7.36	15.52	9.4%	19.8%
6/17/2005	11.96	11.96	46.10	43.10	110.6	103.4	11.5	10.5	137.54	125.58	26.90	22.14	19.6%	17.6%
6/20/2005	10.80	10.80	37.00	36.20	88.8	86.9	10.0	10.0	108.00	108.00	19.20	21.12	17.8%	19.6%
6/21/2005	10.80	10.80	37.60	36.70	90.2	88.1	11.0	10.0	118.80	108.00	28.56	19.92	24.0%	18.4%
6/22/2005	10.80	10.80	39.50	38.50	94.8	92.4	10.3	10.5	110.70	113.40	15.90	21.00	14.4%	18.5%
6/23/2005	10.80	10.80	34.30	50.20	82.3	120.5	8.8	13.0	94.50	140.40	12.18	19.92	12.9%	14.2%
6/28/2005	10.80	10.80	22.50	35.90	54.0	86.2	6.3	10.0	68.04	108.00	14.04	21.84	20.6%	20.2%
6/29/2005	10.80	10.80	37.00	34.50	88.8	82.8	10.0	10.0	108.00	108.00	19.20	25.20	17.8%	23.3%
6/30/2005	10.90	10.80	54.00	75.75	129.6	181.8	14.8	20.0	160.78	216.00	31.18	34.20	19.4%	15.8%
7/4/2005	10.80	10.80	39.00	59.90	93.6	143.8	11.0	16.0	118.80	172.80	25.20	29.04	21.2%	16.8%
7/7/2005	9.00	8.50	45.80	39.10	109.9	93.8	14.0	14.0	126.00	119.00	16.08	25.16	12.8%	21.1%
7/10/2005	9.00		74.55		178.9		25.2		226.50		47.58		21.0%	
7/12/2005	9.00	7.12	30.50	41.00	73.2	98.4	10.0	15.5	90.00	110.36	16.80	11.96	18.7%	10.8%

	Q ozone flow, mg O_3/min		V titrate reading, mL		y_R residue of ozone, mg		t time of ozonation, min		y_A ozone applied, mg		y_C ozone consumed, mg		η efficiency, %	
	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4	R2	R4
					$y_R = V*N*24$				$y_A = Q*t$		$y_C = y_A - y_R$		y_C/y_A	
7/13/2005	10.00	10.00	44.00	39.20	105.6	94.1	12.0	12.0	120.00	120.00	14.40	25.92	12.0%	21.6%
7/14/2005	10.90	10.90	47.60	42.95	114.2	103.1	12.0	12.0	130.80	130.80	16.56	27.72	12.7%	21.2%
7/15/2005	10.90	10.90	63.90	63.20	153.4	151.7	16.0	16.0	174.40	174.40	21.04	22.72	12.1%	13.0%
7/17/2005	10.90		78.30		187.9		21.0		228.90		40.98		17.9%	
7/18/2005	11.30	11.30	80.50	83.30	193.2	199.9	19.8	20.1	223.18	226.94	29.98	27.02	13.4%	11.9%
7/19/2005	11.30	11.30	43.50	51.70	104.4	124.1	11.0	13.0	124.30	146.90	19.90	22.82	16.0%	15.5%
7/20/2005	11.30	11.30	48.30	57.20	115.9	137.3	12.0	14.5	135.60	163.85	19.68	26.57	14.5%	16.2%
7/21/2005	11.30	11.30	49.50	47.80	118.8	114.7	12.0	12.0	135.60	135.60	16.80	20.88	12.4%	15.4%
7/22/2005	11.20	11.20	43.70	43.50	104.9	104.4	11.0	11.0	123.20	123.20	18.32	18.80	14.9%	15.3%
7/26/2005	10.80	10.80	62.40	58.10	149.8	139.4	16.5	16.0	178.20	172.80	28.44	33.36	16.0%	19.3%
7/27/2005	10.90	10.90	64.40	47.50	154.6	114.0	17.0	12.0	185.30	130.80	30.74	16.80	16.6%	12.8%
7/28/2005	11.20	11.20	40.80	52.50	97.9	126.0	10.0	14.0	112.00	156.80	14.08	30.80	12.6%	19.6%
7/29/2005	11.20	11.20	55.80	65.6	133.9	157.4	14.0	17.0	156.80	190.40	22.88	32.96	14.6%	17.3%
7/30/2005	10.90	10.90	72.60	77.1	174.2	185.0	20.0	20.0	218.00	218.00	43.76	32.96	20.1%	15.1%
8/2/2005	10.90	10.90	61.50	60.3	147.6	144.7	16.0	16.0	174.40	174.40	26.80	29.68	15.4%	17.0%

average	20.9%	21.7%
stdev	11.2%	10.3%
t-test	0.221	

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Appendix 3.2 Total COD in effluent after ozone treatment Soluble (filtered 0.45) COD in effluent

	Aerobic		Alternating		Feed			Aerobic		Alternating	
day	R1	R2	R3	R4			day	R1	R2	R3	R4
11/6/2004	19	23	25	28	601		5/3/2005	49	23	31	30
11/7/2004	22	12	26	21	656		5/4/2005	22	19	30	14
11/8/2004	32	28	39	38	676		5/6/2005	32	28	19	19
11/9/2004	41	39	34	61	711		5/10/2005	43	52	42	29
11/10/2004	42	40	43	39	702		5/11/2005	46	49	49	-
11/11/2004	38	37	39	39	551		5/12/2005	24	12	23	36
11/25/2004	44	33	37	37	704		5/13/2005	18	17	30	41
1/16/2005	30	35	34	33	659		5/16/2005	41	39	34	36
1/17/2005	26	39	28	34	712		5/17/2005	27	21	43	44
1/18/2005	38	46	40	42	702		5/18/2005	-	40	37	42
1/19/2005	29	38	31	33	648		5/19/2005	21	28	35	28
1/20/2005	37	17	23	19	730		5/20/2005	28	31	28	34
1/21/2005	42	43	38	51	719		5/24/2005	33	36	32	32
1/22/2005	41	41	36	42	698		5/25/2005	38	37	39	38
1/24/2005	31	35	42	39	614		5/26/2005	47	29	34	32
1/25/2005	32	23	36	34	634		5/27/2005	27	26	35	33
1/26/2005	32	23	36	34	708		5/30/2005	30	35	34	33
1/27/2005	54	22	31	28	610		5/31/2005	26	39	28	34
2/4/2005	39	52	40	28			6/1/2005	38	46	40	42
2/5/2005	38	47	42	45			6/2/2005	29	38	31	33
5/3/2005	67	46	46	37			6/3/2005	37	17	23	19
5/4/2005	42	25	42	19			6/13/2005	42	43	38	51
5/6/2005	42	32	21	20			6/14/2005	41	41	36	42
5/10/2005	58	62	62	45			6/16/2005	31	35	42	39
5/11/2005	73	74	74	-			6/17/2005	32	23	36	34
5/12/2005	35	18	25	59			6/20/2005	32	23	36	34
5/13/2005	26	22	38	70			6/21/2005	54	22	31	28
5/16/2005	62	60	82	61			6/22/2005	39	52	40	28
5/17/2005	35	28	49	51			6/28/2005	38	47	42	45
5/18/2005	69	68	48	56							
5/19/2005	35	36	42	34							
5/20/2005	40	47	31	46							
5/24/2005	43	64	40	41			average	34	33	34	34
5/25/2005	62	64	57	65			stdev	9	11	7	8
5/26/2005	56	43	45	51				26%	34%	19%	24%
5/27/2005	48	45	40	40			t-test	0.347		0.966	
5/30/2005	30	36	39	28							
5/31/2005	37	54	54	35							
6/1/2005	73	79	48	48							
6/2/2005	49	41	52	57							
6/3/2005	31	60	49	43							
6/13/2005	33	53	19	34							
6/14/2005	35	37	47	22							
6/15/2005	39	19	25	31							
6/16/2005	46	43	23	37							
6/17/2005	30	32	20	35							
6/20/2005	21	21	30	27							
6/21/2005	25	19	12	22							
6/22/2005	13	61	23	9							
6/28/2005	62	73	68	69							
6/29/2005	46	66	43	45							
6/30/2005	39	47	49	26							
7/4/2005	65	74	49	56							
7/7/2005	41	69	76	51							
7/12/2005	19	28	21	21							
7/13/2005	17	16	18								
7/15/2005	50	37	37	26							
7/19/2005	34	32	34	30							
7/22/2005	63	80	90	68							
7/26/2005	37	67	56	55							
average	41	42	40	40	669						
stdev	14	18	16	14	50						
	35%	42%	39%	36%	7%						
t-test	0.295		0.670								

Appendix 3.3   Total and volatile suspended solids in the final effluent after ozone treatment

TSS						VSS			
day	R1	R2	R3	R4		R1	R2	R3	R4
1/24/2005	13	13	27	8		8	8	19	8
2/8/2006	8	14	16	8		8	13	15	7
5/16/2005	22	19	18	16		21	17	16	14
5/27/2005	18	21	15	17		18	21	17	23
6/1/2005	14	14	15	9		9	13	15	8
6/2/2005	12	13	26	8		7	8	19	6
6/10/2005	24	28	-	28		10	13	-	20
7/16/2005	7	14	9	9		7	14	9	9
7/17/2005	19	10	12	14		19	10	12	14
7/20/2005	27	10	21	24		24	10	20	24
7/26/2007	7	17	23	16		5	17	21	16
7/27/2005	19	27	18	22		19	20	18	22

av	15.9	16.5	18.2	14.8	av	13.0	13.6	16.5	13.2
stdev	6.8	5.9	5.6	7.0	stdev	6.7	4.3	3.6	6.9

av	16.3			VSS/TSS	0.82	0.82	0.91	0.89
stdev	6.3							

Calculations of the real SRT (d) in the reactors

	av	stdev	min	max
Xw	450		450	450
Xe	32.66	12.55	45.21	20.10
Xw+Xe	482.66		495.21	470.10
1/SRT=Xw*3/X	0.09		0.09	0.09
SRT	**11.19**		**10.90**	**11.49**
			0.28	0.30

Xw TSS in WAS, mg
Xe TSS in effl., mg
Xw+Xe TSS total wasted from the system, mg

SRT calculated 11.23±0.36 d

Appendix 3.4   pH at the end of the cycle after ozone treatment, 10:15 am   Appendix 3.5   pH in the ozonated portion of RAS before and after ozonation

# reactor	#1	#2	#3	#4	Feed
11/1/2004	7.73	7.75	8.10	8.14	
11/2/2004	7.72	7.67	8.18	7.67	
11/3/2004	7.78	7.69	8.20	8.04	
11/4/2004	7.71	7.63	8.11	8.05	
11/5/2004	7.79	7.69	8.13	7.54	
11/6/2004	7.76	7.67	8.06	7.58	
11/7/2004	7.62	7.54	7.50	7.73	
11/8/2004	7.63	7.55	7.86	7.83	
11/9/2004	7.59	7.43	7.82	7.91	
11/10/2004	7.75	7.64	7.78	8.16	
11/11/2004	7.81	7.66	8.43	8.45	
11/12/2004	7.83	7.74	8.34	8.43	
11/13/2004	7.84	7.64	8.28	8.36	
11/14/2004	7.87	7.65	8.28	8.27	
11/15/2004	7.73	7.53	8.03	8.09	
11/16/2004	7.71	7.58	7.92	8.11	
11/17/2004	7.52	8	7.88	8.07	
11/18/2004	7.59	7.92	7.82	8.08	
11/19/2004	7.56	7.86	7.84	8.07	
11/20/2004	7.71	7.77	7.81	8.07	
11/21/2004	7.66	7.72	8.06	7.83	
11/22/2004	7.72	7.74	8.02	7.62	
11/23/2004	7.44	7.75	7.88	7.91	
11/24/2004	7.42	7.68	8.00	8.02	
1/16/2005	7.89	7.83	8.16	8.14	
1/17/2005	7.95	7.84	8.24	8.23	
1/18/2005	7.97	7.86	8.26	8.23	
1/19/2005	7.94	7.82	8.21	8.17	
1/20/2005	7.89	7.79	8.16	8.12	
1/21/2005	7.85	7.74	8.09	8.06	
1/22/2005	7.82	7.74	8.13	8.07	
1/24/2005	7.85	7.78	8.17	8.11	
1/25/2005	8.02	7.91	8.30	8.20	
1/26/2005	7.94	7.83	8.17	8.05	
1/27/2005	7.88	7.83	8.12	8.00	
1/28/2005	7.94	7.88	8.13	8.02	
1/29/2005	7.85	7.77	8.01	7.90	
2/4/2005	8.00	7.87	8.13	8.22	
2/5/2005	7.92	7.74	8.02	7.91	
2/7/2005	7.94	7.93	8.35	8.43	
2/11/2005	7.95	8.05	8.08	8.29	
2/16/2005	7.96	7.99	7.89	8.13	
2/17/2005	7.83	7.91	8.04	7.95	
2/18/2005	7.93	7.96	8.02	7.69	
2/20/2005	7.87	8.05	8.32	8.22	
2/21/2005	7.91	7.95	8.15	8.45	
2/22/2005	7.8	7.79	8.07	8.09	
2/23/2005	7.75	7.76	7.91	8.18	
2/25/2005	7.94	7.91	7.99	8.26	
2/26/2005	7.58	7.65	7.95	7.91	
2/27/2005	7.79	7.84	8.03	8.07	
3/4/2005	7.75	7.78	8.05	8.15	
3/15/2005	7.93	7.82	8.08	7.87	
3/31/2005	7.93	7.76	8.11	7.89	
5/4/2005	7.82	7.83	8.15	8.19	
5/6/2005	7.87	7.82	8.17	8.08	
5/9/2005	7.78	7.72	8.13	8.05	
5/10/2005	7.87	7.72	8.15	8.05	
5/11/2005	7.73	7.73	7.98	7.92	
5/12/2005	7.53	7.65	7.97	7.7	
5/13/2005	8.03	7.77	8.13	8.15	
5/14/2005	7.76	7.58	7.86	7.99	
5/16/2005	7.65	7.55	7.82	7.66	
5/17/2005	7.56	7.52	7.56	7.5	8.02
5/18/2005	7.57	7.52	7.56	7.22	
5/19/2005	7.64	7.52	7.43	7.59	7.91
5/20/2005	7.66	7.6	7.86	7.66	7.91
5/21/2005	7.58	7.56	7.87	7.69	7.98
5/22/2005	7.54	7.56	7.77	7.61	
5/23/2005	7.63	7.6	7.85	7.75	
5/24/2005	7.6	7.56	7.83	7.69	8.06
5/25/2005	7.58	7.58	7.82	7.75	7.89
5/26/2005	7.23	7.48	7.78	7.59	7.97
5/27/2005	7.74	7.56	7.82	7.71	7.97
5/28/2005	7.61	7.59	7.62	7.43	7.95
5/29/2005	7.71	7.72	7.66	7.75	7.88
5/30/2005	7.82	7.78	7.22	7.72	7.87
5/31/2005	7.82	7.75	7.25	7.66	7.93
6/1/2005	7.88	7.77	7.7	7.66	8.03
6/2/2005	7.68	7.51	7.56	7.64	7.94
6/3/2005	7.5	7.42	7.62	7.61	7.92

# reactor	R1	R2	R3	R4	Feed
6/4/2005	7.52	7.4	8.03	8.07	8.02
6/5/2005	7.55	7.6	8.08	8.15	8.10
6/6/2005	7.61	7.46	8.01	8.03	7.96
6/7/2005	7.65	7.63	8.16	8.12	8.08
6/8/2005	7.74	7.56	8.15	8.13	8.16
6/9/2005	7.72	7.77	8.21	8.17	8.17
6/10/2005	7.71	7.75	8.26	8.31	8.00
6/11/2005	7.68	7.71	8.06	8.09	8.16
6/12/2005	7.59	7.57	7.96	7.98	8.26
6/13/2005	7.63	7.66	8.24	8.23	8.16
6/14/2005	7.61	7.7	8.25	8.27	8.12
6/15/2005	7.55	7.8	8.31	8.25	8.23
6/16/2005	7.3	7.44	8.06	7.99	7.99
6/17/2005	7.19	7.27	7.9	7.82	7.95
6/18/2005	7.1	7.46	8.07	8.01	
6/19/2005	7.36	7.35	7.67	7.68	
6/20/2005	7.46	7.62	8	8.02	8.33
6/21/2005	7.53	7.63	8.08	8.08	8.42
6/22/2005	7.44	7.6	7.99	8.04	8.42
6/23/2005	7.45	7.63	8.08	8.08	8.38
6/24/2005	7.9	8.01	8.19	8.32	8.29
6/27/2005	7.57	7.77	8.01	8.08	8.47
6/28/2005	7.62	7.82	8.09	8.13	8.41
6/29/2005	7.55	7.78	8.11	8.12	7.58
6/30/2005	7.29	7.39	7.93	7.99	7.60
7/1/2005	7.13	7.19	7.87	7.92	7.62
7/4/2005	7.00	7.03	7.73	7.77	7.63
7/5/2005	7.01	7	7.75	7.79	
7/6/2005	7.02	7.03	7.78	7.79	
7/7/2005	7.01	7.08	7.75	7.83	7.64
7/12/2005	7.15	7.14	7.75	7.68	7.94
7/13/2005	7.03	6.99	7.73	7.69	7.90
7/14/2005	7.07	7.02	7.92	7.84	7.99
7/15/2005	6.91	6.87	7.78	7.72	8
7/16/2005	7.64	7.66	8.02	7.93	7.97
7/17/2005	7.82	7.9	8.06	7.92	
7/18/2005	7.76	7.84	8.01	7.95	
7/19/2005	7.82	7.88	8.08	7.92	8.42
7/20/2005	7.66	7.74	8.01	7.57	8.34
7/21/2005	7.63	7.7	7.92	7.81	8.44
7/26/2005	7.71	7.75	7.42	7.57	8.46
7/27/2005	7.8	7.74	8.34	8.17	8.54
7/28/2005	7.78	7.8	8.31	7.96	
7/29/2005	7.81	7.85	8.22	7.94	
8/2/2005	7.79	7.79	7.88	8.02	
8/3/2005	7.84	7.82	7.96	8.01	
average	**7.66**	**7.66**	**7.98**	**7.96**	**8.07**
stdev	**0.25**	**0.23**	**0.23**	**0.24**	**0.24**
t-test	**0.939**		**0.127**		

	#R2 before	#R2 after		#R4 before	#R4 after	
1/16/2005	7.61	7.26	4.6%	7.98	7.6	4.8%
1/17/2005	7.53	7.21	4.2%	7.87	7.59	3.6%
1/18/2005	7.49	7.19	4.0%	7.82	7.54	3.6%
1/19/2005	7.64	7.18	6.0%	7.99	7.53	5.8%
1/20/2005	7.64	7.3	4.5%	8.00	7.57	5.4%
1/21/2005	7.56	7.1	6.1%	7.96	7.55	5.2%
1/22/2005	7.72	7.16	7.3%	8.07	7.57	6.2%
1/24/2005	7.59	7.23	4.7%	7.91	7.56	4.4%
1/25/2005	7.51	7.26	3.3%	7.83	7.7	1.7%
1/26/2005	7.51	7.41	1.3%	7.72	7.64	1.0%
1/27/2005	7.64	7.27	4.8%	7.84	7.68	2.0%
1/28/2005	7.59	7.24	4.6%	7.85	7.65	2.5%
1/29/2005	7.65	7.35	3.9%	8.00	7.91	1.1%
2/3/2005	7.45	7.35	1.3%	7.75	7.72	0.4%
2/4/2005	7.6	7.4	2.6%	7.92	7.62	3.8%
2/6/2005	7.52	7.32	2.7%	7.65	7.6	0.7%
2/10/2005	7.56	7.35	2.8%	7.98	7.75	2.9%
2/11/2005	7.75	7.49	3.4%	8	7.66	4.3%
2/13/2005	7.67	7.38	3.8%	7.83	7.63	2.6%
2/16/2005	7.54	7.27	3.6%	7.78	7.73	0.6%
2/17/2005	7.6	7.26	4.5%	7.77	7.66	1.4%
2/18/2005	7.66	7.28	5.0%	8.05	7.74	3.9%
2/20/2005	7.64	7.2	5.8%	7.81	7.58	2.9%
2/21/2005	7.64	7.2	5.8%	8.03	7.66	4.6%
2/22/2005	7.54	7.31	3.1%	7.82	7.43	5.0%
2/23/2005	7.56	7.27	3.8%	7.79	7.72	0.9%
average	**7.59**	**7.28**		**7.89**	**7.64**	
stdev	**0.07**	**0.09**		**0.11**	**0.09**	
t-test	**0.000**			**0.000**		

Appendix 3.7   SOUR mg O2/g VSS h after ozone treatment, samples from the reactors

	time	R1	R2	R3	R4
3/30/2005	12:15 PM	45.3	42.8	36.5	29.8
2/15/2005	12:30 PM	33.2	38.0	30.8	27.5
2/14/2005	3:35 PM	19.5	19.1	22.9	18.1
3/30/2005	4:00 PM	25.4	22.4	22.0	25.6
3/31/2005	9:00 AM	8.2	4.2	5.7	5.9

Appendix 3.6   Alkalinity in the effluent after ozone treatment and the feed, mg CaCO3/L

	R1	R2	R3	R4	feed
11/3/2004	164	184	296	304	421
11/8/2004	129	182	291	586	
11/15/2004	80	140	209	220	
2/15/2005	122	154	237	264	472
4/4/2005	176	178	314	318	516
4/12/2005	175	144	314	305	495
4/18/2005	162	151	393	274	520
4/26/2005	145	147	284	265	523
5/2/2005	151	156	310	282	530
5/9/2005	151	156	310	282	560
5/4/2005	152	135	301	283	520
5/5/2005	159	151	284	288	506
5/24/2005	207	167	330	345	500
5/25/2005	228	175	331	332	520
5/31/2005	171	153	375	265	513
6/16/2005	209	173	269	287	506
6/21/2005					438
7/14/2005	76	68	222	210	482
7/15/2005					456
7/23/2005					415
8/3/2005	135	123	316	283	500
average	**155**	**152**	**299**	**300**	**494**
stdev	**39**	**27**	**47**	**79**	**38**
t-test	**0.673**		**0.985**		

Appendix 3.8   SOUR mg O2/g VSS h in the ozonated portion of RAS before and after ozonation

	#R2 before	#R2 after	#R4 before	#R4 after
2/10/2005	9.8	12	18.4	14.1
2/13/2005	11.6	10	15	12.1
2/15/2005	9.5	9.9	16.1	13.5
average	**10**	**11**	**17**	**13**
stdev	**1**	**1**	**2**	**1**
t-test	**0.790**		**0.025**	

Appendix 3.9   NO$_3^-$, NO$_2^-$ and NH$_4^+$ (N- mg/L) after ozone treatment at end of the cycle (samples from the reactors before wasting)

	NOx								Aerobic		Alternating		NO2								NH4+							
	R1	R2	R3	R4	NO3 #R1	NO3 #R2	NO3 #R3	NO3 #R4	O3 dose mg/mgTSS i.e.s.	Decrease %	O3 dose mg/mgTSS i.e.s.	Decrease %	R1	R2	R3	R4	NO2 R1	NO2 R2	NO2 R3	NO2 R4	R1	R2	R3	R4	NH4+ R1	NH4+ R2	NH4+ R3	NH4+ R4
					mg/L										mg/L								mg/L					
1/17/2005	58	59.5	43	42	84.13	86.30	62.37	60.92	0.043	-2.6%	0.049	2%									0.2	0.2	0.2	0.2	0.40	0.40	0.40	0.40
1/18/2005	56	60	43	39.5	70.59	75.63	54.20	49.79	0.035	-7.1%	0.036	8%																
1/19/2005	57	59.5	44.5	43	71.85	75.00	56.09	54.20	0.037	-4.4%	0.040	3%																
1/20/2005	58	59.5	54.5	53.5	73.11	75.00	68.70	67.44	0.032	-2.6%	0.036	2%																
1/21/2005	57	59.5	55	53.5	71.85	75.00	69.33	67.44	0.037	-4.4%	0.043	3%																
1/22/2005	57	59.5	56	54.5	71.85	75.00	70.59	68.70	0.037	-4.4%	0.043	3%																
1/24/2005	59.5	61	58.5	55.8	73.36	75.21	72.13	68.80	0.041	-2.5%	0.042	4.6%																
1/25/2005	60.5	62	60.5	59.3	74.60	76.45	74.60	73.12	0.036	-2.5%	0.044	2.0%																
1/26/2005	60	62	60.3	57	73.98	76.45	74.35	70.28	0.019	-3%	0.033	5%																
1/27/2005	59	56.5	60.5	57	72.75	69.66	74.60	70.28	0.025	4%	0.036	6%																
1/28/2005	58	58.2	58	53	71.51	71.76	71.51	65.35	0.035	0%	0.034	9%																
1/29/2005	57	58	56	50.5	70.28	71.51	69.05	62.27	0.042	-2%	0.030	10%																
2/4/2005	53	53.5	49.5	47	67.63	68.27	62.42	59.27	0.051	-1%	0.035	5%																
2/5/2005	52.5	53	38.8	38.3	66.99	67.63	48.93	48.30	0.051	-1%	0.042	1%																
2/7/2005	51.6	51.6	42.5	39.5	65.84	65.84	53.59	49.81	0.039	0%	0.031	7%																
2/11/2005	50.5		52.5	53			74.52	75.23	0.034		0.045	-1%																
2/12/2005	50.3	51	53.8	52.5	71.40	72.39	76.37	74.52	0.051	-1%	0.059	2%	0.9	0.9	1.1	1.1									0.57	0.57	0.69	0.69
2/14/2005	49.3	50.5	53	49.5	69.98	71.68	75.23	70.27	0.043	-2%	0.057	7%	0.3	0.3	0.3	0.3									0.19	0.19	0.19	0.19
2/17/2005	49	51	49.2	42.1	69.56	72.39	69.84	59.76	0.060	-4%	0.051	14%			0.1										0.00	0.00	0.06	0.00
2/19/2005	46	48.2	47.6	36.5	65.30	68.42	67.57	51.81	0.055	-5%	0.064	23%													0.00	0.00	0.00	0.00
2/21/2005	46.5	49	45	41.5	66.01	69.56	63.88	58.91	0.049	-5%	0.060	8%													0.00	0.00	0.00	0.00
2/22/2005	48	49.5	48.2	38.5	68.14	70.27	68.42	54.65	0.062	-3%	0.081	20%													0.00	0.00	0.00	0.00
2/23/2005	40.5	40	25.5	37.2	75.37	74.44			0.034	1%	0.064												31.5		0.00	0.00	18.80	0.00
2/24/2005	40	38.5	22.5	33	74.44	71.65			0.034	4%	0.026												23.5		0.00	0.00	14.03	0.00
2/26/2005	40	38	32.5	33.5	74.44	70.72	60.48	62.34	0.028	5%	0.017	-3%			0.1										0.00	0.00	0.06	0.00
2/28/2005	39.5	36.5	35.5	32	73.51	67.93	66.07	59.55	0.051	8%	0.053	10%			0.1										0.00	0.00	0.06	0.00
3/4/2005			39.5	33.5			57.53	48.79	0.070		0.045	15%																
3/5/2005			39.5	35.5			57.53	51.71	0.057		0.050	10%																
3/9/2005	38.7	38.5	37.4	31	56.37	56.08	54.47	45.15	0.061	1%	0.048	17%																
3/10/2005	44	46.4	37.9	33	64.09	67.58	55.20	48.06	0.066	-5%	0.033	13%																
3/15/2005	45	41	39.6	31.8	69.55	63.37	61.20	49.15	0.050	9%	0.051	20%																
5/13/2005	52.5	58.5	50.5	51.5	62.90	70.08	60.50	61.70	0.024	-11%	0.051	-2%																
5/25/2005	52.8	50	40.3	35	63.25	59.90	48.28	41.93	0.057	5%	0.079	13%																
5/26/2005	38	50.7	37	37			44.33	44.33	0.052		0.056	0%									0.5				0.21			
5/27/2005	34.5	52	39.5	38.5			47.32	46.12	0.067		0.057	3%									1.5				0.64			

Appendix 3.10   TSS and VSS/TSS ratio in the mixed liquor after the ozone treatment, samples before wasting

| | TSS, mg/L | | | | | VSS/TSS | | | |
| | aerobic | | alternating | | | aerobic | | alternating | |
date	R1	R2	R3	R4	date	R1	R2	R3	R4
1/17/2005	1980	1920	1920	1918	1/17/2005	0.84	0.81	0.78	0.76
1/18/2005	1900	1800	1800	1840	1/18/2005	0.91	0.87	0.80	0.80
1/19/2005	1860	1760	1660	1755	1/19/2005	0.87	0.83	0.83	0.81
1/21/2005	1980	1860	1940	1816	1/21/2005	0.84	0.83	0.78	0.75
1/23/2005	1880	1700	1860	1660	1/23/2005	0.87	0.87	0.84	0.82
1/24/2005	2020	1860	1920	1820	1/24/2005	0.84	0.83	0.82	0.77
1/25/2005	2120	1940	1880	1860	1/25/2005	0.81	0.79	0.78	0.78
1/26/2005	1860	1860	1840	1780	1/26/2005	0.82	0.81	0.75	0.74
1/27/2005	1787	1711	1920	1780	1/27/2005	0.82	0.81	0.75	0.74
1/28/2005	1560	1720	1760	1720	1/28/2005	0.82	0.81	0.78	0.72
2/3/2005	1880	1920	1840	1800	2/3/2005	0.83	0.82	0.78	0.78
2/4/2005	2000	1920	1980	1880	2/4/2005	0.84	0.86	0.79	0.77
2/6/2005	1900	1860	1880	1740	2/6/2005	0.88	0.84	0.80	0.77
2/7/2005	1720	1700	1880	1780	2/7/2005	0.79	0.76	0.76	0.72
2/11/2005	1750	1683	1558	1583	2/11/2005	0.82	0.80	0.78	0.72
2/19/2005	2090	2040	1860	1860	2/19/2005	0.81	0.80	0.76	0.75
2/27/2005	1750	1833	1583	1708	2/27/2005	0.86	0.83	0.78	0.75
3/9/2005	1875	1800	1517	1892	3/9/2005	0.82	0.81	0.74	0.72
3/13/2005	2180	2200	1910	1980	3/13/2005	0.78	0.76	0.72	0.73
3/16/2005	1950	2017	1833	2033	3/16/2005	0.78	0.76	0.69	0.70
3/28/2005	2140	2550	2090	2100	3/28/2005	0.86	0.84	0.78	0.76
3/29/2005	2150	2240	2140	1860	3/29/2005	0.86	0.85	0.76	0.78
4/20/2005	1850	2050	1992	1767	4/20/2005	0.85	0.85	0.80	0.76
4/25/2005	2110	2130	2240	2650	4/25/2005	0.83	0.84	0.83	0.76
5/4/2005	2070	2080	2460	2280	5/4/2005	0.84	0.84	0.79	0.76
5/5/2005	1840	2130	2280	2250	5/5/2005	0.87	0.88	0.82	0.77
5/6/2005	1840	1940	2010	1850	5/6/2005	0.84	0.86	0.83	0.77
5/7/2005	2280	2320	2650	2480	5/7/2005	0.75	0.75	0.72	0.70
5/10/2005	1940	1930	1950	1940	5/10/2005	0.90	0.90	0.85	0.82
5/13/2005	2140	1820	2000	2070	5/13/2005	0.89	0.91	0.86	0.81
5/20/2005	1900	1980	1940	2020	5/20/2005	0.91	0.93	0.86	0.89
5/24/2005	1860	1910	1960	2200	5/24/2005	0.95	0.97	0.91	0.87
5/26/2005	1760	1680	1860	1820	5/26/2005	0.86	0.89	0.80	0.78
5/30/2005	2170	2160	2220	2160	5/30/2005	0.82	0.73	0.75	0.69
5/31/2005	1780	1870	2020	1580	5/31/2005	0.93	0.91	0.90	0.91
6/1/2005	2160	1860	2090	1920	6/1/2005	0.88	0.83	0.82	0.79
6/13/2005	1950	1890	1860	2080	6/13/2005	0.84	0.84	0.80	0.82
6/14/2005	1870	1890	1960	2080	6/14/2005	0.86	0.84	0.81	0.86
6/15/2005	1870	1770	2030	1950	6/15/2005	0.90	0.92	0.83	0.77
6/17/2005	1980	1900	2060	2020	6/17/2005	0.90	0.88	0.85	0.80
6/20/2005	1890	1940	1930	1980	6/20/2005	0.79	0.80	0.80	0.76
6/22/2005	1930	1970	1990	1890	6/22/2005	0.95	0.94	0.87	0.81
6/23/2005	1930	1808	1817	1842	6/23/2005	0.88	0.78	0.81	0.86
6/24/2005	1930	1830	2000	2000	6/24/2005	0.86	0.86	0.83	0.83
6/27/2005	1490	1830	1760	1830	6/27/2005	0.88	0.84	0.77	0.76
6/29/2005	1920	2090	2060	2020	6/29/2005	0.74	0.73	0.59	0.72
7/4/2005	1960	2230	2170	2130	7/4/2005	0.90	0.86	0.81	0.80
7/7/2005	2060	2170	2180	1960	7/7/2005	0.93	0.89	0.87	0.85
7/12/2005	2110	2380	2140	2050	7/12/2005	0.79	0.79	0.76	0.74
7/13/2005	1860	1700	1720	1760	7/13/2005	0.83	0.81	0.78	0.77
7/14/2005	1820	1920	2010	1700	7/14/2005	0.70	0.69	0.66	0.62
7/15/2005	2160	2220	2190	1990	7/15/2005	0.87	0.83	0.80	0.79
av	1938	1948	1964	1931	av	0.85	0.83	0.79	0.77
stdev	157	193	205	204	stdev	0.05	0.06	0.06	0.05
t-test	0.619		0.143		t-test	0.000		0.000	

Appendix 3.11   Decrease in TSS after ozone treatment

Aerobic

Parameter	O3 consumed	O3 cons/ (Xras bef O3 *1/12)	Xras befO3	stdev	Xras afterO3	stdev	(Xras bef*0.2 - Xras after*0.2) / (Xras bef *1/12) * 100%	Error
Unit	mg	mg O3/mg TSS initial excess sludge	mg/L	mg/L	mg/L	mg/L	%	%
2/10/2005	14.44	0.034	5100	21	5100	12		
2/11/2005	21.24	0.050	5050	47	5000	71		
2/12/2005	19.51	0.044	5350	12	5200	7	6.7%	0.4%
2/16/2005	26.76	0.060	5325	82	5225	24	4.5%	2.0%
2/17/2005	24.72	0.052	5750	94	5565	0	7.7%	1.6%
2/18/2005	24.69	0.055	5400	35	5225	47	7.8%	1.5%
2/20/2005	21.33	0.050	5150	0	5050	12	4.7%	0.2%
2/21/2005								
2/22/2005	15.36	0.034	5450	0	5250	12	8.8%	0.2%
2/23/2005						59		
2/25/2005								
2/27/2005	23.28	0.051	5500	12	5425	0	3.3%	0.2%
3/3/2005	28.68	0.070	4900	24	4700	24	9.8%	1.0%
3/4/2005	23.52	0.057	4925	82	4800	24	6.1%	2.2%
3/8/2005	27.48	0.061	5375	35	5225	24	6.7%	1.1%
3/9/2005	29.52	0.066	5400	12	5170	71	10.2%	1.6%
3/14/2005	24.00	0.050	5725	118	5540	12	7.8%	2.3%
3/16/2005								
4/11/2005	21.96	0.043	6150	12	6105	0	1.8%	0.2%
4/12/2005	37.60	0.080	5625	47	5400	12	9.6%	1.1%
5/9/2005	32.64	0.063	6175	12	5975	12	7.8%	0.4%
5/10/2005	9.12	0.020	5520	85	5450	0	3.0%	1.5%
5/11/2005	9.12	0.018	6175	59	6075	47	3.9%	1.7%
5/12/2005	11.52	0.024	5800	24	5725	24	3.1%	0.8%
5/13/2005	11.52	0.027	5125	28	4975	12	7.0%	0.8%
5/16/2005								
5/17/2005								
5/18/2005								
5/19/2005								
5/20/2005								
5/24/2005	29.20	0.057	6200	14	6200	0		
5/25/2005	22.56	0.052	5200	28	5025	28	8.1%	1.0%
5/26/2005	28.00	0.067	5000	47	4875	24	6.0%	0.9%
5/27/2005	29.12	0.063	5575	35	5410	12	7.1%	1.9%
5/30/2005	22.26	0.041	6475	14	6325	0	5.6%	0.5%
5/31/2005	22.56	0.060	4500	35	4425	35	4.0%	2.1%
6/1/2005	15.36	0.037	4950	28	4877	14	3.5%	0.8%
6/15/2005	1.84	0.003	6325	12	6208	47	4.4%	1.1%
6/16/2005	7.36	0.015	5925	35	5785	47	5.7%	1.8%
6/17/2005	26.90	0.059	5475	12	5250	118	9.9%	0.3%
6/20/2005	19.20	0.040	5775	12	5675	12	4.2%	0.7%

Alternating

Parameter	O3 consumed	O3 cons/ (Xras bef O3 *1/12)	Xras befO3	stdev	Xras afterO3	stdev	(Xras bef*0.2 - Xras after*0.2) / (Xras bef *1/12) * 100%	Error
Unit	mg	mg O3/mg TSS initial excess	mg/L	mg/L	mg/L	mg/L	%	%
2/10/2005	19.13	0.045	5100	12	4825	8	12.9%	0.4%
2/11/2005	23.40	0.059	4750	35	4430	47	16.2%	1.8%
2/12/2005	24.36	0.057	5100	0	4775	0	15.3%	0.0%
2/16/2005	22.44	0.051	5250	12	4925	24	14.9%	0.7%
2/17/2005	31.84	0.076	5050	33	4635	12	19.7%	0.9%
2/18/2005	28.05	0.064	5275	12	4800	12	21.6%	0.5%
2/20/2005	25.17	0.060	5075	12	4750	35	15.4%	1.0%
2/21/2005	33.28	0.081	4950	24	4450	24	24.2%	1.0%
2/22/2005	26.40	0.064	4950	24	4600	24	17.0%	1.0%
2/23/2005	11.28	0.026	5125	12	4825	35	14.0%	1.0%
2/25/2005	7.47	0.017	5275	12	5100	24	8.0%	0.7%
2/27/2005								
3/3/2005	18.66	0.045	4950	14	4600	0	17.0%	0.3%
3/4/2005								
3/8/2005	21.78	0.048	5400	47	5100	12	13.3%	1.1%
3/9/2005								
3/14/2005	23.94	0.051	5600	0	5125	12	20.4%	0.2%
3/16/2005								
4/11/2005								
4/12/2005	44.09	0.086	6150	12	5575	0	22.4%	0.2%
5/9/2005	15.12	0.029	6275	12	6150	12	4.8%	0.4%
5/10/2005	8.88	0.018	5950	0	5600	28	14.1%	0.5%
5/11/2005	6.24	0.029	6550	47	6155	35	14.5%	1.3%
5/12/2005	27.36	0.051	6450	24	6095	47	13.2%	1.1%
5/13/2005	14.40	0.031	5525	12	5410	24	5.0%	0.7%
5/16/2005	36.24	0.069	6275	59	6000	24	10.5%	1.3%
5/17/2005	36.00	0.086	5000	28	4650	14	16.8%	0.9%
5/18/2005	22.20	0.046	5775	24	5660	12	4.8%	0.6%
5/19/2005	18.60	0.047	4700	0	4500	57	10.2%	1.3%
5/20/2005	34.80	0.076	5475	71	5175	12	13.2%	1.5%
5/24/2005	39.52	0.079	5975	21	5750	0	9.0%	0.4%
5/25/2005	26.64	0.056	5750	57	5450	0	12.5%	1.0%
5/26/2005	26.16	0.057	5550	12	5360	35	8.2%	0.9%
5/27/2005	27.20	0.060	5425	59	5190	24	10.4%	1.5%
5/30/2005	17.15	0.030	6900	71	6645	71	8.9%	2.1%
5/31/2005	34.62	0.084	4925	6	4600	12	15.8%	0.4%
6/1/2005	30.54	0.074	4950	28	4775	28	8.5%	1.2%
6/15/2005	0.80	0.001	6550	47	6550	24		
6/16/2005	15.52	0.029	6350	24	6350	59		
6/17/2005	22.14	0.047	5600	71	5505	35	4.1%	1.9%
6/20/2005	21.12	0.043	5875	94	5875	12		

Appendix 3.12 Increase in soluble COD in the RAS after ozone treatment

Aerobic

Parameter	O3 cons	X was	O3 cons/ (Xwas 0.25)	sCOD bef	sCOD bef stdev	sCODafter	sCODafter stdev	(sCOD after - sCOD bef)/sCOD bef	Error
Unit	mg	mg/L	mg O3/mg TSS initial excess sludge	mg/L	mg/L	mg/L	mg/L	%	%
2/3/2005	22.80	1780	0.051	50	13	100	7	99%	33.9%
2/4/2005	23.20	1860	0.050	36	2	77	34	115%	49.6%
2/6/2005	17.40	1780	0.039	56	18	157	24	180%	47.4%
2/10/2005	14.44	1700	0.034	50	14	64	17	30%	55.4%
2/11/2005	21.24	1680	0.051	22	5	65	19	194%	51.9%
2/13/2005	19.51	1817	0.043	50	21	115	6	129%	47.6%
2/16/2005	26.76	1775	0.060	44	13	73	11	66%	44.1%
2/17/2005	24.69	1917	0.052	58	19	81	8	39%	41.4%
2/18/2005	24.93	1800	0.055	69	11	112	12	63%	27.0%
2/20/2005	21.33	1717	0.050	67	7	147	17	120%	22.3%
2/21/2005	26.08	1675	0.062	55	2	145	24	164%	19.1%
2/22/2005	15.36	1817	0.034	75	11	173	11	131%	20.8%
2/23/2005	22.80	1817	0.050	66	3	193	27	192%	18.5%
2/25/2005	13.17	1875	0.061	95	4	171	11	80%	10.7%
2/26/2005	23.28	1833	0.051	95	4	211	10	122%	9.3%
3/3/2005	28.68	1633	0.070	59	10	183	30	212%	32.7%
3/4/2005	23.52	1642	0.057	97	8	229	25	136%	19.3%
3/8/2005	27.48	1792	0.061	66	29	181	24	174%	57.6%
3/9/2005	29.52	1800	0.066	41	5	136	6	229%	16.3%
3/14/2005	24.00	1908	0.050	86	1	187	23	118%	13.0%
3/16/2005	26.37	2017	0.052	68	31	199	7	192%	49.5%
4/11/2005	21.96	2050	0.043	15	2	22	2	47%	22.4%
5/9/2005	32.64	2058	0.063	59	19	119	17	102%	46.4%
5/10/2005	9.12	1840	0.020	81	27	113	22	39%	53.3%
5/11/2005	9.12	2058	0.018	80	6	134	23	68%	23.9%
5/12/2005	11.52	1933	0.024	86	11	198	16	129%	20.5%
5/13/2005	11.52	1708	0.027	58	12	137	19	135%	35.2%
5/16/2005									
5/17/2005									
5/18/2005									
5/19/2005									
5/20/2005									
5/24/2005	29.20	2009	0.057	56	8	125	9	124%	22.3%
5/25/2005	22.56	1708	0.052			97	15		
5/26/2005	28.00	1658	0.067	64	11	113	19	77%	33.6%
5/27/2005	29.12	1841	0.063	55	3	90	9	62%	15.6%
5/30/2005	22.26	2128	0.041	57	9	93	18	62%	34.4%
5/31/2005	22.56	1494	0.060	52	12	125	21	140%	40.9%
6/1/2005	15.36	1638	0.037	67	24	150	12	124%	43.6%
6/15/2005	1.84	2101	0.003	55	14	74	19	33%	51.9%
6/16/2005	7.36	1945	0.015	28	5	35	17	24%	64.4%
6/17/2005	26.90	1796	0.059	38	13				
6/20/2005	19.20	1785	0.027	51	5	67	8	31%	22.5%

Alternating

O3 cons	WAS, mg/L	O3 cons/ (Xwas 0.25)	sCOD bef	sCOD bef stdev	sCODafter	sCODafter stdev	(sCOD after - sCOD bef)/sCOD bef	Error
mg	mg/L	mg O3/mg TSS initial excess sludge	mg/L		mg/L		%	%
15.60	1800	0.035	50	39	92	11	82%	89.7%
18.45	1740	0.042	36	13	133	14	274%	46.6%
14.04	1800	0.031	65	17	161	6	148%	30.1%
19.13	1700	0.045	53	14	136	136	159%	126.0%
23.40	1583	0.059	33	4	125	1	274%	11.9%
24.36	1700	0.057	62	10	165	11	167%	23.0%
22.44	1750	0.051	40	10	113	16	184%	40.3%
31.84	1683	0.076	50	10	266	14	431%	25.2%
28.05	1758	0.064	73	16	208	8	186%	26.2%
25.17	1692	0.060	59	7	197	4	232%	13.7%
33.28	1650	0.081	43	4	197	6	364%	11.3%
26.40	1650	0.064	77	6	244	19	217%	15.1%
11.28	1708	0.026	57	6	116	12	105%	21.5%
7.47	1758	0.017	54	6	125	17	133%	25.6%
22.56	1708	0.053	95	13	288	14	204%	18.2%
18.66	1650	0.045	51	8	151	14	196%	24.7%
18.18	1450	0.050	71	14	182	10	157%	25.8%
21.78	1800	0.048	49	14	133	15	168%	38.6%
15.54	1892	0.033	41	17	118	17	188%	55.0%
23.94	1867	0.051	72	17	154	154	114%	123.6%
18.89	2033	0.037	73	16	184	23	152%	34.1%
8.75	1767	0.020	20	17	49	49	145%	184.9%
15.12	2092	0.029	96	20	111	8	15%	28.1%
8.88	1983	0.018	66	8	104	15	57%	26.2%
6.24	2183	0.029	99	22	211	16	113%	29.5%
27.36	2150	0.051	114	8	267	21	134%	14.6%
14.40	1842	0.031	69	27	93	10	35%	50.7%
36.24	2074	0.069	66	13	125	13	90%	30.1%
36.00	1667	0.086	64	18	163	13	156%	35.6%
22.20	1925	0.046	73	8	119	10	64%	18.8%
18.60	1567	0.047	66	7	102	6	54%	16.3%
34.80	1805	0.076	66	12	140	5	114%	22.5%
39.52	1936	0.079	60	17	139	12	130%	36.6%
26.64	1897	0.056	53	4	141	5	167%	10.3%
26.16	1837	0.057	52	10	104	23	99%	41.6%
27.20	1793	0.060	53	11	98	11	85%	31.8%
17.15	2283	0.030	41	8	95	16	132%	36.6%
34.62	1640	0.084	37	4	103	11	177%	21.8%
30.54	1638	0.074	58	3	166	11	185%	11.3%
0.80	2196	0.001	58	20	71	10	22%	47.7%
15.52	2068	0.029	28	5	61	16	115%	43.2%
22.14	1860	0.047	34	10	100	11	195%	41.4%
21.12	1817	0.043	37	10	65	13	76%	45.3%

Appendix 3.13   COD in the reactors after ozonation and feding (time 0) for two periods of research

	Aerobic				Alternating		
	COD R1	COD R2	(COD R2-COD		COD R3	COD R4	(COD R4-COD
	control	ozonated	R1)/COD R1		control	ozonated	R3)/COD R3
	mg/L	mg/L	%		mg/L	mg/L	%
11/29/2004	559	659	17.9%		496	589	19%
11/30/2004	582	595	2.2%		592	688	16%
12/1/2004	495	524	5.9%		483	581	20%
12/2/2004	465	500	7.6%		464	505	9%
12/3/2004	475	525	10.4%		445	587	32%
12/7/2004	478	503	5.3%		511	559	9%
1/16/2005	574	594	3.4%		491	544	11%
1/17/2005	413	480	16.2%		399	540	35%
1/18/2005	476	566	18.9%		460	520	13%
1/19/2005	495	526	6.3%		465	576	24%
1/20/2005	467	494	5.8%		453	597	32%
1/21/2005	454	517	13.8%		489	575	18%
1/23/2005	430	512	19.1%		474	592	25%
1/24/2005	472	524	11.0%		490	584	19%
1/25/2005	471	512	8.7%		501	606	21%
1/26/2005	484	498	2.9%		510	681	34%
1/27/2005	532	586	10.2%		540	689	28%
1/28/2005	538	601	11.7%		560	608	9%
1/29/2005	548	620	13.1%		582	673	16%

average	495	544		average	495	594	
stdev	48	51		stdev	48	54	

t test	0.000		t test	0.000	

6/23/2005	396	399	0.8%		367	398	8.3%
6/27/2005	450	482	7.0%		441	471	6.8%
7/7/2005	398	400	0.5%		364	405	11.2%
7/14/2005	291	364	25.4%		345	387	12.0%
7/15/2005	455	513	12.6%		513	553	7.7%
7/20/2005	391	401	2.5%		397	444	11.6%
7/21/2005	404	451	11.7%		474	547	15.3%
7/27/2005	436	466	6.9%		360	431	19.8%
7/28/2005	395	426	7.9%		365	563	54.2%
7/29/2005	400	466	0.0%		353	397	12.6%
8/2/2005	351	367	4.7%		302	336	11.3%

average	397	430		average	389	448	
stdev	46	49		stdev	62	76	

t test	0.001		t test	0.002	

Appendix 3.14   COD removal during treatment in aerobic (R1, R2) and alternating (R3, R4) reactors, ozone applied

4/11/2005

time		aer	alt	R1	R2	R3	R4
12.75	12.42	0.00	0.00	330	372	291	332
13.00	12.75	0.25	0.33	182	204	160	173
13.50	13.00	0.75	0.58	137	140	150	154
14.08	13.50	1.33	1.08	111	102	134	140
14.60	14.08	1.85	1.67	98	101	113	131
15.12	14.60	2.37	2.18	86	98	93	125
15.67	15.12	2.92	2.70	85	95	89	112
16.08	15.67	3.33	3.25	78	86	81	93
16.55	16.08	3.80	3.67	77	77	79	85
17.00	16.55	4.25	4.13	78	78	78	79
17.47	17.00	4.72	4.58	78	80	77	77
18.00	17.47	5.25	5.05	71	82	72	76
18.45	18.00	5.70	5.58	70	82	68	72
18.95	18.45	6.20	6.03	70	82	68	74
	18.95		6.53			69	74
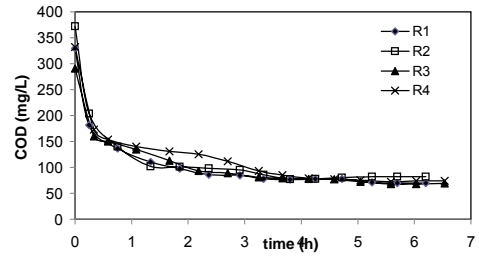
5/12/2005

time		aer	alt	R1	R2	R3	R4
12.75	12.53	0.00	0.00	284	303	269	295
13.00	13.00	0.25	0.47	158	163	148	146
13.31	13.31	0.56	0.77	120	107	121	132
14.03	14.03	1.28	1.50	94	80	120	121
14.42	14.42	1.67	1.88	73	77	107	103
15.00	15.00	2.25	2.47	75	76	88	82
15.43	15.43	2.68	2.90	76	67	80	80
16.00	16.00	3.25	3.47	72	72	72	74
16.47	16.47	3.72	3.93	76	72	64	77
17.00	17.00	4.25	4.47	66	72	64	69
17.47	17.47	4.72	4.93	66	72	64	69
17.97	17.97	5.22	5.43	65	68	63	68
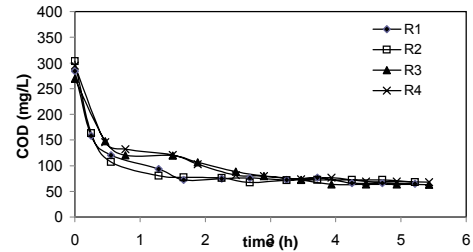
6/23/2005

time		aer	alt	R1	R2	R3	R4
12.43	12.25	0.00	0.00	304	357	301	355
12.88	12.88	0.45	0.63	154	187	231	222
13.58	13.58	1.15	1.33	111	167	165	178
14.18	14.18	1.75	1.93	105	151	108	162
14.75	14.75	2.32	2.50	103	143	109	160
15.38	15.38	2.95	3.13	102	137	103	145
15.75	15.75	3.32	3.50	99	124	91	135
16.50	16.50	4.07	4.25	91	123	92	128
17.00	17.00	4.57	4.75	85	124	91	117
17.50	17.50	5.07	5.25	83	124	96	99
18.00	18.00	5.57	5.75	81	118	94	102
18.50	18.50	6.07	6.25	82	108	97	97
19.00	19.00	6.57	6.75	81	96	96	87
19.50	19.50	7.07	7.25	80	91	89	88
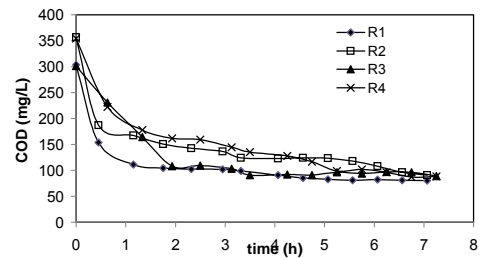
Appendix 3.15   Soluble COD in reactors after ozonation and feeding: in time 0 and after 15 min. biomass-feed contact

day	COD R1			COD R2			COD R3			COD R4		
	t=0	t=15 min		t=0	t=15 min		t=0	t=15 min		t=0	t=15 min	
5/4/2005	291	215	26%	371	227	39%	353	190	46%	368	157	57%
5/10/2005	341	196	43%	360	227	37%	323	170	47%	357	141	61%
5/11/2005	323	190	41%	412	280	32%	322	158	51%	330	133	60%
5/13/2005	323	169	48%	352	199	43%	295	163	45%	391	166	57%
5/16/2005	329	185	44%				378	176	53%	339	132	61%
5/17/2005	386	236	39%				312	164	47%	326	198	39%
5/18/2005	388	199	49%				325	177	46%	374	228	39%
5/19/2005	333	208	38%				278	146	47%	356	188	47%
5/20/2005	325	142	56%				388	178	54%	427	183	57%
5/24/2005	343	227	34%	349	219	37%	386	244	37%	436	164	62%
5/25/2005	372	247	33%	341	216	37%	327	191	42%	375	121	68%
5/26/2005	335	211	37%	363	196	46%	323	181	44%	325	153	53%
5/27/2005	353	240	32%	369	245	34%	309	162	48%	338	186	45%
5/30/2005	444	209	53%	461	181	61%	350	129	63%	419	195	53%
5/31/2005	275	203	26%	386	216	44%	346	208	40%	350	145	59%
6/1/2005	256	110	57%	393	124	68%	347	177	49%	351	120	66%
6/15/2005	349	184	47%	350	153	56%	361	100	72%	350	120	66%
6/16/2005	284	140	51%	349	98	72%	275	110	60%	362	278	23%
6/20/2005	340	195	42%	443	124	72%	273	111	59%	360	208	42%
6/21/2005	308	186	40%	322	135	58%	363	152	58%	364	121	67%
6/22/2005	249	114	54%	323	78	76%	268	163	39%	389	192	51%
6/28/2005	331	196	41%	337	201	40%	322	135	58%	337	133	61%
6/29/2005	311	161	48%	345	208	40%	278	106	62%	416	201	52%
6/30/2005	267	126	53%	262	121	54%	304	187	38%	418	140	66%
7/4/2005	367	167	55%	335	113	66%	242	111	54%	378	143	62%
7/12/2005	242	101	58%	233	67	71%	238	121	49%	325	121	63%
7/13/2005	341	151	56%	384	164	57%	316	163	48%	405	142	65%
7/18/2005	301	193	36%	330	156	53%	341	161	53%	341	136	60%
7/19/2005	317	166	48%	309	133	57%	270	120	56%	388	233	40%
7/22/2005	367	110	70%	314	128	59%	296	136	54%	349	229	34%
7/26/2005	261	95	64%	374	89	76%	340	190	44%	387	260	33%
average	324		45.7%	353		53.3%	318		50.5%	369		53.8%
stdev	46		10.5%	49		14.2%	40		8.1%	33		11.7%

Appendix 3.16   Ammonia uptake rates, Nitrite +nitrate production and utilization rates for ozonated and control reactors: results from kinetic studies

			AEROBIC		
		ozone dose	R1	R2	change
		mg O3/mg TSS	mg/g VSS h	mg/g VSS h	%
AUR	6-Feb-05	0.039	5.20	4.38	15.8%
	20-Feb-05	0.049	5.31	3.86	27.4%
	27-Feb-05	0.051	4.94	3.65	26.1%
	9-Mar-05	0.066	4.79	4.03	15.8%
	16-Mar-05	0.052	3.94	3.39	14.0%
	11-Apr-05	0.043	4.86	3.96	18.6%
	12-May-05	0.024	5.21	4.23	18.9%
	23-Jun-05	0.020	4.79	4.49	6.3%
	av		4.88	4.00	18%
	stdev		0.43	0.37	7%

			ALTERNATING		
		ozone dose	R3	R4	change
		mg O3/mg TSS	mg/g VSS h	mg/g VSS h	%
AUR	6-Feb-05	0.031	6.66	6.25	6.1%
	20-Feb-05	0.060	6.29	5.90	6.1%
	27-Feb-05	0.053	6.20	5.78	6.8%
	9-Mar-05	0.033	6.16	5.50	10.8%
	16-Mar-05	0.037	n/a	5.64	n/a
	11-Apr-05	0.020	6.12	5.71	6.7%
	12-May-05	0.051	5.56	5.82	n/a
	23-Jun-05	0.044	5.71	5.42	5.0%
	av		6.10	5.75	7%
	stdev		0.37	0.26	2%

NPR	6-Feb-05	0.039	5.88	5.33	9.4%
	20-Feb-05	0.049	6.09	4.42	27.4%
	27-Feb-05	0.051	6.97	4.31	38.1%
	9-Mar-05	0.066	5.35	4.30	19.5%
	16-Mar-05	0.052	6.38	4.68	26.6%
	11-Apr-05	0.043	5.72	4.20	26.5%
	12-May-05	0.024	5.45	4.21	22.8%
	23-Jun-05	0.020	6.06	5.93	2.1%
	av		5.99	4.67	22%
	stdev		0.52	0.63	11%

NPR	6-Feb-05	0.031	9.20	9.02	2.0%
	20-Feb-05	0.060	7.85	7.79	0.7%
	27-Feb-05	0.053	7.65	7.26	5.0%
	9-Mar-05	0.033	7.26	7.16	1.3%
	16-Mar-05	0.037	n/a	6.74	n/a
	11-Apr-05	0.020	7.87	7.38	6.2%
	12-May-05	0.051	7.15	6.71	6.2%
	23-Jun-05	0.044	7.25	6.86	5.3%
	av		7.75	7.37	4%
	stdev		0.71	0.76	2%

NUR	6-Feb-05	0.031	6.07	7.52	24.0%
	20-Feb-05	0.060	4.30	6.88	59.8%
	27-Feb-05	0.053	5.88	8.57	45.8%
	9-Mar-05	0.033	4.82	6.3	30.7%
	16-Mar-05	0.037	6.41	8.04	25.4%
	11-Apr-05	0.020	6.48	7.27	12.2%
	12-May-05	0.051	6.17	8.73	41.4%
	23-Jun-05	0.044	6.12	8.07	31.9%
	av		5.78	7.67	34%
	stdev		0.79	0.84	15%

Appendix 3.17   Ozone batch test: COD and ammonia production

5/4/2005

Aerated mixed biomass, synthetic wastewater

After 3 h of aerobic reaction,   20% of biomass withdrawn, ozonated
and returned to the reactor

oznone dose: 0.055 mg O_3/mg initial excess sludge

time		COD		NH4+		NOx-
10:20	0.33	237	65.5	38.46	11	28.10
10:55	0.92	222	65	38.16	8.5	21.71
11:30	1.50	212	65.9	38.69	9	22.99
12:15	2.25	177	62.9	36.93	10	25.55
12:45	2.75	159	60	35.23	13.1	33.46
12:45	2.75	200	61.6	36.17	12.2	31.16
13:10	3.17	170	68.5	40.22	12.8	32.70
13:45	3.75	159	57.3	33.64	13.5	34.49
14:45	4.75	120	48.1	28.24	19	48.54

Direct change (%) after ozonation:

	26%	3%	-7%

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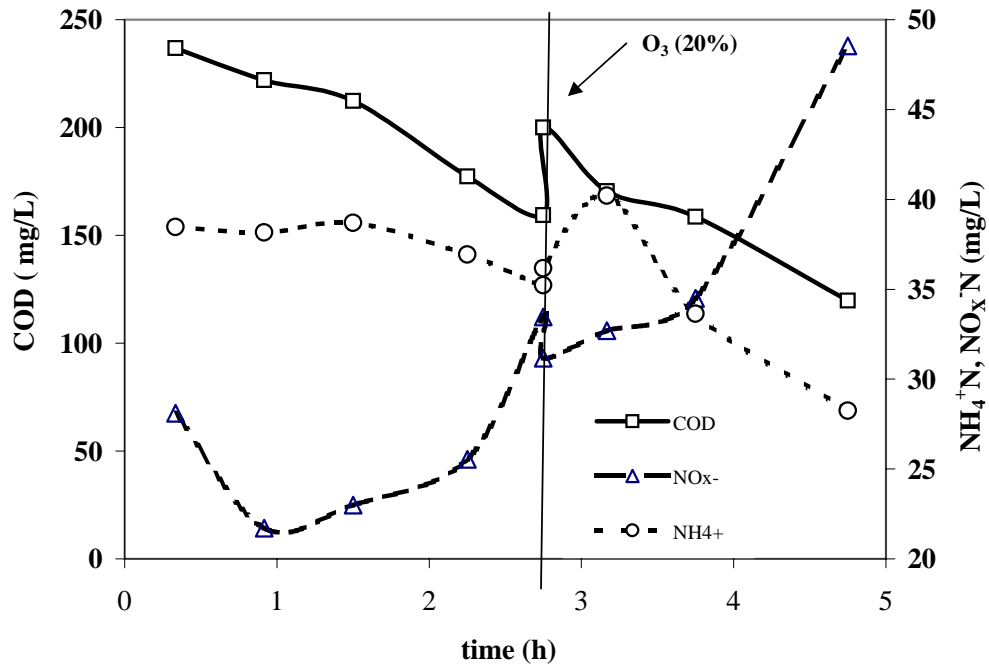
Appendix 4.1   Total (unwashed) EPS in control reactors and in ozonated reactors before and after ozone treatment

EPS, mg/gVSS

date	dose	R1	R2	R2 after O3	increase, %	dose	R3	R4	R4 after O3	increase, %
6/27/2005		433	485				401	494	-	-
6/28/2005		-	-			0.043		518	559	8.0%
6/29/2005	0.032		429	494	15.0%	0.049		492	569	15.6%
6/30/2005	0.057		388	529	36.4%	0.067		460	565	23.0%
7/4/2005	0.046		357	407	14.1%	0.062		498	564	13.3%
7/7/2005	0.030		358	416	16.3%	0.061		419	469	11.9%
7/12/2005	0.029		455	471	3.5%	0.023		573	598	4.4%
7/13/2005	0.033		451	476	5.4%	0.028		500	532	6.4%
7/14/2005	0.030		364	411	12.8%	0.050		515	541	4.9%
7/15/2005	0.040		536	574	7.2%	0.050		526	540	2.6%
7/16/2005		411	422				433	456		
7/18/2005	0.054		448	572	27.8%	0.060		580	620	6.8%
7/19/2005	0.046		497	571	15.0%	0.048		531		
7/20/2005	0.039		348	468	34.4%	0.062		416	430	3.3%
7/21/2005	0.037	429	432	581	34.5%	0.052	382	517	626	21.0%
7/22/2005	0.037	335	335	424	26.4%	0.041	386	413	440	6.7%
7/26/2005	0.048	437	406	535	31.7%	0.058	376	434	463	6.8%
7/27/2005	0.058	341	429	526	22.5%	0.033	425	441	484	9.7%
7/28/2005	0.042	361	428	523	22.2%	0.054	382	437	521	19.3%
7/29/2005	0.039	489	421	484	14.8%	0.063	371	467	541	15.7%
8/2/2005	0.055	337	352	419	18.9%	0.063	271	508	596	17.3%
8/3/2005		399	493				356	545		
average		397	421	493			378	488	537	
stdev		52	55	61			45	49	59	
		13%	13%	12%			12%	10%	11%	

Appendix 4.2   Soluble (washed) EPS in control reactors and in ozonated reactors before and after ozone treatment

EPS, mg/gVSS

date	dose	R1	R2	R2 after O3	dose	R3	R4	R4 after O3
7/16/2007		275.13	359.79	0.00		216.46	285.71	
7/18/2005	0.054	0.00	288.56	323.85	0.060	0.00	345.68	256.05
7/19/2005	0.046	0.00	378.70	248.73	0.048	0.00	291.67	294.55
7/20/2005	0.039	0.00	288.56	200.61	0.062	0.00	265.90	265.64
7/21/2005	0.037	282.80	271.60	246.89	0.052	274.35	349.65	366.55
7/22/2005	0.037	242.09	223.35	189.47	0.041	198.04	264.55	208.50
7/26/2005	0.048	260.71	294.42	323.21	0.058	213.41	338.62	324.34
7/27/2005	0.058	243.66	282.93	273.51	0.033	307.86	307.69	220.91
7/28/2005	0.042	243.66	275.86	304.92	0.054	176.00	260.15	282.54
7/29/2005	0.039	287.36	224.72	288.22	0.063	200.80	294.42	200.61
8/2/2005	0.055	211.54	259.07	251.31	0.063	145.83	293.79	292.40
8/3/2005		258.64	299.08	0.00		277.78	392.16	0.00
average		256	287	265		223	307	271
stdev		24	46	47		53	41	52
		9%	16%	18%		24%	13%	19%

Appendix 4.3 Bound to total (washed to unwashed) EPS ratio

date	dose	R1	R2	R2 after O3		dose	R3	R4	R4 after O3
7/16/2005	0.000	0.67	0.85	-		0.000	0.50	0.63	-
7/18/2005	0.054	-	0.64	0.57		0.060	-	0.60	0.41
7/19/2005	0.046	-	0.76	0.44		0.048	-	0.55	-
7/20/2005	0.039	-	0.83	0.43		0.062	-	0.64	0.62
7/21/2005	0.037	0.66	0.63	0.42		0.052	0.72	0.68	0.59
7/22/2005	0.037	0.72	0.67	0.45		0.041	0.51	0.64	0.47
7/26/2005	0.048	0.60	0.72	0.60		0.058	0.57	0.78	0.70
7/27/2005	0.058	0.71	0.66	0.52		0.033	0.73	0.70	0.46
7/28/2005	0.042	0.68	0.65	0.58		0.054	0.46	0.60	0.54
7/29/2005	0.039	0.59	0.53	0.60		0.063	0.54	0.63	0.37
8/2/2005	0.055	0.63	0.74	0.60		0.063	0.54	0.58	0.49
8/3/2005	0.000	0.65	0.61	-		0.000	0.78	0.72	-

		R1	R2	R2 after O3			R3	R4	R4 after O3
average		66%	69%	52%			59%	64%	52%
stdev		5%	9%	8%			12%	7%	10%
		7%	13%	15%			19%	10%	20%

Appendix 4.4 Total EPS in the effluent and the feed

	EPS, mg/gVSS				
date	R1	R2	R3	R4	feed
7/29/2005	172.84	148.15	160.49	135.80	179.01
8/2/2005	145.90	138.56	140.23	141.92	141.98
8/3/2005	168.21	151.97	151.21	127.11	129.63

average	162	146	151	135	150
stdev	14	7	10	7	26
	9%	5%	7%	6%	17%

Appendix 4.5   Sludge volume index at the end of cycle (before wasting), SVI, mL/g

Day	R1	R2	R3	R4
7/23/2005	60.1	47.1	62.7	32.3
7/24/2005	62.5	49.0	64.5	34.2
7/25/2005	57.2	45.5	58.0	28.1
7/26/2005	61.5	45.7	56.2	26.2
7/27/2005	53.0	46.3	59.5	29.0
7/28/2005	65.8	41.8	50.4	32.5
7/29/2005	55.5	41.0	52.5	33.8
7/30/2005	56.2	36.5	56.2	35.2
7/31/2005	64.1	39.3	60.2	29.7
8/1/2005	61.3	38.2	62.5	26.5
8/2/2005	59.3	43.7	63.7	28.5
8/3/2005	56.5	44.2	64.5	27.4

	R1	R2	R3	R4
average	59.4	43.2	59.2	30.3
stdev	3.8	3.9	4.7	3.2
t-test	0.000		0.000	

Appendix 4.6   Capillary Suction Time CST in RAS (TSS=5400 - 6000 mg/L)

Day	R1	R2	R3	R4
7/23/2005	8.2	6.5	7.9	6.3
7/24/2005	7.6	9.1	6.0	6.0
7/25/2005	8.0	6.7	5.6	
7/26/2005	8.2	8.3	5.8	6.6
7/27/2005	6.6	6.3	6.0	6.1
7/28/2005	6.3	7.1	5.6	6.1
7/29/2005	5.6	6.6	5.7	6.1
7/30/2005	7.0	6.4	5.2	6.2
7/31/2005	6.5	6.3	6.0	5.7
8/1/2005	6.3	7.8	5.4	5.6
8/2/2005	6.2	6.5	5.8	7.3
8/3/2005	5.7	5.9	5.8	6.1

	R1	R2	R3	R4
average	6.9	7.0	5.9	6.2
stdev	0.9	1.0	0.7	0.5
t-test	0.717		0.297	

Appendix 4.7   Flocculation test with high COD content (after ozonation and feeding)

Test performed by settling of biomass in vials 5 min.

4/15/2005

		Aerobic	
		control	ozonated
10.75	0.05	61	61
11.00	0.30	62	58.5
11.53	0.83	65	64.5
11.83	1.13	72	65
12.50	1.80	69	65
12.92	2.22	69.5	65
13.58	2.88	75	67.5
14.00	3.30	74	68
15.17	4.47	73	69
15.67	4.97	77	71
16.17	5.47	76	74.5
16.58	5.88	76	74.5
16.92	6.22	75.5	75
19.48	8.78	76	75
20.00	9.30	79	77
20.42	9.72	79	74
34.50	23.80	76	69
35.30	24.60	73	74

4/18/2005

		Alternating	
		control	ozonated
11.08	0.02	61	61
11.17	0.27	61	59
11.67	0.77	63	61
12.33	1.43	65	62
12.92	2.02	69	65
13.75	2.85	70	65
14.33	3.43	70.5	66
15.17	4.27	72	69
15.53	4.63	72	71
15.92	5.02	76	73
16.37	5.47	77	74
16.75	5.85	77.5	74.5
17.17	6.27	78	75
19.33	8.43	82	83
19.67	8.77	83	83
20.17	9.27	84	84.5
20.58	9.68	85	84

Appendix 5.1 Nitrification batch experiments (no COD and anoxic zone) with ammonia addition

experiment #		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Date		8-Mar-06	17-Mar-06		20-Mar-06		29-Jul-06		3-Aug-06		6-Aug-06
Biomass type		**aerobic**	**aerobic**	**alternating**	**aerobic**	**alternating**	**alternating**		**aerobic**	**alternating**	**alternating**
ammonia added	mg/L	62.4	26.82	24.52	52.44	51.29	14.7	22.5	8.14	5.99	11.14
average pH during nitrification	-	7.95	7.69	7.96	7.63	7.85	7.55	7.50	7.42	7.69	7.86
initial pH	-	7.58	7.73	8.01	7.71	8.04	7.58	7.51	7.43	7.54	7.89
max. pH	-	8.12	7.79	8.03	7.76	8.04	7.60	7.55	7.46	7.55	7.90
min. pH	-	7.82	7.53	7.87	7.42	7.70	7.51	7.49	7.34	7.51	7.78
AUR,	mg/gVSS h	**2.56**	**2.92**	**4.25**	**3.87**	**5.70**	**3.89**	**5.11**	**2.88**	**2.19**	**2.57**
NO3 PR	mg/gVSS h	nm	nm	nm	4.44	3.28	na	na	na	na	na
NO2 PR,	mg/gVSS h	nm	nm	nm	**0.80**	**2.27**	na	na	na	na	na
NH4 conc, initial	mg/gVSS	**27.74**	**8.91**	**9.69**	**36.54**	**28.34**	**7.43**	**11.28**	**3.99**	**2.98**	**4.44**
NH4 conc, final	mg/gVSS	8.69	0.76	4.24	25.16	11.65	0.52	3.35	0.39	0.28	0.00
NO2 conc, initial	mg/gVSS	nm	nm	nm	0.00	0.00	na	na	na	na	na
NO2 conc., final	mg/gVSS	nm	nm	nm	2.38	6.28	na	na	na	na	na
NO2 accumulation	mg/gVSS	nm	nm	nm	2.38	6.28	na	na	na	na	na
DO	mg/L	3.0-5.5	2.5-7	3.5-7.1	2.1-6.4	3.3-6.3	6.2-6.6	4.6-6.6	5.6-6.7	4.9-7.1	3.5-5.4
VSS	mg/L	1385	3010	2530	1435	1810	1980	2000	2040	2006	2510

experiment #		#10	#11	#12	#13	#14	#15	#16	#17	#18	#19
Date		5-Aug-06		7-Aug-06		9-Aug-06		6-Dec-06		11-Dec-06	
Biomass type		**alternating**		**alternating**		**aerobic**		**alternating**		**alternating**	
ammonia added	mg/L	67.76	82.67	57.31	63.6	18.77	78.1	3.62	17.42	14.02	67.4
average pH during nitrification	-	7.90	7.89	7.87	7.91	7.51	7.90	8.43	8.42	8.02	7.96
initial pH	-	7.78	7.81	7.88	7.79	7.43	7.65	8.54	8.64	8.63	8.26
max. pH	-	8.13	8.03	8.06	8.05	7.58	8.01	8.54	8.64	8.63	8.26
min. pH	-	7.51	7.61	7.73	7.82	7.47	7.84	8.27	8.22	7.67	7.79
AUR,	mg/gVSS h	**6.26**	**5.57**	**7.31**	**7.24**	**3.44**	**4.56**	**2.18**	**5.32**	**5.53**	**6.45**
NO3 PR	mg/gVSS h	na	na	na	na	na	na	nm	nm	nm	nm
NO2 PR,	mg/gVSS h	na	na	na	na	na	na	**na**	**2.44**	**2.58**	**3.20**
NH4 conc, initial	mg/gVSS	**35.95**	**46.18**	**31.15**	**34.76**	**10.43**	**44.37**	**2.29**	**11.06**	**9.87**	**47.63**
NH4 conc, final	mg/gVSS	23.54	35.85	9.79	13.58	3.07	34.33	0.00	6.48	1.15	39.21
NO2 conc, initial	mg/gVSS	na	na	na	na	na	na	1.29	0.07	1.48	2.00
NO2 conc., final	mg/gVSS	na	na	na	na	na	na	0.00	2.18	5.34	6.38
NO2 accumulation	mg/gVSS	na	na	na	na	na	na	2.11	2.18	5.34	6.38
DO	mg/L	5.9-6.9	4.7-6.4	6.3-7.2	4.9-7	5.9-7.1	6.4-6.8	5.2-7.8	6.8-7.8	3.2-6.9	1.8-3.6
VSS	mg/L	1885	1790	1840	1830	1800	1760	1580	1580	1420	1415

nm - not measured   na - not aplicable

Appendix 5.2   Nitrification batch experiments (no COD and anoxic zone) with alkalinity addition

experiment #		#1	#2	#3	#4	#5	#6	#7	#8
Date		10-Mar-06		22-Mar-06		29-Mar-06		4-Apr-06	
Biomass type		**aerobic**		**alternating**		**aerobic**		**alternating**	
ammonia added	mg/L	36.7	33.24	37.62	37.99	38.96	38.16	53.43	50.09
bicarbonate added	g/L	0.5	0.65	0.5	0.7	0.5	0.67	0.5	0.7
alkalinity added calculated	mg CaCO3/L	450	585	450	630	450	603	450	630
average pH during nitrification	-	**7.80**	**8.15**	**7.65**	**7.95**	**7.43**	**7.73**	**7.64**	**7.89**
initial pH	-	7.55	7.55	7.65	7.93	7.58	7.58	7.74	7.78
max. pH	-	7.93	8.18	7.78	8.33	7.63	8.03	7.86	8.25
min. pH	-	7.40	7.98	7.45	7.84	7.15	7.56	7.39	7.75
AUR,	mg/gVSS h	**2.33**	**2.54**	**5.33**	**5.62**	**3.47**	**2.19**	**6.54**	**7.46**
NO3 PR	mg/gVSS h	3.34	3.31	5.10	5.40	4.05	2.72	4.19	4.48
NO2 PR,	mg/gVSS h	**0.27**	**0.36**	**1.64**	**4.99**	**0.68**	**0.17**	**3.26**	**3.72**
NH4 conc, initial	mg/gVSS	**27.91**	**29.03**	**17.38**	**19.30**	**9.92**	**9.48**	**17.07**	**16.61**
NH4 conc, final	mg/gVSS	11.16	10.24	0.00	0.00	0.00	0.00	0.15	0.00
NO2 conc., initial	mg/gVSS	0.26	0.20	0.00	0.00	0.21	0.18	0.14	0.20
NO2 conc., final	mg/gVSS	0.00	0.08	2.32	4.84	1.91	0.58	6.41	4.64
NO2 accumulation	mg/gVSS	2.31	2.57	4.46	5.66	1.75	0.58	7.95	6.84
DO	mg/L	3.5-5	3.5-5	3.5-5	3.5-5	3.5-5	error! Low DO<1.5	3.4-6	2.5-7
VSS	mg/L	1315	1145	2165	1969	3925	4025	3130	3015

Appendix 5.3   Nitrification batch experiments (no COD and anoxic zone) with acetate addition

na - not aplicable

experiment #		#1	#2	#3	#4	#5	#6
Date		22-Apr-06		25-Apr-06		5-May-06	
Biomass type		**aerobic**		**aerobic**		**alternating**	
ammonia added	mg/L	52.59	52.85	57.08	47.9	60.68	59.77
COD resulting from acetate	mg/L	**-**	**333**	**-**	**827**	**-**	**584.8**
sodium acetate added	g/L	**-**	**0.7**	**-**	**1.1**	**-**	**1.0**
average pH during nitrification	-	7.88	8.46	7.63	8.54	7.37	8.10
initial pH	-	7.76	7.74	7.47	7.64	7.72	7.69
max. pH	-	7.96	8.81	7.71	8.89	7.80	8.60
min. pH	-	7.75	8.45	7.52	8.44	7.16	8.01
AUR,	mg/gVSS h	**2.62**	**2.40**	**2.55**	**2.26**	**6.27**	**4.76**
NO3 PR	mg/gVSS h	2.06	na-denitrification	2.67	na-denitrification	3.95	na-denitrification
NO2 PR,	mg/gVSS h	**0.72**	**2.79**	**0.19**	**2.87**	**2.76**	**0.91**
NH4 conc, initial	mg/gVSS	**19.26**	**18.66**	**23.73**	**19.79**	**19.42**	**19.34**
NH4 conc, final	mg/gVSS	10.53	10.55	13.77	11.79	3.04	3.66
NO2 conc., initial	mg/gVSS	1.58	1.45	0.32	0.30	0.50	0.25
NO2 conc., final	mg/gVSS	4.00	3.97	1.03	2.19	7.58	4.85
NO2 accumulation	mg/gVSS	4.00	4.55	1.03	4.95	7.58	4.85
DO	mg/L	4.7-6.0	0.4-5.9	3.3-4.5	0.2-6.2	2.2-6.5	0.2-6.2
VSS	mg/L	2730	2832	2405	2420	3125	3090
			8.3%		11.3%		24.1%

Appendix 5.4　　　　　　　　Nitrification batch experiments (no COD and anoxic zone) with nitrite addition

experiment #		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11
Date		9-Feb-07				23-Feb-07			11-May-07			
Biomass type		**alternating**				**alternating**			**alternating**			
ammonia added	mg/L	46.66	50.08	54.95	50.8	47.95	50.1	48.92	48.85	49.75	47.46	49.3
average pH during nitrification	-	8.09	8.15	8.20	8.14	8.28	8.37	8.29				
initial pH	-	7.93	8.01	7.96	7.95	8.05	8.15	7.92	7.63	7.46	7.49	7.47
max. pH	-	8.17	8.21	8.28	8.21	8.38	8.47	8.43	7.96	7.77	7.55	7.67
min. pH	-	7.93	8.01	7.96	7.95	8.05	8.15	7.92	7.30	7.20	7.17	7.24
AUR,	mg/gVSS h	**5.67**	**5.67**	**4.85**	**4.55**	**5.52**	**5.12**	**4.87**	**6.02**	**5.86**	**5.45**	**6.09**
NH4 conc, initial	mg/gVSS	32.86	36.28	38.95	36.82	37.76	36.44	36.24	24.49	23.86	24.98	26.72
NH4 conc, final	mg/gVSS	15.47	19.08	23.90	22.95	24.43	23.51	24.74	8.13	8.31	9.97	9.30
NO2 conc, initial	mg/gVSS	**0**	**20.3**	**40.22**	**58.45**	**16.90**	**30.68**	**46.06**	**0.38**	**9.83**	**13.97**	**17.58**
NO2 conc., final	mg/gVSS	9.63	28.55	43.45	65.99	70.66	65.27	66.47	nm	nm	nm	nm
NO2 accumulation	mg/gVSS	9.63	28.55	44.42	65.99	70.66	65.27	66.47	nm	nm	nm	nm
DO	mg/L	6.0-7.4	5.8-7.5	7.5-7.6	6.7-7.4	7.5-8.0	7.7-8.1	6.4-7.8	4.2-5.4	3.3-4.2	2.1-4.2	1.8-4.0
VSS	mg/L	1420	1380	1410	1360	1270	1375	1350	1995	2085	1900	1845

experiment #		#12	#13	#14	#15	#16	#17	#18	#19	#20	#21
Date		26-Mar-07	15-Feb-07				20-Feb-07		28-Feb-07		
Biomass type		**alternating**	**aerobic**				**aerobic**		**aerobic**		
ammonia added	mg/L	40.49	50.08	49.05	54.92	54.57	37.38	34.77	44.57	41.05	39.13
average pH during nitrification	-	7.65	7.76	7.79	7.87	7.70	7.56	7.68	7.72	7.46	7.44
initial pH	-	7.67	7.76	7.74	7.76	7.69	7.55	7.55	7.83	7.87	7.85
max. pH	-	7.72	7.84	7.87	7.93	7.75	7.65	7.92	7.87	7.87	7.85
min. pH	-	7.57	7.63	7.67	7.78	7.60	7.39	7.55	7.52	7.12	7.01
AUR,	mg/gVSS h	**5.59**	**1.37**	**2.59**	**1.58**	**1.77**	**2.38**	**2.44**	**3.58**	**2.63**	**2.51**
NH4 conc, initial	mg/gVSS	24.54	24.31	24.40	28.16	27.98	17.23	14.83	21.90	19.59	17.20
NH4 conc, final	mg/gVSS	8.04	16.73	18.63	24.34	23.81	9.61	6.42	13.46	13.23	10.87
NO2 conc, initial	mg/gVSS	**8.21**	**0.00**	**11.22**	**20.71**	**34.70**	**44.60**	**59.85**	**0.00**	**9.07**	**16.5**
NO2 conc., final	mg/gVSS	6.53	1.49	11.78	25.13	37.84	66.90	87.36	3.63	13.81	22.28
NO2 accumulation	mg/gVSS	8.21	1.76	13.58	25.85	39.98	66.90	87.36	3.63	13.81	22.28
DO	mg/L	3.0-5.2	6.2-8.2	6.6-7.8	6.6-7.8	5.6-7.6	4.3-6.4	4.0-6.4	6.6-7.3	3.7-7.5	3.8-7.6
VSS	mg/L	1650	2060	2010	1950	1950	2170	2345	2035	2095	2275

nm - not measured

Appendix 5.5   Nitrification batch experiments (no COD and anoxic zone) with nitrite addition

experiment #		#1	#2	#3	#4	#5	#6
Date		6-Feb-07			11-Feb-07		18-Feb-07
Biomass type		aerobic			aerobic		aerobic
average pH	-	7.86	7.91	8.13	7.91	7.91	7.71
NO2UR,	mg/gVSS h	**2.33**	**2.06**	**1.71**	**2.97**	**2.75**	**3.57**
NO2 conc., initial	mg/gVSS	**12.41**	**20.78**	**35.00**	**1.81**	**3.35**	**2.03**
NO2 conc., final	mg/gVSS	4.76	15.66	30.37	0.06	0.88	0.00
DO	mg/L	7.7-7.9	8-8.4	8.1-8.5	8.2-8.6	8.1-8.4	7.4-8
VSS	mg/L	2050	2105	2110	2075	2045	1575

experiment #		#7	#8	#9	#10	#11	#12	#13
Date		6-Dec-06	31-Jan-07		3-Feb-07		7-Feb-07	
Biomass type		alternating	alternating		alternating		alternating	
average pH	-	8.37	8.28	8.18	8.16	8.18	8.27	8.26
NO2UR,	mg/gVSS h	**1.27**	**3.99**	**4.34**	**3.62**	**4.03**	**1.69**	**2.76**
NO2 conc., initial	mg/gVSS	**1.34**	**16.87**	**42.56**	**10.12**	**32.37**	**1.29**	**2.83**
NO2 conc., final	mg/gVSS	0.00	0.63	27.53	0.00	21.14	0.00	0.09
DO	mg/L	5.2-7.8	6.3-7.9	4.5-7.7	7.2-7.7	7.4-7.8	8.2-8.3	8.1-8.2
VSS	mg/L	1580	1475	1495	1526	1547	1530	1640

Appendix 5.6   Nitrite production rate vs. ammonia
uptake rate - batch tests results

	Aerobic	
	AUR	NO2 PR
	mg NH_4^+-N/ gVSS h	mg NO_2^--N/ gVSS h
3/10/2006	2.33	0.27
3/10/2006	2.54	0.36
3/29/2006	3.47	0.68
3/29/2006	2.19	0.17
3/20/2006	3.87	0.80
4/22/2006	2.62	0.72
4/25/2006	2.55	0.19
2/15/2007	3.34	0.81

	Alternating	
	AUR	NO2 PR
	mg NH_4^+-N/ gVSS h	mg NO_2^--N/ gVSS h
3/22/2006	5.33	1.64
3/22/2006	5.62	1.985
4/4/2006	6.54	3.26
4/4/2006	7.46	3.72
3/20/2006	5.7	2.27
5/5/2006	6.27	2.7578
12/11/2006	5.53	2.58
12/11/2006	6.45	3.2
12/6/2006	5.32	2.44
2/9/2007	5.67	2.31

Appendix 5.7   Ammonia uptake rate vs. initial ammonia content, results

batch tests (no COD, anoxic zone)

	influent ammonia content	AUR	
		aerobic	alternating
	mg NH_4^+-N/ gVSS	mg NH_4^+-N/ gVSS h	
8-Mar-06	27.74	2.56	
10-Mar-06	27.91	2.33	
10-Mar-06	29.03	2.54	
17-Mar-06	8.91	2.92	
17-Mar-06	9.69		4.25
20-Mar-06	28.34		5.70
20-Mar-06	36.54	3.87	
22-Mar-06	17.38		5.33
22-Mar-06	19.3		5.62
29-Mar-06	9.92	3.47	
29-Mar-06	9.48	2.19	
4-Apr-06	17.07		6.54
20-Apr-06	23.73	2.55	
22-Apr-06	19.26	2.62	
5-May-06	19.42		6.27
29-Jul-06	7.43		3.89
29-Jul-06	11.28		5.11
3-Aug-06	3.99	2.88	
3-Aug-06	2.98		2.19
5-Aug-06	46.18		5.57
5-Aug-06	35.95		6.26
6-Aug-06	4.44		2.57
7-Aug-06	31.15		7.31
7-Aug-06	34.76		7.24
9-Aug-06	44.37	4.56	
9-Aug-06	10.43	3.44	
6-Dec-06	2.29		2.18
6-Dec-06	11.06		5.32
11-Dec-06	9.87		5.53
11-Dec-06	47.63		6.45
4-Jun-05	23.54	3.15	
9-Feb-07	34.16		6.05
15-Feb-07	24.66	3.34	
1-Mar-07	2.37	3.03	
average		3.03	6.23
stdev		0.63	0.96

main reactors

	influent ammonia content	AUR	
		aerobic	alternating
	mg NH_4^+-N/ gVSS	mg NH_4^+-N/ gVSS h	
6-Feb-05	24.30		6.66
9-Mar-05	30.65		6.16
11-Apr-05	23.46		6.12
12-May-05	33.42		5.56
27-Feb-05	27.93		6.20
20-Feb-05	26.59		6.29
23-Jun-05	27.94		5.71
8-Aug-06	21.11		6.46
8-Aug-06	21.61		6.27
8-Aug-06	14.19	3.19	
8-Aug-06	12.40	3.2	
17-Nov-06	15.70	3.14	
17-Nov-06	14.17	2.79	
30-Nov-06	15.40	2.78	
30-Nov-06	15.10	2.59	
9-Jan-07	20.61	3.01	
9-Jan-07	19.82	3.56	
9-Jan-07	33.00		8.86
9-Jan-07	26.26		6.54
average		3.03	6.44
stdev		0.31	0.87

Appendix 5.8   Nitrite uptake rate vs. initial nitrite content - batch tests results

	influent nitrite content	NO2UR	
		aerobic	alternating
	mg NO_2^--N/ gVSS	mg NO_2^--N/ gVSS h	
6-Dec-06	1.34		1.27
31-Jan-07	16.87		3.99
31-Jan-07	42.56		4.34
3-Feb-07	10.12		3.62
3-Feb-07	32.37		4.03
6-Feb-07	12.41	2.33	
6-Feb-07	20.78	2.06	
6-Feb-07	35.00	1.71	
7-Feb-07	1.29		1.69
7-Feb-07	2.83		2.76
11-Feb-07	1.81	2.97	
11-Feb-07	3.35	2.75	
18-Feb-07	2.03	3.57	

Appendix 5.9   Ammonia uptake rate vs. initial nitrite content - batch tests results

	influent nitrite content	AUR aerobic
	mg NO_2^--N/ gVSS	mg NH_4^+-N/ gVSS h
2/15/2007	0.00	3.37
2/28/2007	0.00	3.58
2/15/2007	11.22	2.59
2/28/2007	9.07	2.63
2/28/2007	16.50	2.51
2/15/2007	20.71	1.58
2/15/2007	34.7	1.77
2/20/2007	44.60	2.38
2/20/2007	59.85	2.44

	influent nitrite content	AUR alternating
	mg NO_2^--N/ gVSS	mg NH_4^+-N/ gVSS h
5/11/2007	0.38	6.02
5/11/2007	9.83	5.86
5/11/2007	13.97	5.45
5/11/2007	17.58	6.09
2/9/2007	0	5.67
2/23/2007	16.90	5.52
3/26/2007	8.21	5.59
2/9/2007	20.3	5.67
2/23/2007	30.68	5.12
2/9/2007	40.2	4.85
2/23/2007	46.06	4.87
2/9/2007	58.5	4.55

Appendix 6.1   FISH analyses - average DAPI area per image /μm^2/

Aerobic		
AOB Cy3	29 III	84 126
	10 IV	102 253
	10 IV	56 713
	2 V	51 563
AOB FITC	29 III	92 822
	10 IV	87 427
	10 IV	112 597
	2 V	87 116
NOB Cy3	3 IV	95 917
	4 IV	44 664
	18 IV	54 251
	8 V	47 294
NOB FITC	3 IV	107 514
	4 IV	91 509
	17 IV	60 651
	26 IV	51 597
Neg DAPI	2 V	80 374

Alternating		
AOB Cy3	27III	119 964
	28 III	106 923
	11 IV	40 596
	24 IV	70 566
	8 V	82 336
AOB FITC	26III	123 501
	28 III	64 206
	11 IV	33 062
	24 IV	56 260
NOB Cy3	5 IV	101 187
	18 IV	31 094
	19 IV	54 140
	8 V	65 747
NOB FITC	5 IV	80 562
	18 IV	55 076
	19 IV	64 280
	26 IV	53 156

av	76 964		av	67 668
stdev	22 837		stdev	26 012
	29.7%			38.4%

t-test 0.485

number of images	298		number of images	314

Appendix 6.2   FISH analyses - positive and negative controls for purchased bacteria culture and autofluorescence

	Control / Day	Bacteria / Probe	Culture	Culture + aerobic sludge (half-half)	Aerobic sludge only
area Cy3/DAPI %	POSITIVE /AOB/ 02/05/2007	*Nitrosomonas europea* **Nsm 156**	40.4%	15.4%	6.3%
DAPI area/ image, μm^2			77 427	72 675	51 563
number of images			24	20	28
area FITC/DAPI %	POSITIVE /NOB/ 26/04/2007	*Nitrobacter vinogradskij* **NIT3**	64.3%	31.9%	2.5%
DAPI area/ image, μm^2			31 008	42 237	68 796
number of images			16	12	16
area Cy3/DAPI %	NEGATIVE 25/04/2007	*Escherichia coli* **Nsm 156**	0.0%	-	-
DAPI area/ image, μm^2			nm	-	-
number of images			8	-	-
area FITC/DAPI %	NEGATIVE 17/04/2007	*Escherichia coli* **NIT3**	0.0%	-	-
DAPI area/ image, μm^2			nm	-	-
number of images			8	-	-
area Cy3/DAPI %	NEGATIVE 02/05/2007	**no probe**	-	-	0.0%
DAPI area/ image, μm^2			-	-	80 374
number of images			-	-	10
area FITC/DAPI %	NEGATIVE 02/05/2007	**no probe**	-	-	0.0%
DAPI area/ image, μm^2			-	-	80 374
number of images			-	-	10

Appendix 6.3   FISH analysis - the summary of results

AOB aerobic   148 images

NO$_2^-$ max	AUR 20 °C	T
mg/L	mg/g VSS h	°C
4.8	3.42	21.3
-	-	-
7.8	2.97	24.7
5.8	1.57	24.7

	av	23.6
	stdev	2.0

Nitrosomonas

Day	area Cy3/DAPI %	number of images	total DAPI area µm²	average DAPI per image µm²
29 III	2.4%	10	841 263	84 126
10 IV	1.9%	18	1 840 550	102 253
10 IV	4.7%	18	1 020 834	56 713
2 V	6.3%	28	1 443 778	51 563

Nitrosomonas

av	**3.8%**		73 664
stdev	**2.1%**		23 823
count	4		
	54%		32%
t-test	0.007		

Nitrosospira

Day	area FITC/DAPI %	number of images	total DAPI area µm²	average DAPI per image µm²
29 III	18.5%	10	928 220	92 822
10 IV	12.1%	18	1 573 681	87 427
10 IV	19.4%	19	2 139 340	112 597
2 V	14.6%	27	2 352 138	87 116

Nitrosospira

av	**16.1%**		94 990
stdev	**3.4%**		12 026
count	4		
	21%		13%

AOB alternating   142 images

NO$_2^-$ max	AUR 20 °C	T
mg/L	mg/g VSS h	°C
-	-	-
16.5	5.20	24.7
-	4.52	24.5
25.0	-	23.7

	av	24.3
	stdev	0.5

Nitrosomonas

Day	area Cy3/DAPI %	number of images	total DAPI area µm²	average DAPI per image µm²
28 III	22.6%	12	1 283 076	106 923
11 IV	21.2%	26	1 055 497	40 596
24 IV	19.9%	29	2 999 104	103 417
8 V	20.8%	25	1 465 399	58 616

Nitrosomonas

av	**21.1%**		77 388
stdev	**1.1%**		32 944
count	4		
	5%		43%
t-test	0.001		

Nitrosospira

Day	area FITC/DAPI %	number of images	total DAPI area µm²	average DAPI per image µm²
26 III	3.2%	11	1 358 506	123 501
28 III	6.0%	12	770 476	64 206
11 IV	5.2%	26	859 600	33 062
24 IV	5.1%	26	1 462 767	56 260

Nitrosospira

av	**4.8%**		69 257
stdev	**1.2%**		38 501
count	4		
	25%		56%

NOB aerobic   140 images

NO$_2^-$ max	AUR 20 °C	T
mg/L	mg/g VSS h	°C
7.9	2.33	22.4
-	-	-
6.4	2.15	23.5
na	1.99	23.7

	av	23.2
	stdev	0.7

Nitrospira

Day	area Cy3/DAPI %	number of images	total DAPI area µm²	average DAPI per image µm²
3 IV	4.9%	10	959 169	95 917
4 IV	8.7%	19	848 624	44 664
18 IV	7.8%	26	1 410 522	54 251
8 V	10.1%	15	709 405	47 294

Nitrospira

av	**7.9%**		60 531
stdev	**2.2%**		23 934
count	4		
	28%		40%
t-test	0.013		

Nitrobacter

Day	area FITC/DAPI %	number of images	total DAPI area µm²	average DAPI per image µm²
3 IV	1.6%	10	1 075 137	107 514
4 IV	4.8%	19	1 738 678	91 509
17 IV	2.8%	25	1 516 285	60 651
26 IV	2.5%	16	825 547	51 597

Nitrobacter

av	**2.9%**		77 818
stdev	**1.3%**		26 150
count	4		
	46%		34%

NOB alternating   172 images

NO$_2^-$ max	AUR 20 °C	T
mg/L	mg/g VSS h	°C
23.9	-	23.5
23.2	4.77	25.0
-	-	-
-	-	-

	av	24.3
	stdev	1.1

Nitrospira

Day	area Cy3/DAPI %	number of images	total DAPI area µm²	average DAPI per image µm²
5 IV	0.9%	26	2 630 860	101 187
18 IV	5.2%	26	808 451	31 094
19 IV	2.8%	18	974 517	54 140
8 V	4.3%	16	1 051 946	65 747

Nitrospira

av	**3.3%**		63 042
stdev	**1.9%**		29 225
count	4		
	57%		46%
t-test	0.007		

Nitrobacter

Day	area FITC/DAPI %	number of images	total DAPI area µm²	average DAPI per image µm²
5 IV	9.1%	26	2 094 601	80 562
18 IV	11.9%	26	1 431 978	55 076
19 IV	11.7%	18	1 157 039	64 280
26 IV	8.8%	16	850 489	53 156

Nitrobacter

av	**10.4%**		63 268
stdev	**1.7%**		12 509
count	4		
	16%		20%

Appendix 6.4.   FISH analysis - detailed counting results

Cy3, FITC, DAPI - area μm^2

Aerobic   AOB   Nsm 156

3/29/2007	Cy3/DAPI %	Cy3	DAPI
R1-1	0.0%	0	86 592
R1-2	0.0%	0	104 826
R1-3	6.9%	17 519	252 755
R1-4	0.0%	0	119 067
R1-5	3.1%	876	28 596
R1-6	0.0%	0	23 536
R1-7	0.0%	0	23 891
R1-8	0.0%	0	62 442
R1-9	2.0%	1 619	79 390
R1-10	0%	0	60 168
	2.4%	**20 014**	**841 263**

4/10/2007	Cy3/DAPI %	Cy3	DAPI
R1-1	0.0%	0	77 217
R1-2	0.0%	0	110 356
R1-3	5.2%	4 638	90 000
R1-4	6.4%	4 996	78 124
R1-5	1.9%	2 253	117 336
R1-6	0.0%	0	94 579
R1-7	8.1%	3 193	39 303
R1-8	3.8%	4 587	120 810
R1-9	4.0%	4 574	113 559
R1-10	0.0%	0	125 101
R1-11	0.0%	0	47 848
R1-12	3.5%	3 713	104 772
R1-13	0.0%	0	28 931
R1-14	0.0%	0	79 000
R1-15	0.4%	753	188 083
R1-16	0.0%	0	171 164
R1-17	2.6%	3 503	136 639
R1-18	2.0%	2 379	117 727
	1.9%	**34 589**	**1 840 550**

4/10/2007	Cy3/DAPI %	Cy3	DAPI
R1-19	8.7%	12 311	141 617
R1-20	0.0%	0	52 284
R1-21	0.0%	0	122 092
R1-22	1.7%	2 091	122 044
R1-23	6.5%	2 907	45 036
R1-24	20.4%	12 896	63 077
R1-25	0.0%	0	19 198
R1-26	10.2%	3 421	33 539
R1-27	3.9%	2 976	75 802
R1-28	2.6%	708	26 885
R1-29	18.3%	10 218	55 710
R1-30	0.0%	0	42 488
R1-31	0.0%	0	29 478
R1-32	0.0%	0	66 743
R1-33	0.0%	0	12 321
R1-34	0.0%	0	26 560
R1-35	0.0%	0	45 007
R1-36	0.0%	0	40 953
	4.7%	**47 528**	**1 020 834**

5/2/2007	Cy3/DAPI %	Cy3	DAPI
R1-1	19.6%	27 118	138 322
R1-2	0.0%	0	63 523
R1-3	0.0%	0	64 925
R1-4	0.0%	0	93 299
R1-5	0.0%	0	99 023
R1-6	0.0%	0	66 826
R1-7	0.0%	0	81 471
R1-8	0.0%	0	32 097
R1-9	31.1%	13 057	42 037
R1-10	0.0%	0	31 180
R1-11	0.0%	0	27 281
R1-12	0.0%	0	29 824
R1-13	39.0%	18 797	48 147
R1-14	0.0%	0	37 431
R1-15	0.0%	0	37 453
R1-16	0.0%	0	50 501
R1-17	0.0%	0	48 197
R1-18	26.0%	14 632	56 345
R1-19	0.0%	0	38 296
R1-20	0.0%	0	41 181
R1-21	13.4%	7 792	57 980
R1-22	0.0%	0	27 759
R1-23	0.0%	0	39 495
R1-24	0.0%	0	37 080
R1-25	0.0%	0	26 618
R1-26	0.0%	0	47 955
R1-27	0.0%	0	39 525
R1-28	23.5%	9 385	40 009
	6.3%	**90 781**	**1 443 778**

Aerobic   AOB   Nsv 443

3/29/2007	FITC/DAPI %	FITC	DAPI
R1-1	19%	21 056	113 558
R1-2	8%	9 149	114 236
R1-3	27%	12 846	48 263
R1-4	7%	5 669	79 197
R1-5	14%	17 895	129 990
R1-6	9%	5 317	61 646
R1-7	20%	29 839	146 039
R1-8	64%	39 069	60 670
R1-9	12%	17 049	145 033
R1-10	46%	13 464	29 589
	18.5%	**171 353**	**928 220**

4/10/2007	FITC/DAPI %	FITC	DAPI
R1-1	6%	4 266	73 397
R1-2	0%	0	79 168
R1-3	14%	3 636	25 399
R1-4	0%	0	69 802
R1-5	11%	10 247	91 138
R1-6	22%	9 836	44 524
R1-7	69%	33 674	49 091
R1-8	34%	20 042	58 859
R1-9	10%	4 585	46 787
R1-10	10%	4 701	46 356
R1-11	0%	0	133 979
R1-12	0%	0	50 526
R1-13	1%	1 870	200 063
R1-14	26%	9 580	36 726
R1-15	0%	0	131 501
R1-16	0%	0	172 317
R1-17	0%	0	145 951
R1-18	74%	87 980	118 097
	12.1%	**190 417**	**1 573 681**

4/10/2007	FITC/DAPI %	FITC	DAPI
R1-19	0%	0	137 331
R1-20	3%	4 505	158 726
R1-21	37%	72 769	194 915
R1-22	38%	67 724	179 907
R1-23	37%	106 660	291 556
R1-24	32%	8 979	28 122
R1-25	0%	0	103 225
R1-26	8%	5 761	70 370
R1-27	67%	15 171	22 740
R1-28	16%	7 157	45 176
R1-29	18%	3 815	20 627
R1-30	69%	40 350	58 072
R1-31	0%	0	90 033
R1-32	18%	18 896	106 622
R1-33	0%	0	111 109
R1-34	0%	0	151 123
R1-35	24%	28 414	119 753
R1-36	17%	11 381	65 547
R1-37	12%	22 438	184 385
	19.4%	**414 020**	**2 139 340**

5/2/2007	FITC/DAPI %	FITC	DAPI
R1-1	0%	0	68 506
R1-2	33%	4 071	12 446
R1-3	53%	1 905	3 616
R1-4	4%	9 755	230 070
R1-5	30%	12 131	40 635
R1-6	27%	13 614	50 987
R1-7	47%	54 173	116 177
R1-8	0%	0	57 802
R1-9	0%	0	77 012
R1-10	0%	0	59 165
R1-11	0%	0	76 746
R1-12	61%	106 844	175 103
R1-13	50%	47 624	94 485
R1-14	0%	0	74 304
R1-15	55%	13 644	24 932
R1-16	38%	36 072	95 746
R1-17	0%	0	283 259
R1-18	0%	0	70 565
R1-19	0%	0	73 711
R1-20	0%	0	34 642
R1-21	1%	2 636	247 945
R1-22	0%	0	103 144
R1-23	35%	19 075	55 119
R1-24	29%	18 577	64 619
R1-25	0%	0	97 598
R1-27	0%	0	49 687
R1-28	27%	3 836	14 117
	14.6%	**343 957**	**2 352 138**

Alteranating   AOB   Nsm 156

3/28/2007	Cy3/DAPI %	Cy3	DAPI
R3-1	7%	8 371	112 392
R3-2	19%	21 409	112 206
R3-3	25%	12 413	48 866
R3-4	4%	1 643	44 393
R3-5	32%	19 847	61 860
R3-6	31%	41 107	131 044
R3-7	13%	14 214	107 751
R3-8	0%	0	117 985
R3-9	19%	42 072	222 753
R3-10	79%	121 671	153 907
R3-11	15%	6 731	46 160
R3-12	0%	0	123 760
	22.6%	**289 479**	**1 283 076**

4/11/2007	Cy3/DAPI %	Cy3	DAPI
R3-1	45%	18 393	41 311
R3-2	53%	31 828	60 221
R3-3	0%	0	31 942
R3-4	0%	0	14 356
R3-5	45%	8302	18450
R3-6	5%	1 147	25 338
R3-7	10%	4 589	48 076
R3-8	14%	6 507	46 924
R3-9	0%	0	6 254
R3-10	0%	0	9919.03533
R3-11	0%	0	21 557
R3-12	0%	0	58 256
R3-13	0%	0	21 645
R3-14	0%	0	23 280
R3-15	21%	13453	63091.00883
R3-16	0%	0	18 950
R3-17	65%	15 108	23 411
R3-18	26%	5 257	20 017
R3-19	0%	0	33 836
R3-20	11%	6674	62096
R3-21	0%	0	63 328
R3-22	0%	0	39 074
R3-23	27%	9 933	36 944
R3-24	41%	29 937	73 825
R3-25	43%	73139	171204
R3-26	0%	0	22 192
	21.2%	**224 267**	**1 055 497**

4/24/2007	Cy3/DAPI %	Cy3	DAPI
R3-1	0%	0	49 639
R3-2	0%	0	32 049
R3-3	0%	0	55 367
R3-4	0%	0	78 225
R3-5	0%	0	155332
R3-6	14%	12 899	93 343
R3-7	4%	3 630	90 010
R3-8	0%	0	87 747
R3-9	0%	0	61 373
R3-10	61%	98 950	162 118
R3-11	57%	12850	22 542
R3-12	22%	7 831	36 318
R3-13	18%	44 441	240 570
R3-14	48%	55 837	116 192
R3-15	22%	4 213	19 006
R3-16	39%	17 888	45 824
R3-17	27%	9341	34 854
R3-18	16%	8 290	51 285
R3-19	27%	16 440	61 774
R3-20	18%	17 521	98 332
R3-21	45%	15 162	33 824
R3-22	15%	4 277	28 332
R3-23	42%	9435	22647
R3-24	52%	2 536	4 883
R3-25	4%	3 153	84 074
R3-26	0%	0	90 384
R3-27	0%	0	15 053
R3-28	34%	59 731	175 312
R3-29	42%	5 062	11 987
	19.9%	**409 487**	**2 058 396**

5/8/2007	Cy3/DAPI %	Cy3	DAPI
R3-1	0%	0	55 487
R3-2	0%	0	49 137
R3-3	0%	0	8 645
R3-4	0%	0	115 511
R3-5	0%	0	276 303
R3-6	0%	0	44 862
R3-7	0%	0	82 980
R3-8	45%	3 872	8 627
R3-9	42%	5 829	14 042
R3-10	68%	6 993	10 312
R3-11	66%	6 171	9 385
R3-12	64%	8 646	13 422
R3-13	70%	16 659	23 959
R3-14	67%	17 143	25 611
R3-15	40%	10 037	25 182
R3-16	51%	6 140	11 972
R3-17	45%	8 742	19 547
R3-18	0%	0	16 970
R3-19	5%	2 413	51 486
R3-20	90%	7 721	8 561
R3-21	56%	130 596	232 575
R3-22	37%	53 992	144 641
R3-23	0%	0	108 029
R3-24	0%	0	82 380
R3-25	74%	19 198	25 773
	20.8%	**304 152**	**1 465 399**

Alteranating AOB   Nsv 443

3/26/2007	FITC/DAPI %	FITC	DAPI
R3-1	0%	0	184 203
R3-2	0%	0	166 411
R3-3	0%	0	195 801
R3-4	0%	0	5 352
R3-5	0%	0	49 538
R3-6	0%	0	151 378
R3-7	34%	42 832	126 080
R3-8	0%	0	143 286
R3-9	0%	0	210 725
R3-10	0%	0	33 360
R3-11	0%	0	92 372
	3.2%	**42 832**	**1 358 506**

3/28/2007	FITC/DAPI %	FITC	DAPI
R3-1	36%	18 225	50 935
R3-2	58%	17 150	29 431
R3-3	5%	621	13 613
R3-4	0%	0	29 721
R3-5	15%	3 133	20 590
R3-6	12%	5 598	48 417
R3-7	1%	795	114 202
R3-8	3%	395	11 531
R3-9	0%	0	49 280
R3-10	0%	0	65 535
R3-11	0%	0	133 083
R3-12	0%	0	204 138
	6.0%	**45 917**	**770 476**

4/11/2007	FITC/DAPI %	FITC	DAPI
R3-1	0%	0	58 850
R3-2	0%	0	35 921
R3-3	0%	0	26 870
R3-4	0%	1 464	6 919
R3-5	0%	0	106 408
R3-6	0%	0	19 747
R3-7	0%	2 993	14 116
R3-8	0%	0	13 142
R3-9	0%	6 418	18 128
R3-10	0%	0	54 687
R3-11	0%	2 188	21 241
R3-12	0%	1 669	19 515
R3-13	0%	16 703	23 122
R3-14	0%	0	47 192
R3-15	0%	0	42 342
R3-16	0%	0	30 659
R3-17	0%	0	11 097
R3-18	0%	0	30 260
R3-19	0%	0	12 340
R3-20	0%	0	24 377
R3-21	0%	12 263	51 236
R3-22	0%	0	23 755
R3-23	0%	0	80 486
R3-24	0%	0	41 862
R3-25	0%	0	30 147
R3-26	0%	931	15 181
	5.2%	**44 629**	**859 600**

4/24/2007	FITC/DAPI %	FITC	DAPI
R3-1	0%	0	118 659
R3-2	64%	33 199	51 866
R3-3	7%	2 849	40 727
R3-4	5%	2 145	47 037
R3-5	0%	0	65 079
R3-6	0%	0	70 352
R3-7	0%	0	100 477
R3-8	0%	0	102 356
R3-9	0	0	80815
R3-10	0%	0	91 094
R3-11	0%	0	69 254
R3-12	0%	0	86 331
R3-13	14%	2 695	19 154
R3-14	0%	0	13 004
R3-15	18%	11 579	65 507
R3-16	0%	0	17 636
R3-17	7%	4 606	61 500
R3-18	0%	0	43 361
R3-19	0%	0	19 592
R3-20	0%	0	61 483
R3-21	24%	10 087	41 531
R3-22	0%	0	28 174
R3-23	32%	6 761	21 015
R3-24	0%	0	43 315
R3-25	0%	0	72 672
R3-26	0%	0	30 776
	5.1%	**73 921**	**1 462 767**

Aerobic   NOB   Ntspa 662

5/8/2007	Cy3/DAPI %	Cy3	DAPI
R1-1	32.2%	6 282	19 534
R1-2	86.2%	3 887	4 509
R1-3	39.3%	18 355	46 725
R1-4	0.0%	0	27 817
R1-5	27.6%	8 919	32 356
R1-6	25.3%	18 366	72 663
R1-7	0.0%	0	91 760
R1-8	0.0%	0	52 176
R1-9	17.3%	6 480	37 441
R1-10	0.0%	0	106 243
R1-11	0	0	67276
R1-12	45.5%	9 194	20 218
R1-13	0.0%	0	66 356
R1-14	0.0%	0	25 390
R1-15	0.0%	0	38 941
	10.1%	**71 483**	**709 405**

5/3/2007	Cy3/DAPI %	Cy3	DAPI
R1-1	2.5%	1 662	65 243
R1-2	26.5%	32 380	122 203
R1-3	0.0%	0	71 743
R1-4	6.0%	8 569	143 756
R1-5	0.0%	0	49 776
R1-6	0.0%	0	185 427
R1-7	0.0%	0	70502
R1-8	0.0%	0	148 197
R1-9	7.3%	3 937	53 899
R1-10	0.0%	0	48 423
	4.9%	**46 548**	**959 169**

5/4/2007	Cy3/DAPI %	Cy3	DAPI
R1-11	3.7%	930	25 375
R1-12	28.7%	2 524	8 807
R1-13	8.3%	7 522	90 540
R1-14	10.2%	3792	37209
R1-15	7.1%	3 883	54 345
R1-16	0.0%	0	24 010
R1-17	5.3%	3 472	65 318
R1-18	7.2%	3256	45504
R1-19	3.0%	1 149	38 608
R1-20	13.5%	14 219	104 951
R1-21	0.9%	616	71 442
R1-22	0.0%	0	71124
R1-23	4.8%	1 998	41 460
R1-24	6.3%	3 923	61 922
R1-25	35.8%	24 594	68 700
R1-26	23.0%	1809.557	7853.982
R1-27	0.0%	0	24 477
R1-28	0.0%	0	6 290
R1-29	49.5%	340	687
	8.7%	**74028.04**	**848 624**

4/18/2007	Cy3/DAPI %	Cy3	DAPI
R1-1	7.1%	1 152	16 174
R1-2	29.6%	19 628	66 316
R1-3	1.5%	1 735	116 620
R1-4	0.0%	0	39 996
R1-5	21.7%	19 084	88 098
R1-6	35.2%	13 416	38 148
R1-7	10.8%	6 571	60 803
R1-8	0.0%	0	45 000
R1-9	16.0%	8 873	55 473
R1-10	0.0%	0	41 967
R1-11	4.1%	1 457	35 777
R1-12	0.0%	0	74 708
R1-13	17.3%	5 856	33 819
R1-14	6.1%	2 129	34 888
R1-15	30.7%	3 388	11 020
R1-16	17.3%	6 244	36 181
R1-17	11.1%	4 473	40 385
R1-18	9.9%	3 663	37 103
R1-19	0.0%	0	50 530
R1-20	0.0%	0	67 525
R1-21	0.0%	0	67 191
R1-22	5.7%	3 392	59 826
R1-23	0.0%	0	61 228
R1-24	0.0%	0	89 011
R1-25	0.0%	0	49 748
R1-26	9.5%	8 851	92 989
	7.8%	**109 912**	**1 410 522**

Aerobic NOB Nit3

5/3/2007	FITC/DAPI %	FITC	DAPI
R1-1	0.0%	0	129 323
R1-2	0.0%	0	174 578
R1-3	26.5%	3 632	13 702
R1-4	0.5%	254	53 161
R1-5	7.5%	9 336	124 411
R1-6	0.0%	0	45 156
R1-7	1.4%	2 642	187 758
R1-8	0.2%	609	302 032
R1-9	7.7%	616	7 961
R1-10	0.0%	0	37 055
	1.6%	**17 089**	**1 075 137**

5/4/2007	FITC/DAPI %	FITC	DAPI
R1-11	0.0%	0	94 837
R1-12	0.0%	0	159 221
R1-13	0.4%	1 134	267 115
R1-14	0.0%	0	134 424
R1-15	0.0%	0	61 367
R1-16	0.0%	0	81 300
R1-17	3.1%	1 509	49 374
R1-18	0.0%	0	92 313
R1-19	0.0%	0	92 144
R1-20	5.5%	9 020	164 076
R1-21	0.0%	0	42 578
R1-22	0.0%	0	120 833
R1-23	14.7%	5 092	34 553
R1-24	61.0%	46 937	76 935
R1-25	5.4%	3 121	58 307
R1-26	1.7%	714	42 370
R1-27	5.3%	2 290	43 012
R1-28	4.0%	2 199	55 172
R1-29	15.6%	10 750	68 748
	4.8%	**82 766**	**1 738 678**

4/17/2007	FITC/DAPI %	FITC	DAPI
R1-1	0.0%	0	19 256
R1-2	53.0%	8 353	15 769
R1-3	7.1%	2 011	28 164
R1-4	31.6%	1 349	4 268
R1-5	0.0%	0	108 672
R1-6	15.9%	1 540	9 698
R1-7	16.8%	1 800	10 721
R1-8	0.0%	0	48 594
R1-9	0.0%	0	51 101
R1-10	0.0%	0	197 929
R1-11	0.0%	0	21 128
R1-12	0.0%	0	204 495
R1-13	0.0%	0	72 948
R1-14	0.0%	0	31 749
R1-15	3.3%	547	16 576
R1-16	24.9%	13 910	55 909
R1-17	0.0%	0	47 258
R1-18	0.0%	0	79 167
R1-19	11.2%	1 669	14 947
R1-20	0.6%	531	93 923
R1-21	0.0%	0	33 214
R1-22	9.3%	4 329	46 390
R1-23	0.0%	0	68 339
R1-24	19.7%	5 958	30 191
R1-25	0.0%	0	172 530
	2.8%	**41 997**	**1 482 937**

4/26/2007	FITC/DAPI %	FITC	DAPI
R1-1	0.0%	0	49 997
R1-2	0.0%	0	135 413
R1-3	26.6%	6 767	25 479
R1-4	0.0%	0	50 654
R1-5	4.9%	1 873	38 496
R1-6	13.6%	4 475	32 811
R1-7	14.4%	4 778	33 244
R1-8	17.7%	2 830	15 989
R1-9	0.0%	0	21 156
R1-10	0.0%	0	115 483
R1-11	0.0%	0	61 218
R1-12	0.0%	0	95 831
R1-13	0.0%	0	30 705
R1-14	0.0%	0	88 148
R1-15	0.0%	0	24 211
R1-16	0.0%	0	6 712
	2.5%	**20 723**	**825 547**

Alternating   NOB   Ntspa 662

4/5/2007	Cy3/DAPI %	Cy3	DAPI
R3-1	1.1%	531	48 352
R3-2	0.0%	0	14 889
R3-3	0.0%	0	17 525
R3-4	0.0%	0	78 122
R3-5	0.0%	0	205 806
R3-6	0.0%	0	48 987
R3-7	0.8%	1 018	123 546
R3-8	4.7%	1 257	26 962
R3-9	2.4%	1 527	62 920
R3-10	0.0%	0	119 706
R3-11	0.0%	0	212 451
R3-12	0.0%	0	51 811
R3-13	0.0%	0	189 120
R3-14	0.0%	0	32 258
R3-15	3.6%	4 131	115 850
R3-16	0.0%	0	55 760
R3-17	0.0%	0	122 040
R3-18	0.0%	0	105 239
R3-19	0.7%	1 313	182 966
R3-20	0.0%	0	86 705
R3-21	0.0%	0	151 356
R3-22	4.5%	6 552	145 013
R3-23	5.2%	5 216	99 968
R3-24	0.0%	0	105 253
R3-25	1.1%	1 831	172 430
R3-26	0.0%	0	55 825
	0.9%	**23 376**	**2 630 860**

4/18/2007	Cy3/DAPI %	Cy3	DAPI
R3-1	0.0%	0	69 087
R3-2	0.0%	0	24 208
R3-3	0.0%	0	37 977
R3-4	0.0%	0	39 724
R3-5	0.0%	0	36 906
R3-6	5.7%	1 988	34 964
R3-7	20.2%	16 237	80 580
R3-8	23.1%	2 675	11 605
R3-9	21.8%	4 832	22 129
R3-10	0.0%	0	24 891
R3-11	0.0%	0	24 426
R3-12	0.0%	0	27 255
R3-13	0.0%	0	19 842
R3-14	0.0%	0	22 836
R3-15	32.4%	3 420	10 555
R3-16	0.0%	0	15 877
R3-17	0.0%	0	18 503
R3-18	0.0%	0	25 636
R3-19	0.0%	0	44 037
R3-20	0.0%	0	45 940
R3-21	0.0%	0	27 259
R3-22	0.0%	0	24 180
R3-23	26.0%	9 725	37 398
R3-24	0.0%	0	31 028
R3-25	0.0%	0	33 304
R3-26	17.5%	3 210	18 305
	5.2%	**42 087**	**808 451**

4/19/2007	Cy3/DAPI %	Cy3	DAPI
R3-1	0.0%	0	82 964
R3-2	2.3%	972	42 364
R3-3	2.0%	3 209	162 388
R3-4	12.5%	15 878	126 794
R3-5	0.0%	0	18 674
R3-6	19.1%	4 652	24 305
R3-7	0.0%	0	22 223
R3-8	0.0%	0	34 993
R3-9	0.0%	0	93 453
R3-10	0.0%	0	24 846
R3-11	0.0%	0	34 628
R3-12	0.0%	0	26 781
R3-13	6.5%	2 802	43 056
R3-14	0.0%	0	59 076
R3-15	0.0%	0	71 218
R3-16	0.0%	0	50 278
R3-17	0.0%	0	36 125
R3-18	0.0%	0	20 351
	2.8%	**27 513**	**974 517**

5/8/2007	Cy3/DAPI %	Cy3	DAPI
R3-1	34.6%	13 213	38 135
R3-2	38.1%	10 583	27 765
R3-3	0.0%	0	24 485
R3-4	0.0%	0	128 053
R3-5	0.0%	0	41 636
R3-6	0.0%	0	69 704
R3-7	0.0%	0	86 027
R3-8	0.0%	0	72 937
R3-9	30.5%	5 108	16 758
R3-10	0.0%	0	74 995
R3-11	1.0%	679	69 757
R3-12	7.7%	4 977	64 695
R3-13	6.4%	6 200	96 441
R3-14	0.0%	0	75 314
R3-15	0.0%	0	109 171
R3-16	8.1%	4 514	56 073
	4.3%	**45 274**	**1 051 946**

Alternating   NOB   Nit 3

4/5/2007	Cy3/DAPI %	FITC	DAPI
R3-1	0.0%	0	214 213
R3-2	11.7%	7 969	68 034
R3-3	0.0%	0	89 700
R3-4	55.2%	65 104	118 017
R3-5	4.1%	2 978	72 660
R3-6	1.2%	1 320	107 397
R3-7	21.7%	5 534	25 514
R3-8	19.4%	19 760	102 048
R3-9	3.0%	5 082	167 061
R3-10	27.7%	6 590	23 764
R3-11	23.2%	11 800	50 820
R3-12	0.0%	0	52 566
R3-13	4.4%	1 963	44 866
R3-14	21.5%	3 421	15 881
R3-15	0.0%	0	16 577
R3-16	23.7%	5 774	24 348
R3-17	7.9%	6 986	88 758
R3-18	0.0%	0	26 863
R3-19	0.0%	0	48 898
R3-20	0.0%	0	48 393
R3-21	6.8%	5 840	85 553
R3-22	3.3%	1 563	48 038
R3-23	32.0%	15 664	48 941
R3-24	0.0%	0	239 295
R3-25	12.0%	21 253	177 619
R3-26	2.8%	2 476	88 776
	9.1%	**191 078**	**2 094 601**

4/18/2007	Cy3/DAPI %	FITC	DAPI
R3-1	1.2%	707	58 053
R3-2	0.0%	0	25 017
R3-3	34.4%	11 398	33 146
R3-4	98.6%	18 029	18 288
R3-5	16.9%	5 948	35 168
R3-6	63.8%	46 013	72 065
R3-7	0.0%	0	73 667
R3-8	18.4%	1 985	10 794
R3-9	1.0%	305	29 367
R3-10	15.6%	5 219	33 433
R3-11	39.4%	43 035	109 123
R3-12	0.0%	0	73 860
R3-13	18.9%	3 399	18 012
R3-14	34.5%	30 989	89 880
R3-15	0.0%	0	159 845
R3-16	0.0%	0	27 630
R3-17	0.0%	0	27 826
R3-18	0.0%	0	101 434
R3-19	4.1%	746	18 136
R3-20	0.0%	0	54 383
R3-21	1.5%	582	38 511
R3-22	0.0%	0	7 809
R3-23	0.0%	0	83 053
R3-24	0.6%	1 186	205 870
R3-25	0.0%	0	21 785
R3-26	15.3%	893	5 823
	11.9%	**170 434**	**1 431 978**

4/19/2007	Cy3/DAPI %	FITC	DAPI
R3-1	0.0%	0	19 743
R3-2	27.0%	3 803	14 085
R3-3	0.0%	0	50 128
R3-4	0.0%	0	55 934
R3-5	0.0%	0	85 916
R3-6	14.0%	2 929	20 950
R3-7	0.0%	0	56 354
R3-8	36.0%	46 850	130 120
R3-9	22.1%	14 467	65 372
R3-10	16.7%	17 520	105 052
R3-11	8.0%	3 911	48 633
R3-12	10.2%	7 836	77 005
R3-13	7.5%	12 827	171 032
R3-14	1.7%	1 145	66 075
R3-15	22.4%	23 851	106 272
R3-16	0.0%	0	28 008
R3-17	0.0%	0	24 252
R3-18	0.0%	0	32 109
	11.7%	**135 139**	**1 157 039**

4/26/2007	Cy3/DAPI %	FITC	DAPI
R3-1	0.0%	0	9 807
R3-2	4.3%	1 463	33 844
R3-3	13.9%	5 848	41 928
R3-4	13.3%	12 925	97 528
R3-5	18.2%	11 386	62 579
R3-6	65.9%	42 925	65 129
R3-7	0.0%	0	19 568
R3-8	0.0%	0	91 030
R3-9	0.0%	0	26 284
R3-10	0.0%	0	2 731
R3-11	0.0%	0	100 556
R3-12	0.0%	0	91 740
R3-13	0.0%	0	36 215
R3-14	0.0%	0	61 263
R3-15	0.0%	0	50 670
R3-16	0.0%	0	59 617
	8.8%	**74 547**	**850 489**

Appendix 7.1   Fate of the estrogens EE2, E2, and the metabolite E1 in activated sludge batch experiments under aerobic and anoxic conditions (Experiment 1)

Experiment #1, July 27 2005

Total and volatile suspended solids in the beginning of the reaction, mg/L

	aerobic #1	anoxic #2
TSS	4450	4520
stdev	42	14
%	0.9	0.3
VSS	3525	3530
stdev	7	0
%	0.2	0.0
VSS/TSS	0.79	0.78

pH

time		pH
10.92	0	7.73
11.42	0.50	7.71
11.88	0.97	7.60
12.40	1.48	7.52
12.90	1.98	7.43
13.40	2.48	7.45
13.90	2.98	7.48
14.88	3.97	7.29
15.87	4.95	7.34
16.88	5.97	7.47
17.87	6.95	7.59

DO, mg/L

time		DO
10.92	0	1.3
11.42	0.50	1.9
11.88	0.97	5.2
12.40	1.48	4.1
12.90	1.98	5.9
13.40	2.48	3.1
13.90	2.98	5.3
14.88	3.97	5.2
15.87	4.95	7.5
16.88	5.97	7.3
17.87	6.95	7.4

NH$_4^+$, mg/L

time			NH4+
10.92	0	54.1	27.42
11.42	0.50	67.7	34.31
11.88	0.97	70.2	35.58
12.40	1.48	57.4	29.09
12.90	1.98	26	13.18
13.40	2.48	13	6.59
13.90	2.98	1.2	0.61
14.88	3.97	0	0.00
15.87	4.95	0	0.00
16.88	5.97	0	0.00
17.87	6.95	0	0.00

Aerobic reactor pH and ammonia

y = -18.365x + 53.372
R^2 = 0.9678
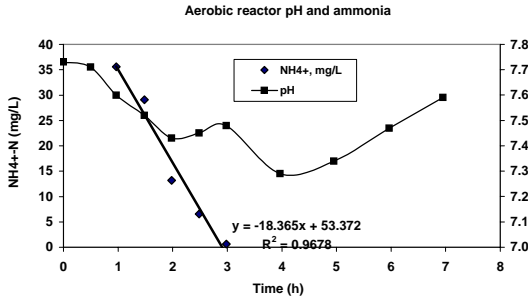
COD (feed)   495 mg/L
F/M   aerobic   0.14 g/g d
       anoxic   0.14 g/g d

AUR   18.365 mg/Lh
       5.21 mg/gVSS h

ESTROGEN REMOVAL:

AEROBIC   mg/L

	E2	EE2	E1	E2+E1	log E2
0.00	5.56	4.56	0.50		
0.00	3.92	5.80	1.94		
0.00	4.74	5.18	1.22	5.96	0.676
0.25	2.90	4.66	2.10	5.00	0.462
0.50	1.40	5.40	3.14	4.54	0.146
0.75	1.26	6.16	3.74	5.00	0.100
1.00	0.62	7.60	4.06	4.68	-0.208
1.50	0.48	4.36	3.86	4.34	-0.319
2.00	0.32	4.22	3.88	4.20	-0.495
2.50	0.36	4.82	3.80	4.16	-0.444
3.00	0.22	3.80	3.60	3.82	-0.658
4.00	0.46	4.40	4.06	4.52	-0.337
5.00	0.20	6.50	4.16	4.36	-0.699
6.00	0.22	4.14	3.80	4.02	-0.658
7.00	0.14	4.04	3.44	3.58	-0.854

ANOXIC   mg/L

		E2	EE2	E1	E2+E1	log E2
	0.00	4.40	1.38	0.40		
	0.00	3.82	1.58	0.44		
av	0.00	4.11	1.48	0.42	4.53	0.614
	0.25	2.82	2.76	1.86	4.68	0.450
	0.50	1.16	4.14	2.80	3.96	0.064
	0.75	1.10	3.68	3.24	4.34	0.041
	1.00	1.92	3.54	3.42	5.34	0.283
	1.50	0.26	3.56	3.52	3.78	-0.585
	2.00	0.42	4.20	3.42	3.84	-0.377
	2.50	0.44	3.84	3.46	3.90	-0.357
	3.00	0.42	3.78	3.48	3.90	-0.377
	4.00	0.42	3.92	3.62	4.04	-0.377
	5.00	0.44	3.86	3.58	4.02	-0.357
	6.00	0.42	3.78	3.40	3.82	-0.377
	6.92	0.54	3.92	3.56	4.10	-0.268

AEROBIC

	INFL	EFL	REMOV
EE2	5.18	4.04	22%
E1	1.22	3.44	
E2	4.74	0.14	97%
E2+E1	5.96	3.58	40%
log E2		0.409	

ANOXIC

	INFL	EFL	REMOV
EE2	4.14	3.92	5%
E1	0.42	3.56	
E2	4.11	0.42	90%
E2+E1	4.53	3.98	12%
log E2		0.332	

y = -0.3323x + 0.3977
R^2 = 0.6786

y = -0.409x + 0.4407
R^2 = 0.8748

Estrogen removal in an aerobic reactor

y = -0.972x + 6.7565
R^2 = 0.4712

y = -0.3696x + 4.9854
R^2 = 0.7983

y = -0.1015x + 4.0052
R^2 = 0.332

Aerobic:

	Removal mg/L h	mg/gVSS h
EE2	0.9720	0.276
E1	0.1015	0.029
E1+E2	0.3696	0.105
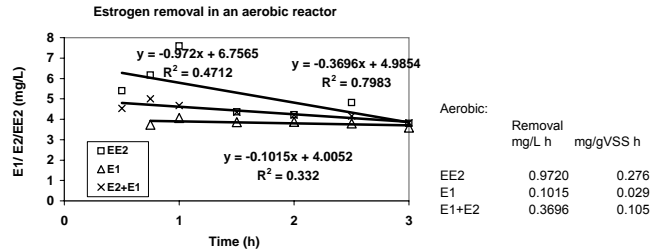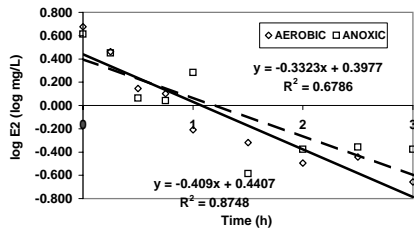
Appendix 7.2   Fate of the estrogens EE2, E2, and the metabolite E1 in activated sludge batch experiments under aerobic and alternating anoxic/aerobic conditions (Experiment 2).

Experiment #2, August 03, 2005

Total and volatile suspended solids
in the beginning of the reaction, mg/L

	aerobic	alternating
TSS	2190	2650
stdev	71	71
%	3.2	2.7
VSS	1890	2090
stdev	99	14
%	5.2	0.7
VSS/TSS	0.86	0.79

	aerobic	alternating	
AUR	9.5232	13.499	mg/Lh
	5.04	6.46	mg/gVSS h

COD (feed)		500 mg/L	
F/M	aerobic	0.26	g/g d
	alternating	0.24	g/g d

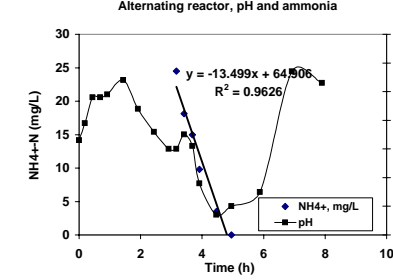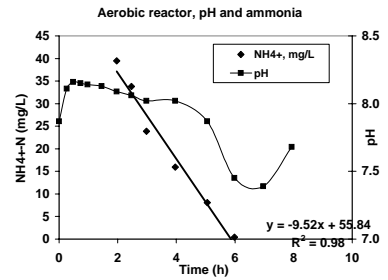|				pH		DO, mg/L		NH₄⁺, mg/L			
time				aerobic	alternating	aerobic	alternating	aerobic	alternating	aerobic	alternating
9.77	9.90	0.00	0.00	7.87	8.03	1.9	0.1	57.6	-	28.66	-
10.03	10.08	0.27	0.18	8.11	8.09	5.1	0.1	69.2	-	34.43	-
10.25	10.33	0.48	0.43	8.16	8.18	5.4	0.1	70.9	-	35.28	-
10.50	10.58	0.73	0.68	8.15	8.18	6.4	0.1	68.3	-	33.99	-
10.73	10.82	0.97	0.92	8.14	8.19	6.2	0.2	74	-	36.82	-
11.22	11.33	1.45	1.43	8.13	8.24	6.5	0.1	75.9	-	37.77	-
11.73	11.82	1.97	1.92	8.09	8.14	6.3	0.1	79.3	-	39.46	-
12.23	12.33	2.47	2.43	8.06	8.06	6.4	0.1	68	-	33.84	-
12.75	12.82	2.98	2.92	8.02	8.00	6.7	0.1	48	-	23.88	-
13.73	13.07	3.97	3.17	8.02	8.00	7.2	3.2	32	49.2	15.92	24.48
14.82	13.32	5.05	3.42	7.87	8.05	7.1	3.1	16.2	36.4	8.06	18.11
15.75	13.58	5.98	3.68	7.45	8.01	7.0	3.3	0.8	30.1	0.40	14.98
16.73	13.82	6.97	3.92	7.39	7.88	7.3	3.7	0	19.7	0.00	9.80
17.70	14.37	-	4.47	7.68	7.77	7.7	4.0	0	7	0.00	3.48
-	14.85	-	4.95	-	7.80	-	4.0	-	0	-	0
-	15.78	-	5.88	-	7.85	-	5.1	-	-	-	0
-	16.82	-	6.92	-	8.27	-	6.9	-	-	-	0
-	17.80	-	7.90	-	8.23	-	6.9	-	-	-	0

Aerobic reactor, pH and ammonia

$y = -9.52x + 55.84$
$R^2 = 0.98$

Alternating reactor, pH and ammonia

$y = -13.499x + 64.906$
$R^2 = 0.9626$

AEROBIC

Time	E2	EE2	E1	E1+E2	log E2
9.75	0	6.2	5.6	1		
9.75	0	4.2	3	1.2		
9.75	0	5.2	4.3	1.1	6.3	0.716
10	0.25	2.4	5.6	2.4	4.8	0.380
10.25	0.5	1.6	6	3.4	5	0.204
10.5	0.75	0.8	4.8	3	3.8	-0.097
10.75	1	0.8	5	4	4.8	-0.097
11.25	1.5	0.8	5.4	4	4.8	-0.097
12.25	2.5	0.4	3.4	2.4	2.8	-0.398
12.75	3	0.6	4	3.6	4.2	-0.222
13.75	4	0.6	4	4.2	4.8	-0.222
14.75	5	0.6	3.6	4	4.6	-0.222
15.75	6	0.4	3.2	3.8	4.2	-0.398
16.75	7	0.4	2.4	1.6	2	-0.398
17.75	8	0.4	3.4	2.6	3	-0.398
33.75	24	1.2	2	2.2	3.4	0.079

ALTERNATING

Time	E2	EE2	E1	E1+E2	metabolite	peak area	log E2
9.83	0	4	3.4	0			
9.83	0	4.4	3.8	0			
9.83	0	4.2	3.6	0	4.2	0.12	0	0.623
10.08	0.25	1.8	4.2	2.2	4	4.03	59859	0.255
10.33	0.5	0.6	4	2.8	3.4	11.80	178727	-0.222
10.58	0.75	0.8	3.8	3.4	4.2	16.02	243310	-0.097
10.83	1	1	4	3.4	4.4	23.10	351663	0.000
11.33	1.5	1.2	4	2.6	3.8	19.29	293360	0.079
11.83	2	1	3.6	2.2	3.2	14.20	215477	0.000
12.33	2.5	1	3	1.4	2.4	10.75	162703	0.000
12.83	3	1	3.6	1.6	2.6	13.33	202205	0.000
13.08	3.25	0.6	4.4	3.6	4.2	9.67	146166	-0.222
13.33	3.5	0.8	4	3	3.8	1.24	17189	-0.097
13.58	3.75	1	4	3.8	4.8	0.42	4615	0.000
13.83	4	0.8	3.8	3.4	4.2	0.94	12468	-0.097
14.33	4.5	0.4	4	3.6	4	0.12	0	-0.398
14.83	5	0.6	3.8	3.8	4.4	1.07	14592	-0.222
15.83	6	1	3.6	3.4	4.4	0.66	8253	0.000
16.83	7	0.8	3.4	3.6	4.4	1.70	24226	-0.097
17.83	8	1.6	3.6	3.6	5.2	1.73	24619	0.204
33.83	24	0	2.6	1	1	2.00	28712	

AEROBIC

	INFL	EFL	REMOV
EE2	4.3	3.4	21%
E1	1.1	2.6	
E2	5.2	0.4	92%
E2+E1	6.3	3.0	52%
log E2			0.387

ALTERNATING

		INFL	EFL	REMOV
TOTAL:	EE2	4.4	3.4	23%
	E1	0	3.6	
	E2	4.2	0.8	81%
ANOXIC:	E2	4.2	1	76%
	E2+E1	4.2	2.6	38%
	EE2	3.6	3.6	0%
	log E2			0.184

aerobic

$y = -0.2051x + 4.741$
$R^2 = 0.2294$

$y = -0.099x + 3.798$
$R^2 = 0.073$

$y = -0.429x + 5.519$
$R^2 = 0.796$

anoxic   aerobic

$y = -0.0541x + 4.4054$
$R^2 = 0.0048$

$y = -0.720x + 6.660$
$R^2 = 0.853$

$y = 0.02x + 3.4$
$R^2 = 0.0057$
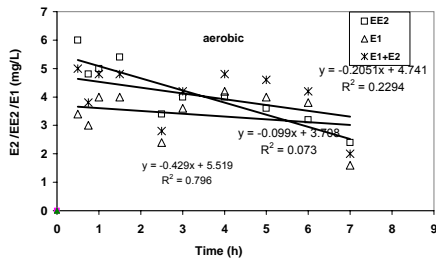
	Removal mg/L h	mg/gVSS h
E1+E2	0.205	0.109
E1	0.099	0.052
EE2	0.429	0.227
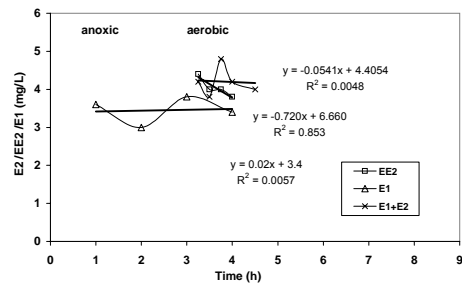
	Removal mg/L h	mg/gVSS h
E1+E2	N/A	N/A
E1	N/A	N/A
EE2	0.720	0.344

Appendix 7.3   Fate of the estrogens EE2, E2, and the metabolite E1 in activated sludge batch experiments under aerobic and alternating anoxic/aerobic conditions for starved biomass (Experiment 3).

Experiment #3, August 12, 2005

Total and volatile suspended solids in the beginning of the reaction, mg/L

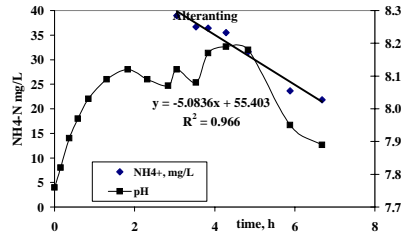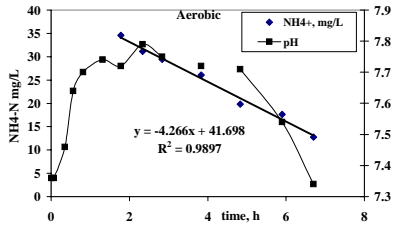|	aerobic	alternating
TSS	2230	2040
stdev	42	57
%	1.9	2.8
VSS	1830	1710
stdev	156	14
%	8.5	0.8
VSS/TSS	0.82	0.84

AUR	aerobic	alternating	
	4.266	5.084	mg/Lh
	2.33	2.97	mg/gVSS h

COD (feed)		1200 mg/L
F/M	aerobic	0.66 g/g d
	alternating	0.70 g/g d

time				pH		DO, mg/L		NH₄⁺, mg/L			
				aerobic	alternating	aerobic	alternating	aerobic	alternating	aerobic	alternating
9.67	9.70	0	0.00	7.36	7.76	1.4	0.1	17.1	17.2	22.49	22.62
9.73	9.85	0.07	0.15	7.36	7.82	1.0	0.1	19.2		25.25	
10.02	10.07	0.35	0.37	7.46	7.91	0.4	0.1	21.8	19.8	28.67	26.04
10.23	10.28	0.57	0.58	7.64	7.97	1.5	0.1	23.4		30.78	
10.48	10.55	0.82	0.85	7.70	8.03	1.8	0.2	23.4	22.6	30.78	29.73
10.98	11.00	1.32	1.30	7.74	8.09	2.7	0.1	23.7	26.8	31.17	35.25
11.45	11.53	1.78	1.83	7.72	8.12	3.4	0.1	26.3		34.59	
12.00	12.02	2.33	2.32	7.79	8.09	4.2	0.1	23.7	27.8	31.17	36.57
12.50	12.53	2.83	2.83	7.75	8.07	4.6	0.1	22.4	28.9	29.46	38.01
	12.75		3.05		8.12		1.0		29.6		38.93
	13.00		3.53		8.08		4.5		27.9		36.70
13.50	13.53	3.83	3.83	7.72	8.17	4.4	4.4	19.8	27.7	26.04	36.43
	13.98		4.28		8.19		4.6		27		35.51
14.50	14.53	4.83	4.83	7.71	8.18	5.2	5.1	15.1	24	19.86	31.57
15.57	15.58	5.90	5.88	7.54	7.95	4.9	4.6	13.4	18	17.63	23.68
16.37	16.38	6.70	6.68	7.34	7.89	4.2	4.1	9.7	16.6	12.76	21.83

Aerobic: $y = -4.266x + 41.698$, $R^2 = 0.9897$

Alternating: $y = -5.0836x + 55.403$, $R^2 = 0.966$

AEROBIC

Time		E2	EE2	E1	E1+E2	log E2
9.67	0.00	7.72	9.12	0.00	7.72	0.8876
9.73	0.07	4.26	4.76	0.42	4.68	0.6294
10.02	0.35	3.24	4.00	1.20	4.44	0.5105
10.23	0.57	2.84	4.22	1.82	4.66	0.4533
10.48	0.82	2.20	4.26	2.26	4.46	0.3424
10.98	1.32	1.38	4.42	2.92	4.30	0.1399
11.45	1.78	1.30	4.36	3.20	4.50	0.1139
12.00	2.33	0.60	4.40	3.44	4.04	-0.2218
12.50	2.83	0.78	4.50	3.60	4.38	-0.1079
13.50	3.83	0.58	4.42	3.60	4.18	-0.2366
14.50	4.83	0.42	4.48	3.68	4.10	-0.3768
15.57	5.90	0.78	4.38	3.66	4.44	-0.1079
16.37	6.70	0.34	4.16	3.52	3.86	-0.4685
33.50	23.83	0.14	1.58	0.66	0.80	-0.8539
33.52	23.85	0.34	3.76	2.66	3.00	-0.4685

ALTERNATING

Time		E2	EE2	E1	E1+E2	metabolite	peak area	log E2
9.70	0.00	4.84	5.18	0.00	4.84	0.105	13880	0.6848
9.85	0.15	4.06	3.74	0.78	4.84	0.410	61580	0.6085
10.07	0.37	3.76	3.80	1.36	5.12	1.289	199203	0.5752
10.28	0.58	2.32	3.82	1.80	4.12	2.208	342984	0.3655
10.55	0.85	1.96	3.92	2.20	4.16	3.253	506496	0.2923
11.00	1.30	1.56	4.08	2.80	4.36	4.188	652754	0.1931
11.53	1.83	0.98	4.10	3.16	4.14	4.149	646683	-0.0088
12.02	2.32	1.74	4.28	3.38	5.12	3.698	576187	0.2405
12.53	2.83	0.70	4.40	3.56	4.26	3.436	535091	-0.1549
12.75	3.05	0.78	4.40	3.64	4.42	2.870	446562	-0.1079
13.00	3.30	0.46	4.30	3.60	4.06	1.973	306238	-0.3372
13.23	3.53	0.42	4.46	3.78	4.20	1.005	154716	-0.3768
13.53	3.83	0.56	5.56	3.72	4.28	0.088	11236	-0.2518
14.53	4.83	0.46	4.72	3.72	4.18	0.275	40505	-0.3372
15.58	5.88	0.18	4.10	3.58	3.76	0.061	6910	-0.7447
16.38	6.68	0.18	4.04	3.56	3.74	0.078	9602	-0.7447
33.50	23.80	0.10	1.64	0.58	0.68	0.081	10056	-1.0000
33.52	23.82	0.48	3.78	2.56	3.04	0.188	26787	-0.3188

AER

	INFL	EFL	REMOV
EE2	4.76	4.16	13%
E1	0	3.52	
E2	4.26	0.34	92%
	1	0.8	20%
	4.4	0.8	82%
E2+E1	4.26	3.86	9%
log E2			0.236

ANOX

	INFL	EFL	REMOV
EE2	5.18	4.04	22%
E1	0	3.56	
E2	4.84	0.18	96%
	4.84	0.48	90%
E2+E1	4.84	3.74	23%
	4.84	4.42	9%
log E2			0.212

$y = -0.072x + 4.734$, $R^2 = 0.668$
$y = -0.075x + 4.552$, $R^2 = 0.249$
$y = -0.009x + 3.654$, $R^2 = 0.047$

$y = -0.160x + 4.801$, $R^2 = 0.749$
$y = -0.151x + 5.182$, $R^2 = 0.167$
$y = -0.0312x + 3.7959$, $R^2 = 0.2718$

$y = -0.2124x + 0.5424$, $R^2 = 0.9255$
$y = -0.2355x + 0.595$, $R^2 = 0.8649$
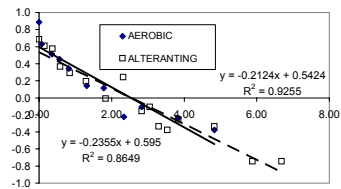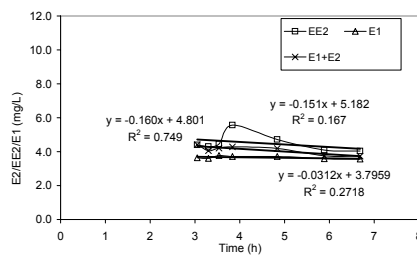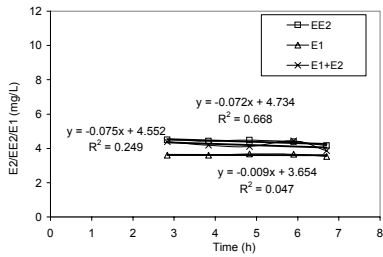
Removal	mg/L h	mg/gVSS h
E1+E2	0.075	**0.041**
E1	0.009	**0.005**
EE2	0.072	**0.039**

Removal	mg/L h	mg/gVSS h
E1+E2	0.160	**0.093**
E1	0.031	**0.018**
EE2	0.151	**0.088**

Appendix 7.4   Transformation of estrogens in aerated deionized water
(negative control) and sludge (positive control)

6/23/2005

Negative control - transformation of estrogens in aerated dH_2O

Time, min.	Concentration (mg/L)		
	E2	EE2	E1
0	3.840	8.940	0.000
3	3.900	9.160	0.000
6	3.900	9.340	0.000
9	3.780	9.180	0.000
12	4.200	8.160	0.000
15	3.340	8.880	0.000
	3.83	**8.94**	**0.00**
	0.31	**0.47**	**0.00**

Positive control - transformation of estrogens in aerated sludge

Time, min.	Concentration (mg/L)		
	E2	EE2	E1
0	5.90	7.42	0.36
3	3.24	7.70	0.48
6	2.10	6.98	1.16
9	2.58	7.30	1.52
12	2.10	8.10	1.92
15	1.58	7.46	1.96
	2.92	**7.49**	**1.23**
	1.43	**0.35**	**0.63**

Appendix 8.1   Transformation of estrogens in ozonated deionized water and sludge

6/24/2005

Ozonated water and estrogens   Concentration (mg/L)

Time			E2	EE2	E1
0.0	0.00		1.68	4.37	0.00
0.5	1.03		1.52	3.95	0.00
1.0	2.05		0.76	1.39	0.00
1.5	3.08		0.01	0.74	0.00
2.0	4.10		0.00	0.18	0.00
2.5	5.13		0.00	0.21	0.00
3.0	6.16		0.00	0.28	0.00
3.5	7.18		0.02	0.14	0.00
4.0	8.21		0.00	0.00	0.00
4.5	9.23		0.00	0.00	0.00
5.0	10.26		0.13	0.00	0.00
6.0	12.31		0.11	0.00	0.05
7.0	14.36		0.11	0.00	0.03
8.0	16.42		0.00	0.02	0.00
9.0	18.47		0.03	0.00	0.00
10.0	20.52		0.17	0.00	0.00
11.0	22.57		0.07	0.00	0.00
12.0	24.62		0.07	0.00	0.06
13.0	26.68		0.00	0.00	0.12
14.0	28.73		0.05	0.00	0.00
15.0	30.78		0.08	0.00	0.00

Ozonated Sludge and estrogens   Concentration (mg/L)

Time, min	O3, mg	dose	E2	EE2	E1
0.00	0.00	0.0000	2.16	7.93	0.12
0.50	1.03	0.0017	1.95	6.90	0.23
1.00	2.05	0.0034	0.47	3.12	0.00
1.50	3.08	0.0050	0.25	1.92	0.00
2.00	4.10	0.0067	0.19	1.29	0.05
2.50	5.13	0.0084	0.12	0.75	0.00
3.00	6.16	0.0101	0.27	0.48	0.00
3.50	7.18	0.0117	0.01	0.26	0.00
4.00	8.21	0.0134	0.04	0.26	0.08
4.50	9.23	0.0151	0.00	0.26	0.00
5.00	10.26	0.0168	0.12	0.16	0.00
6.00	12.31	0.0201	0.00	0.19	0.00
7.00	14.36	0.0235	0.00	0.00	0.00
8.00	16.42	0.0268	0.00	0.00	0.03
9.00	18.47	0.0302	0.00	0.00	0.04
10.00	20.52	0.0335	0.00	0.00	0.09
11.00	22.57	0.0369	0.00	0.00	0.00
12.00	24.62	0.0403	0.02	0.05	0.00
13.00	26.68	0.0436	0.01	0.00	0.00
14.00	28.73	0.0470	0.00	0.00	0.03
15.00	30.78	0.0503	0.00	0.00	0.03

TSS   2447 mg/L

Appendix 8.2   Estrogen removal through partial sludge ozonation.

Calculations neglecting the wastage decrease due to solids compensation (Assumption 3)

**1) Assumption: Estrogens remain in the liquid phase (no adsorption to the solids)**

It.	control				ozonated (20% RAS)				BENEFIT		
	INFLUENT	EFFLUENT	WAS	RAS	INFLUENT	EFFLUENT	WAS	RAS	EFFLUENT	WAS	RAS
0	3.00	1.75000	0.25	1.00000	3.00	1.75000	0.25	0.800000	0.0%	0.0%	20.0%
1	4.00	2.33333	0.33333	1.33333	3.80	2.21667	0.316667	1.013333	5.0%	5.0%	24.0%
2	4.33	2.52778	0.36111	1.44444	4.01	2.34111	0.334444	1.070222	7.4%	7.4%	25.9%
3	4.44	2.59259	0.37037	1.48148	4.07	2.37430	0.339185	1.085393	8.4%	8.4%	26.7%
4	4.48	2.61420	0.37346	1.49383	4.09	2.38315	0.340449	1.089438	8.8%	8.8%	27.1%
5	4.49	2.62140	0.37449	1.49794	4.09	2.38551	0.340787	1.090517	9.0%	9.0%	27.2%
6	4.50	2.62380	0.37483	1.49931	4.09	2.38613	0.340876	1.090804	9.1%	9.1%	27.2%
7	4.50	2.62460	0.37494	1.49977	4.09	2.38630	0.3409	1.090881	9.1%	9.1%	27.3%
8	4.50	2.62487	0.37498	1.49992	4.09	2.38635	0.340907	1.090902	9.1%	9.1%	27.3%
9	4.50	2.62496	0.37499	1.49997	4.09	2.38636	0.340908	1.090907	9.1%	9.1%	27.3%
10	4.50	2.62499	0.37500	1.49999	4.09	2.38636	0.340909	1.090909	9.1%	9.1%	27.3%
11	4.50	2.62500	0.37500	1.50000	4.09	2.38636	0.340909	1.090909	9.1%	9.1%	27.3%
12	4.50	2.62500	0.37500	1.50000	4.09	2.38636	0.340909	1.090909	9.1%	9.1%	27.3%

A. EFFLUENT

$a_n = 1.75$
$a_{n+1} = a_n/3 + 7/4$
$q = 1/3$
$S = \lim S_n = 1.75/(1-1/3) = 2.625$

$a_n = 1.75$
$a_{n+1} = a_n*0.8/3 + 7/4$
$q = 0.8/3$
$S = \lim S_n = 1.75/(1-0.8/3) = 2.3864$
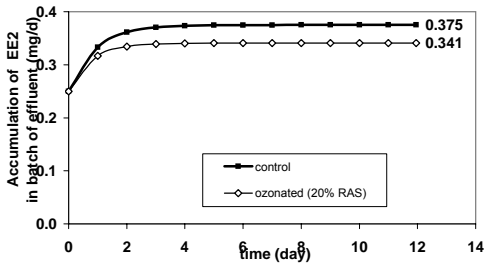
$\eta_1 = (2.625-2.387)/2.625 = 9.1\%$
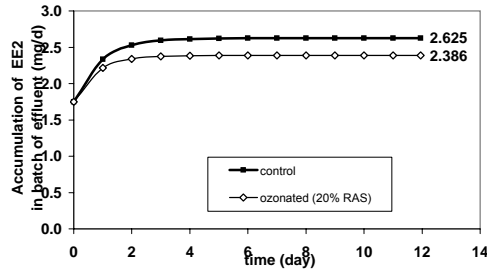
B. WAS

$a_n = 0.25$
$a_{n+1} = a_n/3 + 3/12$
$q = 1/3$
$S = \lim S_n = 0.25/(1-1/3) = 0.375$

$a_n = 0.25$
$a_{n+1} = a_n*0.8/3 + 3/12$
$q = 0.8/3$
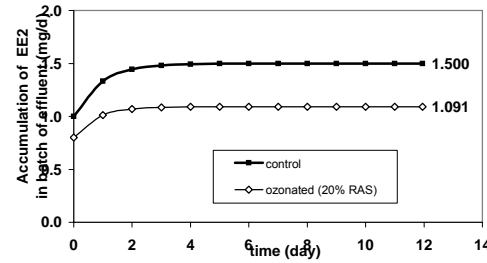$S = \lim S_n = 0.25/(1-0.8/3) = 0.341$

$\eta_1 = (0.375-0.341)/0.375 = 9.1\%$

C. RAS

$a_n = 1$
$a_{n+1} = (a_n+3)/3$
$q = 1/3$
$S = \lim S_n = 1/(1-1/3) = 1.50$

$a_n = 0.8$
$a_{n+1} = (a_n+3)*0.8/3$
$q = 0.8/3$
$S = \lim S_n = 0.8/(1-0.8/3) = 1.091$

$\eta_1 = (1.5-1.091)/1.51 = 27.3\%$

2) Assumption: Estrogens are sorbed to the solids and not present in the effluent.

It.	control				ozonated (20% RAS)				BENEFIT		
	INFLUENT	EFFLUENT	WAS	RAS	INFLUENT	EFFLUENT	WAS	RAS	EFFLUENT	WAS	RAS
0	3.00	0.00000	0.25000	2.75000	3.00	0.00000	0.25000	2.200000	-	0.0%	20.0%
1	5.75	0.00000	0.47917	5.27083	5.20	0.00000	0.43333	3.813333	-	9.6%	27.7%
2	8.27	0.00000	0.68924	7.58160	6.81	0.00000	0.56778	4.996444	-	17.6%	34.1%
3	10.58	0.00000	0.88180	9.69980	8.00	0.00000	0.66637	5.864059	-	24.4%	39.5%
4	12.70	0.00000	1.05832	11.64148	8.86	0.00000	0.73867	6.500310	-	30.2%	44.2%
5	14.64	0.00000	1.22012	13.42136	9.50	0.00000	0.79169	6.966894	-	35.1%	48.1%
6	16.42	0.00000	1.36845	15.05291	9.97	0.00000	0.83057	7.309056	-	39.3%	51.4%
7	18.05	0.00000	1.50441	16.54850	10.31	0.00000	0.85909	7.559974	-	42.9%	54.3%
8	19.55	0.00000	1.62904	17.91946	10.56	0.00000	0.88000	7.743981	-	46.0%	56.8%
9	20.92	0.00000	1.74329	19.17617	10.74	0.00000	0.89533	7.878919	-	48.6%	58.9%
10	22.18	0.00000	1.84801	20.32816	10.88	0.00000	0.90658	7.977874	-	50.9%	60.8%
11	23.33	0.00000	1.94401	21.38414	10.98	0.00000	0.91482	8.050441	-	52.9%	62.4%
12	24.38	0.00000	2.03201	22.35213	11.05	0.00000	0.92087	8.103657	-	54.7%	63.7%

	control				ozonated (20% RAS)				BENEFIT		
It.	INFLUENT	EFFLUENT	WAS	RAS	INFLUENT	EFFLUENT	WAS	RAS	EFFLUENT	WAS	RAS
13	25.35	0.00000	2.11268	23.23945	11.10	0.00000	0.92530	8.142682	-	56.2%	65.0%
14	26.24	0.00000	2.18662	24.05283	11.14	0.00000	0.92856	8.171300	-	57.5%	66.0%
15	27.05	0.00000	2.25440	24.79843	11.17	0.00000	0.93094	8.192287	-	58.7%	67.0%
16	27.80	0.00000	2.31654	25.48189	11.19	0.00000	0.93269	8.207677	-	59.7%	67.8%
17	28.48	0.00000	2.37349	26.10840	11.21	0.00000	0.93397	8.218963	-	60.6%	68.5%
18	29.11	0.00000	2.42570	26.68270	11.22	0.00000	0.93491	8.227240	-	61.5%	69.2%
19	29.68	0.00000	2.47356	27.20914	11.23	0.00000	0.93560	8.233309	-	62.2%	69.7%
20	30.21	0.00000	2.51743	27.69172	11.23	0.00000	0.93611	8.237760	-	62.8%	70.3%
21	30.69	0.00000	2.55764	28.13407	11.24	0.00000	0.93648	8.241024	-	63.4%	70.7%
22	31.13	0.00000	2.59451	28.53957	11.24	0.00000	0.93675	8.243418	-	63.9%	71.1%
23	31.54	0.00000	2.62830	28.91127	11.24	0.00000	0.93695	8.245173	-	64.4%	71.5%
24	31.91	0.00000	2.65927	29.25200	11.25	0.00000	0.93710	8.246460	-	64.8%	71.8%
25	32.25	0.00000	2.68767	29.56433	11.25	0.00000	0.93721	8.247404	-	65.1%	72.1%
26	32.56	0.00000	2.71369	29.85064	11.25	0.00000	0.93728	8.248096	-	65.5%	72.4%
27	32.85	0.00000	2.73755	30.11308	11.25	0.00000	0.93734	8.248604	-	65.8%	72.6%
28	33.11	0.00000	2.75942	30.35366	11.25	0.00000	0.93738	8.248976	-	66.0%	72.8%
29	33.35	0.00000	2.77947	30.57419	11.25	0.00000	0.93741	8.249249	-	66.3%	73.0%
30	33.57	0.00000	2.79785	30.77634	11.25	0.00000	0.93744	8.249449	-	66.5%	73.2%
31	33.78	0.00000	2.81469	30.96164	11.25	0.00000	0.93745	8.249596	-	66.7%	73.4%
32	33.96	0.00000	2.83014	31.13151	11.25	0.00000	0.93747	8.249704	-	66.9%	73.5%
33	34.13	0.00000	2.84429	31.28721	11.25	0.00000	0.93748	8.249783	-	67.0%	73.6%
34	34.29	0.00000	2.85727	31.42995	11.25	0.00000	0.93748	8.249841	-	67.2%	73.8%
35	34.43	0.00000	2.86916	31.56078	11.25	0.00000	0.93749	8.249883	-	67.3%	73.9%
36	34.56	0.00000	2.88007	31.68072	11.25	0.00000	0.93749	8.249914	-	67.4%	74.0%
37	34.68	0.00000	2.89006	31.79066	11.25	0.00000	0.93749	8.249937	-	67.6%	74.0%
38	34.79	0.00000	2.89922	31.89144	11.25	0.00000	0.93749	8.249954	-	67.7%	74.1%
39	34.89	0.00000	2.90762	31.98382	11.25	0.00000	0.93750	8.249966	-	67.8%	74.2%
40	34.98	0.00000	2.91532	32.06850	11.25	0.00000	0.93750	8.249975	-	67.8%	74.3%
41	35.07	0.00000	2.92237	32.14612	11.25	0.00000	0.93750	8.249982	-	67.9%	74.3%
42	35.15	0.00000	2.92884	32.21728	11.25	0.00000	0.93750	8.249987	-	68.0%	74.4%
43	35.22	0.00000	2.93477	32.28251	11.25	0.00000	0.93750	8.249990	-	68.1%	74.4%
44	35.28	0.00000	2.94021	32.34230	11.25	0.00000	0.93750	8.249993	-	68.1%	74.5%
45	35.34	0.00000	2.94519	32.39711	11.25	0.00000	0.93750	8.249995	-	68.2%	74.5%
46	35.40	0.00000	2.94976	32.44735	11.25	0.00000	0.93750	8.249996	-	68.2%	74.6%
47	35.45	0.00000	2.95395	32.49340	11.25	0.00000	0.93750	8.249997	-	68.3%	74.6%
48	35.49	0.00000	2.95778	32.53562	11.25	0.00000	0.93750	8.249998	-	68.3%	74.6%
49	35.54	0.00000	2.96130	32.57432	11.25	0.00000	0.93750	8.249998	-	68.3%	74.7%
50	35.57	0.00000	2.96453	32.60979	11.25	0.00000	0.93750	8.249999	-	68.4%	74.7%
51	35.61	0.00000	2.96748	32.64231	11.25	0.00000	0.93750	8.249999	-	68.4%	74.7%
52	35.64	0.00000	2.97019	32.67212	11.25	0.00000	0.93750	8.249999	-	68.4%	74.7%
53	35.67	0.00000	2.97268	32.69944	11.25	0.00000	0.93750	8.250000	-	68.5%	74.8%
54	35.70	0.00000	2.97495	32.72449	11.25	0.00000	0.93750	8.250000	-	68.5%	74.8%

A. WAS

$a_n = 0.25$
$a_{n+1} = a_n * 11/12 + 3/12$
$q = 11/12$
$S = \lim S_n = 0.25/(1-11/12) = 3$

$a_n = 0.25$
$a_{n+1} = a_n * 0.8 * 11/12 + 3/12$
$q = 0.8 * 11/12$
$S = \lim S_n = 0.25/(1-8.8/12) = 0.9375$

$\eta_2 = (3 - 0.9375)/3 = 68.8\%$
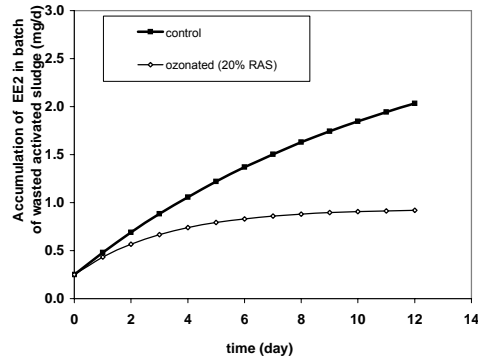
B. RAS

$a_n = 2.75$
$a_{n+1} = (a_n + 3) * 11/12$
$q = 11/12$
$S = \lim S_n = 2.75/(1-11/12) = 33$

$a_n = 2.20$
$a_{n+1} = (a_n + 3) * 0.8 * 11/12$
$q = 0.8 * 11/12$
$S = \lim S_n = 2.20/(1-0.8*11/12) = 8.25$

$\eta_2 = (33 - 8.25)/33 = 75\%$
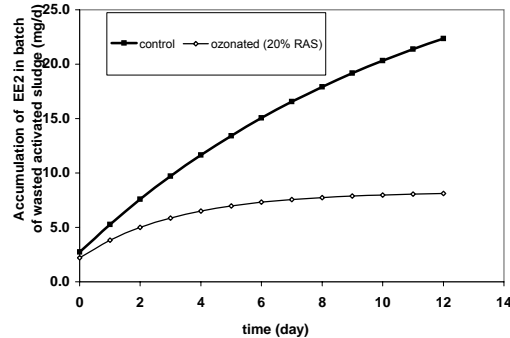
C. EFFLUENT

N/A

Appendix 8.3   Estrogen removal through partial sludge ozonation.
Calculations including WAS decrease in the ozonated train due to solids compensation

**1) Assumption: Estrogens remain in the liquid phase (no adsorption to the solids)**

It.	control				ozonated (20% RAS)				BENEFIT		
	INFLUENT	EFFLUENT	WAS	RAS	INFLUENT	EFFLUENT	WAS	RAS	EFFLUENT	WAS	RAS
0	3.00	1.75000	0.25	1.00000	3.00	1.7667	0.2250	0.8083	-1.0%	10.0%	19.2%
1	4.00	2.33333	0.33333	1.33333	3.81	2.2427	0.2856	1.0261	3.9%	14.3%	23.0%
2	4.33	2.52778	0.36111	1.44444	4.03	2.3709	0.3020	1.0848	6.2%	16.4%	24.9%
3	4.44	2.59259	0.37037	1.48148	4.08	2.4055	0.3064	1.1006	7.2%	17.3%	25.7%
4	4.48	2.61420	0.37346	1.49383	4.10	2.4148	0.3075	1.1049	7.6%	17.6%	26.0%
5	4.49	2.62140	0.37449	1.49794	4.10	2.4173	0.3079	1.1060	7.8%	17.8%	26.2%
6	4.50	2.62380	0.37483	1.49931	4.11	2.4180	0.3080	1.1063	7.8%	17.8%	26.2%
7	4.50	2.62460	0.37494	1.49977	4.11	2.4182	0.3080	1.1064	7.9%	17.9%	26.2%
8	4.50	2.62487	0.37498	1.49992	4.11	2.4182	0.3080	1.1065	7.9%	17.9%	26.2%
9	4.50	2.62496	0.37499	1.49997	4.11	2.4182	0.3080	1.1065	7.9%	17.9%	26.2%
10	4.50	2.62499	0.37500	1.49999	4.11	2.4182	0.3080	1.1065	7.9%	17.9%	26.2%
11	4.50	2.62500	0.37500	1.50000	4.11	2.4183	0.3080	1.1065	7.9%	17.9%	26.2%
12	4.50	2.62500	0.37500	1.50000	4.11	2.4183	0.3080	1.1065	7.9%	17.9%	26.2%

A. EFFLUENT

$a_n = 1.75$

$a_{n+1} = a_n/3 + 7/4$

$q = 1/3$

$S = \lim S_n = 1.75/(1-1/3) = 2.625$

$a_n = 1.7667$

$a_{n+1} = a_n*(0.8/3 + 0.1/12*1/3) + 7/4 + 2*0.1/12$

$q = 9.7/12/3$

$S = \lim S_n = 1.7667/(1-9.7/12/3) = 2.4183$
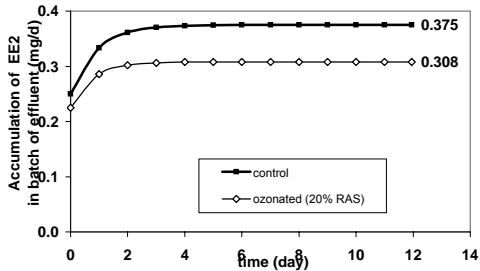
$\eta_1 = (2.625 - 2.4183)/2.625 = 7.9$

B. WAS

$a_n = 0.25$

$a_{n+1} = a_n/3 + 3/12$

$q = 1/3$
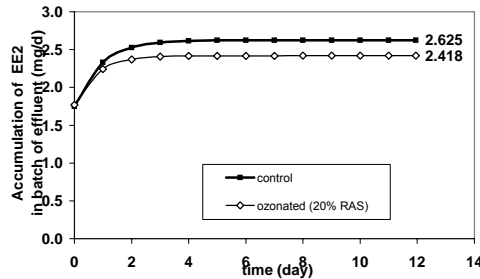
$S = \lim S_n = 0.25/(1-1/3) = 0.375$

$a_n = 0.225$

$a_{n+1} = a_n*(0.8/3 + 0.1/12*1/3) + 0.9/4$

$q = 9.7/12/3$

$S = \lim S_n = 0.225/(1-9.7/12/3) = 0.3080$

$\eta_1 = (0.375 - 0.3080)/0.375 = 17.9\%$
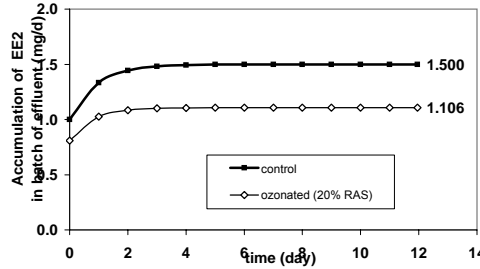

C. RAS

$a_n = 1$

$a_{n+1} = (a_n + 3)/3$

$q = 1/3$

$S = \lim S_n = 1/(1-1/3) = 1.50$

$a_n = 0.8083$

$a_{n+1} = (a_n + 3)*(0.8/3 + 0.1/12/3)$

$q = 9.7/12$

$S = \lim S_n = 0.8083/(1-9.7/12/3) = 1.064$

$\eta_1 = (1.5 - 1.064)/1.51 = 26.2\%$

2) Assumption: Estrogens are sorbed to the solids and not present in the effluent.

It.	control				ozonated (20% RAS)				BENEFIT		
	INFLUENT	EFFLUENT	WAS	RAS	INFLUENT	EFFLUENT	WAS	RAS	EFFLUENT	WAS	RAS
0	3.00	0.00000	0.25000	2.75000	3.00	0.00000	0.225	2.225000	-	10.0%	19.1%
1	5.75	0.00000	0.47917	5.27083	5.23	0.00000	0.391875	3.875208	-	18.2%	26.5%
2	8.27	0.00000	0.68924	7.58160	6.88	0.00000	0.515641	5.099113	-	25.2%	32.7%
3	10.58	0.00000	0.88180	9.69980	8.10	0.00000	0.607433	6.006842	-	31.1%	38.1%
4	12.70	0.00000	1.05832	11.64148	9.01	0.00000	0.675513	6.680075	-	36.2%	42.6%
5	14.64	0.00000	1.22012	13.42136	9.68	0.00000	0.726006	7.179389	-	40.5%	46.5%
6	16.42	0.00000	1.36845	15.05291	10.18	0.00000	0.763454	7.549713	-	44.2%	49.8%
7	18.05	0.00000	1.50441	16.54850	10.55	0.00000	0.791228	7.824371	-	47.4%	52.7%
8	19.55	0.00000	1.62904	17.91946	10.82	0.00000	0.811828	8.028075	-	50.2%	55.2%
9	20.92	0.00000	1.74329	19.17617	11.03	0.00000	0.827106	8.179156	-	52.6%	57.3%
10	22.18	0.00000	1.84801	20.32816	11.18	0.00000	0.838437	8.291207	-	54.6%	59.2%
11	23.33	0.00000	1.94401	21.38414	11.29	0.00000	0.846841	8.374312	-	56.4%	60.8%

	control				ozonated (20% RAS)				BENEFIT		
It.	INFLUENT	EFFLUENT	WAS	RAS	INFLUENT	EFFLUENT	WAS	RAS	EFFLUENT	WAS	RAS
12	24.38	0.00000	2.03201	22.35213	11.37	0.00000	0.853073	8.435948	-	58.0%	62.3%
13	25.35	0.00000	2.11268	23.23945	11.44	0.00000	0.857696	8.481661	-	59.4%	63.5%
14	26.24	0.00000	2.18662	24.05283	11.48	0.00000	0.861125	8.515566	-	60.6%	64.6%
15	27.05	0.00000	2.25440	24.79843	11.52	0.00000	0.863667	8.540711	-	61.7%	65.6%
16	27.80	0.00000	2.31654	25.48189	11.54	0.00000	0.865553	8.559361	-	62.6%	66.4%
17	28.48	0.00000	2.37349	26.10840	11.56	0.00000	0.866952	8.573193	-	63.5%	67.2%
18	29.11	0.00000	2.42570	26.68270	11.57	0.00000	0.867989	8.583451	-	64.2%	67.8%
19	29.68	0.00000	2.47356	27.20914	11.58	0.00000	0.868759	8.591060	-	64.9%	68.4%
20	30.21	0.00000	2.51743	27.69172	11.59	0.00000	0.869329	8.596703	-	65.5%	69.0%
21	30.69	0.00000	2.55764	28.13407	11.60	0.00000	0.869753	8.600888	-	66.0%	69.4%
22	31.13	0.00000	2.59451	28.53957	11.60	0.00000	0.870067	8.603992	-	66.5%	69.9%
23	31.54	0.00000	2.62830	28.91127	11.60	0.00000	0.870299	8.606294	-	66.9%	70.2%
24	31.91	0.00000	2.65927	29.25200	11.61	0.00000	0.870472	8.608001	-	67.3%	70.6%
25	32.25	0.00000	2.68767	29.56433	11.61	0.00000	0.8706	8.609268	-	67.6%	70.9%
26	32.56	0.00000	2.71369	29.85064	11.61	0.00000	0.870695	8.610207	-	67.9%	71.2%
27	32.85	0.00000	2.73755	30.11308	11.61	0.00000	0.870766	8.610903	-	68.2%	71.4%
28	33.11	0.00000	2.75942	30.35366	11.61	0.00000	0.870818	8.611420	-	68.4%	71.6%
29	33.35	0.00000	2.77947	30.57419	11.61	0.00000	0.870857	8.611803	-	68.7%	71.8%
30	33.57	0.00000	2.79785	30.77634	11.61	0.00000	0.870885	8.612087	-	68.9%	72.0%
31	33.78	0.00000	2.81469	30.96164	11.61	0.00000	0.870907	8.612298	-	69.1%	72.2%
32	33.96	0.00000	2.83014	31.13151	11.61	0.00000	0.870922	8.612454	-	69.2%	72.3%
33	34.13	0.00000	2.84429	31.28721	11.61	0.00000	0.870934	8.612570	-	69.4%	72.5%
34	34.29	0.00000	2.85727	31.42995	11.61	0.00000	0.870943	8.612656	-	69.5%	72.6%
35	34.43	0.00000	2.86916	31.56078	11.61	0.00000	0.870949	8.612720	-	69.6%	72.7%
36	34.56	0.00000	2.88007	31.68072	11.61	0.00000	0.870954	8.612767	-	69.8%	72.8%
37	34.68	0.00000	2.89006	31.79066	11.61	0.00000	0.870958	8.612803	-	69.9%	72.9%
38	34.79	0.00000	2.89922	31.89144	11.61	0.00000	0.87096	8.612829	-	70.0%	73.0%
39	34.89	0.00000	2.90762	31.98382	11.61	0.00000	0.870962	8.612848	-	70.0%	73.1%
40	34.98	0.00000	2.91532	32.06850	11.61	0.00000	0.870964	8.612862	-	70.1%	73.1%
41	35.07	0.00000	2.92237	32.14612	11.61	0.00000	0.870965	8.612873	-	70.2%	73.2%
42	35.15	0.00000	2.92884	32.21728	11.61	0.00000	0.870965	8.612881	-	70.3%	73.3%
43	35.22	0.00000	2.93477	32.28251	11.61	0.00000	0.870966	8.612886	-	70.3%	73.3%
44	35.28	0.00000	2.94021	32.34230	11.61	0.00000	0.870966	8.612891	-	70.4%	73.4%
45	35.34	0.00000	2.94519	32.39711	11.61	0.00000	0.870967	8.612894	-	70.4%	73.4%
46	35.40	0.00000	2.94976	32.44735	11.61	0.00000	0.870967	8.612896	-	70.5%	73.5%
47	35.45	0.00000	2.95395	32.49340	11.61	0.00000	0.870967	8.612898	-	70.5%	73.5%
48	35.49	0.00000	2.95778	32.53562	11.61	0.00000	0.870967	8.612899	-	70.6%	73.5%
49	35.54	0.00000	2.96130	32.57432	11.61	0.00000	0.870967	8.612900	-	70.6%	73.6%
50	35.57	0.00000	2.96453	32.60979	11.61	0.00000	0.870968	8.612901	-	70.6%	73.6%
51	35.61	0.00000	2.96748	32.64231	11.61	0.00000	0.870968	8.612902	-	70.6%	73.6%
52	35.64	0.00000	2.97019	32.67212	11.61	0.00000	0.870968	8.612902	-	70.7%	73.6%
53	35.67	0.00000	2.97268	32.69944	11.61	0.00000	0.870968	8.612902	-	70.7%	73.7%
54	35.70	0.00000	2.97495	32.72449	11.61	0.00000	0.870968	8.612903	-	70.7%	73.7%

A. WAS

$a_n = 0.25$

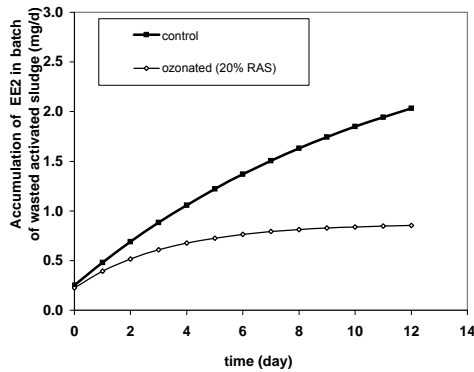$a_{n+1} = a_n * 11/12 + 3/12$

$q = 11/12$

$S = \lim S_n = 0.25/(1-11/12) = 3$

$a_n = 0.225$

$a_{n+1} = a_n * (0.8*11/12 + 0.1/12) + 3/12$

$q = 0.8*11/12 + 0.1/12 = 8.9/12$

$S = \lim S_n = 0.225/(1-8.9/12) = 0.8709$

$\eta_2 = (3-0.8709)/3 = 70.97\%$

B. RAS

$a_n = 2.75$

$a_{n+1} = (a_n + 3) * 11/12$
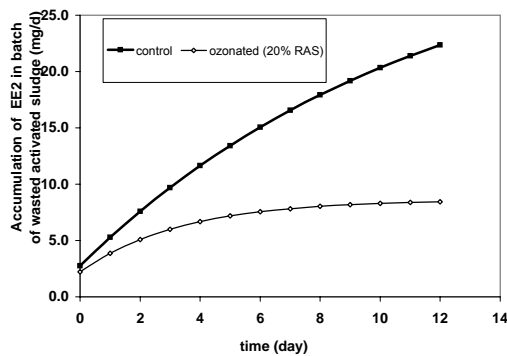
$q = 11/12$

$S = \lim S_n = 2.75/(1-11/12) = 33$

$a_n = 2.225$

$a_{n+1} = (a_n + 3)(0.8*11/12 + 0.1/12)$

$q = 0.8*11/12 + 0.1/12 = 8.9/12$

$S = \lim S_n = 2.225/(1-8.9/12) = 8.6129$

$\eta_2 = (33-8.6129)/33 = 73.9\%$

C. EFFLUENT

N/A

Appendix 9.1.   Calculation of autotrophic growth rate for aerobic and alternating
anoxic/aerobic system

Symbols like in eq. 9.2, §9.2

b (1/d) = 0.153
S (mg/L) = 37
c (mg/L)= 104.37

b (1/d) = 0.058
S (mg/L) = 37
c (mg/L)= 112.72

day	Aerobic	
	AUR, mg/L h	μ max, 1/d
Jan-09	4.52	1.04
Jan-09	5.44	1.25
Nov-30	4.96	1.14
Nov-30	4.83	1.11

	4.94	**1.14**
	0.38	**0.09**

day	Alternating	
	AUR, mg/L h	μ max, 1/d
Jan-09	9.32	1.98
Jan-09	10.04	2.14
Mar-26	8.58	1.83
Mar-16	8.92	1.90

	9.22	**1.96**
	0.63	**0.13**