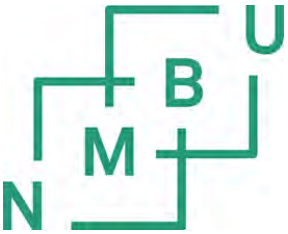
Source separating sanitary systems - energy efficient treatment of blackwater and minimizing greenhouse gas emissions

Kildeseparerende sanitærsystemer - minimering av energiforbruk og lystgassutslip ved biologisk rensing av svartvann

Philosophiae Doctor (PhD) Thesis

Daniel Todt

Department of Environmental Sciences
Norwegian University of Life Sciences



Year: 2015

Title: Source separating sanitary systems - energy efficient treatment of blackwater and minimizing greenhouse gas emissions

Author: Daniel Todt

Thesis Nr 2015:31

Supervision Petter D. Jenssen, Peter Dörsch

Publisher: Norwegian University of Life Sciences
Department of Environmental Sciences
P.O. box 5003
N-1430 Ås

Funding: EU FP-7 (Grant No. 232274)
Norwegian Research Council (Skattefunn-226774)
Innovation Norway (FoU støtte)

ISSN: 1894-6402

ISBN: 978-82-575-1281-1

Print: 07 Media, Oslo, Norway

Abstract

The goal of this study was to evaluate different treatment options for blackwater in a source separating sanitary system for mountain huts and other decentralized applications. In a first step source-separated wastewater (greywater and blackwater) originating from 24 residential flats was analyzed for the content of organic matter and nutrients as well as typical key parameters for microbial processes used in the treatment and reuse of wastewater. In accordance to earlier findings, blackwater was the major contributor to the total load of organic matter and nutrients in the wastewater, accounting for 69% of the chemical oxygen demand (COD), 83% of the total nitrogen (N) and 87% of the phosphorus (P). The high concentration of the nutrients (1.4-1.7 g N L^{-1} and 0.15-0.2 g P L^{-1}) in blackwater from low flushing (vacuum) toilets opens for new technologies regarding nutrient reuse as well as more energy efficient design of traditional removal approaches, especially for nitrogen.

In the second part a primary treatment method based on filtration technology for blackwater was developed. A particle size fractioning analysis was performed, which showed that approximately half of the total suspended solids are attributed to the supra-colloidal fraction (1-100µm). This fraction that is difficult to remove with traditional sedimentation based on gravity. Filtration of blackwater using an organic percolation filter, on the other hand, showed promising results of more than 80% TSS removal efficiency. There was no significant difference in removal performance between 15 and 30cm filter depth, so that more compact filters can be built. However, both this study as well as others pointed to the limited hydraulic capacity of the organic filter systems. To overcome this hydraulic obstacle, a two-step mechanical filter in series was developed. The first step was a traditional drum screen and the second a novel type of counter-flow in which an organic filter matrix is continuously added and renewed. The two-step mechanical filter unit showed a TSS removal efficiency comparable than a static percolation filter. This filter was tested at a mountain hut in the Alps, were it worked satisfactorily receiving peak loads of up to 70 toilet flushes per hour.

In the third part of the thesis, an aerobic moving bed biofilm reactor system (MBBR) was evaluated as a potential secondary treatment step for blackwater. The target application of a decentralized sanitation system at mountain lodge would often have the main focus on organic matter removal. However, in conditions with substrate limitation regarding the heterotrophic activity on the outermost biofilm layer, an establishment of ammonia oxidizing bacteria (AOB) in deeper layers is likely to occur. Nitrification is therefore an inevitable side-process in an aerobic biofilm system loaded with blackwater. The high free ammonia content of the blackwater was further shown to cause a partial inhibition of nitrite oxidizing bacteria (NOB) so that nitrite accumulates in the system. An experiment with different mixtures of greywater and blackwater showed substantial N_2O emissions. N_2O is suspected to be 300 times more potent compared to CO_2 as a greenhouse gas and in addition involved in the depletion of the ozone layer and needs therefore closer attention in a process evaluation. The emission factor (N_2O in % of biological N turnover) in the experimental MBBR system substantially increased with a higher proportion of blackwater, reaching 8.5% when loaded with pure blackwater compared to 0.7% when loaded with a household wastewater mixture of 20% blackwater and 80% greywater. These N_2O emissions could be related to accumulation of nitrite, which results in nitrosative stress and N_2O production by AOB, but also heterotrophic denitrifiers. Electron donor limitation of the heterotrophic denitrifiers in anoxic biofilm zones might also have been a factor that leading to the notable production rates of N_2O.

The two-step mechanical filtration device developed by this study might be an interesting alternative to traditional primary settling or septic tanks especially considering the high dry matter content of 13-20% in the retentate. However, when processing blackwater the liquid effluent needs further treatment. The high energy consumption and notable N_2O emissions of the aerobic biofilm system tested herein questions the sustainability of MBBR system as secondary treatment step. Other, especially anaerobic methods, should therefore be evaluated if an energy efficient method that also allows for recycling of nutrients to agricultural production is to be obtained.

Sammendrag

Formålet med dette studie var å evaluere forskjellige behandlingsmetoder for svartvann i kildespeparende sanitærsystemer spesielt med tanke på bruk for turisthytter og andre desentrale bruksområder. Som første steg ble de to kloakkfraksjonene, gråvann og svartvann, fra et kildeseparende sanitærsystem analysert for organisk stoff, næringstoffer og nøkkelparametere for mikrobielle renseprosseser. I tråd med funn fra andre studier var mesteparten av organisk stoff (69% av kjemisk oksygenforburk KOF) og næringstoffer (83% av nitrogen og 87% av fosfor) i svartvannsfraksjon. Næringsstoffer i svartvann fra vannsparende toaletter, som vakuumtoaletter, er i tillegg har høy konsentrasjon (1.4-1.7 g N L^{-1} and 0.15-0.2 g P L^{-1}) i forhold til vanlig avløpsvann. Dette er interessant i sammenheng med kretsløpsteknologier og gjenbruk av næringsstoffer til matproduksjon, eller med hensyn på nye metoder for en mer energieffektiv nitrogenfjerning.

Etter analysen av svart- og gråvannet sammensetning ble en primærrensemetode basert på filtrerings gjennom et organisk filter bestående av torv og blanding av torv og høvelspon testet. Partikkelfordelingsanlaysen av svartvann viste at omtrent halvparten av partikkelmassen tilhørete til den såkalte supra-colloidale fraksjonen (1-100µm) som er vanskelig å fjerne i et tradisjonelt system basert på sedimentering ved gravitasjon. En filtrering gjennom et organisk filter viste seg derimot å være en effektivt og en rensegrad på 80% ble oppnådd for suspandert stoff (SS). Det var ingen signifikant forskjell i renseeffekt i mellom 15 og 30cm filterdypde, men filtersystemet hadde en begrenset hydraulisk kapasitet. For å eliminere denne ulempen ble et nytt mekansisk filtersystem har blitt utviklet. Dette er basert på en to filtertrinn koblet i serie. Det første trinnet var et modifisert tradisjonelt trommelfilter etterfulgt av et motstrømsfilter der filtermediet blir kontinuerlig automatisk fornyet. To-trinnsfilteret oppnådde neste samme TSS rensegrad som et statisk perkolasjonsfilter. Dette systemet som hadde stor hydraulisk kapasitet ble også testet på et turisthytte i Alpene der det fungerte meget tilfredstillende, med en belastning på opp til 70 toalettspylinger per time.

Studiets siste fase har vært en uttesting av en aerob moving bed biofilm reaktor (MBBR) som et potensielt sekundærrensetrinn til filtrert svartvann. Sekundærrensingen var først og fremst tenkt for å fjerne organisk stoff (KOF). Det må imidlertid påregnes at en substratbegrensning til hetertropfe bakterier i de ytre biofilmsonene kan oppstå slik at ammonikk oxiderende bakterier (AOB) klare å etablere seg dypere i biofilmen. Nitrifikasjon er derfor en neste uungåelig bioprossess i en aerob biofilm som behandler svartvann. Svartvann inneholder mye fri ammoniakk (NH_3) som har en hemmende virking på nitrit oxiderende bakterier (NOB). Dette fører til at nitrit akkumuleres i systemet. Et eskperiment der en MBBR har blitt belastet med forskjellige blandinger av svartvann og gråvann viste en kraftig økning i lystgass (N_2O) utslipp med høyere andel svartvann i kloakken. Lystgass er mistenkt å ha en 300 ganger kraftigere drivhusgassvirking enn CO_2 og er også antatt å være involvert i nedbryting av stratosferisk oson. Utslippsfaktoren (N_2O i % av biologisk nitrogenomsettning) økte fra 0.7 med en blanding av 20% svartvann og 80% gråvann til 8.5% med rent svartvann. Denne lystgassproduksjon er antagelig foråsaket av nitrittakumulering, men en mulig kritisk faktor kan være begrenset tilgang til elektrondonorsubstrat for heterotrofe denitrifikanter i dypere anoxiske biofilmsoner.

Det 2-trinns mekansisk filtersystemet som er utviklet kan være et alternativ til en tradisjonell slamavskiller, særlig med tanke på det høye tørrstoffinnhold (13-20%) som er oppnådd i retentatet. Når filteret belastes med svartvann er utløpet fortsatt å regne for konsentrert kloakk som trenger en videre behandling. De store lystgassutslippene, men også energiforbruket setter spørsmålstegn ved hvor bærekraftig bruk av et aerobt biofilmsystem er som potensiell løsning. En literaturstudie av andre prosjekter som har evaluert løsninger til behandling av svartvann tyder på at anaerobe metoder der organisk stoff kan bli konvertert til biogass og gjenbrukt som energi og gjødsel kan være interessante.

Acknowledgments

First I will thank to my main supervisor Petter D. Jenssen for introducing the ideas of source separation, reuse and ecological sanitation and motivate me to work on this topic. His visions and ideas on novel, more sustainable approaches to deal with water and nutrient inspired me throughout my research work and writing process. Petter has also great skills to balance hard research work and scientific writing with relaxing activities such as cross-country skiing, a working philosophy I very much appreciated.

A big thank goes to my co-supervisor Peter Dörsch for the fantastic supervision of my work on biological nitrogen conversation. I really appreciated our numerous deep and fruitful discussions on enzymatic reactions and biofilms in the transient boundary between soil microbiology and wastewater engineering. I also want to thank for the numerous inputs in academic writing and drawing of figures.

A special thank also the Assistant Prof. Arve Heistad for providing the lab facilities at the Department of Mathematical Science and Technology (IMT) as well as for the numerous, motivating discussions on my experiments. A big thank goes also to Tore Ensby and Bjørn Brenna from IMT department for helping with the construction of the prototype for the counter-flow filter. A great thank also to Linda Hink from the University of Aberdeen for reviewing the chapter on the enzymatic processes and giving high valuable feedbacks to my review manuscripts.

Great thanks to my present employer Ecomotive AS, Runde (Norway) and its holding Jets AS, Hareid (Norway) for financing the last two years of my study. I have got a great support from our managing director Ottar Håkonsholm and my colleagues Melesse Eshetu and Jostein Grevsgård. A special thank goes to our former managing director Kåre Haddal who unfortunately passed away last year. I hope his visionary ideas on implementing source separating sanitation will continue to succeed in future.

A thank goes also to the partners that were involved in the EU FP7 project SANBOX: Jets AS, Hareid (Norway), Seecon GmbH, Wolhusen (Switzerland), Gysi+Berglas AG, Baar (Switzerland), SiA Norplast, Saldus (Latvia), Bioazul, Campanillas (Spain), University of Ljubljana (Slowenia), University of Tartu (Estonia), TTZ Bremerhaven (Germany). A special thank goes also to the companies and people that were involved in the onsite testing of the pilot blackwater treatment system at Britannia hut. We enjoyed a great time at the Britannia lodge appreciated the magnificent hospitality of the crew of Therese Andenmatten. I want to thank for the patience she and her staff devoted to our test sanitary system, which unfortunately did not always worked perfectly. A special thank goes also the Gysi+Berglas AG (Baar, Switzerland) and their managing director Markus Berglas for establishing the nice shelter for our blackwater treatment system in a challenging environment at 3030 meter above sea level. A lot of thanks also to Marc Bachmann, Viesmann AG, Spreitenbach (Switzerland) and Herold Zurbriggen and Dario Andenmatten, Herivan AG, Saas-Fee (Switzerland) for the great support to supply our sanitation system with solar heat. Last but not least also big thank to the Swiss Alpine Club and its representatives Christian Fux, Hans Jungen, Christiane Ody, Jaques Bondallaz and Marc Renaud.

Installation of the shelter box in front of Britannia hut

A great thanks goes further to the Institute for Natural Resource Sciences at the Zürich University for Applied Sciences (ZAHW) for providing me access to their lab facilities. A warm thank to may former colleagues Andreas Graber, Andreas Schönborn, Prof. Ranka Junge and Prof. Jean Bernard Bächtiger for this help with data analysis as well as the great discussions.

I also have to thank the MSc-students, PhD-candidate colleagues and trainees who contributed with term papers, diploma thesis or hourly-based work to the study. These are Anne-Guri Weihe (MSc, NMBU), Olga Popovitch (trainee, NMBU), Fikre Assafa (MSc, NMBU), Tilahun Megnstab (MSc, NMBU), Pavlo Kozminykh (PhD-cand., NMBU), Nataliia Sivchenko (PhD-cand., NMBU), Annina Walter (BSc, ZAHW) and Risch Tratschin (trainee, Seecon GmbH).

Britannia lodge at its 100 year celebration

Table of contents

List of appendix papers

Paper	Title	Bibliography
Paper 1	Load and distribution of organic matter and nutrients in a separated household wastewater stream	Todt D., Heistad A., Jenssen P.D. (2015) Environmental Technology 36:1584-1593
Paper 2	Removal of particles in organic filters in experimental treatment systems for domestic wastewater and black water	Todt, D., Jenssen, P. D., Klemencic, A. K., Oarga, A. & Bulc, T. G. (2014) Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering 49:948-954.
Paper 3	Particle removal in a novel sequential mechanical filter system loaded with blackwater	Todt D., Jenssen P.D. (2015) Water Science and Technology 71:1407-1413
Paper 4	Mechanism leading to N_2O production in waste water treating biofilm systems – a review	Todt D. Dörsch P. (in preparation) (manuscript for review article)
Paper 5	N_2O emissions in a biofilm loaded with different mixtures of concentrated household wastewater	Todt D, Dörsch P. (2015) International Journal of Environmental Science and Technology DOI 10.1007/s13762-015-0778-1

1 Introduction

Despite highly-developed sewer infrastructure in the countries of the Alps, wastewater facilities at remote tourism businesses are often violating present environmental standards. Studies in Austria, Germany, Italy and Switzerland claims that at least 60% of the over 2500 official mountain lodges will need to upgrade their sanitation systems in the next 10 years (Abegglen, 2004, Günthert and Narr, 2002, Günthert and Rauch, 2007). And the situation looks similar among the numerous summer mountain farms (Almen, Alpages) dealing with agro-tourism (Arnberger et al., 2006), especially with respect for the trend for converting alpine farm buildings into tourist accommodations (Wessely and Güthler, 2004). For the development of contemporary high alpine sanitation solutions various projects were started in the last decade (Abegglen, 2004, Andreottola et al., 2003, Günthert and Rauch, 2007, Todt, 2012). Different solutions were developed and tested ranging from advanced dry toilets to highly sophisticated decentralized wastewater treatment systems. Dry sanitary solution are principally easier to operate and less energy demanding. However, an increasing demand for comfort among tourists resulted into a trend towards the installation of water flush toilets, especially in larger mountain lodges with high visitor frequency. Compared to dry toilet solutions, water flush toilets provide more flexibility in the architectural integration and are apprehended as more comfortable by many tourists. However, the installation of flushing toilets will increase the water consumption of the refuge substantially and discharge a considerable amount of concentrated wastewater, which again requires advanced, often high energy demanding treatment systems (Abegglen, 2004, Günthert and Rauch, 2007).

This study was attached to the EU FP7-project SANBOX, which belongs to the most recent attempts to develop contemporary high alpine sanitation systems. It is based on source separation and reuse of water and nutrients, a novel integrating sanitation approach that emerged in recent years (Jenssen et al., 2009, Masi et al., 2010, Langergraber and Muellegger, 2005). The idea of source separating and reusing resources are also suggested as a sustainable solution for the future for mountain huts (Langergraber and Muellegger, 2005). While a reuse of nutrients may be difficult to obtain at a mountain lodge, the reuse of water gives advantages, especially for lodges located in environments with long freezing periods and limited access to fresh water. SANBOX was therefore treated greywater from kitchen and washing facilities and blackwater from the toilets separately and reused the treated greywater for toilet flushing. For this purpose different treatment components were developed and evaluated in a pilot-scale application at Britannia lodge in Saas-Fee, Switzerland (Andreottola et al., 2003). This study is focusing on the treatment of the blackwater fraction, which was shown to be a challenging task due the high concentration load of organic matter and ammonia. The main objective was therefore to evaluate and develop a robust and energy efficient treatment solution for the blackwater fraction that can be implemented in source separating sanitation systems on mountain lodges and other remote tourist facilities.

1.1 Background and research targets of this study

As outlined in the introduction, this study contributed with the evaluation of a blackwater treatment method to a EU FP7 project for the development of a source separating sanitation system for mountain lodges and other types of remote tourist facilities. With respect to its main objective to evaluate and develop robust and energy efficient treatment technologies for the removal of organic matter, this study was focusing on a classical two-step treatment approach consisting of a primary treatment unit for removal of particulate matter followed by a secondary treatment unit for the removal of soluble and colloidal organic components. Based on this approach, a pilot-scale system was developed and tested at Britannia lodge (Figure 1).

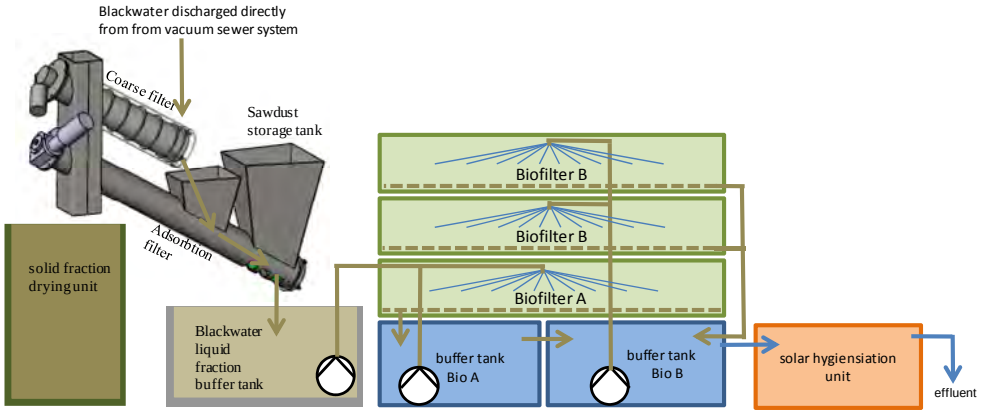


Figure 1: The proposed idea for a blackwater treatment system for the pilot application at Britannia lodge

As visualized in Figure 2, the research of this study is structured into three main pillars starting with the characterization of the blackwater (paper 1) followed by the development of the primary treatment unit (paper 2, paper 3) and the critical evaluation of different secondary treatment technologies including the assessment of greenhouse gas emissions. In a first stage we were characterizing the blackwater from student dormitories at the Norwegian University for Life Sciences in Ås, Norway. These results are presented in paper 1 including a comparison to other blackwater data found in the literature. Section 3.2 further provides some supplementary data and information on the size distribution of colloidal and supra-colloidal particulate matter which was shown to have a high relevance for the substrate availability in a biofilm, which again is discussed in section 5.2.

The development of the mechanical filter system for the primary treatment step started with the evaluation of different technologies summarized in section 1.2, as well as initial experiments, which results are presented in section 4.1, 4.2 and paper 2. The results of the pilot-scale testing in the laboratory are presented in paper 3 and supplemented with the available data material from the onsite testing period at Britannia lodge in section 4.4. Section 4.5 provides further the results of a supplementary initial study for an integration of precipitation of the nutrients N and P into struvite.

The secondary treatment step that was originally developed and tested at Britannia lodge did not show a sufficient treatment performance under the high loading rates the system received during the seasonal peaks. Its technical evaluation, including the experiences from the onsite testing at Britannia lodge, is summarized in section 1.1. This study was however focusing on the evaluation of an alternative solution consisting of a moving bed biofilm reactor (MBBR), which was suggested in the final report of SANBOX project (2001/77/EC). The main focus of this additional research was to assess potential critical side

processes related to the biological conversion of ammonia and potential greenhouse gas emission. It started with a comprehensive literature review (paper 4), followed by a pilot-scale experiment with different wastewater mixtures from the student dormitories (paper 5). Chapter 6 synthesizes the outcomes of these two studies with focus on blackwater treatment. The last chapter (6) presents a critical evaluation of the outcomes of this study with respect to alternative blackwater treatment methods that were found in the literature.

Blackwater characterization	**Primary treatment**	**Secondary treatment**
organic matter and main nutrients (paper 1)	evaluation of technology (section 1.2)	evaluation of technology (section 1.1)
process parameters (paper 1)	preliminary experiments (paper 2; chapter 1)	summarizing results onsite test Britannia lodge (section 5.2)
mass loading rates per capita (paper 1)	pilot-scale test in laboratory (paper 4)	evaluation of an intensive biofilm system including risk for greenhouse gas emissions (paper 4; paper 5; section 5.5)
particle-size distribution (paper 1)	summarizing results of onsite test at Britannia lodge (section 4.4)	literature review on the degradation of different types of organic substrates in a biofilm (section 5.2)
supplementary data on size distribution of colloidal and supra-colloidal matter (section 3.1)	supplementary preliminary study for an integration of N and P precipitation (section 4.4)	critical evaluation of results and suggestion for further research (chapter 6)

Figure 2: Structural overview over the content of this study with respect to the main objective to evaluate different methods to treat blackwater onsite at remote locations.

1.2 Primary treatment for removal of particulate matter

A majority of the present wastewater treatment systems have a primary treatment step which is either based on sedimentation or filtration or a combination of these two (Tchobanoglous et al., 2002, ATV, 1997). For the target application on mountain lodges, a selected technology primarily needs to be robust and simple to operate. Technologies that require complex controlling systems and highly skilled staff should be avoided. For mountain lodges located in high alpine environments, which are characterized by exposed rock and permafrost, also space requirement become an important issue (Abegglen, 2004). A majority of the mountain lodges across the Alps have a limited accessibility, the sewage is either disposed locally or transported to the valley by helicopter (Abegglen, 2004, Arnberger et al., 2006, Günthert and Rauch, 2007). It is therefore advantageous to apply particle separation systems that produce a retentate with high dry matter content (Günthert and Rauch, 2007). This resulted in the selection of filtration as primary treatment process. Based on these specifications, a compact two-step mechanical filter system was developed and tested in both lab-scale (paper 3) as well as in pilot-scale at Britannia lodge in Saas-Fee, Switzerland (Todt, 2012).

1.3 Secondary treatment for removal of soluble organics and nutrients

A notable fraction of apparently soluble organic matter (determined as filtrated chemical oxygen demand COD), as well as dissolved nutrients (Total Ammonoious Nitrogen TAN; orthophosphate PO_4^{3-}) were detected in the blackwater of Kaja student dormitories (paper 1) and also by others (Oldenburg et al., 2008, Zeeman et al., 2008). Different methods were applied to recover or remove dissolved nitrogen (N) and phosphorous (P) compounds, ranging from classical nutrient removal systems based on microbial N conversion (Gujer, 2010, Kuenen, 2008, Udert and Jenni, 2013) and chemical coagulation and precipitation of P (Tchobanoglous et al., 2002). In recent time, novel more reuse focused methods are developed such as stripping (Siegrist et al., 2013) or microbial fuel cells (Kuntke et al., 2012) for N recovery. Removal and recovery of soluble organic matter on the other hand is mainly performed within a microbial conversion process (Tchobanoglous et al., 2002, Larsen et al., 2013). Chemical wet oxidation (sometimes also called advanced oxidation) is another method for organic matter removal often used in drinking water treatment. However, a direct oxidation with radicals (free $Cl^{\cdot}$, ozone) is significantly more energy demanding than an enzyme based microbial oxidation and was shown produce highly toxic byproducts (e.g. trihalomethanes) when applied to waste- or natural water having a high organic content (von Gunten, 2013, Kalibbala et al., 2011). A supporting application of small ozone doses to a bioreactor system on the other hand was shown to have a beneficial effect by enhancing hydrolysis and subsequent biodegradation of medical residuals and other slowly degradable compounds (von Gunten, 2013, Zimmermann et al., 2011). However, a proper and environmentally friendly removal of the highly concentrated dissolved organic matter present in blackwater requires a microbial process as a main treatment step (Zeeman and Kujawa-Roeleveld, 2011, Otterpohl, 2002, Regelsberger et al., 2007, von Gunten, 2013).

The high concentration of degradable organic matter in the blackwater makes it interesting for anaerobic treatment methods, especially, because of low energy consumption (Halalsheh and Wendland, 2008, Zeeman et al., 2008). As demonstrated by Zeeman and Kujawa-Roeleveld (2011), it is possible to remove a majority of organic matter present in blackwater in an Upstream Anaerobic Sludge Blanked (UASB) and use the produced methane (CH_4) to provide the heat needed to facilitate high degradation rates, so that the system could be operated without external energy. However, it is important to ensure a proper capturing and usage of the produced CH_4 to avoid secondary environmental impacts, since CH_4 was identified as a strong greenhouse gas with a 3.7 times greater global warming potential than CO_2 (Lashof and Ahuja, 1990). An uncontrolled accumulation of CH_4 represents further a considerable risk for explosion. Due to the complexity and risks related to CH_4 handling in small decentralized applications it was decided to focus on aerobic treatment methods.

The aerobic treatment methods applied to blackwater are unsaturated vertical flow constructed wetlands (Masi et al., 2010) , trickling filters (Otterpohl, 2002) as well as membrane bioreactors (MBR) (Knerr et al., 2011). The former two natural-based approaches have the advantage of low, almost negligible energy consumption, but the disadvantage of large space requirements (Masi et al., 2010), which limits its potential applications in high alpine environments (Abegglen, 2004, Günthert and Rauch, 2007). MBR are compact systems, but have the disadvantage of high energy consumption as well as the need for a more or less continuous operation also during season breaks (Günthert and Rauch, 2007). For our pilot system at Britannia lodge we therefore decided to test an unsaturated vertical-flow fixed-film biofilter that represent a more advanced type of trickling filter. Fixed-film biofilm filters were further pointed out as a robust and energy saving advanced treatment method for mountain lodges (Günthert and Rauch, 2007).

At Britannia lodge the mechanical filter system released an average COD load of 0.2 -1.3 kg O_2 d^{-1} to the subsequent biofilter, which corresponds to a volumetric load of 2-13 kg O_2 d^{-1} per m^3 filter media (Todt, 2012). This measured load was notably over the recommended 1.2-2.4 kg O_2 d^{-1} per m^3 filter media (ATV, 1997), which resulted into a relatively poor treatment efficiency with only 5-10% COD reduction, especially during the season peaks. The main reason for this massive overload of the filter system was an underestimation of the daily blackwater load at Britannia lodge and is discussed more in detail by Todt

(2012). Hence, a larger biofilter or alternatively a secondary treatment system with greater volume efficiency would be required. For Britannia lodge and most of the other high mountain lodges it would be sufficient to focus the wastewater treatment on organic matter (COD) removal while nitrogen removal is secondary (Abegglen, 2004, Günthert and Rauch, 2007). At Britannia lodge this might be obtained by using a south oriented moraine slope as a natural, extensive gravel-bed filter (Todt, 2012). However, in the future Swiss authorities probably will require a higher effluent quality that can be controlled by regular sampling before any discharge into a natural ecosystem (Abegglen, 2004). For this situation, the wastewater would need to be treated within the given space in the basement of the building and a more volume efficient system will be required (Todt, 2012). An MBR as installed at the nearby Monte-Rosa lodge (Abegglen, 2004) maybe a potential solution, but proper operation at a mountain lodge with long season breaks and high loading variations is challenging with respect to membrane fouling. A moving bed biofilm reactor MBBR with subsequent gravity sedimentation (Odegaard et al., 1994) may be a more robust alternative to tackle the large variation in load as well as season breaks given very limited available space (Todt, 2012, Andreottola et al., 2003). However, intensive aerobic biological wastewater treatment systems reached an emerging attention in terms of greenhouse gas emissions (e.g. Kampschreur et al., 2009, Wunderlin et al., 2012), especially for N_2O, which is suspected to have a 300 times greater greenhouse impact than CO_2 (ICCP, 2001) and to be involved in the ozone layer depletion (Ravishankara et al., 2009). To evaluate this issue more in detail, the present literature was reviewed on critical factors that may lead to N_2O emissions in an aerobic wastewater treatment processes with focus on biofilm system (paper 4). The suggested MBBR reactor system was further evaluated in a comprehensive pilot-scale experiment with different mixtures of household wastewater with focus on N_2O emissions (paper 5).

2 Material and Methods

The study was mainly based on different bench-scale and pilot-scale laboratory experiments that were conducted at the Norwegian University for Life Sciences in Ås, Norway using source separated household wastewater (blackwater, greywater) from student dormitories. Details of this sewer system are outlined in paper 1. Details of the method description to the experiments are outlined in the corresponding paper. Details on smaller supplementary experiments were integrated into the corresponding figure or table captions. The methods related to the larger supplementary experiments on nutrient recovery and substrate analysis are given in the corresponding sections 4.5 and 5.3, respectively.

Due to the general high variability of wastewater, the data in this study are usually given as a range, which is representing the interval between the 25% and 75% percentiles. Statistical significant tests are based on none-parametric Wilcoxon signed-ranked tests unless otherwise is declared. The limit for a significant difference was defined at a p-value <0.1, If no significant difference was found the determined p-value is declared.

3 Characterization of blackwater

3.1 Characteristic and distribution of suspended solids in blackwater

The study (paper 1) gives insights into the composition and distribution of different size fraction of suspended solids in the blackwater discharged from Kaja student dormitories. The suspended solids represent a substantial fraction of the total organic load, accounting for 67-76% of total COD. The high proportion of 88-93% volatile compounds (paper 1) indicates that suspended solids mainly consist on organic matter. A sequential filtration over different filter nets with defined mesh size identified two major size fractions for the suspended solids in this particular blackwater: coarse particles (>1mm) and small-sized supra-colloidal particles in a size of a few micrometers (1-10 µm). The coarse particles accounted for of 23-37% SS and 28-38% of COD. The content on N and P was with 20-50 mg N g^{-1} and 3-10 mg P g^{-1} relatively low in these large-sized particles compared to the content of 33-66 mg N g^{-1} and 18-21 mg P g^{-1} that was determined for the total suspended solids (paper 1). Hence, coarse suspended solids seem to a large extent to consist of carbohydrates which would point towards toilet paper as a possible source., Toilet paper consist of 98% organic compounds and have a negligible N and P content (Jönsson et al., 2005). The second major size-fraction of suspended solids in blackwater, accounting for 10-23% SS and 13-16% of COD, was attributed to micro-sized particles in a range of 1-10 µm (paper 1). This smallest fraction of so-called supra-colloidal solids range from 1-100 µm (Balmat, 1957) and showed a considerably higher content of nutrients accounting for 100-120 mg N g^{-1} and 21-25 mg P g^{-1}, respectively (data not shown). These figures are within a typical N and P content of 120 mg N g^{-1} TS and 20 mg P g^{-1} TS reported for bacteria biomass in wastewater (Comeau, 2008), which indicates that these small supra-colloidal solids mainly represent living or death bacteria biomass, likely originated from fecal matter or intestinal flora.

The blackwater analyzed by our study passed a grinder pump (Vacuumarator^{TM} 25MB, Jets, Hareid), that is likely to impact the particulate matter. The particle-size distribution might therefore be significantly different in another type of sewer system. However, a recent study identified a similar fractionation of suspended solids in municipal wastewater as in our blackwater, by identifying two distinct major types of particles: large-sized particles (>63µm) mainly consisting on carbohydrates and supra-colloidal particulate matter with high protein content as typically found in living or death biomass (Sophonsiri and Morgenroth, 2004). Hence, regardless the sewer system, suspended solids seem to consist of two major types of particles: a) coarse particles, mainly consisting on carbohydrates, likely originated from toilet paper and b) supra colloidal particles with high content on N and P, likely attributed to biomass originated from excreta. Depending on its purpose, particle separation in wastewater treatment needs thereby to consider the specific characteristics of each of these particle fractions. If the main purpose is to reduce the load on particulate organic matter, as typically in primary treatment applications (Tchobanoglous et al., 2002) a focus on the coarse particle fraction, which is likely easier to remove, may be appropriate. In our blackwater, a removal of those large-sized solids (>1mm) would approximately reduce one third of the COD load. If the major purpose is to reach a high reduction of organic matter (COD, BOD) as well as a notable retention of particle bound nutrients, a strong focus need also be taken on removal of supra-colloidal particles.

3.2 Supplementary analysis of apparently soluble solids and nutrients

In our analysis of the blackwater the observed concentration of total apparently soluble N and P (filtrated) was notably higher than the identified soluble compounds TAN and PO_4^{3-}, respectively. This points towards the presence of other, apparently soluble N and P compounds. We hypothesized that N and P bound to colloidal solids may account for a considerable fraction of those unidentified N and P compounds, since they are smaller than the apparent pore size (1.2 µm) of the microfilter (Whatmann GF-C) used for distinguishing between particulate and soluble wastewater components (paper 1). The assumption that colloidal solids may account for a notable fraction of apparently soluble solids is supported by a study of

municipal wastewater (Dulekgurgen et al., 2006, Sophonsiri and Morgenroth, 2004) as well as the data from a supplementary laser diffraction analysis of a blackwater samples. Both these investigations show that a considerable fraction (26%) of particles < 100 µm) were smaller than the apparent pore size of the microfilter (Figure 3).

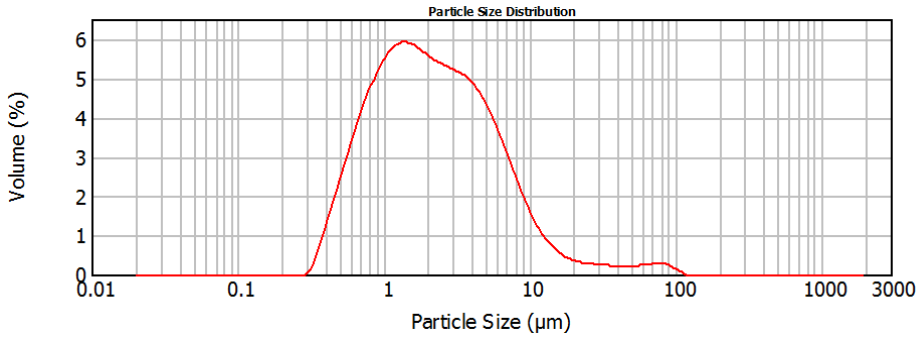


Figure 3: Distribution of colloidal and supra-colloidal particles in a selected blackwater sample. The particle size distribution was determined with help of a laser difractometer (Mastersizer 2000, Malvern, Worcestershire UK). Detection limit of the apparatus was 0.3 µm. Precedent to this procedure the sample was prepared by filtrating across a 100 µm net and diluting with distilled water (1:1).

As hypothesized in the previous section, supra-colloidal particles are most likely attributed to living or death biomass. Also the none-identified soluble N and P compounds may be in part be attributed to biomass, since free bacteria cells can be considerably smaller-sized than 1 µm (Comeau, 2008). The N/P ratio in the unidentified apparently soluble compounds ranged from 14-52, which is notably higher than the N/P ratio reported for biomass ranges from 5 (Comeau, 2008) to 14 (Keskitalo et al. 2010). Hence, a considerable fraction of the unidentified apparently soluble N seems to be attributed to by other compounds than biomass or biomass residuals. Nitrogen contained in blackwater mainly orginated from urine (Jönsson et al., 2005), which means that unhydrolized urea may represent a substantial source of apparently soluble N in addition to TAN and collidal biomass residuals. In fresh urine, urea represents a major fraction (>90%) of N, which is subsequently hydrolyzed to ammonia by urease-active bacteria, also called ureolysis, that normally happens in sewer systems (Mobley and Hausinger, 1989, Udert et al., 2003a). In urine collecting pipes, urea was shown to become completely hydrolyzed within 1-2 days (Udert et al., 2003a). Considering the estimated retention time of 36-48 hours for the blackwater in the Kaja sewer system (paper 1), a majority of the urea is probably hydrolyzed, as supported by the high proportion of TAN to total N. The high variation in the above estimated retention time in our sewer system on the other hand likely resulted in varying degrees of ureolysis from almost complete to a still notable presence of residual urea. The high variation in the detected unidentified apparently soluble N ranging from 67 to 162 mg L^{-1} would support this hypothesis. However additional experiments as done in the above cited study (Udert et al., 2003a) need to be performed to determine the exact rates and degrees of ureolysis at different points of the Kaja sewer system.

4 Removal of particulate matter from blackwater with mechanical filtration

This chapter presents the mechanical filter system that was developed within the SANBOX project as well as this study. The first two sections explore the filter mechanism with respect to the two major groups of suspended solids identified for each of the two sequentially applied technologies. The subsequent section assesses the performance and reliability of the integrated two-step mechanical filter system compared prelim experiments and other particle removal systems presented in the literature. In a last section, the results of an additional initial experiment are presented to assess the opportunity for an integrated phosphorous removal by struvite precipitation in the system.

4.1 Removal of coarse particulate fraction with screen filtration

The coarse particulate fraction can be retained with simple mechanical screening, which is a relatively wide-spread primary treatment method in municipal wastewater treatment plants (Tchobanoglous et al., 2002). Such pre-screening applications typically use coarse screens with an opening size of 5-10 mm (Tchobanoglous et al., 2002), but also fine-screen systems with opening-size down to 350 µm are used at some places (Rusten and Odegaard, 2006). Fine-screens with a small opening size usually need a backwashing system, which increases the system complexity and usually also reduces the dry matter in the retentate (Tchobanoglous et al., 2002). To assess the impact on opening-size on TSS retention and hydraulic capacity a small experiment was conducted in column filters with different screen-sizes loaded with blackwater (Table 1). The results showed that textile nets with opening sizes ranging from 100 to 1000 µm started to pond after a load corresponding to 57 mm, while the two columns with metal screens having opening sizes of 3 and 5 mm, could be loaded with, 84 mm, and >100 mm, respectively, before ponding occurred. The filter nets and the 3 mm screen showed a comparable TSS removal efficiency, ranging from 65 to 71%, while TSS retention in the 5 mm screen was almost negligible (<10%). These results indicate that a) a majority of the particles were smaller than 5 mm and b) particle retention on the screens seems mainly to be determined by the filter-cake. The latter started to cover the whole filter area after a load corresponding to 29-57 mm on all of the filter columns, except the 5 mm screen, on which no filter-cake formation was observed (Table 1). Accordingly observations that filter-cake has a significant impact on particle retention were also made by others (Faure et al., 2006, Rusten and Odegaard, 2006). Hence, filtration across a static screen or textile net is a complex function of retention and accumulation of particles.

In a mechanical drum-screen occurrence of filter-cake is principally antagonized with a screw conveyor, which is continuously removing the retentate (Tchobanoglous et al., 2002). Some more advanced belt-filter systems aim to control filter-cake accumulation on a certain level which is facilitating retention of smaller particles, but still below a critical limit for serious losses of hydraulic capacity (Rusten and Odegaard, 2006). Both principles presumes that clogging is limited to the void channels within the filter-cake, which can be mechanically removed, while the void channels inside the screen itself sustain its hydraulic capacity (Tchobanoglous et al., 2002). To check this internal void channel clogging, an optical inspection of the textile nets and metal screens was performed after removal of the filter-cake with help of a hand scraper, which is simulating the mechanical impact of a screw conveyor. In the two micro screen textile nets (100; 500 µm), almost all void channels (>95%) where clogged, while the 1mm net showed a minor fraction (20%) of still open channels (Table 1). In all textile nets, removal of the accumulated material inside the void channels was only possible by washing under a water tap. In the 3 mm metal screen on the other hand, approximately 40% of the void channels were still open and the clogged channels could be opened by applying relatively low forces in emptying small amounts of water from a beaker glass. In the 5mm screen more than 90% of the channels were still open (Table 1). The 3 mm opening size was shown to facilitate filter-cake formation and associated additional retention of particles smaller than the apparent opening size

to some extent, while internal clogging of the void channels seems not to be a critical issue. Screens with smaller opening sizes likely need a backwash device to antagonize clogging of void channels, while large opening sizes seem to result in considerably lower particle retention with the tested type of wastewater. Hence, for a mechanical screening of the blackwater used in our study, a 3 mm opening size was chosen as the most appropriate for mechanical screening. However, since the optimal opening size is likely highly dependent on the average size of the coarse particle fraction, it may differ considerably for other types of wastewater. More bench-scale filtration experiments with different mesh-sizes are therefore recommended when a broader application of the drum-screen used in first filtration step in the mechanical filter system (Figure 4) should be evaluated.

Table 1: Initial experiment for evaluation of the optimal opening size for a mechanical screen to remove the coarse particle fraction. Five parallel columns with an apparent filter area of 0.126 m^2 were loaded batch-wise with 0.9 liter raw blackwater in 90 sec intervals. Totally 15 batch loads where loaded corresponding to a load of 105 mm. After all sewage passed the filter, filter-cake was measured and removed with help of a hand scraper. The data are based on two sequential repetitions. The fraction of apparently clogged void channels was approached with help of visual estimation.

Opening size	**100 µm**	**500 µm**	**1000 µm**	**3000 µm**	**5000 µm**
filter fabricate	SEFAR PETEX100/32	SEFAR PETEX500/39	SEFAR PETEX1000/39	metal screen	metal screen
apparent opening area	32%	39%	45%	ca. 40%	ca. 40%
thickness of screen	125 µm	620 µm	1000 µm	1500 µm	1500 µm
load until apparent filter-cake occurred	21 mm	29 mm	36 mm	57 mm	>105 mm
Load until apparent ponding occurred	57 mm	57 mm	57 mm	84 mm	>105 mm
final thickness filter-cake	25-26 mm	23-24 mm	23-24 mm	15-18 mm	0 mm
TSS reduction efficiency	85-90%	80-85%	60-65%	60-65%	5%
fraction of apparently clogged void channels	>95%	>95%	70-80%	50-60%	<10%

4.2 Removal of micro-particle fraction with filtration across a percolation matrix

Micro-sized particles will not be retained on a mesh with a typical opening size as used in mechanical screens and the retention of supra-colloidal particles, especially, would require microfiltration. However, such a membrane filtration was shown to struggle with serious clogging and fouling when applied to concentrated raw sewage (Zhang et al., 2012) and thereby not appropriate for a direct filtration of blackwater. An alternative to membrane- or screen filtration is a filtration over a complex percolation matrix as applied in form of sand filters or organic filter beds, which are featuring tortuous void channels that facilitate a broad number of concurrent processes related to physical retention and chemical adsorption of organic compounds and nutrients as well as biological immobilization and decomposition of organic matter (Gajurel et al., 2003a, Kõiv et al., 2009, Taylor et al., 2003). Especially biological immobilization was shown to have a significant capacity for retaining particles in a size of several order of magnitudes

smaller than the average opening size of the void channel (Zhao et al., 2009). A lot of work has been done to investigate retention of particles, nutrients and organic pollution in peat, the most common media used in organic filter beds (Corley et al., 2006, Kõiv et al., 2009, Novoselova and Sirotkina, 2008). However, the void channels of peat are relatively small which limits the limited hydraulic capacity when loaded with higher strength wastewaters (Corley et al., 2006). The high concentration of biodegradable organics present in raw blackwater will likely fuel biofilm development in the void channels, which was pointed out as a main factor for a biological immobilization of supra-colloidal particles but also clogging and serious losses of hydraulic capacity (Zhao et al., 2009, Hua et al., 2013b).

To gather more knowledge on these issues, we conducted an initial study (paper 2) where the filtration processes across an organic filter matrix with different types of filter media was investigated more in detail. The study focused on two different applications of matrix percolation filters: 1) primary treatment of blackwater and 2) polishing of effluent from an SBR package treatment plant. More detailed information and results from this experiment are given in paper 2, while in this chapter the outcomes of the primary treatment application experiment with settled blackwater. are discussed. The settled blackwater showed a TSS varying from 400 to 1600 mg L^{-1}, which is slightly lower than the TSS for the experiment outlined in Table 1. The settled blackwater was further characterized by a low proportion of coarse particles (>100 µm), which accounted only for 16% of TSS, and a high proportion 67%, of supra-colloidal particles (1-10 µm) (paper 2). This particle-size distribution was again assumed to be comparable to the effluent of the proposed prescreening unit.

The results of our experiments confirmed our assumption that the hydraulic capacity of a static percolator column was considerably lower when loaded with settled blackwater compared to a load with secondary effluent of a biological treatment step (diluted secondary sludge). We further compared two different types of filter media: peat as used by most of other studies and a mixture of peat and sawdust to provide a coarser structure and larger void channels. The peat-sawdust mixture showed a clearly better hydraulic capacity but surprisingly also a statistically significant ($p<0.1$) greater particle retention (89% versus 74%, respectively). We assume that this greater filter performance was a result of a greater turtousity provided by the mixed media. Our second experiment showed further that the TSS removal efficiency did not differ statistically significant ($p=0.54$) between 15 cm and 30 cm filter depth (paper 2). This irrelevance of filter depth within the tested range (15-30 cm) was likely attributed to the observed unilateral accumulation of the retained particles in the uppermost 5 cm of the filter matrix (paper 2), which is in accordance to the observations of other researchers (Taylor et al., 2004). It may therefore be possible build a percolation column considerably smaller than the typical filter depth of 30-100 cm without losing significant TSS removal efficiency.

Regardless the used filter media (peat, peat-sawdust mixture), permanent ponding started to occur after only three days loading with blackwater at a rate of 72 cm d^{-1} (paper 2). Such serious hydraulic limitations were also pointed out by earlier studies applying percolator filters to raw municipal wastewater (Lens et al., 1994). Hence, a matrix percolation filter is only applicable to raw sewage and especially raw blackwater when clogging of the void channels can be antagonized in an efficient way. Some researchers facilitated a development of a compost worm population in the filter matrix, which are mechanically impacting its structure and continuously establishing new void channels to sustain hydraulic capacity (Gajurel et al., 2003a, Taylor et al., 2003). However, such so-called vermicompost filters are still relatively large-sized extensive systems and the filter matrix needs to be renewed on a regular basis, which is usually a dirty, unpopular routine for the operation staff (Gajurel et al., 2003b). We decided therefore to develop a mechanical method to antagonize void channel clogging, which we tested in our third initial experiment with a manual mixing of the uppermost 5 cm of the filter matrix. This manual mixing resulted into a temporary disappearance of ponding and by performing it on a daily basis corresponding to an interval of 72 cm hydraulic load, the hydraulic capacity could be sustained over a period of 7 days without renewing the filter matrix. The mixing disturbed the matrix structure considerably, but it showed no significant ($p=0.68$) negative effect on the TSS reduction efficiency (paper 2). The negative impact of the mechanical

disturbance on the matrix structure might have been compensated by an increasing presence of biofilm fragments across the filter matrix. Biofilm was shown to enhance TSS retention by immobilizing supra-colloidal particles by sorption into the biofilm matrix consisting largely of extracellular polymeric substance (EPS) (Zhao et al., 2009). In addition to establishing new void channels the mixing might have moved biofilm fragments in deeper filter layers and thereby enhanced the bio-immobilization of supra-colloidal particles. However, further research will be needed to investigate this hypothesis more in detail.

4.3 Lab test experiment of the integrated mechanical filter system

The results from the different initial trials for gathering knowledge on filtrations processes of different particle-size fractions where used to develop a prototype mechanical two-step filtration system (Figure 4). The laboratory experiments showed that it is possible to remove a substantial fraction of 78-85% suspended solids (TSS), 60-80% of COD and 31-36% of phosphorous (paper 3). This overall removal efficiency obtained with the mechanical filter system is comparable to the removal efficiencies obtained with the passive percolator columns in the previous experiment (paper 2) and others (e.g. Lens et al., 1994). However, the inlet TSS concentration in the blackwater applied to this laboratory test experiment (paper 3) was substantially higher than the settled blackwater loaded to the passive percolator columns in our previous experiment (paper 2) or municipal wastewater used by the other above cited studies. The results of the laboratory experiments indicated at the same time a dependency of TSS removal efficiency on TSS inlet concentration, which points towards a reduced filter performance with lower wastewater concentration. This section will therefore investigate this dependency as well as the retention mechanism in each of the filtration steps more in detail with help of the outcomes from the blackwater analysis and preliminary experiments in static filter columns discussed in the preceding sections.

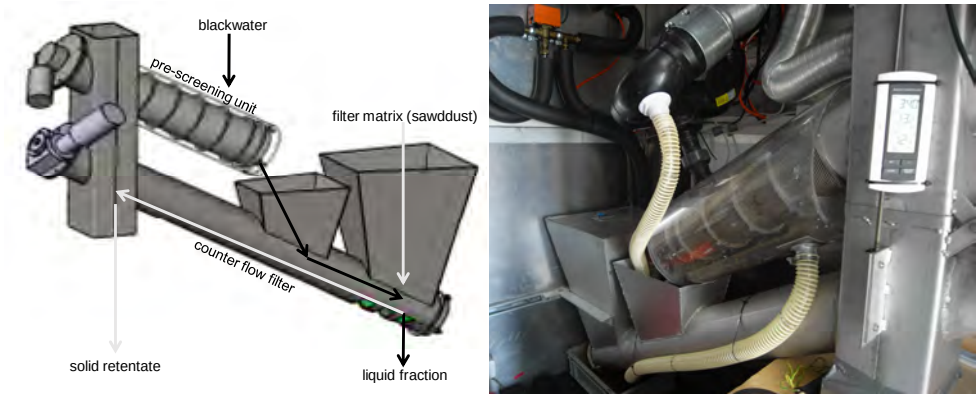


Figure 4: Mechanical two-step filtration unit (technical drawing adapted from paper 3) used in the lab test experiment and onsite test at Britannia lodge (Saas-Fee, Switzerland).

The TSS removal efficiency of 58-72% (paper 3) in the drum screen used in the first filtration step was in a range comparable to the 60-65% obtained with a static filtration across a flat metal screen of the same opening size (3 mm) in the initial experiment (Table 1). These findings indicated that the goal of controlling the balance between filter-cake accumulation and filter-cake removal worked as expected under those particular loading conditions. However, it is important to note that the TSS in the blackwater used for the laboratory test experiment, which was determined in a range of 6-9 g L^{-1} (paper 3), was considerably higher than the range of TSS (4.7-6.1 g L^{-1}) in the samples taken at Kaja (paper 1). The reason for this

notable variation in blackwater quality between the two experiments using blackwater from the same source (Kaja dormitories) was an inadvertent up-concentration in the holding tank used for the laboratory test experiments of the mechanical filter (paper 3). After discovering this source of bias, the tank was replaced with a new one having a larger volume and an improved mixing device. For the characterization of source-separated sewage presented in paper 1, only the samples from the new holding tank were used. The up-concentration in the old holding tank seems not only to have impacted the TSS, but also the particle size distribution as indicated by a considerably higher proportion of 62-79% (supplementary analysis on n=3 samples) large sized solids (>1mm) than the 23-37% determined in unbiased blackwater samples from Kaja dormitories (paper 1). This larger proportion of large-sized particles in the blackwater used for the laboratory test experiment might have enhanced the TSS removal efficiency of the drum filter compared to a load with a normal particle-size distribution determined for Kaja (paper 1) due to; a) a greater fraction of TSS that is easily retained on a 3mm screen and b) greater filter-cake development. These assumptions would point towards a notable lower TSS removal efficiency when the system was loaded with an unbiased blackwater having a normal particle-size distribution. Additional experiments are therefore needed to clarify this issue.

The TSS removal efficiency of the counter-flow filter used in the second filtration step was with 14-21% (paper 3) considerably lower than the removal efficiency of 60-74% obtained with the static percolator columns (paper 2). This finding points towards a significant greater negative impact on the filter structure and particle retention mechanism by the rotation of the conveyor than the manual mixing in the preliminary experiment (paper 2). One main difference between the two mechanical mixing methods was in the mechanically affected filter cross-section. The hand mixing in the preliminary experiment only affected the uppermost 5-10 cm or 30-60% of the filter depth (paper 2). The rotating conveyor in the drum-screen on the other hand had impact on the whole filter cross-section due to rotation of the filter matrix. This probably decreased the particle retention capacity, but also resulted into outwashing of retained particles as well as fractions of the filter matrix. The data on phosphorous retention, (n=12, paper 3) give some additional information on the type of retained particles in the two filtration steps. Based on the data presented in paper 3 and additional measurements of orthophosphate (not shown), the P-content of the retained suspended solids in the mechanical filter system was calculated to be in a range of 11-12 mg P g^{-1} TS. This is close to the 3-10 mg P g^{-1} TS determined for coarse particulate material (section 3.1, paper 1), which again points towards that mainly large-sized particles where retained in both filtration steps, while a majority of the supra colloidal matter seemed to have passed through. Hence, the anticipated bio-immobilization of small particles in the second filtration step seems only to have taken place to a minor extent.

An insufficient biofilm development within the filter matrix may also be attributed to the relatively low retention time of the filter matrix in the percolation zone of the filter tube defined as the distance between the inlet funnel where the pre-filtrated blackwater infiltrates the matrix and the effluent grid at the lower end of the tube. Despite a high load of readily degradable organics as well as continuous supply with putatively active heterotrophic biomass from the blackwater, the retention time of potentially built up smaller sessile biomass spots was likely not sufficient to develop larger biofilm spots. According to the literature (Zhao et al., 2009) as well as our own experience from the experiments (paper 2), a development of a significant biofilm mass takes at least 2-3 days even with high substrate availability. Both the liquid flow (Hua et al., 2013b), but in our system also the movement of the conveyor may antagonize biofilm development by forcing detachment events. Hence, a significant bio-immobilization of supra-colloidal solids within the counter-flow filter matrix likely requires a significantly longer solid matrix retention time. This may be obtained by reduced conveying speed, which was shown in previous experiments (paper 3) to result in serious decrease of the hydraulic capacity. Increase of the length of the percolation zone may be another possible solution to facilitate a greater biofilm development in the system, but this might also impact the hydraulic capacity of the system. More research is therefore needed to optimize the design.

4.4 Onsite test at Britannia lodge

In order to test the mechanical filter system under "real-life" onsite conditions, it was installed and operated for two seasons at the Britannia lodge. The filter was directly connected to two guest toilets using a vacuum-on-demand system (VOD^{TM}, Jets AS, Hareid, Norway). At Britannia lodge the loading frequency was determined by the number guests and their toilet habits. Logging of toilet flush events identified two distinct day peaks (morning and evening) with extremely high flushing frequencies, up to 70 flushes per hour, for the two toilets used by this study (Todt, 2012). This is almost two fold the frequency of 40 flushes per hour applied in the laboratory experiment (paper 3). Multiplying the flushing volume of 0.5 L d^{-1} and the determined number of toilet flushes resulted in a hydraulic loads up to 150 L d^{-1} on peak days and 108 L d^{-1} in average during the high season period (Figure 5, upper panel). The hydraulic loading rates during the season peak were notably higher than the loading rate of 66 L d^{-1} applied in the laboratory test experiment.

Due to direct connection of the filter to the discharge pipe of the vacuum pump, sampling of the inlet was not possible and inlet concentration for TSS, COD and TAN was estimated with help of the flushing volume of the toilets (0.5 L) and the (unbiased) data from the blackwater at Kaja dormitories (paper 1). In the laboratory experiment, TAN was shown to remain unchanged in the filter system (not shown) and the TAN measured in the effluent of the filter system at Britannia lodge was comparable to the calculated values, indicating that the utilized extrapolation approach is a realistic estimate (Todt, 2012). The calculated inlet concentrations were 8000 -10'000 mg L^{-1} for TSS and 15'000-20'000 mg O_2 L^{-1} for COD (Todt, 2012). This was comparable to the up-concentrated blackwater used for the laboratory test experiment (paper 3). The liquid fraction discharged by the system, sampled below the effluent of the second filtration step showed a TSS concentration of 1430-1770 mg L^{-1} and a COD concentration of 7100-8200 mg O_2 L^{-1} (Figure 5), which was slightly higher than the effluent concentration ranges reached in the laboratory test experiment for those two parameters (paper 3). Taking the above extrapolated inlet concentration range this would result in a removal efficiency of 81% for TSS and 56% for COD (based on median values), which is comparable to the removal efficiencies obtained in the preceding laboratory test experiment (paper 3). However, considering the reported variation in the composition of blackwater between different locations (paper 1) greater uncertainties need to be taken into account in the used approach to extrapolate the inlet concentrations. Calculating a conservative estimate for the removal efficiencies by taking the 1st quartile of the estimated inlet and 3rd quartile of the determined outlet would give a removal efficiency of 78% for TSS and 45% for COD, which is still on a comparable level to the results obtained in the lab test experiment. Assuming that the inlet at Britannia had a comparable distribution of small and large particles to the unbiased blackwater at Kaja, these estimated removal efficiencies indicates that the particle size distribution may not have such a high impact on the filtration performance as anticipated in the previous section (4.3). This might be related to the partial contribution in each filtration step to the total TSS removal efficiency and its dependency on the inlet concentration. The outcomes of the lab test experiment indicates, that a majority (67-85%) of the removed suspended solids were retained by the first filtration step in which only a weak dependency between removal efficiency and inlet concentration could be found (paper 3).

The obtained results at Britannia lodge indicate that the tested mechanical two-step filtration system can work satisfactory under the rough conditions at a high mountain lodge with periodically high toilet flushing frequencies. However, the tested device was a prototype, which faced diverse teething problems. Especially the wood-shaving uptake in the counter-flow filter was hampered by frequent clogging in the storage funnel and thus the needs to be improved. It is also important to point out that the filtration device is a primary treatment unit and not a complete sewage treatment system. Regardless the relatively high removal efficiency, the effluent was still a high concentrated wastewater in terms of both particulate matter and COD, especially, and further treatment would be necessary. An appropriate secondary treatment step would therefore be required to remove the remaining soluble and small particulate organic matter, as well as nutrients, as discussed more in depth in the subsequent chapter.

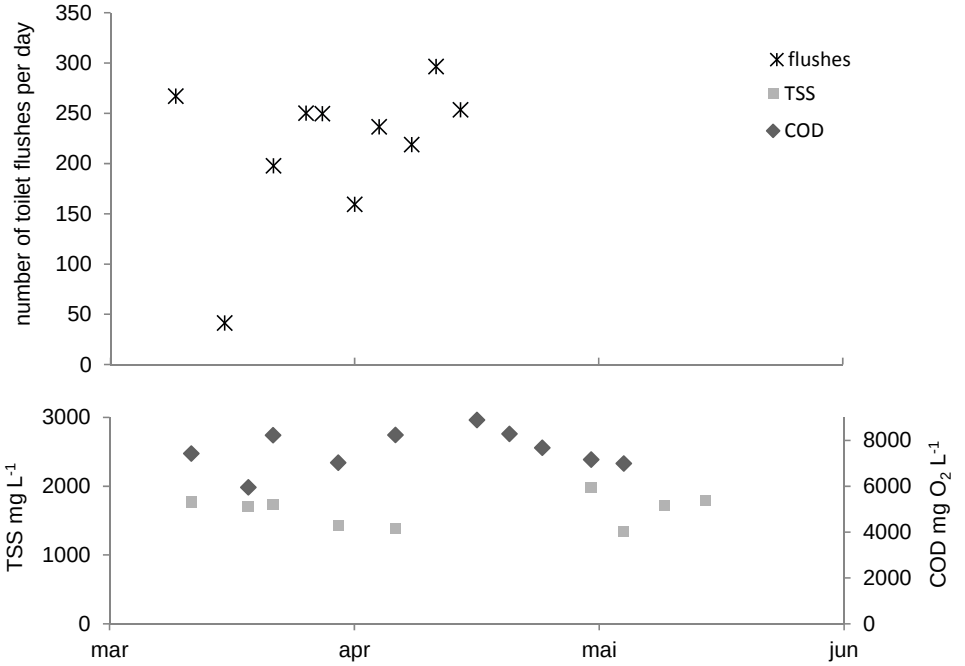


Figure 5: Upper panel: determined number of toilet flushes for a selected number of days. Lower panel: concentration of COD and TSS in the effluent of the mechanical two step filtration unit during the onsite test at Britannia lodge, sampled during the spring season 2011. The samples were taken as grab samples.

4.5 Initial experiment to explore a potential precipitation of struvite

In recent years, struvite ($MgNH_4PO_4{}^*6H_2O$), has become highly interesting for recovering P and N from blackwater (de Graaff et al., 2011, Tervahauta et al., 2013, Zeeman and Kujawa-Roeleveld, 2011) or urine (Liu et al., 2013, Morales et al., 2013, Latifian et al., 2014). In both of these two household wastewater fractions, conditions were shown to be favorable for a precipitation of struvite (Udert et al., 2003b, Zeeman and Kujawa-Roeleveld, 2011), a mineral consisting on the main elements Mg, P and N ($MgNH_4PO_4{}^*6H_2O$). Compared to conventional P precipitation processes does struvite precipitation also recover a fraction of NH_4^+ in addition to PO_4^{3-} (Zeeman and Kujawa-Roeleveld, 2011). In blackwater and urine, struvite precipitation is usually limited by the amount of the precipitant Mg^{2+} present (Udert et al., 2003b, Zeeman and Kujawa-Roeleveld, 2011), thus struvite precipitation is usually triggered by adding of Mg^{2+} (Zeeman and Kujawa-Roeleveld, 2011). To explore the potential of blackwater from Kaja regarding struvite precipitation a small initial experiment was conducted using the mechanical filter system (paper 3) through enriching the wood-shaving with Mg^{2+}.

The experiment was conducted sequentially starting with a control period with standard operation of the mechanical filter system in accordance to the methods described in paper 3 and an experimental period with Mg^{2+} enrichment of wood-shaving. To exclude potential side effects by a pH change as enhanced stripping of NH_3, or precipitation of other types of phosphates, different available Mg-compounds were screened regarding solubility and impact on pH when added to blackwater. Based on this pre-screening Mg-hydroxide-carbonate $(MgCO_3)_4{}^*Mg(OH)_2{}^*5H_2O$ (Merck, Darmstadt, Germany) was selected because it showed a good solubility and little impact on pH of the blackwater (changes < 0.1). The theoretical amount of Mg-hydroxide-carbonate which is needed to be dosed into the wood-shaving can be calculated based on the wood-shaving uptake rate, which was determined to be 1 L per 100 loading batches (paper 3),

and the range for PO_4^{3-} of 70-110 mg P L^{-1} detected during the control period in the blackwater (Figure 6a). With these assumptions, 30-50 g Mg^{2+} or 120-200g Mg-hydroxide-carbonate would be required per liter wood-shaving to ensure a molar Mg:N:P ratio of 1:1:1. Since the practical limit for enrichment was shown to be 20g Mg-hydroxide-carbonate L^{-1} wood-shaving, the conveyor speed was 10 times increased to 1L wood-shaving per 10 loading batches to ensure a sufficient Mg^{2+} availability in the filter matrix.

In the preceding control period without addition of Mg no significant change was found for PO_4^{3-} ($p=0.13$) and TAN ($p=0.42$) when the blackwater passed the mechanical filter system (Figure 6a,c). With addition of Mg, on the other hand, an almost complete disappearance was observed for PO_4^{3-} (Figure 6b) as well as a partial disappearance for TAN (Figure 6d). The molar ratio of the removed fraction of PO_4^{3-} to the removed TAN was close to 1:1 (Figure 7a), which indicates that struvite precipitation might have taken place in the filter system. In urine, PO_4^{3-} was shown to precipitate also with Ca^{2+} to calcium-phosphates, especially hydroxyapatite (HAP) $Ca_{10}(PO_4)_6(OH)_2$ (Udert et al., 2003b). However, only Mg^{2+} was added and no apparent change in pH took place, so the increased removal of P is most likely an effect of the Mg^{2+} addition only. Hence, Ca^{2+} probably did not played a significant role in the observed PO_4^{3-} reduction. Two minerals were identified that may be a result of a precipitation of PO_4^{3-} with Mg^{2+} struvite and newberyite ($MgHPO_4*3H_2O$) (Udert et al., 2003a). However, newberyite unlikely occurs in notable amounts when pH >8 (Abbona et al., 1982) as measured in our blackwater (paper 1). Hence struvite is likely the major Mg^{2+} based precipitate, which again supports the findings in Figure 7a.

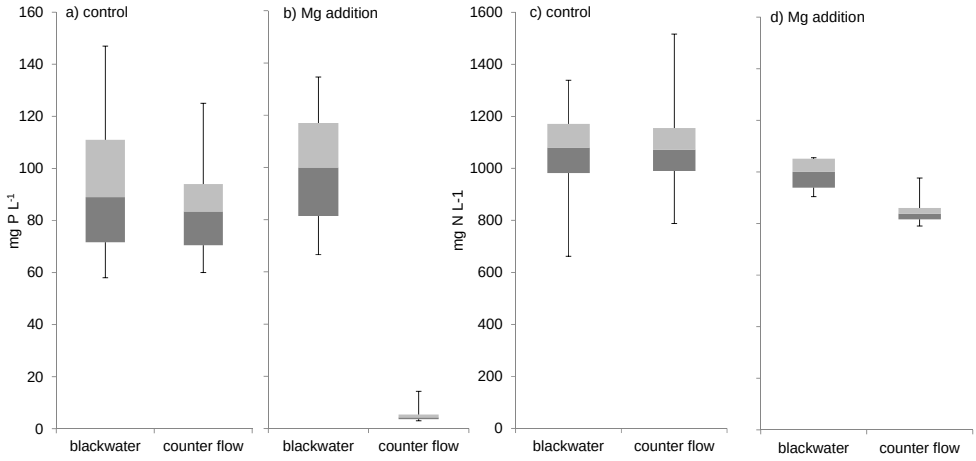


Figure 6: orthophosphate (PO_4^{3-}) (a,b) and total ammonia nitrogen (TAN) (c,d) in the raw blackwater (n=11) and composite sample in the effluent of the mechanical filter system (n=9) under standard operation (a, c) and with (b, d) Mg-enriched wood-shaving in the second filtration step.

The data in Figure 6 provide however no information if putative precipitated struvite was retained within the wood-shaving retentate. Struvite crystals were shown to be in a size of 40-80 µm (Morales et al., 2013) and thereby belonging to the supra-colloidal solids (Balmat, 1957), which might be washed out from the sawdust matrix in the filter. The available data material from our initial experiment is not comprehensively enough to give a clear answer to this question, since struvite was not directly measured. However, a few samples (n=6) were analyzed for total-P. These results indicate that a greater P retention took place during the test period compared to the control period (Figure 7c). However, especially when considering the observed difference in the P-content in the raw blackwater (Figure 7b), the apparent increase of P reduction

across the filter system by adding Mg^{2+} seems not as high as it might have been expected by the remarkable observed disappearance of PO_4^{3-} (Figure 6b). Hence, a considerable fraction of the precipitated struvite likely was washed out into the filtrate where it again appeared as total-P. More research is needed to clarify the precipitation and accumulation mechanism more in detail also with respect to kinetics and hydraulic retention time in the filter system.

Considering that the mechanical filter system is thought as a primary treatment step an integrated struvite precipitation may result in a critical P limitation for a subsequent biological degradation step, which is needed for a proper treatment of the liquid effluent as discussed in the following chapter. To ensure the growth of heterotrophic biomass, which is needed to sustain a degradation of organic matter on a sufficient rate, the ratio between organic substrate, or degradable COD respectively, and P should be below a critical threshold (Keskitalo et al., 2010, Jefferson et al., 2001) which was determined in range of 72-200, depending on the composition of biomass (Keskitalo et al., 2010). The biodegradable fraction of COD in municipal wastewater typically accounts for 92-97% for apparently soluble organics and 87% for particulate organics (Henze and Comeau, 2008). It is therefore reasonable to assume that at least 90% of the residual COD in a blackwater is biodegradable after having passed the mechanical filter system. Taking the determined effluent COD in a range of 4.6-5.4 g O_2 L^{-1} (paper 3) and the highest reported threshold of 200 for the COD/P ratio (Keskitalo et al., 2010), a P content of at least 25 mg P L^{-1} would be required to ensure a proper biodegradation of these residual organics in the filtrate. This is below the measured total P content (Figure 7c) but notable higher than the measured more readily bio-available PO_4^{3-} content (Figure 6b) in the effluent of the filter system with Mg^{2+} enriched wood-shaving. Taking a lower threshold for the COD/P ratio of 72 would require 68 mg P L^{-1}, which is in the range of the measured total P. A struvite precipitation in a primary treatment step as thought with the mechanical filter system is therefore questionable. This is also in accordance to the recommendations to implement a traditional P precipitation in subsequence to those reactor compartments where the major biological degradation of organic matter takes place (ATV, 1997). However, struvite precipitation may be an alternative P and N removal method to the traditional Fe^{3+} or Al^{3+} based P precipitation in subsequence to a biological treatment step as done in other projects (Zeeman and Kujawa-Roeleveld, 2011).

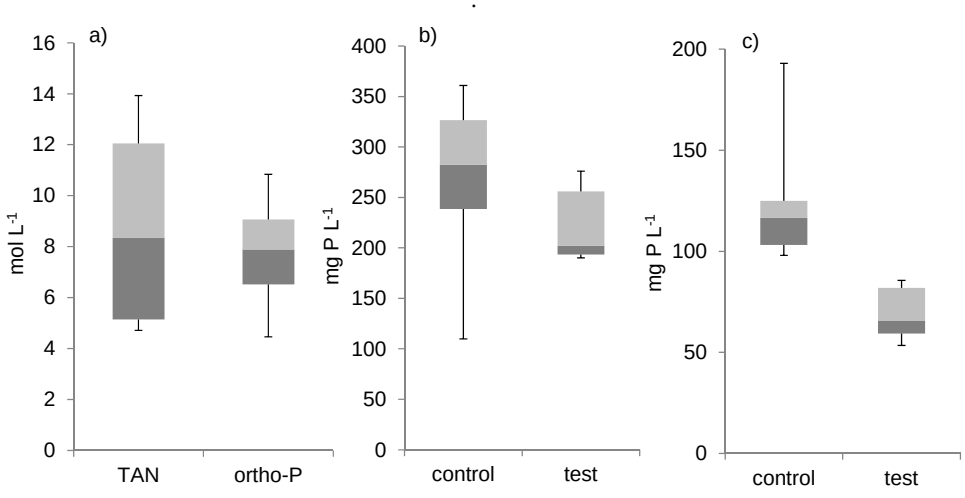


Figure 7: a) Molar difference in TAN and PO_4^{3-} of samples before and after filtration across the mechanical filter system with Mg-enriched wood-shaving b) Total-P determined in the raw blackwater and c) Total-P in the composite samples in the effluent of the mechanical filter system for control period (control) and test period (test) with Mg-enriched wood-shaving.

5 Assessment of critical processes and conditions that may facilitate N_2O emissions in a biofilm system treating blackwater

Based on the experiences and results from the onsite testing at Britannia Lodge, a moving bed biofilm reactor was evaluated as a possible secondary treatment step in succeeding the mechanical filter system for the removal of small particulate and soluble organic matter (paper 5). Organic matter (BOD, COD) removal was the main requirement at Britannia lodge. In an intensive biofilm systems however, also nitrification will likely occur as an inevitable side effect (Daude and Stephenson, 2003, Odegaard et al., 1993, Andreottola et al., 2003), especially when loaded with blackwater (paper 5). Nitrification activity in wastewater treatment systems loaded with blackwater has been pointed out as a potential source for N_2O generation (de Graaff et al., 2010, Itokawa et al., 2001). N_2O is suspected to be a strong greenhouse gas as well as involved in the ozone layer depletion (Ravishankara et al., 2009). Hence, for a proper evaluation of an MBBR as a potential secondary treatment step for blackwater treatment in alpine onsite applications, the occurring biological transformation processes need closer attention. To explore the mechanism controlling the occurrence of concomitant nitrification with potentially concurring N_2O production, a literature review was performed regarding relevant microbiological processes (paper 4) and an experiment with a pilot-scale two stage MBBR reactor conducted (paper 5).

5.1 Substrate and biomass dynamics in a biofilm

In suspended biomass cultures (e.g. activated sludge), the magnitude of the nitrification activity is mainly determined by the biomass retention time or sludge age (Ekama and Wentzel, 2008). In a fixed biofilm system, the biomass retention time is usually higher and thereby not the main limiting factor for nitrification (Morgenroth, 2008b). The occurrence and magnitude of the nitrification activity in a biofilm system is defined by an internal competition between heterotrophic and autotrophic biomass (Gujer and Boller, 1986, Logan et al., 1987). A biofilm system, which is loaded with both organic substrate and ammonia, usually develops a heterogeneous biofilm, containing both heterotrophic and autotrophic biomasses that are competing for oxygen (Wanner and Gujer, 1985, Morgenroth, 2008b). In a biofilm were oxygen is the only growth limiting factor, autotrophic biomass development was depressed due to higher growth rates of the heterotrophs. Biofilms highly loaded with organic substrate therefore have an almost homogenous biomass composition with strong heterotrophic dominance. Ammonia oxidation organism (AOB) activity and nitrification, will not reach significant rates (Wanner and Gujer, 1985). These are the conditions that basically would have been expected in the first of the two sequential complete stirred reactor tanks (CSTR) in our pilot-scale experiment. However, during certain periods a greater ammonia oxidation activity than expected was observed in the high loaded biofilm system, which could be attributed to a temporary substrate limitation of the heterotrophic activity (paper 5). Under these conditions, oxygen is not completely depleted by the heterotrophic activity and becomes available to nitrifying biomass in deeper layers (Wanner and Gujer, 1985).

The depletion and availability of a compound in different biofilm zones can be modeled with help of a diffusive-reactive mass balance equation. The partial differential equation describes the balance between accumulation, diffusion and reaction for a single compound or substrate (Eq. 1). C_F is the compound concentration in the biofilm (mg L^{-1}) x the distance from the surface (l), D_F the diffusion coefficient for the compound (l^2 t^{-1}) and R_F reaction rate (g l^{-3} t^{-1}) for the compound of interest (Morgenroth, 2008b):

$$\underbrace{\frac{\partial C_F}{\partial t}}_{accumulation} = \underbrace{D_F \frac{\partial^2 C_F}{\partial x^2}}_{diffusion} - \underbrace{r_F}_{reaction}$$

Eq. 1 (Morgenroth, 2008b)

To assess a potential oxygen limitation of the deeper located nitrifiers, modelling need to focus on the heterotrophic activity, which is determining the oxygen availability in a stratified heterogeneous biofilm. For the reaction term the standard model for heterotrophic growth as used in the activated sludge models (ASM) is usually assigned. The standard model for heterotrophic growth describes the conversion of the compounds of organic substrate (S_{org}) and O_2 into heterotrophic biomass and CO_2 using Monod kinetics (Table 2). In the well aerated rector systems, a majority of the produced CO_2 is immediately stripped into the atmosphere and thereby often omitted in the models. This was also done in Table 2. However, in low aerated systems CO_2 may dissolve into carbonic acid (H_2CO_3), which again can impact the carbonate equilibrium and pH in bulk liquid and may thereby need to be considered as an additional compound in the system.

Table 2: Standard model for the process of heterotrophic growth (IWAQ ASM) in its original form (Gujer et al., 1999, Henze et al., 1987) and resolved for C_{Sorg}. The model consists on the following parameters: C_{Sorg}: organic substrate expressed in COD (mg O_2 L^{-1}), C_{O2}: oxygen concentration (mg O_2 L^{-1}); X_H: heterotrophic biomass expressed in COD (mg O_2) K_S: half saturation constant for organic substrate (mg O_2 L^{-1}); K_{O2}: half saturation constant for oxygen (mg O_2 L^{-1}); μ,*max*: max growth rate (1/d).

	C_{Sorg} (O_2)	C_{O2}	X_H (O_2)	process rate (1/d)
standard	$-\frac{1}{Y_H}$	$-\frac{1-Y_H}{Y_H}$	+1	$\mu, max \frac{C_{S_{org}}}{K_S + C_{S_{org}}} \frac{C_{O_2}}{K_{O_2} + C_{O_2}} X_H$
resolved	-1	-(1-Y_H)	+Y_H	$\mu, max \frac{C_{S_{org}}}{K_S + C_{S_{org}}} \frac{C_{O_2}}{K_{O_2} + C_{O_2}} X_H$

Due to the mass transport resistance, mainly soluble organic substrates will react with biofilm systems and will be relevant for a further assessment of a potential oxygen limitation. The determination of soluble wastewater components is usually done by micro-filtration with 0.45-1.2 µm opening size, with the result that the resulting apparently soluble organic matter (filtrated COD) will be comprised of both molecular dissolved compounds and colloidal organic matter (paper 1). This is also supported by the results on the particle-size distribution in our blackwater (Figure 3), as well as more sophisticated analysis done by others on domestic wastewater (Dulekgurgen et al., 2006, Logan et al., 1987, Sophonsiri and Morgenroth, 2004, Levine et al., 1985). However, only low molecular compounds (e.g. glucose, acetate), which microorganisms can readily take up across the cell membrane and convert into energy or biomass via intracellular enzymatic processes, account for readily degradable substrates in a microbiological terminology (Kappeler and Gujer, 1992, Larsen and Harremoës, 1994, Levine et al., 1985, Morgenroth et al., 2002). Degradable larger-sized organic molecules (>1 kDa), colloidal-bound or suspended organic matter, on the other hand, need first to undergo an extracellular hydrolysis, which may have a different kinetic than the uptake of low molecular weight substrates for aerobic growth, especially in the mass-transport limited biofilm systems (Confer and Logan, 1997). A closer attention is therefore needed for the determination of S_{org} (Table 2), which is discussed in the subsequent sections.

5.2 Challenges with parameterization of organic substrates for a biofilm model

According to the present practice, readily degradable soluble organic substrates (S_{org}, Table 2) are usually determined in an incubation trial with raw (none-filtrated) wastewater in a suspended biomass culture (activated sludge) as developed by Kappeler and Gujer (1992). From the respiration curve describing the oxygen uptake rate (OUR), S_{org} is calculated by subtracting the baseline-respiration (flat part of the respiration curve) from the total respiration:

$$S_{org} = \frac{\int total\ respiration - \int baseline\ respiration}{(1 - Y_H)}$$ Eq. 2 (Kappeler and Gujer, 1992)

In such an incubation of suspended biomass, however, a larger fraction of high molecular and colloidal-bound organic matter are immobilized relatively quickly into biomass flocs and subsequently hydrolyzed into smaller substrates (Makowska and Spychala, 2014, Levine et al., 1985). Hydrolysis of larger molecules as well as particular colloidal substrates (e.g. starch) was shown to reach comparable or even greater rates than the uptake rates of low molecular readily degradable substrates for heterotrophic growth (Kommedal et al., 2006). Hence, the apparent degradation kinetics for particular colloidal substrates reflected in the respiration curve will not differ to the degradation kinetics of low molecular dissolved organic substrates (Makowska and Spychala, 2014). For activated sludge systems, this respiratory approach of Kappeler and Gujer (1992), which associates low molecular substrates as well as readily hydrolysable high molecular or colloidal-bound substrates to the parameter S_{org}, is therefore reasonable.

For biofilm systems, however, the apparent degradation rate of a particular substrate is not only dependent on its apparent biodegradability, but also on its mass transport limitation across the biofilm (Logan et al., 1987, Wanner and Gujer, 1986, Kommedal et al., 2006). This mass transport limitation is determined by the diffusivity D_F (Eq. 1) that was shown to have an inverse relationship to the molecule-size of a particular substrate (Kommedal et al., 2006, Logan et al., 1987). For biofilm modeling, the classical substrate fractionation into soluble readily degradable (S_S or S_{org}) and particulate slow degradable (X_S) (as used in activated sludge models) (Gujer et al., 1999, Henze et al., 1987, Henze et al., 1995) is therefore insufficient and a more detailed distinguishing between different substrate qualities is needed (Morgenroth et al., 2002). Based on a literature review we identified four possible main substrate groups of that are listed in Table 3 and outlined more in detail in the subsequent sections.

Table 3: Identified substrate groups that should be considered in a biofilm system

Substrate		Molecule size	Transportation mechanism	Degradation mechanism and kinetics
A	Readily degradable low molecular substrates and small-sized readily hydrolysable colloids	0-500 kDa	Diffusion and advection	Rapid hydrolysis or direct cell uptake for growth
B	Colloidal well hydrolysable substrates	500 kDa – 1µm	Advection, mainly in macro pores	Intermediate hydrolysis rates
C	Slow hydrolysable soluble and colloidal substrates	0 – 1µm	Diffusion and advection	Slow hydrolysis
D	Slow hydrolysable particulate substrates	> 1µm	Only advection in bulk liquid and surface reaction with biofilm	Slow hydrolysis

5.2.1 Readily degradable low molecular substrates and small-sized readily hydrolysable colloids (A)

As outlined in the previous section, the apparent degradation kinetics of readily hydrolysable higher molecular and small-sized colloidal substrates (e.g. starch, proteins) was shown to be comparable to low molecular readily degradable substrates (Kommedal et al., 2006, Mosquera-Corral et al., 2003, Makowska and Spychala, 2014). Hence, the hydrolysis process can basically be ignored, with the result that the reaction term in Eq. 1 can be approached with the same stoichiometric relations and kinetics (Michaelis-Menten) as used for low molecular substrates. The different diffusivities for low and high molecular substrates, however, may need to be considered in the diffusion term of Eq.1. This can be done by modeling parallel substrates of different molecule sizes with corresponding D_F as done by Kommedal et al. (2006) or modeling only one substrate based on an average molecule size for soluble and small-colloidal degradable organic matter as done by our study (paper 5). This approach was shown to be valid up to a molecular weight of 500 kDa (Kommedal et al., 2006), which compares to a molecule length of 5-20 nm (Erickson, 2009, Levine et al., 1985).

5.2.2 Colloidal well hydrolysable substrates (B)

For colloidal organic matter with molecular weight of 500 kDa (approximating to 5-20 nm) or higher, the Brownian diffusion, on which the diffusion term in Eq. 1 is based on, does no longer apply. The transportation of these larger-sized substrates into a biofilm is therefore limited to advective processes in macro pores (Logan et al., 1987, Larsen and Harremoës, 1994). Hence, hydrolysis of these larger-sized, none-diffusible compounds is limited to biomass located at the biofilm surface or along macro pores and free enzymes in the bulk liquid (Mosquera-Corral et al., 2003, Henze and Comeau, 2008). The location and magnitude of hydrolysis of readily hydrolysable colloids seem to depend on the type of biofilm system as indicated by two studies working with the same type of colloidal substrate (starch), but different types of biofilm reactors (Table 4). Larsen and Harremoës (1994) attributed a major proportion of the hydrolysis activity in the degradation of starch to free extracellular enzymes in the bulk liquid. Mosquera-Corral et al. (2003) the other hand did not find a significant hydrolysis activity in the bulk liquid and attributed the majority of starch hydrolysis to membrane-bound extracellular enzymes on the surface and along macro pores inside the biofilm.

Comparing the reactor conditions and loading rates of these two studies (Table 4) indicate that surface load and specific surface area impact the location as well as the rate of hydrolysis. Larsen and Harremoës (1994) were using a reactor with relatively low specific surface area attached to an apparently static carrier (even though rotating, the drum was not changing its position relative to the reactor geometry) and relatively high surface, but low volume loading rates (Table 4). In this system, collision events of colloidal substrates in the wastewater with the biofilm surface was likely limited so that only a smaller fraction of colloids was immobilized into the biofilm, hence the major hydrolysis activity took place in the bulk liquid. The system from Mosquera-Corral et al. (2003), on the other hand, provided a significant larger specific surface area attached to small carriers having a continuing high turbulent movement across the reactor volume. This resulted in a relatively low surface loading rate even though the volume loading rate was significantly higher than in the other study (Table 4). In this suspended biofilm system, collision events and subsequent biofilm immobilization of colloidal substrate particles probably took frequently place with the result that hydrolysis of starch was dominated by membrane-bound enzymes. Comparing the substrate fluxes obtained by these two studies (Table 4) indicates that this membrane-bound hydrolysis is more efficient than a hydrolysis performed by free enzymes in the bulk liquid. This is also reflected by the difference in the obtained starch removal efficiency which was closed to 100% in the system of Mosquera-Corral et al. (2003) compared to only 15-29% in the system of Larsen and Harremoës (1994).

The kinetics for hydrolysis seem further to vary considerably between different types of colloidal substrates (Morgenroth et al., 2002). Starch, which was investigated by the above discussed studies (Table 4), showed high hydrolysis rates by a sequential breaking up into smaller compounds that can diffuse deeper into the

biofilm (Morgenroth et al., 2002). This is supported by the results of Mosquera-Corral et al. (2003) who did not find an apparent difference in the degradation kinetics of relatively large-sized (10^{14}-10^{17} kDa) starch polymers, to the its corresponding low molecular monomer glucose in a biofilm system. Hence, starch and other larger-sized readily hydrolysable colloidal substrates may account the substrate group A (Table 3) for biofilm systems providing a high specific surface area. The average hydrolysis rate for colloidal substrates contained in wastewater, however, was shown to be markedly slower than for low molecular readily degradable compounds (Balmat, 1957, Dimock and Morgenroth, 2006).

In addition to biofilm conditions and the composition of a single substrate, also substrate interactions may impact the hydrolysis rates. A coinciding presence of low molecular degradable substrates may introduce a catabolite repression of the hydrolysis enzyme production needed to decompose larger-sized substrates (Morgenroth et al., 2002). The findings of our literature review point out that substrate group B is not well-defined and its magnitude is dependent on the substrate composition as well as the hydraulic conditions within a particular biofilm system. The latter needs to be considered when conducting off-situ studies of full-scale or pilot-scale systems, as discussed later in the subsequent section 5.3.

Table 4: Reactor type, specific biofilm surface area and organic loading rate (OLR) and substrate flux into the biofilm and degradation rate per reactor volume unit for two studies with contradictory findings regarding the hydrolysis of the colloidal substrate starch. Load (OLR), flux and degradation rate of the organic substrate are given in COD equivalents.

	Mosquera-Corral et al. (2003)	Larsen and Harremoës (1994)
Reactor type	biofilm airlift suspension	rotating drum
Biofilm carriers	basalt (0.3 mm)	cylindrical surface
Specific biofilm surface area	650-2000 m^2 m^{-3}	166 m^2 m^{-3}
OLR per biofilm surface area	25-76 g O_2 m^{-2} d^{-1}	74-158 g O_2 m^{-2} d^{-1}
OLR per reactor volume	50 kg O_2 m^{-3} d^{-1}	12-26 kg O_2 m^{-3} d^{-1}
Substrate flux biofilm	25-76 O_2 m^{-2} d^{-1}	9-25 g O_2 m^{-2} d^{-1}
Degradation rate per reactor volume	50 kg O_2 m^{-3} d^{-1}	1.4-4.3 kg O_2 m^{-3} d^{-1}

5.2.3 Slow hydrolysable soluble and colloidal substrates (C)

Hydrolysis is strictly defined as the decomposition of polymers into smaller units with water. In the subject wastewater treatment however, the definition of hydrolysis is broader and encompasses additional depolymerisation processes that are needed to make slowly degradable substrates available for heterotrophic growth (Morgenroth et al., 2002). These slowly degradable substrates are usually attributed to particulate organic matter in supra-colloidal size (>1µm) (Gujer et al., 1999, Henze et al., 2002). However, slow degradable organic substrates encompass a broader number of organic matter in a size of 1 kDa-100 µm whereas a notable fraction is assumed to be colloidal or molecular dissolved (Morgenroth et al., 2002). This is supported by an earlier study who identified slow biodegradable humic and fulvic acids as a notable fraction of the small and medium-sized organic molecules (20 kDa) present in a wastewater (Levine et al., 1985), which again can reach deeper biofilm layers by Brownian diffusion (Logan et al., 1987).

5.2.4 Slow hydrolysable particulate substrates (D)

The hydrolysis rate of large-sized particulate organic substrates (>1 μm) was shown to be 3 times lower than for larger sized colloidal substrates and 4-5 times lower for smaller-sized colloidal and soluble substrates (Balmat, 1957). The results of kinetic studies conducted with different protein-based substrates (egg white) propose the decomposition of large-sized particles as a sequence of several breakups into smaller particles and molecules (Dimock and Morgenroth, 2006). Large-sized particles are usually too large to enter the macro pores of a biofilm and therefore only interact with a small fraction of the biofilm biomass at the surface (Logan et al., 1987, Oldenburg et al., 2002). Larger-sized organic matter therefore most likely decomposes only to a minor extend in biofilm systems, which is supported by the results of Janning et al. (1998) who found that only 25% of particulate organic matter that has been accumulated in biofilter was hydrolyzed within 24 hours. Protozoa and other macro organism are assumed to significantly contribute to the degradation of particulate organic substrates in addition to enzymatic hydrolysis on the biofilm surface and in the bulk liquid (Morgenroth et al., 2002). These macro organisms are, however, usually not considered in the present modeling of biological wastewater treatment processes.

5.3 Exploration of degradation kinetics for different substrates with ex-situ studies

In our MBBR experiment (paper 5) we tested two different types of ex-situ studies to explore the degradation kinetics of different substrates (organic matter, N-compounds). The following sections will summarize its outcomes with respect to the previously discussed different groups of organic substrates (Table 3).

5.3.1 Determination of respiration kinetics of a diluted blackwater incubated with biofilm media grits

To gather more information on the degradation kinetics a small bench-scale experiment in closed incubation bottles was conducted based on the standard method for the determination of S_{org} for suspended biomass cultures (Kappeler and Gujer, 1992), that we modified for a biofilm system. Raw and filtrated (GF-C 1.2 μm, Whatman, Little Chalfont, UK) blackwater was diluted 1:10 with tap water and incubated together with 2 biofilm media grits into glass bottles (510 ml) that were sealed with a cap that integrated manometric O_2 consumption measurements otherwise used for determination of BOD (Oxitop™, WTW, Weilheim, Germany). In this system, CO_2 produced by the respiratory activity is adsorbed on NaOH sitting in the cap so that the consumed O_2 introduce a pressure drop, which is logged by an electro-mechanical sensor. Each of the prepared blackwater solutions were incubated in four parallels with 22.7 ml diluted blackwater and 2 media grits (BWT-S, Biowater Technology AS, Tønsberg, Norway). This the liquid volume was in accordance to the incubation volume used for high range BOD tests (22.7ml, 0-4000 mg O_2 L^{-1}) when using the Oxitop™ bottle system, so that the respiration curve displayed by the pressure sensor device approximates the oxygen demand of the incubated biofilm in mg O_2 L^{-1} bulk liquid. Nitrification was inhibited with 1 drop of Allylthiourea (ATU) as used in a corresponding BOD test (Oxitop™). ATU is a metal complexing agent that completely inhibits the enzyme ammonia monooxygenase (AMO), which is performing the first step of nitrification (Hyman et al., 1990). The incubation took place in an incubator box at a controlled temperature of 20 ±0.1 ºC. The incubation liquid was continuously stirred with a magnetic stirrer in a range of 180-450 RPM. The RPM was automatically controlled by the stirrer rack (Oxitop™ S6) provided by the manufacturer of the BOD equipment (WTW) and could not be impacted by the authors.

The plotted accumulative respiration curves (Figure 8) showed three distinct apparent respiration rates as indicated by the fitted linear regression lines. These three apparent respiration rates are likely referring to the degradation of three different types of substrates. The highest apparent respiration rates were observed during the first 5-6 hours of incubation accounting for 46 and 39 O_2 h^{-1} for raw and filtrated blackwater, respectively. After 5-6 hours, a distinct decrease in the apparent respiration rate could be identified (Figure 8), which is likely attributed to the depletion of readily degradable or hydrolysable substrates (group A,

Table 3, section 1). In the subsequent 12-18 hours the apparent respiration rates kept relatively constant on 20 and 28 mg O_2 h^{-1} for raw and filtrated blackwater, which is approximately 50% lower than in the preceding hours, but still notably higher than the background respiration towards the end of the experiment (Figure 8). This period may reflect the degradation of colloidal readily hydrolysable substrates. In our system the degradation of these colloidal substrates was likely limited by the mass transport into the biofilm (group B, Table 3, section 5.2.2), which is reflected by a notable lower respiration rate compared to substrate group A. After 18-24 hours, again a relatively distinct decrease in the apparent respiration rate occurred down to a level of 15 and 12 mg O_2 h^{-1} for raw and filtrated blackwater, respectively, and remained constant for the remaining experimental time (Figure 8). For the filtrated blackwater, this apparent background respiration was likely attributed to endogenous respiration of the biofilm and degradation of slow hydrolysable apparently soluble substrates (group C, Table 3, section 5.2.3). The greater apparent respiration rate of the raw compared to the filtrated sample likely reflects the degradation of particulate substrates (group D, Table 3, section 5.2.4).

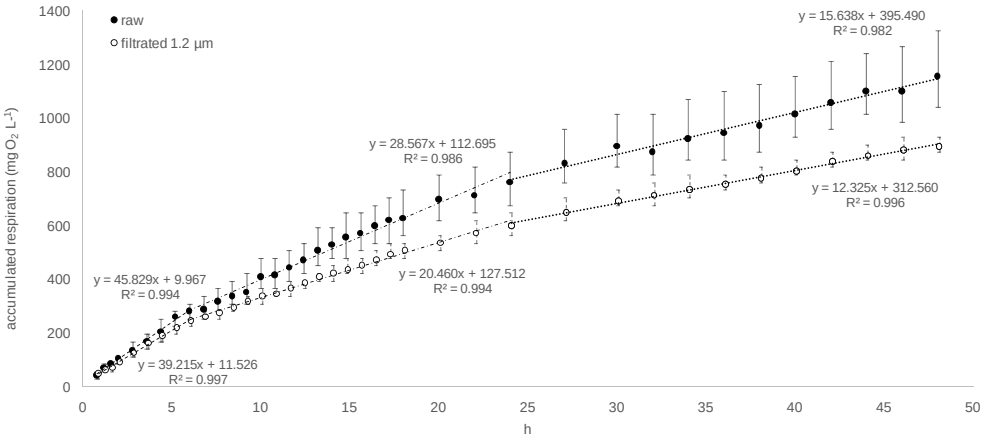


Figure 8: Respiration curves of diluted blackwater that was incubated with 3 biofilm media grits for 48 hours. The error bars are showing the min/max values of four parallels. The linear regression lines where fitted to the average.

By assuming that the degradation of all four substrates took place coincidently at constant rates until substrate A and B were depleted, the net respiration was estimated for each of the substrates (Table 5) with help of the inclination of the three linear phases in the apparent respiration curve (Figure 8). Subtracting the inclination of the second from the first regression line would reflect the net respiration for substrate A, presupposed that a potential catabolite repression of the hydrolase enzymes was negligible. The net respiration of substrate B can be estimated by subtracting the inclination of the third from the second regression line. The difference in the apparent respiration rate between filtrated and raw blackwater in the last 24 hours accounting for 3.3 mg O_2 h^{-1} (Figure 8), was probably attributed to the degradation of slow hydrolysable particulate substrates (group D, Table 3, section 5.2.4). The difference in the apparent respiration rate between the filtrated and raw sample was most distinct in the period of h 5-24 where it accounted for 8.1 mg O_2 h^{-1} compared to 3.3 mg O_2 h^{-1} in the subsequent and 6.2 O_2 h^{-1} in the first 5-6 hours (Figure 8). This might be attributed to a partial retention of readily degradable colloidal substrates on the glass fiber filter that might have occurred due to a filter-cake effect. The slightly lower difference during the first 5-6 hours might have been a result of reduced hydrolysis activity due to catabolitic repression introduced by the coinciding presence of readily degradable low molecular substrates.

With the above assumptions, the y-axis intercept of 312 mg O_2 L^{-1} for the third regression line (hours 18-24) of the filtrated incubations (Figure 8), would reflect the net O_2 consumption for the degradation of substrates A and B, which likely accounted for a major fraction of the depleted 411 ±9 O_2 L^{-1} apparently soluble COD (data not shown). For the filtrated incubation, this would translate to a yield coefficient (Y_H) of 0.2 in average, which is notably lower than a typical Y_H of 0.4 as typically reported for biofilm systems (Morgenroth, 2008b). This may point towards a scaling error in the displayed figures by the BOD measure device (Oxitop™) for this particular experiment, which however could not be verified by the authors due to lacking background information by the manufacturer on the exact algorithm used by the device to transform the determined pressure data into a BOD concentration. By assuming such a scaling-error based bias in the displayed respiration curve and taking an Y_H of 0.4, the obtained respiration rates would need to be corrected with a factor 0.8 as considered in the embraced figures in Table 5. With those obtained corrected net respiration rates and the calculated protected surface area of $1.83*10^{-3}$ for each of the tree incubated media grits, it further possible to estimate the flux, which occurred into the biofilm for the substrates A and B. This estimated average flux for the substrates A and B was calculated to be 2.4 g O_2 d^{-1} , which is in the lower end of recommended BOD surface loading rates for the dimensioning of biofilm systems in municipal treatment (Morgenroth, 2008a).

Table 5: Estimated net respiration rates and flux for the different substrates present in raw and filtrated (1.2 µm) diluted blackwater based on apparent respiration rates obtained in the incubation experiment (Figure 8). The figures in the brackets are corrected for a putative scaling error of the BOD measure device.

Substrate		Estimated net respiration rate (mg O_2 h^{-1})	Estimated substrate flux or degradation rate
A	Small-sized readily hydrolysable and/or degradable molecules and colloids	19 (14)	154 mg O_2 m^{-2} h^{-1}
B	Colloidal well hydrolysable substrates	8 (10)	67 mg O_2 m^{-2} h^{-1}
C	Slow hydrolysable soluble and colloidal substrates	n.a.	n.a.
D	Slow hydrolysable particulate substrates	3 (2)	3.3 mg O_2 L^{-1} h^{-1} *

*hydrolysis rate in bulk liquid per reactor volume.

The incubation trials provide an indication on the characteristic degradation times for the different groups of substrates contained in diluted blackwater (Figure 9). For substrates A and B, the characteristic degradation times (Figure 9) refer to the length of the first two regression lines (Figure 8). For substrate C and D the total degradation time exceeded the incubation time of the experiment (Figure 9). All of these putative substrates showed apparent zero order respiration rates (Figure 8), which is likely due to initial substrate concentrations far beyond a typical half saturation constant of 2 mg O_2 L^{-1} for heterotrophic growth (Gujer et al., 1999). The initial concentration of the apparently soluble substrates degraded within the incubation time can be approached by the difference in soluble COD in the beginning and end of the incubation which accounted in average for 396 and 411 mg O_2 L^{-1} for raw and filtrated blackwater, respectively (data not shown). Periods with substrate limitation and corresponding half or first order kinetics most likely took place in the transient phases from the first to the second regression line where substrate A was depleted. This transient phase in which substrate A was below a critical concentration may however have been relatively short so that it was not captured with the temporal resolution of the measure points.

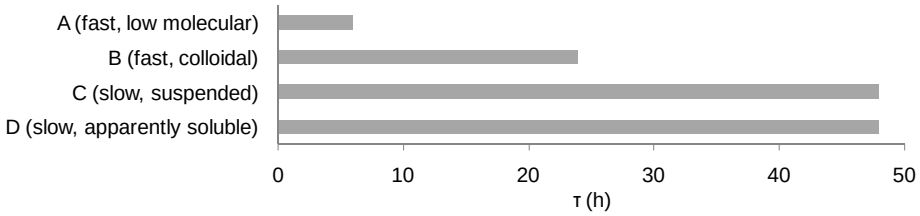


Figure 9: Estimated specific reaction times (τ) and hydraulic retention time for the first of the two sequential reactor tanks of the MBBR system used for the experiment in paper 5 for during first loading period when the system was loaded with a mixture of 20% blackwater and 80% greywater at a hydraulic loading rate of 1487 L d^{-1}.

With the characteristic times for the substrates A and B (τ A,B), the apparent respiration rates for substrates A and B (rO_2 A,B) and the background respiration rate (r_b,O_2): it is possible to estimate the proportional distribution of the two main groups of readily degradable substrates (A, B, Table 3).

$$\text{proportion substrate A} = (rO_2,A - r_b,O_2)* \tau A / ((rO_2,A - r_b,O_2)* \tau A + (rO_2,B - r_b,O_2)* \tau B) \quad \text{Eq. 3}$$

Taking characteristic times of 6 and 18h for substrate A and B, respectively would give a proportional distribution of 44% substrate A; 56% substrate B in the raw, and 52% substrate A; 48% substrate B in the filtrated sample. The difference between the filtrated and raw sample may be attributed to the earlier discussed partial retention of substrate B in the filtration process due to filter-cake effect. However, the results indicate that approximately half of the substrates that degraded within 24h seem to be mass transport limited. This is in accordance to the results of a recent study on the particle-size distribution in municipal wastewater, which showed that approximately 50% of the apparently soluble degradable COD (<1.6 µm) is smaller than 13nm (Dulekgurgen et al., 2006) which approximates to our defined limit of 500 kDa for substrate A. To distinguish between endogenous respiration and O_2 consumption by degradation of substrate C on the other hand was not possible to obtain with a respiration curve over 48 hours.

5.3.2 Exploring substrate groups and its degradation kinetics in a biofilm reactor with ex-situ studies in open batch experiments

To use ex-situ studies in open batch experiments in a bench-scale reactor and plotting the depletion of apparently soluble COD is a method to explore substrate groups and their degradation kinetics in a biofilm system: We used this approach in our MBBR experiment outlined in paper 5. The batch experiments plotted in Figure 10 were started with a 1:1 mixture of bulk liquid from each of the two sequential CSTR in our pilot-scale MBBR system to simulate the loading conditions in the second reactor tank (paper 5). Hence, the initial concentration for the substrates was depending on the particular conditions in the reactor tanks at the days the liquid was sampled. With this method differences in degradation kinetics for filtrated COD could be identified in course of the batch experiment (Figure 10). These kinetic differences over time are likely attributed to different types of apparently soluble substrates (substrates A-C, Table 3). Figure 10 shows the apparent degradation kinetics for filtrated COD in the second reactor tank for two loading periods with different mixtures of greywater and blackwater. Similar to the respiration curves of the closed bottle incubations (Figure 8), the depletion of apparently soluble COD could be described with a series of linear regression lines (Figure 10). The observed high degradation rates between the first two data points to a presence of readily degradable substrates in the incubation liquid (paper 5). Considering that the degradation of all substrates took coinciding place on constant reaction rates from the beginning of the experiment until particular substrates where depleted, the net degradation rate of the putative substrate A can be calculated by subtracting the rate obtained by substrate B and C approached by the second regression line. With these assumptions the system obtained a net degradation rate for substrate A of -33 and -75 mg O_2 L^{-1} (Figure 10), which translates to a COD flux of 284 and 152 mg O_2 m^{-2} h^{-1} for period 1

and period 2, respectively. These figures are in a reasonable range in terms of the degradation rate determined for substrate A in the respiratory experiment with diluted blackwater (Table 5). The second regression line from hour 2 to 24 likely points to the degradation of substrates belonging to the group B, which took place on a rate of -3 and -1.8 mg O_2 L^{-1} (Figure 10) translating to a COD flux of 9 and 19 mg O_2 m^{-2} h^{-1} for period 1 and period 2, respectively. These substrate fluxes obtained in h 2-24 in the batch experiment are notably lower than the substrate fluxes that were estimated for the substrate group B with help of the respiration curves of the incubation experiment (Table 5).

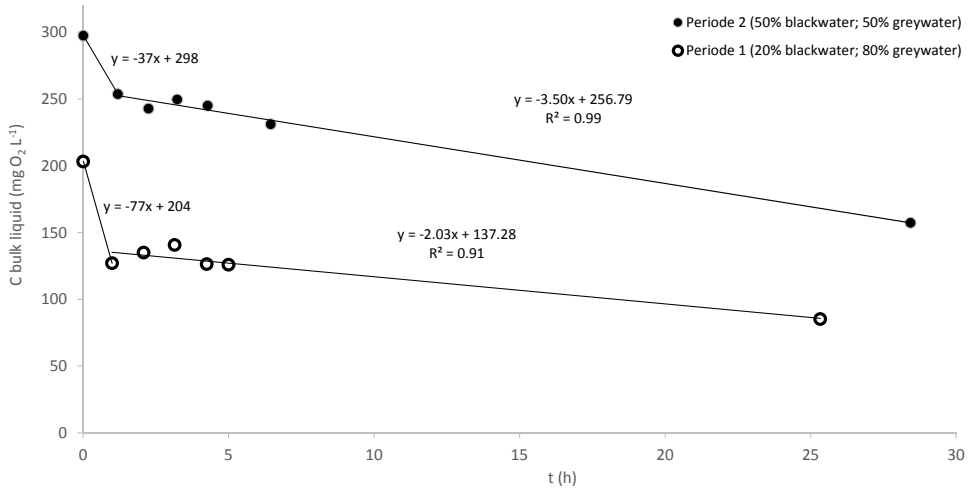


Figure 10: Apparent degradation of filtrated (apparently soluble) COD in an open batch experiment with a bench-scale MBBR reactor.

A reason for the discrepancy in the estimated flux of substrate B between the bottle incubation and the batch experiment maybe found in different loading and reactor conditions. The organic surface load (OLR per surface area) to the biofilm of the bottle incubation was approximately fivefold the organic surface load applied in the batch experiment (Table 6). The significantly lower surface load in the batch experiment might have resulted in higher availability of hydrolysis enzymes per g colloidal substrates (group B, Table 3) with the result that a greater fraction of well hydrolysable colloids (e.g. starch) may have degraded within the first hour and thereby appeared as substrate A in the degradation curve. This hypothesis is partially supported by the earlier discussed results from Mosquera-Corral et al. (2003) who did not find an apparent difference between large-sized starch polymers and its monomer glucose in a biofilm reactor with relatively low surface load compared to other studies (Table 4). Hence, the degradation which took place during hours 2-24 in the batch experiments might have represented substrates belonging to the slow hydrolysable group C (Table 3) mainly. This would indicate that comparably rapid hydrolysis of the substrate group B took place in the pilot-scale MBBR, where the substrate for the batch experiment were taken from. This hypothesis may be again partially challenged by the high surface load of the pilot-scale reactor, which was almost tenfold the surface load applied in the batch experiments and still notable higher than in bottle incubation experiment (Table 6). However, as discussed earlier, the degradation of well hydrolysable colloidal substrates depends not only on the surface load, but also on probability of collision events of colloidal particle the biofilm surface (section 5.3.2). This probability seems to be significantly impacted by the specific surface area (section 5.3.2), which was dedicated highest in the pilot-scale system (Table 6). This again would support the outcomes of section 5.3.2 where the degradation rate of substrate B as well as its distinctness to substrate A may vary considerably between different biofilm systems.

Regardless of which substrate group the observed disappearance of filtrated COD in the batch experiments exactly points to, the results in (Figure 10) point to a greater activity related to potentially slow degradable soluble substrates with an increased proportion blackwater in the wastewater mixture. This would indicate that blackwater contains a greater proportion slow degradable apparently soluble substrates than greywater or a mixed household wastewater (paper 5). More research will be needed to confirm this hypothesis and underlying indicative data material. It is less laborious to assess substrate kinetics with bottle incubations than with batch experiments in an open reactor. We therefore propose to perform additional bottle experiments by incubating parallels with different mixtures of grewater and blackwater. To also investigate the impact of the specific surface area on the degradability of different substrates, we further propose to incubate three parallels having 2, 3 and 4 biofilm media grits in 22.7 ml, corresponding to a specific surface area of 162, 243 and 324 m^2 m^{-3}, respectively.

Table 6: Comparison of biofilm area, organic loading rates (ORL) and degradation rate of putative substrate B for apparently soluble degradable organics in the bottle incubation with diluted (1:10) blackwater (Figure 8); batch experiment with 1:1 mixture from the sequential reactor tanks of the pilot-scale reactor that was loaded with 2 different mixtures (P1, P2) of household wastewater (Figure 10); and pilot-scale reactor (paper 5). For the bottle incubations and batch experiments, the ORL was calculated based on the apparently soluble COD that was degraded within 24h. For the pilot-scale experiment, the ORL was calculated based on the difference of apparently soluble COD between inlet and the bulk liquid of the second complete stirred reactor tank multiplied with the hydraulic loading rate. The flux of substrate A in the pilot-scale system was approached with the COD flux obtained in the first of the two sequential reactor tanks at a hydraulic retention time of 3.2 h and 7.7 h for P1 and P2, respectively (more details paper 5).

	Bottle incubation	Batch P1	Batch P2	Pilot-scale P1	Pilot-scale P2
specific biofilm surface area (m^2 m^{-3})	162	271	237	390	390
OLR per biofilm surface area (g O_2 m^{-2} d^{-1})	2.5	0.4	0.6	5.4	3.3
OLR per reactor volume (kg O_2 m^{-3} d^{-1})	0.41	0.17	0.20	2.1	1.3
flux substrate A (g O_2 m^{-2} h^{-1})	154	284	152	393	255
flux substrate B (g O_2 m^{-2} h^{-1})	67	9	19	(9)*	(19)*

*the fluxes related to substrate group B or C in the second reactor tank of the pilot-scale system were approached with the results of the ex-situ study.

5.3.3 Simplified approximation of S_{org} in the pilot-scale system with the difference in apparently soluble COD between sequential CSTR

For a daily monitoring of the organic substrate concentration in a pilot-scale or full-scale system, both of the previously discussed methods (bottle incubation, ex-situ batch experiment) are time consuming. In our long-term experiment we therefore approached S_{org} in the first of our two sequential CSTR by simply taking the difference in filtrated COD of the second CSTR (paper 5). This long-term experiment comprised three loading periods with different mixtures of blackwater and greywater. For each of these loading periods, the hydraulic loading rate was justified to obtain a BOD load of approximately 1.2 kg O_2 d^{-1}, which again resulted into different hydraulic retention times in the reactor tanks. More detailed outlines on the experiment, wastewater composition in the different loading periods can be found in the method chapter

of paper 5. In the first loading period, the retention time in the second CSTR was relatively short (3h), so that the degraded filtrated COD mainly consisted of readily degradable substrates belonging to the group. In the second and third loading period of the experiment, the HRT in the second CSTR was with 8 and 15h, respectively substantially longer (paper 5). The longer HRT of the second and third loading period facilitated a greater degradation of slow hydrolysable substrates. Hence, the filtrated COD removed in the second CSTR likely comprised a significant fraction of substrate groups B and C in these periods. Summarized, the approach to estimate S_{org} used by our study is simple, but it was likely biased with higher molecular or colloidal substrates that may not reach the whole aerobic biofilm fraction due to the mass transport resistance. This bias challenges the estimation of a potential oxygen limitation of the nitrifying biomass located in the deeper layers, which was done in paper 5 and discussed further in the subsequent section (5.4). For this purpose a more distinct determination of the organic substrates would be desirable. The respiratory determination with help bottle incubations as discussed in section 5.3.1 may be an alternative to obtain a more precise estimate of S_{org}.

5.4 Exploring the competition between heterotrophic and autotrophic biomass as a controlling factor to the nitrification activity with a simplified modeling approach

From the general mass balance equation (Eq. 1) and the standard model for heterotrophic growth (Table 2), a simplified approach to estimate the limiting factor (O_2, organic substrate) of the heterotrophic activity can be derived. For estimating which of these factors that is limiting at a specific time, only the diffusion term and the stoichiometric coefficient linking the reaction rates of organic substrate (S_{org}) and O_2, ($\nu_{S.O2}$), need to be considered, since the kinetic reaction term is irrelevant (Wanner et al., 2006, Henze et al., 2002). Hence, a stoichiometric coefficient can be derived by resolving the reaction model for organic substrate (C_{Sorg}) as outlined in Table 2, giving a ratio between the reaction of S_{org} ($r.S_{org}$) and O_2 ($r.O_2$) determined by the heterotrophic yield coefficient (Y_H) only (Eq. 4). The latter refers to the fraction of S_{org} getting immobilized into new biomass during heterotrophic growth and thereby not oxidized into CO_2.

$$\nu_{S.O_2} = \frac{r.S_{org}}{r.O_2} = \frac{1}{(1 - Y_H)}$$ Eq. 4 (Wanner et al., 2006)

With help of this stoichiometric coefficient $\nu_{S.O2}$, the substrate concentrations (C_{Sorg}) and diffusion coefficients (D_{Sorg}, D_{O2}), the substrate penetration coefficient $\Upsilon_{S.O2}$ can be derived, which describes the penetration of organic substrate relative to the penetration of oxygen (Wanner et al., 2006, Henze et al., 2002):

$$\Upsilon_{S.O2} = \left(1 - Y_{HET,O2}\right) \frac{D_{S_{org}}}{D_{O_2}} \frac{C_{S_{org}}}{C_{O_2}}$$ Eq. 5 (Wanner et al., 2006, Henze et al., 2002)

If $\Upsilon_{S.O2}$ is smaller than 1, O_2 penetrates less than the organic substrate and is thereby the limiting factor giving a so called oxygen limited biofilm. Under these conditions nitrifiers are hardly competing against the faster growing heterotrophs. If $\Upsilon_{S.O2}$ is greater than 1, organic substrate becomes the limiting factor in deeper biofilm layers where O_2 is present, but no organic substrate so that heterotrophs are no longer able to outcompete nitrifiers or other types of aerobic slow growing organisms. Hence, in such, so called, substrate limited biofilm, nitrification may takes place in the zones where heterotrophs are limited by organic substrate as long and O_2 is present. A $\Upsilon_{S.O2}$ around 1 will result in shifting conditions between substrate and oxygen limitation and usually allow a moderate growth and establishment of nitrifying organisms, but to a significantly lower extent than in a truly substrate limited biofilm having a $\Upsilon_{S.O2} >> 1$ (Wanner et al., 2006, Henze et al., 2002).

As outlined in the previous section, the determined S_{org} probably consisted of a broad number of components with different reaction kinetics and molecular size. This needs to be considered in the parameterization of Eq. 5. The S_{org}-mix determined via the degradation of apparently soluble COD in the

second CSTR most likely consisted of readily degradable low molecular substrates (group A, Table 3) but also larger-sized readily hydrolysable substrates (group B, Table 3). Taking into account this relatively wide range of different substrates, we assumed an average molecule size of 100-500 kDa for the S_{org} mix in our reactor tank (paper 5). Based on this paramterization we calculated a $\Upsilon_{S.O2}$ ranging from 0.6 to 1.3 for the first of our two CSTR and determined an ammonia flux (J_{NH4}) ranging from 2 to 18 mg N m^{-2} h^{-1}. By comparing the relation between $\Upsilon_{S.O2}$ and J_{NH4} in our study with data reported from another study (60 mg N m^{-2} h^{-1} for a $Y_{.SO2}$ of 0.3 and 8 mg N m^{-2} h^{-1} for $Y_{S.O2}$ of 1.1) (Elenter et al., 2007), our figures seem to be within a reasonable range (paper 5). Parameterizing D_{Sorg} for the low molecular substrate acetate, on the other hand, would have resulted into a $Y_{.SO2}$ ranging from 1- 2.9 (not shown). Such a high $Y_{S.O2}$ would have pointed to longer periods without detectable nitrification which is not in accordance to the measured fluxes for TAN, NO_2^- and NO_3^- (paper 5), which again supports the assumption that our determined S_{org} mix did not only contain low molecular, readily degradable substrates.

D_{Sorg} differs not only between different organic compounds, it is also dependent on the media in which diffusion takes place. A biofilm represents a complex matrix of cells, extracellular polymeric substances (EPS) and interstitial water, which results in a lower diffusivity inside the biofilm (D_f) than in water (D_{aq}) where the diffusion coefficients are usually determined (Stewart, 1998). In general, the ratio D_f/D_{aq} does not differ significantly among none-polar solutes of different molecular size (Stewart, 1998). Most present studies therefore apply a unified correction factor D_f/D_{aq} for different substrate to consider the lower diffusivity in a biofilm. The correction factor D_f/D_{aq} depends further on the selected modelling approach and literature values thereby range from 0.3 (Kommedal et al., 2006) to 0.6 (Morgenroth, 2008b). In our study we assumed a unified correction factor D_f/D_{aq} for both organic substrate (S_{org}) and O_2. With this assumption, the correction factor could be ignored in the calculation of $Y_{S.O2}$, since it would apply in both nominator and denominator of Eq. 5. However, the literature provides a weak indication that D_f/D_{aq} is lower for high molecular none-polar organic compounds (>1kDa) than for O_2 or low molecular none-polar organic compounds (e.g. acetate) (Stewart, 1998). This would again point towards an overestimation of $Y_{.SO2}$ with our approach, considering that average molecule size of the soluble substrates in the wastewater mixtures of our study might have been greater than 1kDa (paper 5).

In the second of our CSTR the concentration of S_{org} was expected to be relatively low in a range of 5-10 mg L^{-1} . This was supported by the two batch experiments that were conducted to explore the kinetics of the liquid compounds. With a DO ranging from 3-6 mg O_2 L^{-1} and the S_{org} of 5-10 mg L^{-1} , $\Upsilon_{S.O2}$ would range from 0.08 to 0.3, pointing clearly to a thoroughly substrate limitation in the biofilm. The net nitrification rate however, was only slightly higher than in the first CSTR. We attribute this surprisingly low net nitrification rate in the second CSTR, to an assumed large O_2 consumption of the fluffy biofilm by immobilizing and degrading particulate organic substrates (paper 5). This reaction with particulate substrates was likely facilitated by the lower turbulence that was observed compared to the first CSTR. Our hypothesis that the physical impact of the turbulence in the reactor tank was an additional factor determining the magnitude of nitrification is also supported by recent research on biofilm dynamics, which encompasses the detachment of biomass as well as attachment or immobilization of particulate matter from the bulk liquid (Derlon et al., 2013, Elenter et al., 2007). Frequent detachment events were shown to facilitate nitrifiers, especially ammonia oxidizing bacteria (AOB), even on conditions with $\Upsilon_{S.O2}$ >>1, where nitrification activity is not necessary expected (Elenter et al., 2007).

In biofilm systems receiving a high load of organic substrate, nitrification does often not reach significant reaction rates, even if ammonia is present on a notable concentration due to an out-competition of nitrifying organism by the faster growing heterotrophs (Tchobanoglous et al., 2002, Wanner and Gujer, 1985). However, as outlined in the previous paragraphs, the occurrence of nitrification is determined by the oxygen penetration depth relative to the substrate penetration depth into the biofilm rather than the organic loading rate per reactor volume (Eq. 5). This relative oxygen penetration depth or $Y_{S.O2}$, respectively, again depends on the substrate and oxygen concentration in the bulk liquid as well as the average molecule size

of the organic substrate. The assumed high proportion of high molecular compounds (paper 5) as well as colloidal solids (Figure 3) in the filtrated COD of blackwater points to a correspondingly high average molecular size of the organic substrate. In a biofilm system loaded with blackwater, nitrification may therefore coincide with heterotrophic degradation on organic loading rates where it might not have been expected in a load with mixed domestic wastewater. The measured fluxes for TAN, NO_2^- and NO_3^- , which reached notable levels in both reactor tanks of our MBBR system on a relatively high load of degradable organic matter (BOD) support this hypothesis (paper 5).

A wastewater treatment plant needs to be sized based on the expected maxima loading rates from a particular sewer system with the result that the average organic mass loading rate is most likely considerably lower than the mass load the system is sized for. Especially low loading periods will therefore facilitate conditions with a low $Y_{S.O2}$ and high nitrification activity. Nitrification is therefore an inevitable side-process in a biofilm system loaded with blackwater and needs a closer attention to antagonize negative side-effects of the treatment process (e.g. sludge floatation due to N-gas production by denitrification in a subsequent gravity settler) as well as potential greenhouse gas emissions. The latter are discussed more in detail in the subsequent section (5.5).

5.5 Mechanism behind N_2O emissions in a biofilm system loaded with blackwater

Paper 4 comprehensively reviews different mechanism of N_2O production and its underlying factors in a biofilm (Figure 11). The outcomes of the reviewed microbial pathways, indicate for the production of N_2O either a presence of oxidized N compounds (NO_2^-, NO_3^-, NO) or an active biological ammonia oxidation process is required within the systems boundaries (paper 4). In raw blackwater, the oxidized N-compounds NO_2^- and NO_3^- were below the detection limit (paper 1), hence, the presence of nitrification activity is a mandatory factor for the occurrence of N_2O in a biological treatment process loaded with blackwater. In an intensive biofilm system (MBBR), as assed by this study nitrification was shown to occur with high likelihood, regardless the system is designed for biological N-removal or not (section 5.4). Even though the fluxes for NO_2^- and NO_3^- in our reactor system did not reach significant rates in terms of N removal, they were shown to be connected to a notable N_2O production and need closer attention (paper 5).

The outcomes of our literature review (paper 4) identified the observed accumulation of NO_2^- as a potential factor, which is mediating N_2O production by ammonia oxidizing bacteria (AOB) and heterotrophic denitrifiers that are likely present in a heterogeneous biofilm systems (Figure 11). These findings were confirmed by the observed N_2O emission peaks in our MBBR experiment that coincided with enhanced presence of NO_2^- in the reactor liquid. NO_2^- accumulation takes place when nitrification is imbalanced so that the NO_2^- production rate by AOB is greater than the NO_2^- oxidation rate by nitrite oxidizing bacteria (NOB) (paper 4). Among known factors leading to an unilateral inhibition of NOB (more details are given in paper 3), the presence of free ammonia (NH_3) was identified as the most critical factor leading to imbalanced nitrification and NO_2^- accumulation for a biofilm system loaded with raw blackwater (paper 5). Having a NH_3 concentration of 70-200 mg L^{-1} (paper 1), raw blackwater is expected to inhibit NO_2^- oxidation by NOB for 77-90% (Anthonisen et al., 1976). This is confirmed by the observed data in the reactor tanks from the loading phase with pure blackwater, where NO_2^- accounted for 7-18% only of the oxidized N compounds (NO_2^- and NO_3^-) measured in the bulk liquid (paper 5). A tendency towards incomplete nitrification and accumulation of NO_2^- was also reported from a similar MBBR system, which is treating a mixed wastewater of a high mountain lodge (Andreottola et al., 2003). The accumulation of NO_2^- seems therefore to be a critical issue in biofilm reactor systems loaded with concentrated household wastewater. Regardless if nitrogen removal is required or not, biological nitrogen conversation needs therefore to be considered in the design and operation of biofilm reactor systems to antagonize NO_2^- accumulation. Otherwise greenhouse gas emissions may outweigh the ecological benefit of the treatment process.

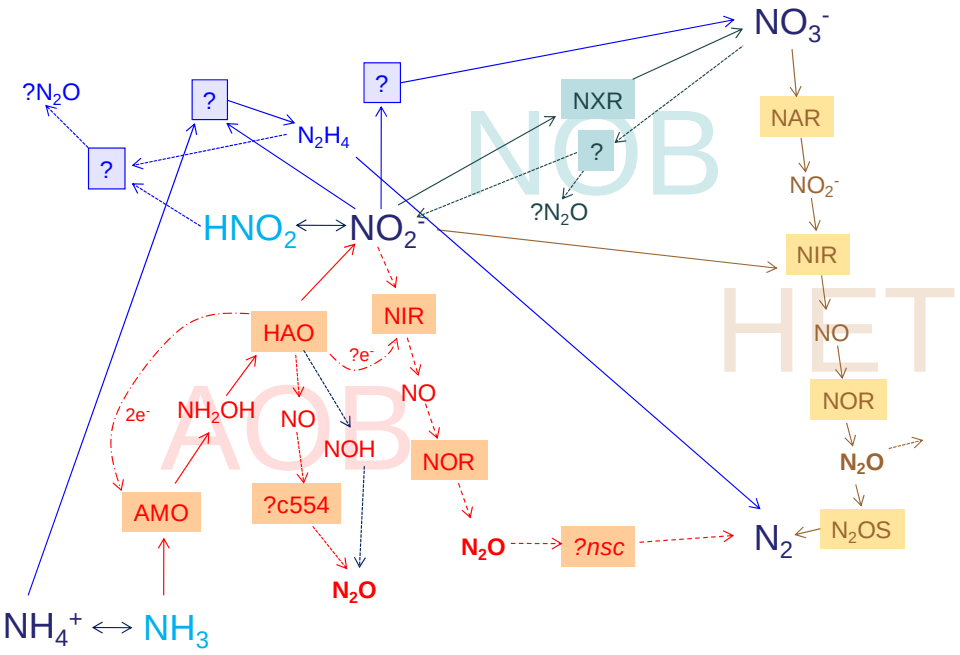


Figure 11: Pathways of enzymatic nitrogen conversation by the four main groups of organisms in wastewater treatment systems: ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), heterotrophic denitrifiers (HET) and anammox bacteria (AMX). The AOB metabolism consists of the enzymes ammonia monoxygenase (AMO), hydroxylamine dehydrogenase (HAO), the heme protein cytrochrome c554 (c554, which is mediating the electron transport and has in addition a putative NO reductase function. AOB consists further of the so-called nitrifier denitrification chain consisting of the enzymes nitrite reductase (NIR) and nitric oxide reductase (NOR) and a putative N_2O reducing *nsc* protein. The NOB metabolism consists of the nitrite oxidoreductase (NXR and a so far little investigated respiratory metabolism with NO_3^- as electron acceptor. Heterotrophic denitrification involves the reductase enzymes nitrate reductase (NAR) nitrite reductase (NIR), nitric oxide reductase (NOR) and N_2O reductase N_2OR. The anammox metabolism is so far little investigated. At AOB, N_2O is mainly produced via intermediate releases from HAO with high reaction rates or nitrosative stress and via nitrifier denitrification with O_2 tension. Heterotrophic denitrifiers produce N_2O under transient microaerobic conditions, in presence of free NO or HNO_2 or at electron donor limitation (paper 4)

In addition to nitrosative stress mediated by high concentrations of NO_2^-, a second N_2O producing pathway associated to incomplete heterotrophic denitrification may be critical in an aerobic biofilm reactor loaded with blackwater (Itokawa et al., 2001, paper 5). Incomplete heterotrophic denitrification can occur when readily degradable organic substrates are depleted so that the electron donor availability is limited to slow degradable substrates (Itokawa et al., 2001, Pan et al., 2013). In the second of the two sequential CSTR in our pilot-scale system, a larger proportion of the reduced filtrated COD degraded on a relatively low reaction rate (Figure 10). These findings point to a high proportion of slow degradable compounds on the total available degradable apparently soluble substrates in this particular reactor tank. The degradability of the available organic substrates determines the biomass competition between heterotrophic and autotrophic organisms in a biofilm. If slow degradable organic compounds (Group C, Table 3) are the dominating organic substrate, the growth rate of heterotrophs will not reach a level which is needed to compete with nitrifiers with a coinciding ample presence of NH_3 in the system (Jin et al., 2012, Wang et al., 2010, Lackner and Horn, 2012). With this substrate mix (slow degradable organic substrates, ample presence of NH_3), nitrifiers likely dominate the aerobic biofilm zones regardless the organic substrate concentration in

the bulk liquid. Due to the low degradability and low reaction rates (r_F, Eq. 1), slow molecular compounds belonging to substrate group C (Table 3) (e.g. humic acids) may penetrate the whole biofilm cross-section. In presence of ample electron acceptors (NO_2^- or NO_3^-) produced by the nitrifiers located in the aerobic biofilm layer, these slow degradable low molecular substrates will fuel incomplete heterotrophic denitrification when reaching anoxic biofilm zones (paper 4, paper 5).

For a definitive confirmation of the above outlined hypothesis that a biofilm system loaded with blackwater may facilitate a notable incomplete heterotrophic denitrification activity, additional research is needed. As a next step, we suggest additional incubation experiments in vaccine bottles in accordance to the method described in paper 5 with parallels were AOB activity is inhibited. Acetylene (C_2H_2) and allylthiourea (ATU) are the most frequently used AOB inhibitors. However, both of these substances may partially impact heterotrophic denitrification, which needs to be closer assessed. Acetylene inhibits the N_2O reducing enzyme (N_2OS) in the heterotrophic denitrification reaction chain (Jensen et al., 2007). Hence, it is not possible to distinguish N_2O from putative incomplete heterotrophic denitrification provoked by slowly degradable substrates. ATU was shown to slightly reduce the denitrification rates, but had no significant affect on the proportion of N_2O found in the total gas emission (Jensen et al., 2007). ATU, a none-competitive inhibitor to the ammonia monooxygenase (AMO) enzyme of AOB (Hyman et al., 1990) may therefore work better for our purpose than acetylene. No information was found on the potential impact on the nitrifier denitrification reaction chain by these inhibitors. For a proper distinguishing of N_2O produced by incomplete heterotrophic denitrification from N_2O produced by the respiratory AOB enzymes, the complete AOB metabolism should ideally be inhibited. Nitrifier denitrification can use NH_3 (Schmidt, 2009) or particular low molecular readily degradable organic compounds as electron donors (Bock et al., 1995, Schmidt et al., 2001). Latter substrates are unlikely present in notable quantities under conditions that facilitate incomplete heterotrophic denitrification. The usage of NH_3 as electron donor on the other hand needs an active nitrification process, which again is suppressed at an inhibition of the AMO enzyme. Hence, an inhibition of the AMO enzyme with ATU should be an appropriate approach to distinguish a potential N_2O contribution of heterotrophic denitrification in deeper biofilm layers from N_2O produced by AOB.

6 Evaluation of the outcomes in terms of resource recovery

The mechanical filter system that was developed and tested within this study showed relatively high removal efficiency for suspended solids and COD (paper 3). It may be possible to optimize the particle retention further by evaluating alternative types of filter media (paper 2, paper 3) or improving the mechanical conveying process (paper 3). As shown in our preliminary experiment outlined in chapter 4.5, an additional retention of dissolved nutrients may be obtainable by amending the filter media (wood-shavings) with Mg^{2+}. However, blackwater has a high load of apparently soluble organics (paper 1) of which again a remarkable fraction seem to be attributed to molecular dissolved compounds (e.g. acetate) as shown by other studies on domestic sewage (Dulekgurgen et al., 2006, Sophonsiri and Morgenroth, 2004). These soluble components retain only to a limited extent in the filter system. Hence, even with substantial improved filtration efficiency, the mechanical filter system still represents a primary step in a multi-stage treatment process, which needs to be succeeded by a secondary treatment step for soluble organic compounds. As discussed in the previous chapter (6) such a secondary treatment step has to facilitate a biological degradation process, which can be done in extensive, natural based systems (e.g. wetland) or in more intensive bioreactor systems (e.g. activated sludge, MBBR).

An energy-saving alternative to the intensive aerobic bioreactor system assessed by this study maybe a single-pass trickling filter or constructed wetland as successfully applied to blackwater in the Mediterranean area (Masi et al., 2010, Regelsberger et al., 2007). Nowadays, little information is available on potential N_2O emissions from these extensive systems loaded when high concentration wastewater. Gravel beds (Logan et al., 1987), but also constructed wetlands (Hua et al., 2013a) are principally systems that facilitate an attached growth biomass, similar to the biofilm reactor systems discussed in the previous chapter. Hence, the identified critical key factors of NO_2^- accumulation and electron donor limited denitrification (paper 5) may also occur in such more extensive types of biofilm systems. However, a detailed modeling and assessment of the process conditions within a wetland system are behind the scope of this study and needs further research.

For locations with limited space availability or unfavorable ambient climatic conditions, extensive systems are difficult to implement and an intensive reactor-based secondary treatment step needs to be evaluated as shown on the example of Britannia lodge. In our study we therefore tested an intensive aerobic biofilm reactor (MBBR), which could be associated to notable risks for uncontrolled nitrogen conversation processes and subsequent N_2O emissions (paper 5). These findings clearly indicate a limited applicability of traditional aerobic biological treatment methods for concentrated blackwater. Calculations done for a two-stage treatment process for Britannia lodge comprising the mechanical filter system for suspended solid removal and an MBBR for a proper treatment of soluble organic compounds further show that the system will require electrical energy corresponding to 0.1-0.5 kWh d^{-1} per person (Todt, 2012). A 50 PE system will therefore require 5-10 kWh d^{-1}, which is a remarkable energy footprint and difficult to supply with solar power as originally intended at Britannia lodge. A majority of the present blackwater applications in Europe use therefore anaerobic methods (e.g. UASB) to remove organic matter (Table 7). Anaerobic reactor systems provide the advantage to extract and utilize the energy contained in the organic matter of the blackwater, so that a treatment process can become self-sustaining or even provide surplus energy (Otterpohl et al., 1997).

For blackwater treatment, anaerobic methods seem therefore to be clearly more appropriate than intensive aerobic treatment processes, also for locations where space is a limiting factor. The gas handling seem rather not as complex as thought in the beginning of this study. The produced CH_4 can relatively simply be converted to heat with a standard gas burner and a preceding simple charcoal filter (Zeeman and Kujawa-Roeleveld, 2011). Recent studies showed that passing the gas through an adsorption filter filled with zeolite (Alonso-Vicario et al., 2010) or activated carbon (de Arespacochaga et al., 2014) provides a sufficient degree of purification for a proper burning process or even more advanced use. For a simple heat

production or a cooking device, the gas may even be used directly without purification step, as commonly practiced in developing projects (e.g. Lebofa and Huba, 2011, Zurbrügg et al., 2011). If the gas is then burned in real time no gas storage is necessary and this can be an important safety measure considering small decentralized installations. An anaerobic treatment of blackwater and kitchen wastes was also successfully applied to Refugio Casera Bosconero (Langergraber and Müllegger, 2011), a similar alpine accommodation place to Britannia lodge discussed by this study. However, a detailed evaluation of the safety and applicability of simple small-scale biogas applications is beyond the scope of this study and further research is recommended to get more knowledge on that issue.

Table 7: Projects on source separating sanitation with blackwater treatment in Europe and applied technologies

Project, location	Technologies	Size
ZeroM, Turkey	UASB (Halalsheh and Wendland, 2008, Regelsberger et al., 2007)	unknown
Flintenbreite, Lübeck, Germany	Vacuum toilets (Oldenburg et al., 2002, Otterpohl, 2002, Otterpohl et al., 1997), Rottebehälter (Gajurel et al., 2003a, Gajurel et al., 2003b), UASB (Elmitwalli and Otterpohl, 2007), MBR (Li et al., 2008)	400 PE
School, Netherland	UASB, MBR (van Voorthuizen et al., 2008)	20-25 l/day (experimental scale)
Camp ground, Spain	MBR (Schories, 2008)	50 PE
Wetshus, Netherland	UASB (Elmitwalli et al., 2006), Anammox (de Graaff et al., 2010)	lab scale experiments
Sneek, Netherland	Vacuum toilets (1 liter flush), UASB (Zeeman et al., 2008), struvite precipitation, Anamox (Zeeman and Kujawa-Roeleveld, 2011)	150 PE

An anaerobic reactor provides reducing conditions with the result that nitrogen is either reduced to ammonia or N_2, depending on its original form in the inlet. The nitrogen in raw blackwater was shown to appear either as ammonia or organic bound N. Oxidized N compounds (NO_2^-, NO_3^-) on the other hand could not be detected (paper1). Organic bound N may be reduced to ammonia under anaerobic conditions, but a further reduction to N_2 is unlikely to take place at significant rates in absence of NO_2^-, which would be needed for a anaerobic NH_4^+ oxidation to N_2 (Arrigo, 2005). Nitrogen contained in the effluent of an anaerobic reactor treating raw blackwater is expected to appear mainly as ammonia at a high concentration level (TAN 1-1.5 g N L^{-1}) (paper 1). This high concentration may open for novel, highly efficient nutrient recovery options (paper 1), e.g. struvite precipitation as discussed in section 4.5 or microbial fuel cells (Kuntke et al. 2012). For decentralized applications however, these options may be technically too sophisticated, especially for remote applications as mountain huts.

If N-removal is required a more conventional microbiological approach may be easier to implement. Considering the high TAN content, N-removal from anaerobic treated blackwater likely meets the same challenges with NO_2^- accumulation and subsequent N_2O emissions as raw blackwater, as discussed in section 5.5. Readily degradable organic substrates (substrate group A, Table 3) will to a large extend be depleted in an anaerobic degradation process. Heterotrophic denitrification is therefore difficult to obtain without dosing an external electron donor. The latter will increase the ecological footprint of the treatment substantially and is therefore not an appropriate alternative. A process utilizing the group of Anammox bacteria (Jetten et al., 1997) was shown to produce less N_2O, if properly designed (paper 4) and may

therefore be more appropriate for the treatment of blackwater. In recent years Anammox processes reached an increasing popularity for the treatment of anaerobic digestate with high ammonia content as produced in sludge treatment (e.g. Fux et al., 2004b, Strous et al., 1997), but also effluent of UASB fed with blackwater (de Graaff et al., 2010, Zeeman and Kujawa-Roeleveld, 2011). Anammox may even be an option for less concentrated, anaerobically pre-treated mixed sewage (Malovanyy et al., 2015). Anammox bacteria reach its maximum growth (Lotti et al., 2014) and N-conversation rates (Dosta et al., 2008) under mesophilic conditions at 35-40 °C. Anammox based wastewater treatment processes are therefore typically operated at enhanced temperatures of 25 °C or higher (Lackner and Horn, 2012), which would coincide with the typical temperature range in anaerobic organic matter removal processes (e.g. UASB) (e.g. Elmitwalli et al., 2006, Zeeman et al., 2008). The main challenge in sustaining an Anammox based N removal process seems to be the minimizing of NOB activity, which is competing with Anammox regarding the substrate NO_2^-. The high NH_3 concentration in blackwater was shown to mediate a partial nitrification by inhibiting NOB (paper 1, paper 5). However, a long term suppression of NO_2^- oxidation via the inhibitory impact of NH_3 seems difficult due to adaption of NOB to enhanced NH_3 concentration with time. Maintaining a stable partial nitrification therefore requires more sophisticated process control strategies (Malovanyy et al., 2015, Fux et al., 2004a). Additional research is therefore needed to evaluate the feasibility of Anammox based N-removal for an application in smaller-scale, decentralized sanitary systems, where an advanced process control is difficult.

7 Conclusions

7.1 Characterisation of blackwater

- The blackwater contributed 69% of the total COD load, 83% of the total N load and 87% for total P load of the combined wastewater (grey- and blackwater) from Kaja student dormitories. This corresponds to findings of other studies and confirms the stated high recovery potential for organic matter and nutrients with a source separating sanitation approach (paper 1).

- A high fraction of 37-47% of the suspended solids contained in the blackwater could be attributed to supra-colloidal solids having a size of 1-100 µm, which are hardly retained in a sedimentation process. Other particle separation methods are therefore needed, especially for primary treatment.

- The filtrated or apparently soluble COD of blackwater seems to contain a notable proportion of colloidal solids (20-100 nm). In opposite to low molecular dissolved compounds, these colloidal solids are too large for a transport via Brownian diffusion, which needs to be considered in the modeling and design of a biofilm system.

- The low COD/N ratio (6-7) and high content of free ammonia (50 mg L^{-1}) in blackwater is a challenge for traditional biological nitrogen removal approaches (paper 1). The low COD/P ratio (175-214) in greywater may be critical in terms of P-limitation of a subsequent biological treatment process for removal of organic matter (paper 1).

- The high concentration of nitrogen and phosphorous in blackwater from vacuum or low-flush toilets, amounting for 1.4-1.7 g N L^{-1} and 150-200 mg P L^{-1}, respectively, open up for novel reuse options as for example struvite precipitation (paper 1).

7.2 Removal of particulate matter from blackwater with mechanical filtration

- The results assessment of different organic filter media show that mixtures containing a wider grain size range results a in higher tortuosity, a higher hydraulic capacity and significant greater removal efficiency (paper 2).

- The thickness of traditional organic filters can be halved from 30 to 15 cm without a significant performance loss in terms of particle (TSS) removal when loaded with concentrated blackwater. These finding can be used to design a novel mechanical filter system (paper 2).

- A novel mechanical filtration system was developed as a primary treatment system for the blackwater from vacuum or other types of low-flush toilets. The filtration unit showed a removal capacity of 78-85% for TSS, 60-80% for COD and 42-57% for phosphorous, which is comparable to the performance of organic filter systems loaded with municipal wastewater (paper 3).

- The TSS removal efficiency of the mechanical filtration system was dependent on the TSS inlet concentration. A closer analysis of the hydraulics indentified the peak flow rate given by the number of toilet flushes per time unit as the most critical factor. With a peak flow rate of 0.73 L min^{-1}, corresponding to 40 toilet flushes (1.1 liter) per hour, as applied in our laboratory experiment, a daily load of at least 310 toilet flushes or 340 L d^{-1} was shown to be possible (paper 3).

- Amending the wood-shavings that are used as filter matrix in the second step of the mechanical filter system with Mg^{2+} (Mg-hydroxide-carbonate) resulted into a coinciding reduction of NH_4^+ and PO_4^{3-}. This is likely connected to a struvite precipitation that took place in the filter media and maybe an option for N and P recovery.

7.3 Aerobic biological treatment options for blackwater and risk for greenhouse gas emissions

- Nitrification is a process that likely will occur in an intensive biofilm system if ample ammonia substrate is present: This is the case in blackwater, even with high organic loading rates. Nitrification was at the same time identified as a basic prerequisite for N_2O emission and therefore needs closer attention also in systems having organic matter removal as the solely purpose (paper 5).

- The accumulation of NO_2^- was identified as a main factor for N_2O emissions. In biofilm systems loaded with concentrated household wastewater or blackwater, an accumulation of NO_2^- will high likely take place if no denitrification step is integrated. Hence, regardless if nitrogen removal is required or not, biological nitrogen transformations needs to be considered in the design and operation of biofilm reactor systems to antagonize critical greenhouse gas emissions (paper 4).

- A unilateral inhibition of nitrite oxidizing bacteria by a critical high presence of free NH_3 was shown to be the most critical factor leading to NO_2^- accumulation in an aerobic reactor system loaded with concentrated household wastewater (paper 4, paper5).

- Blackwater can result in a high presence of slow degradable low molecular organic substrates in particular sections of a biofilm reactor system, and this may fuel N_2O emissions by incomplete heterotrophic denitrification in presence of oxidized N (NO_2^-, NO_3^-) (paper 4; paper 5).

7.4 Overall conclusion

The tested approach consists of a primary treatment step with a mechanical filter system followed by a biological treatment of the liquid fraction in a biofilm system. The system showed potential to reach high treatment efficiencies if implemented properly. However, the high energy consumption as well as the risk for greenhouse gas emissions in the secondary aerobic biological treatment questions the sustainability of this treatment approach. Anaerobic methods such as upstream anaerobic sludge blanked reactor systems may be more appropriate for removal of organic matter with respect to resource recovery and zero emission. Also recovering of nutrients needs further research efforts with focus on novel, innovative approaches, especially for nitrogen.

8 List of abbreviations

AOB	Ammonia Oxidizing Bacteria
AMO	Ammonia monoxygenase (enzyme in ammonia oxidation bacteria)
ATU	Allylthiourea (inhibitor of ammonia oxidation)
$BOD_{(5)}$	Biological Oxygen Demand (mg O_2 L^{-1}), in this study referring to 5 days (BOD_5)
CSTR	Complete Stirred Reactor Tank
COD	Chemical Oxygen Demand (mg O_2 L^{-1})
DO	Dissolved Oxygen (mg O_2 L^{-1})
EPS	Extracellular Polymeric Substances
HRT	Hydraulic Retention Time
MBR	Membrane Bio Reactor
MBBR	Moving Bed Biofilm Reactor
N	nitrogen
N_2	di-nitrogen (atmospheric form of nitrogen)
NH_3	free ammonia
NH_2OH	hydroxylamine
NH_4^+	ammonium (protonized form of ammonia)
NO	nitric oxide
NO_2^-	nitrite
NO_3^-	nitrate
NOB	Nitrite Oxidizing Bacteria
N_2O	Nitrous oxide
N_2OS	Nitrous oxide reductase (enzyme of heterotrophic denitrification)
OLR	Organic loading rate
P	phosphorous
PO_4^{3-}	ortho-phosphate
UASB	Upstream Anaerobic Sludge Blanked (reactor)
TAN	Total Ammonia Nitrogen (mg N L^{-1})
TSS	Total Suspended Solids (mg L^{-1})

9 References

2001/77/EC, D. DIRECTIVE 2001/77/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 September 2001 on the promotion of electricity produced from renewable energy sources in the internal electricity market.

ABBONA, F., LUNDAGER MADSEN, H. E. & BOISTELLE, R. 1982. Crystallization of two magnesium phosphates, struvite and newberyite: Effect of pH and concentration. *Journal of Crystal Growth,* 57**,** 6-14.

ABEGGLEN, C. 2004. Übersicht Abwasserentsorgungssysteme in SAC-Hütten. Zürich: ETH Zürich (Swiss Federal Institute of Technology), Institut für Hydromechanik und Wasserwirtschaft.

ALONSO-VICARIO, A., OCHOA-GÓMEZ, J. R., GIL-RÍO, S., GÓMEZ-JIMÉNEZ-ABERASTURI, O., RAMÍREZ-LÓPEZ, C. A., TORRECILLA-SORIA, J. & DOMÍNGUEZ, A. 2010. Purification and upgrading of biogas by pressure swing adsorption on synthetic and natural zeolites. *Microporous and Mesoporous Materials,* 134**,** 100-107.

ANDREOTTOLA, G., DAMIANI, E., FOLADORI, P., NARDELLI, P. & RAGAZZI, M. 2003. Treatment of mountain refuge wastewater by fixed and moving bed biofilm systems. *Water Science and Technology,* 48**,** 169-177.

ANTHONISEN, A. C., LOEHR, R. C., PRAKASAM, T. B. S. & SRINATH, E. G. 1976. Inhibition of nitrification by ammonia and nitrous-acid. *Journal Water Pollution Control Federation,* 48**,** 835-852.

ARNBERGER, A., MUHAR, A. & STERL, P. 2006. ALP Austria Programm zur Sicherung und Entwicklung der alpinen Kulturlandschaft - Auswirkungen des Tourismus. Wien: Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft.

ARRIGO, K. R. 2005. Marine microorganisms and global nutrient cycles. *Nature,* 437**,** 349-355.

ATV 1997. *Biologische und weitergehende Abwasserreinigung,* Berlin, Ernst & Sohn.

BALMAT, J. L. 1957. Biochemical Oxidation of Various Particulate Fractions of Sewage. *Sewage and Industrial Wastes,* 29**,** 757-761.

BOCK, E., SCHMIDT, I., STUVEN, R. & ZART, D. 1995. NITROGEN LOSS CAUSED BY DENITRIFYING NITROSOMONAS CELLS USING AMMONIUM OR HYDROGEN AS ELECTRON-DONORS AND NITRITE AS ELECTRON-ACCEPTOR. *Archives of Microbiology,* 163**,** 16-20.

COMEAU, Y. 2008. Microbial Metabolism. *In:* HENZE, M., VAN LOOSDRECHT, M. C. M., EKAMA, G. A. & BRDJANOVIC, D. (eds.) *Biological Wastewater Treatment.* London: IWA Publishing.

CONFER, D. R. & LOGAN, B. E. 1997. Molecular weight distribution of hydrolysis products during the biodegradation of model macromolecules in suspended and biofilm cultures. II. Dextran and dextrin. *Water Research,* 31**,** 2137-2145.

CORLEY, M., RODGERS, M., MULQUEEN, J. & CLIFFORD, E. 2006. The performance of fibrous peat biofilters in treating domestic strength wastewater. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering,* 41**,** 811-824.

DAUDE, D. & STEPHENSON, T. 2003. Moving bed biofilm reactors: a small-scale treatment solution. *Water Science and Technology,* 48**,** 251-257.

DE ARESPACOCHAGA, N., VALDERRAMA, C., MESA, C., BOUCHY, L. & CORTINA, J. L. 2014. Biogas deep clean-up based on adsorption technologies for Solid Oxide Fuel Cell applications. *Chemical Engineering Journal,* 255**,** 593-603.

DE GRAAFF, M. S., TEMMINK, H., ZEEMAN, G. & BUISMAN, C. J. N. 2011. Energy and phosphorus recovery from black water. *Water Science and Technology,* 63**,** 2759-2765.

DE GRAAFF, M. S., ZEEMAN, G., TEMMINK, H., VAN LOOSDRECHT, M. C. M. & BUISMAN, C. J. N. 2010. Long term partial nitritation of anaerobically treated black water and the emission of nitrous oxide. *Water Research,* 44**,** 2171-2178.

DERLON, N., COUFORT-SAUDEJAUD, C., QUEINNEC, I. & PAUL, E. 2013. Growth limiting conditions and denitrification govern extent and frequency of volume detachment of biofilms. *Chemical Engineering Journal,* 218**,** 368-375.

DIMOCK, R. & MORGENROTH, E. 2006. The influence of particle size on microbial hydrolysis of protein particles in activated sludge. *Water Research,* 40**,** 2064-2074.

DOSTA, J., FERNÁNDEZ, I., VÁZQUEZ-PADÍN, J. R., MOSQUERA-CORRAL, A., CAMPOS, J. L., MATA-ÁLVAREZ, J. & MÉNDEZ, R. 2008. Short- and long-term effects of temperature on the Anammox process. *Journal of Hazardous Materials,* 154**,** 688-693.

DULEKGURGEN, E., DOGRUEL, S., KARAHAN, Ö. & ORHON, D. 2006. Size distribution of wastewater COD fractions as an index for biodegradability. *Water Research,* 40**,** 273-282.

EKAMA, G. A. & WENTZEL, M. C. 2008. Nitrogen Removal. *In:* HENZE, M., VAN LOOSDRECHT, M. C. M., EKAMA, G. A. & BRDJANOVIC, D. (eds.) *Biological Wastewater Treatment.* London: IWA Publishing.

ELENTER, D., MILFERSTEDT, K., ZHANG, W., HAUSNER, M. & MORGENROTH, E. 2007. Influence of detachment on substrate removal and microbial ecology in a heterotrophic/autotrophic biofilm. *Water Research,* 41**,** 4657-4671.

ELMITWALLI, T. A. & OTTERPOHL, R. 2007. Anaerobic biodegradability and treatment of grey water in upflow anaerobic sludge blanket (UASB) reactor. *Water Research,* 41**,** 1379-1387.

ELMITWALLI, T. A., VAN LEEUWEN, M., KUJAWA-ROELEVELD, K., SANDERS, W. & ZEEMAN, G. 2006. Anaerobic biodegradability and digestion in accumulation systems for concentrated black CD water and kitchen organic-wastes. *Water Science and Technology,* 53**,** 167-175.

ERICKSON, H. P. 2009. Size and Shape of Protein Molecules at the Nanometer Level Determined by Sedimentation, Gel Filtration, and Electron Microscopy. *Biological Procedures Online,* 11**,** 32-51.

FAURE, Y. H., BAUDOIN, A., PIERSON, P. & PLE, O. 2006. A contribution for predicting geotextile clogging during filtration of suspended solids. *Geotextiles and Geomembranes,* 24**,** 11-20.

FUX, C., HUANG, D., MONTI, A. & SIEGRIST, H. 2004a. Difficulties in maintaining long-term partial nitritation of ammonium-rich sludge digester liquids in a moving-bed biofilm reactor (MBBR). *Water Science and Technology,* 49**,** 53-60.

FUX, C., MARCHESI, V., BRUNNER, I. & SIEGRIST, H. 2004b. Anaerobic ammonium oxidation of ammonium-rich waste streams in fixed-bed reactors. *Water Science and Technology,* 49**,** 77-82.

GAJUREL, D. R., BENN, O., LI, Z., BEHRENDT, J. & OTTERPOHL, R. 2003a. Pre-treatment of domestic wastewater with pre-composting tanks: evaluation of existing systems. *Water Science and Technology,* 48**,** 133-138.

GAJUREL, D. R., LI, Z. & OTTERPOHL, R. 2003b. Investigation of the effectiveness of source control sanitation concepts including pre-treatment with Rottebehaelter. *Water Science and Technology,* 48**,** 111-118.

GUJER, W. 2010. Nitrification and me - A subjective review. *Water Research,* 44**,** 1-19.

GUJER, W. & BOLLER, M. 1986. Design of a nitrifying tertiary trickling filter based on theoretical concepts. *Water Research,* 20**,** 1353-1362.

GUJER, W., HENZE, M., MINO, T. & VAN LOOSDRECHT, M. 1999. Activated Sludge Model No. 3. *Water Science and Technology,* 39**,** 183-193.

GÜNTHERT, F. W. & NARR, N. 2002. Klärschlammproblematik im Hochgebirge „Untersuchung und Optimierung der Verwertung bzw. Beseitigung von Abfällen aus der Abwasserbehandlung im Bereich alpiner Hütten des Deutschen Alpenvereins“. Neubiberg: Universität der Bundeswehr München.

GÜNTHERT, F. W. & RAUCH, W. 2007. Abschlussbericht Klärschlammproblematik im Hochgebirge. Deutsche Bundesstiftung Umwelt.

HALALSHEH, M. & WENDLAND, C. 2008. Integrated Anaerobic–Aerobic Treatment of Concentrated Sewage. *In:* OTTERPOHL, R., BAZ, I. A. & WENDLAND, C. (eds.) *Efficient Management of Wastewater.* Springer Berlin Heidelberg.

HENZE, M. & COMEAU, Y. 2008. Wastewater Characterization. *In:* HENZE, M., VAN LOOSDRECHT, M. C. M., EKAMA, G. A. & BRDJANOVIC, D. (eds.) *Biological Wastewater Treatment.* London: IWA Publishing.

HENZE, M., GRADY, C. P. L., GUJER, W., MARAIS, G. V. R. & MATSUO, T. 1987. Activated Sludge Model No. 1. *IAWPRC Scientific and Technical Reports.*

HENZE, M., GUJER, W., MINO, T., MATSUO, T., WENTZEL, M. C. & MARAIS, G. V. R. 1995. Activated Sludge Model No. 2. *IAWQ Scientific and Technical Reports.*

HENZE, M., HARREMOËS, P., JANSEN, J. L. C. & ARVIN, E. 2002. *Wastewater Treatment: Biological and Chemical Processes,* Springer.

HUA, G. F., LI, L., ZHAO, Y. Q., ZHU, W. & SHEN, J. Q. 2013a. An integrated model of substrate clogging in vertical flow constructed wetlands. *Journal of Environmental Management,* 119**,** 67-75.

HUA, G. F., ZHU, W., SHEN, J. Q., ZHANG, Y. H. & ZENG, Y. T. 2013b. The Role of Biofilm in Clogging Process in Vertical Flow Constructed Wetland. *Applied Engineering in Agriculture,* 29**,** 61-66.

HYMAN, M. R., KIM, C. Y. & ARP, D. J. 1990. INHIBITION OF AMMONIA MONOOXYGENASE IN NITROSOMONAS-EUROPAEA BY CARBON-DISULFIDE. *Journal of Bacteriology,* 172**,** 4775-4782.

ICCP 2001. *Climate Change 2001: The Scientific basis,* New York, Cambridge University Press.

ITOKAWA, H., HANAKI, K. & MATSUO, T. 2001. Nitrous oxide production in high-loading biological nitrogen removal process under low COD/N ratio condition. *Water Research,* 35**,** 657-664.

JANNING, K. F., LE TALLEC, X. & HARREMOES, P. 1998. Hydrolysis of organic wastewater particles in laboratory scale and pilot scale biofilm reactors under anoxic and aerobic conditions. *Water Science and Technology,* 38**,** 179-188.

JEFFERSON, B., BURGESS, J. E., PICHON, A., HARKNESS, J. & JUDD, S. J. 2001. Nutrient addition to enhance biological treatment of greywater. *Water Research,* 35**,** 2702-2710.

JENSEN, M. M., THAMDRUP, B. & DALSGAARD, T. 2007. Effects of specific inhibitors on anammox and denitrification in marine sediments. *Applied and Environmental Microbiology*, 73**,** 3151-3158.

JENSSEN, P. D., BERGSTRØM, C. & VATN, A. 2009. From Crap to Food. *The New Economy*.

JETTEN, M. S. M., HORN, S. J. & VANLOOSDRECHT, M. C. M. 1997. Towards a more sustainable municipal wastewater treatment system. *Water Science and Technology,* 35**,** 171-180.

JIN, R. C., YANG, G. F., YU, J. J. & ZHENG, P. 2012. The inhibition of the Anammox process: A review. *Chemical Engineering Journal*, 197**,** 67-79.

JÖNSSON, H., BAKY, A., ULF, J., HELLSTRÖM, D. & KÄRRMAN, E. 2005. Composition of urine, faeces, greywater and biowaste for utilisation in the URWARE model. Gothenburg: Urban Water, Chalmers University of Technology.

KALIBBALA, H. M., KAGGWA, R., WAHLBERG, O. & PLAZA, E. 2011. Characteristics of natural organic matter and formation of chlorination by-products at Masaka waterworks. *Journal of Water Supply Research and Technology-Aqua,* 60**,** 511-519.

KAMPSCHREUR, M. J., TEMMINK, H., KLEEREBEZEM, R., JETTEN, M. S. M. & VAN LOOSDRECHT, M. C. M. 2009. Nitrous oxide emission during wastewater treatment. *Water Research,* 43**,** 4093-4103.

KAPPELER, J. & GUJER, W. 1992. Estimation of Kinetic Parameters of Heterotrophic Biomass under Aerobic Conditions and Characterization of Wastewater for Activated Sludge Modelling. *Water Science and Technology,* 25**,** 125-139.

KESKITALO, J., JANSEN, J. L. & LEIVISKA, K. 2010. Calibration and validation of a modified ASM1 using long-term simulation of a full-scale pulp mill wastewater treatment plant. *Environmental Technology,* 31**,** 555-566.

KNERR, H., RECHENBURG, A., KISTEMANN, T. & SCHMITT, T. G. 2011. Performance of a MBR for the treatment of blackwater. *Water Science and Technology,* 63**,** 1247-1254.

KÕIV, M., VOHLA, C., MÕTLEP, R., LIIRA, M., KIRSIMÄE, K. & MANDER, Ü. 2009. The performance of peat-filled subsurface flow filters treating landfill leachate and municipal wastewater. *Ecological Engineering,* 35**,** 204-212.

KOMMEDAL, R., MILFERSTEDT, K., BAKKE, R. & MORGENROTH, E. 2006. Effects of initial molecular weight on removal rate of dextran in biofilms. *Water Research,* 40**,** 1795-1804.

KUENEN, J. G. 2008. Anammox bacteria: from discovery to application. *Nat Rev Micro,* 6**,** 320-326.

KUNTKE, P., ŚMIECH, K. M., BRUNING, H., ZEEMAN, G., SAAKES, M., SLEUTELS, T. H. J. A., HAMELERS, H. V. M. & BUISMAN, C. J. N. 2012. Ammonium recovery and energy production from urine by a microbial fuel cell. *Water Research,* 46**,** 2627-2636.

LACKNER, S. & HORN, H. 2012. Comparing the performance and operation stability of an SBR and MBBR for single-stage nitritation-anammox treating wastewater with high organic load. *Environmental Technology,* 34**,** 1319-1328.

LANGERGRABER, G. & MUELLEGGER, E. 2005. Ecological Sanitation—a way to solve global sanitation problems? *Environment International,* 31**,** 433-444.

LANGERGRABER, G. & MÜLLEGGER, E. 2011. Examples of source separating sanitation solutions for mountain refuges. *Sustainable Sanitation Practice*.

LARSEN, T. A. & HARREMOËS, P. 1994. Degradation mechanisms of colloidal organic matter in biofilm reactors. *Water Research,* 28**,** 1443-1452.

LARSEN, T. A., UDERT, K. M. & LIENERT, J. 2013. *Source Separation and Decentralization for Wastewater Management*, IWA Publishing.

LASHOF, D. A. & AHUJA, D. R. 1990. Relative contributions of greenhouse gas emissions to global warming. *Nature,* 344**,** 529-531.

LATIFIAN, M., HOLST, O. & LIU, J. 2014. Nitrogen and Phosphorus Removal from Urine by Sequential Struvite Formation and Recycling Process. *CLEAN – Soil, Air, Water,* 42**,** 1157-1161.

LEBOFA, M. M. & HUBA, E. M. 2011. Biogas Systems in Lesotho: an effective way to generate energy while sanitizing wastewater. *Sustainable Sanitation Practice.*

LENS, P. N., VOCHTEN, P. M., SPELEERS, L. & VERSTRAETE, W. H. 1994. Direct treatment of domestic wastewater by percolation over peat, bark and woodchips. *Water Research,* 28**,** 17-26.

LEVINE, A. D., TCHOBANOGLOUS, G. & ASANO, T. 1985. Characterization of the Size Distribution of Contaminants in Wastewater: Treatment and Reuse Implications. *Journal (Water Pollution Control Federation),* 57**,** 805-816.

LI, F. Y., BEHRENDT, J., WICHMANN, K. & OTTERPOHL, R. 2008. Resources and nutrients oriented greywater treatment for non-potable reuses. *Water Science and Technology,* 57**,** 1901-1907.

LIU, X., HU, Z., ZHU, C., WEN, G., MENG, X. & LU, J. 2013. Effect of contact to the atmosphere and dilution on phosphorus recovery from human urine through struvite formation. *Environmental Technology,* 35**,** 271-277.

LOGAN, B. E., HERMANOWICZ, S. W. & PARKER, D. S. 1987. A fundamental model for trickling filter process design. *Journal Water Pollution Control Federation,* 59**,** 1029-1042.

LOTTI, T., KLEEREBEZEM, R., LUBELLO, C. & VAN LOOSDRECHT, M. C. M. 2014. Physiological and kinetic characterization of a suspended cell anammox culture. *Water Research,* 60**,** 1-14.

MAKOWSKA, M. & SPYCHALA, M. 2014. Organic Compounds Fractionation for Domestic Wastewater Treatment Modeling. *Polish Journal of Environmental Studies,* 23**,** 131-137.

MALOVANYY, A., YANG, J., TRELA, J. & PLAZA, E. 2015. Combination of upflow anaerobic sludge blanket (UASB) reactor and partial nitritation/anammox moving bed biofilm reactor (MBBR) for municipal wastewater treatment. *Bioresource Technology,* 180**,** 144-153.

MASI, F., EL HAMOURI, B., SHAFI, H. A., BABAN, A., GHRABI, A. & REGELSBERGER, M. 2010. Treatment of segregated black/grey domestic wastewater using constructed wetlands in the Mediterranean basin: the zer0-m experience. *Water Science and Technology,* 61**,** 97-105.

MOBLEY, H. L. & HAUSINGER, R. P. 1989. Microbial ureases: significance, regulation, and molecular characterization. *Microbiological Reviews,* 53**,** 85-108.

MORALES, N., BOEHLER, M., BUETTNER, S., LIEBI, C. & SIEGRIST, H. 2013. Recovery of N and P from Urine by Struvite Precipitation Followed by Combined Stripping with Digester Sludge Liquid at Full Scale. *Water,* 5**,** 1262-1278.

MORGENROTH, E. 2008a. Biofilm Reactors. *In:* HENZE, M., VAN LOOSDRECHT, M. C. M., EKAMA, G. A. & BRDJANOVIC, D. (eds.) *Biological Wastewater Treatment.* London: IWA Publishing.

MORGENROTH, E. 2008b. Modelling Biofilm. *In:* HENZE, M., VAN LOOSDRECHT, M. C. M., EKAMA, G. A. & BRDJANOVIC, D. (eds.) *Biological Wastewater Treatment.* London: IWA Publishing.

MORGENROTH, E., KOMMEDAL, R. & HARREMOES, P. Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment - a review. 2002. I W a Publishing, 25-40.

MOSQUERA-CORRAL, A., MONTRÀS, A., HEIJNEN, J. J. & VAN LOOSDRECHT, M. C. M. 2003. Degradation of polymers in a biofilm airlift suspension reactor. *Water Research,* 37**,** 485-492.

NOVOSELOVA, L. Y. & SIROTKINA, E. E. 2008. Peat-Based Sorbents for the Purification of Contaminated Environments: A Review. *Solid Fuel Chemistry,* 42**,** 251-262.

ODEGAARD, H., RUSTEN, B. & BADIN, H. 1993. Small waste-water treatment plants based on moving-bed biofilm reactors. *Water Science and Technology,* 28**,** 351-359.

ODEGAARD, H., RUSTEN, B. & WESTRUM, T. 1994. A new moving-bed biofilm reactor - applications and results. *Water Science and Technology,* 29**,** 157-165.

OLDENBURG, M., ALBOLD, A., NIEDERSTE-HOLLENBERG, J. & BEHRENDT, J. Experience with Separating Wastewater Treatment Systems --- The Ecological Housing Estate: Luebeck Flintenbreite. *In:* ERIC, W. S. & WAYNE, C. H., eds., 2002. ASCE, 58.

OLDENBURG, M., ALBOLD, A., WENDLAND, C. & OTTERPOHL, R. 2008. Erfahrungen aus dem Betrieb eines neuen Sanitärkonzepts über einen Zeitraum von acht Jahren. *KA Korrespondenz Abwasser, Abfall,* 10**,** 1100-1105.

OTTERPOHL, R. 2002. Options for alternative types of sewerage and treatment systems directed to improvement of the overall performance. *Water Science and Technology,* 45**,** 149-158.

OTTERPOHL, R., GROTTKER, M. & LANGE, J. 1997. Sustainable water and waste management in urban areas. *Water Science and Technology,* 35**,** 121-133.

PAN, Y. T., NI, B. J. & YUAN, Z. G. 2013. Modeling electron competition among nitrogen oxides reduction and N_2O accumulation in denitrification. *Environmental Science & Technology,* 47**,** 11083-11091.

RAVISHANKARA, A. R., DANIEL, J. S. & PORTMANN, R. W. 2009. Nitrous Oxide (N2O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science,* 326**,** 123-125.

REGELSBERGER, M., BABAN, A., BOUSELMI, L., SHAFY, H. A. & EL HARNOURI, B. 2007. Zer0-M, sustainable concepts towards a zero outflow municipality. *Desalination,* 215**,** 64-72.

RUSTEN, B. & ODEGAARD, H. 2006. Evaluation and testing of fine mesh sieve technologies for primary treatment of municipal wastewater. *Water Science and Technology,* 54**,** 31-38.

SCHMIDT, I. 2009. Chemoorganoheterotrophic Growth of *Nitrosomonas europaea* and *Nitrosomonas eutropha*. *Current Microbiology,* 59**,** 130-138.

SCHMIDT, I., ZART, D. & BOCK, E. 2001. Effects of gaseous NO2 on cells of Nitrosomonas eutropha previously incapable of using ammonia as an energy source. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology,* 79**,** 39-47.

SCHORIES, G. 2008. IWAPIL - innovative wastewater treatment applications for isolated locations. *Desalination,* 224**,** 183-185.

SIEGRIST, H., LAURENI, M. & UDERT, K. M. 2013. Transfer into the gas phase: ammonia stripping. *In:* LARSEN, T. A., UDERT, K. M. & LIENERT, J. (eds.) *Source separation and decentralization for wastewater management.* London: IWA Publishing.

SOPHONSIRI, C. & MORGENROTH, E. 2004. Chemical composition associated with different particle size fractions in municipal, industrial, and agricultural wastewaters. *Chemosphere,* 55**,** 691-703.

STEWART, P. S. 1998. A review of experimental measurements of effective diffusive permeabilities and effective diffusion coefficients in biofilms. *Biotechnology and Bioengineering,* 59**,** 261-272.

STROUS, M., VAN GERVEN, E., ZHENG, P., KUENEN, J. G. & JETTEN, M. S. M. 1997. Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations. *Water Research,* 31**,** 1955-1962.

TAYLOR, M., CLARKE, W. P. & GREENFIELD, P. F. 2003. The treatment of domestic wastewater using small-scale vermicompost filter beds. *Ecological Engineering,* 21**,** 197-203.

TAYLOR, M., CLARKE, W. P., GREENFIELD, P. F. & SWAIN, G. J. 2004. Characterizing the physical and chemical properties of a vermicompost filter bed. *Compost Science & Utilization,* 12**,** 383-391.

TCHOBANOGLOUS, G., BURTON, F. L., STENSEL, H. D. & METCALF & EDDY 2002. *Wastewater Engineering: Treatment and Reuse,* New York, McGraw-Hill Professional.

TERVAHAUTA, T., HOANG, T., HERNÁNDEZ, L., ZEEMAN, G. & BUISMAN, C. 2013. Prospects of Source-Separation-Based Sanitation Concepts: A Model-Based Study. *Water,* 5**,** 1006-1035.

TODT, D. 2012. SANBOX – Entwicklung und Evaluierung von neuen Ansätzen zur Behandlung von Hüttenabwässern. Ås: Norwegian University for Life Sciences.

UDERT, K. M. & JENNI, S. 2013. Biological nitrogen conversion processes. *In:* LARSEN, T. A., UDERT, K. M. & LIENERT, J. (eds.) *Source separation and decentralization for wastewater management.* London: IWA Publishing.

UDERT, K. M., LARSEN, T. A., BIEBOW, M. & GUJER, W. 2003a. Urea hydrolysis and precipitation dynamics in a urine-collecting system. *Water Research,* 37**,** 2571-2582.

UDERT, K. M., LARSEN, T. A. & GUJER, W. 2003b. Estimating the precipitation potential in urine-collecting systems. *Water Research,* 37**,** 2667-2677.

VAN VOORTHUIZEN, E., ZWIJNENBURG, A., VAN DER MEER, W. & TEMMINK, H. 2008. Biological black water treatment combined with membrane separation. *Water Research,* 42**,** 4334-4340.

VON GUNTEN, U. 2013. Chemical oxidation processes. *In:* LARSEN, T. A., UDERT, K. M. & LIENERT, J. (eds.) *Source separation and decentralization for wastewater management.* London: IWA Publishing.

WANG, C. C., LEE, P. H., KUMAR, M., HUANG, Y. T., SUNG, S. W. & LIN, J. G. 2010. Simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD) in a full-scale landfill-leachate treatment plant. *Journal of Hazardous Materials,* 175**,** 622-628.

WANNER, O., EBERL, H. J., MORGENROTH, E., NOGUERA, D. R., PICIOREANU, C., RITTMANN, B. E. & VAN LOOSDRECHT, M. C. M. 2006. Mathematical Modeling of Biofilms. London, UK: IWA Publishing.

WANNER, O. & GUJER, W. 1985. Competition in Biofilms. *Water Science and Technology,* 17**,** 27-44.

WANNER, O. & GUJER, W. 1986. A multispecies biofilm model. *Biotechnology and Bioengineering,* 28**,** 314-328.

WESSELY, H. & GÜTHLER, A. 2004. Alpenpolitik in Deutschland – Anspruch und Realität. *In:* BUND NATURSCHUTZ FORSCHUNG (ed.) *Nr. 8.* Nürnberg: Bund Naturschutz in Bayern e.V.

WUNDERLIN, P., MOHN, J., JOSS, A., EMMENEGGER, L. & SIEGRIST, H. 2012. Mechanisms of N_2O production in biological wastewater treatment under nitrifying and denitrifying conditions. *Water Research,* 46**,** 1027-1037.

ZEEMAN, G. & KUJAWA-ROELEVELD, K. 2011. Resource recovery from source separated domestic waste(water) streams; full scale results. *Water Science and Technology,* 64**,** 1987-1992.

ZEEMAN, G., KUJAWA, K., DE MES, T., HERNANDEZ, L., DE GRAAFF, M., ABU-GHUNMI, L., MELS, A., MEULMAN, B., TEMMINK, H., BUISMAN, C., VAN LIER, J. & LETTINGA, G. 2008. Anaerobic treatment as a core technology for energy, nutrients and water recovery from source-separated domestic waste(water). *Water Science and Technology,* 57**,** 1207-1212.

ZHANG, T. C., SURAMPALLI, R. Y., VIGNESWARAN, S., TYAGI, R. D., ONG, S. L. & KAO, C. M. 2012. *Membrane Technology and Environmental Applications,* Reston, ASCE.

ZHAO, L. F., ZHU, W. & TONG, W. 2009. Clogging processes caused by biofilm growth and organic particle accumulation in lab-scale vertical flow constructed wetlands. *Journal of Environmental Sciences-China,* 21**,** 750-757.

ZIMMERMANN, S. G., WITTENWILER, M., HOLLENDER, J., KRAUSS, M., ORT, C., SIEGRIST, H. & VON GUNTEN, U. 2011. Kinetic assessment and modeling of an ozonation step for full-scale municipal wastewater treatment: Micropollutant oxidation, by-product formation and disinfection. *Water Research,* 45**,** 605-617.

ZURBRÜGG, C., VÖGELI, Y. & ESTOPPEY, N. 2011. Digesting Faeces at Household Level - Experience From a “Model Tourism Village” In South India. *Sustainable Sanitation Practice.*

PAPER 1

Load and distribution of organic matter and nutrients in a separated household wastewater stream

Daniel Todt, Arve Heistad, Petter D. Jenssen

2015

Environmental Technology

Vol. 36, No. 12, 1584-1593

Environmental Technology, 2015
Vol. 36, No. 12, 1584–1593, http://dx.doi.org/10.1080/09593330.2014.997300

Taylor & Francis
Taylor & Francis Group

Load and distribution of organic matter and nutrients in a separated household wastewater stream

Daniel Todt[a*], Arve Heistad[b] and Petter D. Jenssen[a]

[a]*Department of Environmental Sciences, Norwegian University of Life Sciences, Postboks 5003 N-1432, Aas, Norway;*
[b]*Department of Mathematical Sciences and Technology, Norwegian University of Life Sciences, Postboks 5003 N-1432, Aas, Norway*

(*Received 12 September 2014; accepted 6 December 2014*)

Wastewater from a source-separated sanitation system connected to 24 residential flats was analysed for the content of organic matter and nutrients and other key parameters for microbiological processes used in the treatment and reuse of wastewater. Black water (BW) was the major contributor to the total load of organic matter and nutrients in the wastewater, accounting for 69% of chemical oxygen demand (COD), 83% of total nitrogen (N) and 87% of phosphorus (P). With a low COD/N ratio and high content of free ammonia, treating BW alone is a challenge in traditional biological nitrogen removal approaches. However, its high nitrogen concentration (1.4–1.7 g L^{-1}) open up for nutrient reuse as well as for novel, more energy efficient N-removal technologies. Grey water (GW) contained low amounts of nutrients relative to organic matter, and this may limit biological treatment processes under certain conditions. GW contains a higher proportion of soluble, readily degradable organic substances compared with BW, which facilitates simple, decentralized treatment approaches. The concentration of organic matter and nutrients varied considerably between our study and other studies, which could be related to different toilet flushing volumes and water use habits. The daily load per capita, on the other hand, was found to be in line with most of the reported studies.

Keywords: source-separating sanitation; grey water; black water; nutrient load; organic load

Introduction

In recent years, an increasing interest in source-separation sanitary systems has appeared in the literature.[1–7] The segregation of household wastewater into grey water (GW) and black water (BW) opens up new opportunities for water reuse as well as recovery of nutrients and energy contained in the organic matter.[1,8,9] Urine [9] and thereby also BW [1,7,8] were reported to contain the major fraction of nutrients in a relatively concentrated form, thus making BW significantly more suitable for nutrient recovery than mixed wastewater. BW also contains the major amount of organic matter in high concentration, which makes it suitable for novel treatment processes that recover the energy contained in the organic matter.[1,7,8,10] GW consists mainly of readily degradable organic substances,[11] which makes it possible to treat GW into a quality that is suitable for reuse for toilet flush and other applications in simple and energy-saving treatment systems.[2,6,7]

Current data regarding the composition of the two wastewater fractions BW and GW are still limited compared with that of mixed municipal wastewater, and only few studies provide a complete overview of the composition and mass loads in GW and BW from residential homes.[3,9,11,12] The interest in source-separated sanitary systems is increasing. There is therefore a need to expand the database especially regarding typical process parameters used in wastewater treatment. This is particularly important with respect to the design of microbiological processes that play a key role in the present as well as in the future treatment and reuse technologies.[3,9,11]

The aim of this study is to contribute comprehensive additional data on source-separated wastewater streams by analysing the BW and GW produced at the Kaja student dormitory at the Norwegian University of Life Sciences, a residential complex with 48 permanent inhabitants.[1] The Kaja student dormitories are one of the first large-scale projects on source-separating sanitation in Western Europe and has been subject to several research projects.[e.g. 13,14] To this date, no complete overview of the characteristics and mass loads of the two wastewater fractions at Kaja has been published. In this paper, the key parameters that are typically used in the design of biological wastewater processes were analysed and compared with results reported from other studies. Furthermore, the mass loading rates per capita summarizing the discharge of organics and nutrients as well as their distribution in the two wastewater fractions were determined.

*Corresponding author. Email: danitodt@gmail.com

Methods

Source-separated sewer system at Kaja student dormitories

The GW and BW used in this paper originate from a permanently habited student dormitory with 24 flats housing a total of 48 students. The GW is collected via a traditional gravity sewer. The BW is collected using a vacuum sewer system with an integrated grinder pump (Vacuumerator 25MB™, Jets, Hareid, Norway). The flushing volume of the toilet is 1.2 litre. The GW is pumped directly from the inlet chamber of the septic tank to a 1500-litre storage tank in the laboratory. The raw BW is collected in a 500-litre tank and pumped via a 150 m transfer pipe (40 mm diameter) into a 700-litre storage tank in the laboratory. Both storage tanks are equipped with a low speed (30 rpm) rotary mixer. Total retention time of the wastewater in the transfer pipe and buffer tanks is estimated to be in the range of 36–48 h for both the wastewater streams. For transfer to the laboratory facilities, impeller pumps with large opening size (Tsurumi 40U) are installed to minimize potential impacts on particle size distribution.

Flow rate measurements

Total wastewater volume was estimated by monitoring the total fresh water consumption of the dormitories and by daily reading of the flow meter of the freshwater supply over a period of two months. The monitoring was performed in November–December when the outdoor taps were closed and no water consumption for car wash or irrigation was expected. The volume of BW was measured at the inlet of the storage tank in the laboratory using a conductivity-based flow meter (Optiflux2000, Krohne, Duisburg, Germany). To exclude distortion by interval delivery from the dormitories to the laboratory, the daily average BW load was calculated from weekly readings of the accumulated volume. The flow rate for GW was calculated by subtracting the measured BW volume from the total fresh water consumption using random variable algebra.

Sampling and analysis of parameters

A total of 73 BW and 63 GW samples were randomly collected in the period 2010–2013. These samples were collected for different projects, which were focusing on different parameters. Hence only suspended solids (SS) and chemical oxygen demand (COD) were analysed for all the samples. These two parameters were therefore used for performing statistical tests, for assessing differences between periods and for checking of particular subsample sets. If not noted elsewhere, statistical significance is given at a p-value of .01. With respect to the limited available number of samples for some of the parameters, descriptive statistics based on percentiles was chosen for the presentation of results. In figures, 25, 50 and 75 percentiles are displayed as box plots with 5 and 95 percentiles indicated by error bars. The ranges for concentration and ratios presented in the text refer to 25 and 75 percentiles.

The particle size distribution analysis was based on standard fractionation, which distinguishes wastewater into settleable (>100 μm), supra-colloidal (1–100 μm), colloidal (10^{-3}–1 μm) and soluble ($<10^{-3}$ μm) solids.[15] Due to analytical limitations, soluble and colloidal solids are not distinguished in this study; colloidal solids are therefore included to soluble solids in this paper. In addition to these standard solid fractions, we distinguished coarse solids (>1000 μm) and small-sized supra-colloidal solids (1–10 μm). Particle size distribution was determined with the help of sequential filtration which is the method also used in the earlier studies.[16,17] This filtration was performed with calibrated woven textile nets with a mesh size of 1000, 100 and 10 μm (Sefar® PETEX® 07-1000/45; 07-100/32; 07-10/2) on a subset of 16 samples. The relevance of these samples in terms of the overall data set was checked for COD and SS using the Hotellings T_2 test ($p < .1$). For determining the solids, the net weight of the retentate on the filter was measured after drying at 105°C for 4 h. The potential impact of the drying process on the weight of the filter nets was checked by drying some blank nets. The filtrate on each of these mesh sizes was additionally analysed for COD, N_{tot} and P_{tot}.

COD and phosphorous and nitrogen compounds were analysed using the spectrophotometric analysing kits from Hach-Lange®. Total Ammonious N (TAN) includes both dissociated NH_4^+ and undissociated NH_3. In addition, for the spectrophotometric analysis of TAN, the concentration range of the NH_3 fraction was calculated for a selected standard temperature of 15°C using the measured pH values and the equilibrium equation provided by Anthonisen et al.[18] Suspended solids (SS) and volatile suspended solids (VSS) were determined using 1.2 μm glass fibre filters (Whatman GF-C, GE Healthcare, Little Chalfont, UK). The filtrate was used for determining the dissolved compounds. Total solids were determined by air-drying at 105°C. Volatile solids (VS) and VSS were determined by the loss of ignition at 550°C of the dried sample or filter residue, respectively, and inorganic solids (IS) and inorganic suspended solids (ISS) by the corresponding ash residue. Dissolved solids were calculated by subtracting SS from TS, VSS from VS and ISS from IS, respectively.

The most recent European (EN) and US (EPA) standards for water and wastewater examination require a 0.45 μm filtration.[19] However, in relation to high strength wastewaters and reactor liquid, glass fibre filters of 0.7 μm or 1.2 μm are also in widespread use.[14,20] Earlier experiments with BW from Kaja student dormitories showed that the 1.2 μm glass fibre filter gives the best repeatability among parallels.[14] With respect to the boundary of 1

µm between colloidal and supra-colloidal solids, 1.2 µm glass fibre filters are also typically used for particle size fractionation.[16,17] A recent study could rather not determine any significant difference between 0.45 µm membrane filters and 1.2 µm glass fibre filters (Whatman GF-C) in terms of SS.[20] We used, therefore, 1.2 µm glass fibre filters for particle size fractionation as well as for the determination of SS in both wastewater fractions (GW and BW).

Calculation of daily mass loads

The daily load of a particular parameter X ($Load_X$) can be calculated based on hydraulic loading rate (Q), number of inhabitants (PE) and the concentration range of the parameter X (C_X) according to the following equation:

$$\mathbf{Load}_X = \frac{Q * C_X}{\mathbf{PE}}. \quad (1)$$

In this study, hydraulic loading rates were determined based on weekly readings, concentration ranges were determined by the analysis of grab samples and the number of inhabitants was estimated on a qualitative basis. Since the data material available from this study does not allow a quantitative determination of the variance of Q and PE, wastewater production per inhabitant (Q/PE) was calculated as a constant by taking the ratio of the average values estimated for Q and PE. $Load_X$ was calculated by multiplying the random variable C_X by the constant Q/PE.

Results and discussion

Hydraulic loading rates

The average total water consumption of the student dormitories was 5.9 ± 0.6 m^3 d^{-1}. No distinct pattern could be observed between the weekdays (not shown). Shorter, diurnal variations were not captured by this study. Assuming that only a negligible fraction of the total fresh water consumption left the system by other pathways (e.g. human consumption, irrigation of indoor plants and subsequent evaporation), this fresh water consumption corresponded approximately to the total wastewater production. The BW load was 327 ± 26 L d^{-1} in average, which accounts for 5.5% of the total water consumption. Subtracting the measured BW production resulted in an average daily GW production of 5.6 ± 0.6 m^3 d^{-1}, accounting for 94.5% of the total water consumption. In the measuring period, the official number of inhabitants of Kaja dormitories was 48 adult persons. However, vacation leaves or overnight stays of guests are likely to give a variation in the effective number of inhabitants, which again impacts the hydraulic loading rate of the wastewater fractions. Assume that a mean of 45 inhabitants would result in a total wastewater volume of 131 L, where 7.2 L is BW and 123 L is GW. This is approximately 10% below the 150 L defined within the person equivalent (PE) [21] or a recently determined average value of 148 L.[22] The estimated GW production at Kaja was higher than the average (108 L captia^{-1} d^{-1}). Hence, the lower total wastewater production or freshwater consumption can be related to low BW volumes due to the vacuum toilets having a significantly lower flushing volume than standard gravity flush toilets.

Concentration ranges and distribution of organic matter and nutrients N and P

Figure 1 shows the concentration ranges of solids, COD and the nutrients N and P determined in the two wastewater fractions. On a few samples ($n = 5$) supplementary analysis for Kjeldal N, nitrate (NO_3^-) and nitrite (NO_2^-) was performed. NO_2^- was below the detection limit (0.02 mg N L^{-1}) and NO_3^- was around 0.2 mg N L^{-1} (not shown). In both wastewater fractions, no apparent difference was observed between total N and Kjeldahl N, which was subject to additional analysis on selected samples ($n = 3$, not shown). Hence, the presence of oxidized N compounds was negligible in the household wastewater of this study. As indicated in the methods section, the boundary between soluble and particulate matter is an artificial definition in the range of 0.45–1.2 µm depending on the method used for analysis. Referring to the 1.2 µm filtration used in this study, the total 'soluble' fractions for N and P were higher than the dissolved ionic forms of these two nutrients, ammonia (TAN) and PO_4^-, respectively. TAN accounted for 87–95% of the soluble N in BW and 73–85% in GW (Figure 1). In BW the soluble P consisted to a large extend (94–98%) of PO_4^-. In GW, on the other hand, PO_4^- accounted only for 48–76% of the soluble P (Figure 1), which points towards a larger presence of dissolved organic P-containing compounds in GW, most likely originating from household chemicals (e.g. dishwashing agents). These findings further indicate that a considerable fraction of the soluble nutrients and organic substances (COD) may be bound to colloidal particulate matter such as cell residues or viruses. This assumption is supported by a recent study showing that a larger fraction of apparently soluble components as determined by microfiltration (0.45 µm) is in reality associated with colloidal particulate matter.[16]

In BW, the proportion of particulate organic matter of 67–76% (measured as COD) was significantly ($p < .01$) higher than that in GW which accounted for only 50–58%. Another study also found a higher proportion of the total COD bound to particulate matter in BW than in GW.[11] GW therefore contains a larger fraction of soluble organic matter that was shown to be readily degradable.[11] This makes GW feasible for simple, decentralized treatment approaches and local water reuse (e.g. toilet flushing).[2,6,7]

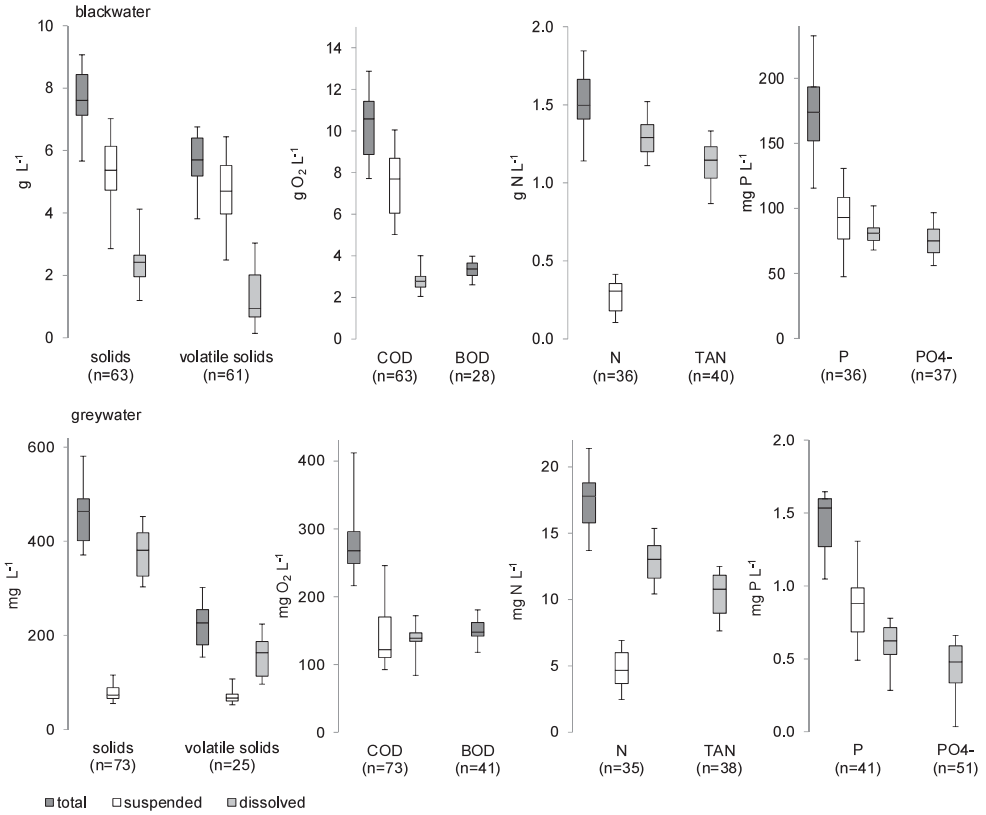


Figure 1. Concentration range of solids, organic matter (COD, BOD) and nutrients (N,P) in BW fraction (upper panel) and GW fraction (lower panel). The boxplots show 25%, 50% and 75% percentiles including 5% and 95% percentiles indicated by error bars.

Approximately half of the phosphorous was particle bound in both wastewater fractions, accounting for 54–61% in GW and 50–64% in BW. Contradictory to P, only a minor fraction of N was found to be particle bound in both wastewater fractions. Particulate nitrogen accounted only for 21–32% of total N in GW and 13–22% in BW (Figure 1, Figure 2(a)).

The high content of particulate matter in BW may challenge certain treatment processes and was therefore analysed in more detail. BW was fractionated, in compliance with earlier studies,[15,17] into settleable solids (> 100 µm) and supra-colloidal solids (1–100 µm). The settleable solids (> 100 µm) accounted for 53–63% and supra-colloidal solids (1–100 µm) for 37–47% of the total suspended solids. The settleable solids in our BW contained 31–45% of total COD (Figure 2), which was in the upper percentile of the range (15–48%) reported for domestic wastewater.[17] A majority (67–76%) of these settleable solids, accounting for 28–38% of total COD, could be attributed to large-sized particles (> 1 mm) (Figure 2) that alternatively could be removable using mechanical screening.[23] The supra-colloidal solids (1–100 µm) contained 34–43% of the total COD (Figure 2), which is high compared with the 12–38% reported for domestic sewage.[17] Hence, the organic load from supra-colloidal organic matter seems to be higher in BW than mixed sewage. In total, 31–49% of the supra-colloidal solids accounted for 13–16% of total COD and could be attributed to small-sized particles (< 10 µm), close to the boundary of the colloidal solids (Figure 2).

Particle bound phosphorous was mainly attached to the smallest supra-colloidal solids (< 10 µm), which is comparable to municipal wastewater.[17] Particulate nitrogen, on the other hand, was more equally distributed over all particle size fractions (Figure 2). Hence, a separation of the largest particles (> 1000 µm) would have retained approximately 40% of the particle bound nitrogen, but only 12% of the particle bound phosphorous (Figure 2). However,

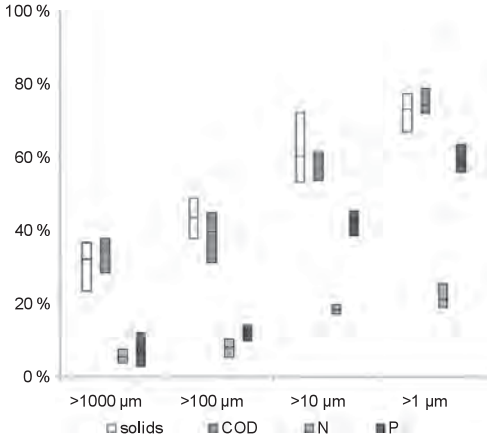


Figure 2. Distribution of SS, COD and nutrients N, P over different particle size fractions in BW analysed on a selected number of samples ($n = 16$). The boxplots show 25, 50 and 75 percentiles.

considering the high proportion of soluble N and P compounds, mechanical removal of coarse particulate material would only result in a minor retention of these nutrients (4–7% for N and 3–12% for P, respectively, Figure 2). It is important to note that the present results refer to BW that has passed a vacuum pump with an integrated macerator. Hence, the determined particle size distribution in our BW may differ considerably from that in BW from traditional gravity sever systems, but it is likely relatively representative of other types of sewer systems with integrated macerator pumps (e.g. pressure sewer system).

Consequences of source separation on treatment process design and reuse

The proportion of biodegradable organic matter differs considerably between the two wastewater fractions. Compared with municipal wastewater,[21] the COD/BOD ratio in our wastewater fractions for BW from Kaja was 2.7–3.4, which is in the upper quartile, and 1.6–2 for GW, which is in the lower quartile, indicating that BW contains a larger proportion of inert or slowly degradable organic matter than GW. Hence, organic substances contained in BW may be more difficult to remove than organic substances in GW, especially when a certain discharge limit in terms of COD needs to be reached. These findings differ from a study of a source-separating sewer system in Turkey showing that 95% of the BW and 93% of the GW are biodegradable with COD/BOD ratios of 3.5 and 2.6, respectively.[11] These differences indicate that the proportion of biodegradable organics seem to be comparable for BW but may differ significantly for GW between different locations and cultural regions.

The pH determined in GW was in the near-neutral range. In the BW samples pH was higher, ranging up to 9 (Figure 3). pH has a strong impact on the equilibrium between ammonium (NH_4^+) and dissociated free ammonia (NH_3). In BW, NH_3 accounted for 6–18% of TAN, while it was almost absent in GW, accounting only for 0.2–0.4% of TAN (Figure 3). The ammonia odour of BW also indicates the presence of free NH_3. In biological N removal processes, the high presence of NH_3 in BW will inhibit nitrite oxidizers (NOB) and thereby facilitate an accumulation of NO_2^-,[18] which attribute to greenhouse gas emissions.[24] The low alkalinity/N ratio of BW (Figure 2) may further lead to an insufficient buffering capacity for the proton release of the nitrification process and subsequent

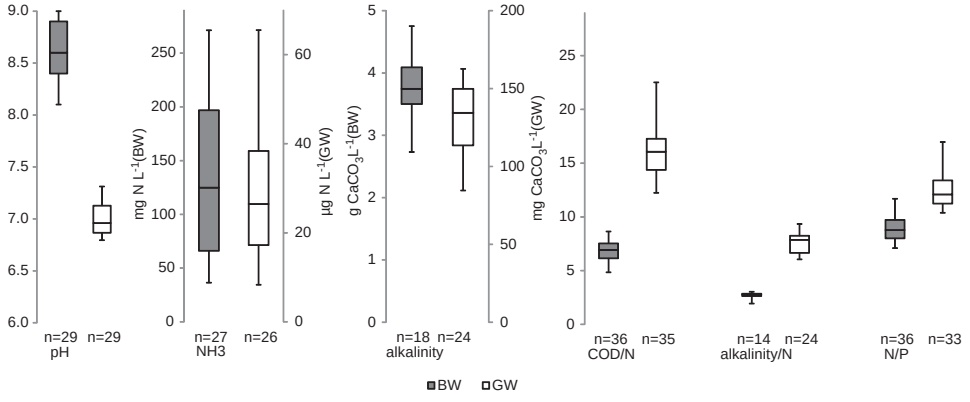


Figure 3. Concentration range of pH, free ammonia (NH_3) and alkalinity and ratios of COD/N, alkalinity/N and N/P for each of the household wastewater fractions of GW and BW. NH_3 was calculated at a temperature of 15°C based on pH and TAN (NH_4^+) using the equilibrium equation from of Anthonisen and Loehr.[11]

Table 1. Reported average concentration ranges of BOD, COD, N and P for our greywater compared with those of selected studies related to residential households in Europe and the US test norm.

Location	BOD_5 (mg O_2 L^{-1})	COD (mg O_2 L^{-1})	Nitrogen (mg N L^{-1})	Phosphorous (mg P L^{-1})
This study (Norway)	140–160	250–300	16–19	1.3–1.6
Great Britain [36]	146	451	8.7	1.4
Sweden [4]	418	588	10	7.5
Germany [37]	n.a.	640	27.2	9.8
The Netherlands [38]	n.a.	724	7.2	26
Average Europe [3]	205–449	350–783	6.7–22	0.4–8.2
Average literature [21]	100–400	200–700	8–30	2–7
US standard for type approval tests [39]	100–300	200–500	3–6	1–4

substantial pH drops in the biological N removal system as reported for urine.[25]

The COD/N/P ratio is important for the evaluation of a potential nutrient limitation in a biological treatment process [11,26] as well as for determining the electron donor limitation in N removal systems using heterotrophic denitrification.[27] The overall COD/N/P ratio (calculated from the total components) was 100/14/1.4 in BW (Figure 3, median values), which is comparable to the ratio of 100/14/2 reported for BW of the study in Turkey.[11] The overall COD/N/P ratio for our GW, 100/6/0.5 (Figure 3, median values), differed significantly from that of the Turkish study, where the ratio was 100/2.5/2.5,[11] and also other comparable studies (Table 1).

Considering the critical COD/N/P ratio of 100/20/1 determining nutrient limitation for biological treatment processes,[12] the COD/N/P ratio for our BW should not limit a biological treatment process. However, for the particulate BW components, the determined COD/N ratio was critically high, especially for large-sized particles (> 1 mm) (Figure 4). Hence, aerobic decomposition (e.g. composting) of separated solids from BW might become N limited. The COD/P ratio, on the other hand, was below the critical limit for all solid fractions in BW (Figure 4). The low overall COD/N ratio determined for BW ranging from 6 to 7 (Figure 3) limits the application of traditional N removal processes based on heterotrophic denitrification, which would require a COD/N ratio of 7.6 or higher.[27] Hence, for the treatment of BW, a recently developed alternative N removal process based on partial nitrification with subsequent anaerobic ammonia oxidation (*Anammox*) may be more appropriate, as also supported by a recent pilot study.[5] The high ammonia concentration in BW from a vacuum sewer or other low flush sanitary systems may further open ways for new, innovative technologies towards nutrient reuse and energy conservation, for example, microbial fuel cells that were successfully applied to urine.[28]

For GW, the COD/N/P ratio may challenge a biological treatment process in terms of nutrient limitation. However, the limit within a particular system can vary markedly, depending on the biomass immobilization for a particular

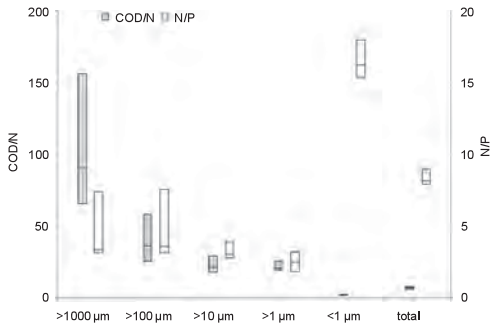


Figure 4. Ratios for COD/N and N/P for different particle size fractions in BW analysed on a selected number of samples ($n = 16$). The boxplots show 25, 50 and 75 percentiles.

nutrient and the yield factor for heterotrophic growth. As shown with wastewater from a paper mill industry, an adapted biomass may perform satisfactorily with a significantly higher COD/N/P ratio than that given by the general limits.[29] The literature reports a biomass immobilization of 0.07 g N g^{-1} biomass COD,[29,30] 0.005–0.013 g P g^{-1} biomass COD [29] and a yield factor of 0.58 g biomass COD g^{-1} degraded COD.[29,30] This gives the critical limit of 25 for N and 72–200 for P. The COD/N ratio in our GW was between 14 and 17 (Figure 3) and clearly below this critical limit, hence N should not be a limiting factor. The determined ratio for COD/P ranging 175–214 (Figure 3) was clearly above the critical limit (72) for systems with high biomass immobilization (0.013 g P g^{-1} biomass COD) and close to the limit (200) for systems with low biomass immobilization (0.005 g P g^{-1} biomass COD). Most of the GW treatment systems consist of a primary settling tank where a major fraction of particulate matter is retained so that the system mainly receives the soluble components, for which the ratios were determined in the range of 10–12 for $COD_{soluble}/N_{soluble}$ and 175–215 for $COD_{soluble}/P_{soluble}$ (Figure 1). Hence, regardless of the degree of particulate matter removal in the primary treatment step, in our GW phosphorous most likely limits biological treatment processes even with a highly

adapted biomass. The outcomes of our calculations on potential nutrient limitations in the biological treatment of GW are further supported by the experimental study of Jefferson and Burgess,[26] which showed that balancing the COD/N/P ratio of GW by adding N and/or P resulted in a significantly greater COD removal rate compared with unmodified GW. Which one of the nutrients (N, P) that is a potential limiting factor strongly depends on the composition of the particular GW,[11,26] and was shown to vary considerably between different locations.[11] Our GW seems mainly to be P-limited, while the GW of Jefferson and Burgess [26] was shown to be mainly N-limited. Among the reported GWs listed in Table 1, the COD/N ratio varies from 13 to 52 and the COD/P from 65 to 322 (calculated based on the figures in Table 1).

The N/P ratio is a key parameter for determining the potential reuse of the BW fraction as fertilizer and is encouraged by an increasing number of authors.[1,7,31] The overall N/P ratio was 8–10 for BW and 11–13 for GW, which is within the normal range for municipal wastewater.[21] The detailed analysis of the BW particle size fractions showed that the N/P ratio can be impacted by using different BW fractions as fertilizers. Applying raw BW as well as the liquid fraction as an organic liquid fertilizer would result in an N-dominated fertilizer, while applying a separated solid fraction would give a more equal amount of the two nutrients (Figure 4).

Variation patterns in terms of wastewater composition

Potential diurnal variations were completely equalized by the sewer system and thus not captured by this study. Comparison of the samples taken in the morning and afternoon of the same day indicates no distinct pattern towards higher concentration ranges on a particular time of day. The overall sample variation (standard deviation in percentage of average) was 20% for SS and 7% for COD in BW and 7% for SS and 16% for COD in GW, which is low compared with that of municipal wastewater.[21] Hence, the buffering capacity of the sewer system and holding tanks was sufficient to equalize short-term variations, which often occurs on a diurnal or weekly basis. There is no distinct seasonal pattern in the distribution of the parameters (Figure 5). This was statistically confirmed by the Hotelling T^2 test showing no significant ($p = .37$ for BW and 0.54 for GW) difference between samples taken in the cold season (November–April) and warm season (May–October). The data of this study therefore clearly indicate the absence of seasonal variation in the organic load of household wastewater, which is often the case in the larger municipal sewer systems.[21] This can be explained by the negligible impact of groundwater intrusion and storm water events (e.g. precipitation periods and snow smelt) on the household wastewater of this study. These factors are usually the main reason for seasonal variation patterns in the composition of municipal wastewater.[21]

Average concentration of key parameters in the wastewater fractions at Kaja compared with that in other locations with source-separated household wastewater

The composition of the GW fraction was shown to be dependent on kitchen and washing infrastructure, type of detergent used in a particular household as well as lifestyle, especially washing and cooking habits of its inhabitants.[32] This dependency explains the relatively wide variation in composition of GW reported from different locations. The GW analysed in this study was in the lower concentration range in terms of organic matter (COD and BOD) and P. The total N concentrations seem, on the other hand, to be in the upper range of the reported values (Table 1).

The low concentration of organic matter may indicate different washing and cooking habits in Norway compared with other places in Europe. In Norway, drinking water is relatively cheap, and except for dry summers, there are

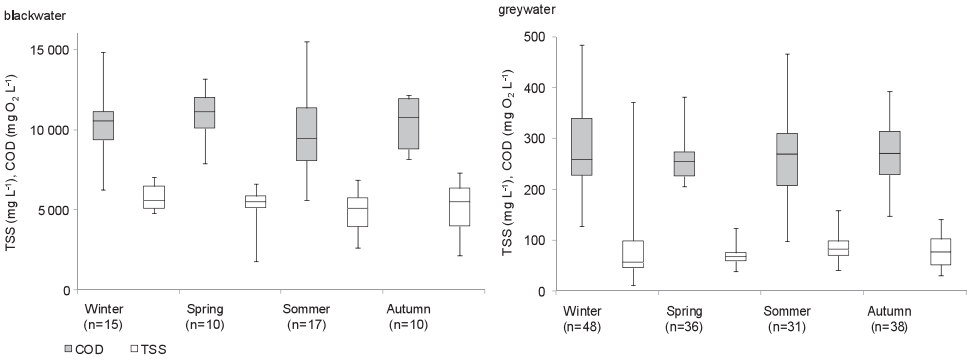


Figure 5. Seasonal variation of SS and COD for each household wastewater fraction (BW and GW). The boxplots show the 25%, 50% and 75% percentiles. The error bars are showing the min/max values.

no restrictions regarding use. Hence, there is little awareness on water conservation among people in their daily routines. Many places with source-separating sanitation reported in the literature are ecovillages, where people may have a considerably higher awareness of fresh water conservation. Hence, GW at Kaja seems to be more diluted than GW in other studies, which is also supported by the 14% higher GW volume produced per capita at Kaja, compared with the average of other studies (see Section 3.1). The students at Kaja also showed a preference for ready-made food products that can be easily prepared by warming in the stove or microwave. This particular cooking habit may reduce the use and subsequent cleaning of frying pans and other cooking utensils and may lower the discharge of grease, resulting in a low organic matter content of the GW.

The GW from Kaja was also low in terms of P concentration, compared with other studies. This may be a result of higher water consumption. Another explanation could be the use of household chemicals. While phosphate has been widely prohibited for laundry detergents, phosphates are still relatively common in dishwashing machines over all Europe. In Norway, the maximum P content in detergents for laundry and manual dishwashing is 0.2%. Detergents for dishwashing machines, however, are allowed up to 3.5% P (FOR-2004-06-01-922, Section 12-2). Since the student dormitories at Kaja are not equipped with dishwashing machines, the P load may be significantly lower than in dwellings with more modern household infrastructure.

The concentration of BW compounds depends strongly on the flushing volume of the installed toilet system. Locations with vacuum flush toilets (VC) display higher concentrations than sites with gravity flush toilets (WC).[8] The concentrations found in this study are comparable to that in other studies having a VC infrastructure (Table 2). The calculated key ratios such as COD/N, N/P are in the same range as most of these studies (Table 2), except the BW from Palmquist and Hanæus,[33] in which the COD/N ratio was considerably higher. These results indicate that BW mainly varies in terms of concentration or due to the dilution by flush water, while its mass composition seems to be comparable over a wide range of locations.

Mass loading per capita and proportion of organic matter and nutrients in GW and BW

Table 3 shows the mass load per capita for organic matter (COD) and nutrients for wastewater fractions from different source-separating sanitation systems in Europe. All of these locations, including our student dormitories, are residential buildings where a majority of the inhabitants are absent part of the day due to work, school or other activities. Hence, most of these persons will also produce wastewater outside their home which is not captured in the figures in Table 3. This means that most of the reported mass loadings (Table 3) are underestimated.[34] This can also explain much of the variations in Table 3, especially the significant lower mass loadings reported for BW in

Table 2. Reported average concentration ranges of BOD, COD, N and P for our blackwater compared with other residential households in temperate and cold climatic areas with gravity flush (WC) and vacuum toilets (VC).

Black water	BOD_5 (g O_2 L^{-1})	COD (g O_2 L^{-1})	Nitrogen (g N L^{-1})	Phosphorous (mg P L^{-1})
This study, VC (Norway)	3.1–3.6	8.9–11.4	1.4–1.7	150–200
Sweden, low flush WC [4]	n.a.	3.0	0.16	28
The Netherlands, VC [40]	n.a.	9.5–19	1.9	110–280
Turkey, WC [11]	0.3	1.2	0.18	25
Germany, VC [34]	n.a.	8.0	1.5	175
Germany [41]	0.3	0.7	0.28	29
Average WC [21]	0.3–0.6	0.9–1.5	0.1–0.3	20–40

Table 3. Estimated mass loading rates per capita for the main nutrients nitrogen and phosphorous in the wastewater fraction of our study compared with literature data from other source-separated sanitation systems in Europe.

	Organics (COD) (g O_2 d^{-1})		Nitrogen (g N d^{-1})		Phosphorous (g P d^{-1})	
Location	BW	GW	BW	GW	BW	GW
This study (Norway)	65–83	31–37	10–12	2–2.5	1.1–1.4	0.15–0.2
Sweden[42]	85	39	4.6	0.6	1.5	0.5
Sweden [35]			12	1.4	1.4	0.4
Germany [34]	40	39	7.5	1.7	0.9	0.6
The Netherlands [10,40]	57–119		11.4		0.7–1.7	
Typical Europe [3]	75	46	11.9	1.5	1.5	0.5
Turkey [11]	90	25	19.6	0.7	3.7	0.8

Germany.[34] The mass loadings for our BW and GW of COD and N represent approximately the average of other GW or BW studies listed in Table 3. Our GW showed a notably lower P load per capita (Table 3), which is most likely a result of the absence of P-containing dishwashing detergents in the student dormitories (see Section 3.5). Regardless of these differences, the variations among the reported mass loadings for COD, N and P (Table 3) were notably smaller than the variations among the reported concentration ranges of these parameters (Tables 1 and 2) for both BW and GW.

In our wastewater samples, BW contributed 69% of the total organic load (COD) per capita, which is close to the average of 66% calculated for the other studies listed in Table 3. The contribution from BW at Kaja to the total N load per capita was 83% close to the average of 88% calculated for the other studies. The BW contribution to the total P load per capita, on the other hand, was 87% and notably higher than the average of 73% calculated for the other studies. However, in general our results as well as the figures from the other studies (Table 3) do not deviate much from the theoretical recovery potential by segregating BW of 59% for COD, 97% for N and 90% for P with phosphate-free detergents.[8] When phosphate-containing detergents are used, the P fraction in BW is reduced to 74%,[35] which likely explains the notable higher P fraction in our BW compared with the average among the other studies.

Conclusions

- In this study, BW contributed 69% of the total COD load, 83% of the total N load and 87% of total P. This corresponds to findings of other studies and confirms the stated high recovery potential for organic matter and nutrients by segregating BW from the household wastewater stream.
- The proportion of particle bound organic matter was 67–76% of total COD in BW and 50–58% of total COD in GW. In the BW of this study, 37–47% of the SS could be attributed to supra-colloidal solids, which are unlikely to be retained in a sedimentation process.
- With a low COD/N ratio (6–7) and high content of free ammonia (50 mg L^{-1}), BW is a challenge to treat by using traditional biological nitrogen removal approaches, but the high nitrogen concentration of 1.4–1.7 g L^{-1} in BW opens up new opportunities for nutrient reuse.
- Fresh water consumption by different human activities and plumbing fixtures resulted in notable variation in the concentrations for organic matter (COD) and nutrients (N, P) in BW and GW, while the mass loading rates were comparable independent of location.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Jenssen PD, Heyerdahl PH, Warner WS, Greatorex JM. Local recycling of wastewater and wet organic waste – a step towards zero emission community. Paper presented at Proceedings of the 8th International Conference on Environmental Science and Technology; 2003 Sep 8–10; Lemnos Island, Greece.
[2] Masi F. Water reuse and resources recovery: the role of constructed wetlands in the Ecosan approach. Desalination. 2009;246:27–34.
[3] Meinzinger F, Oldenburg M. Characteristics of source-separated household wastewater flows: a statistical assessment. Water Sci Technol. 2009;59:1785–1791.
[4] Palmquist H, Hanaeus J. Hazardous substances in separately collected grey- and blackwater from ordinary Swedish households. Sci Total Environ. 2005;348:151–163.
[5] de Graaff MS, Zeeman G, Temmink H, van Loosdrecht MCM, Buisman CJN. Long term partial nitritation of anaerobically treated black water and the emission of nitrous oxide. Water Res. 2010;44:2171–2178.
[6] Larsen TA, Udert KM, Lienert J. Source separation and decentralization for wastewater management. London: IWA Publishing; 2013.
[7] Regelsberger M, Baban A, Bouselmi L, Shafy HA, El Harnouri B, Zero M. Sustainable concepts towards a zero outflow municipality. Desalination. 2007;215:64–72.
[8] Otterpohl R. Options for alternative types of sewerage and treatment systems directed to improvement of the overall performance. Water Sci Technol. 2002;45:149–158.
[9] Larsen TA, Alder AC, Eggen RIL, Maurer M, Lienert J. Source separation: eill we see a paradigm shift in wastewater Handling? Environ Sci Technol. 2009;43:6121–6125.
[10] Zeeman G, Kujawa-Roeleveld K. Resource recovery from source separated domestic waste(water) streams; full scale results. Water Sci Technol. 2011;64:1987–1992.
[11] Hocaoglu SM, Insel G, Cokgor EU, Baban A, Orhon D. COD fractionation and biodegradation kinetics of segregated domestic wastewater: black and grey water fractions. J Chem Technol Biotechnol. 2010;85:1241–1249.
[12] Tchobanoglous G, Burton FL, Stensel HD, Metcalf E. Wastewater engineering: treatment and reuse. 4th ed. New York: McGraw-Hill Professional; 2002.
[13] Heistad A, Paruch AM, Vråle L, Adam K, Jenssen PD. A high performance compact filter system treating domestic wastewater. Ecological Eng. 2006;28:374–379.
[14] Todt D, Jenssen PD, Klemencic AK, Oarga A, Bulc TG. Removal of particles in organic filters in experimental treatment systems for domestic wastewater and black water. J Environ Sci Health Part A – Toxic/Hazardous Subst Environ Eng. 2014;49:948–954.
[15] Balmat JL. Biochemical oxidation of various particulate fractions of sewage. Sewage Ind Wastes. 1957;29:757–761.
[16] Dulekgurgen E, Dogruel S, Karahan Ö, Orhon D. Size distribution of wastewater COD fractions as an index for biodegradability. Water Res. 2006;40:273–282.
[17] Sophonsiri C, Morgenroth E. Chemical composition associated with different particle size fractions in municipal, industrial, and agricultural wastewaters. Chemosphere. 2004;55:691–703.
[18] Anthonisen AC, Loehr RC, Prakasam TBS, Srinath EG. Inhibition of nitrification by ammonia and nitrous-acid. J Water Pollut Control Fed. 1976;48:835–852.

[19] Eaton AD, Clesceri LS, Rice EW. Standard methods for the examination of water & wastewater. 21st ed. Washington (DC): American Public Health Association; 2005.
[20] Helness H, Thorvaldsen G. Karakterisering av avløpsvann for primærrensing. Trondheim: Sintef; 2004.
[21] Henze M, Comeau Y. Wastewater characterization. In: Henze M, van Loosdrecht MCM, Ekama GA, Brdjanovic D, editors. Biological wastewater treatment. London: IWA Publishing; 2008. p. 33–52.
[22] Friedler E, Butler D, Alfiya Y. Wastewater composition. London: Iwa Publishing; 2013.
[23] Rusten B, Odegaard H. Evaluation and testing of fine mesh sieve technologies for primary treatment of municipal wastewater. Water Sci Technol. 2006;54:31–38.
[24] Kampschreur MJ, Temmink H, Kleerebezem R, Jetten MSM, van Loosdrecht MCM. Nitrous oxide emission during wastewater treatment. Water Res. 2009;43:4093–4103.
[25] Udert KM, Jenni S. Biological nitrogen conversion processes. In Larsen TA, Udert KM, Lienert J, editors. Source separation and decentralization for wastewater management. London: IWA Publishing; 2013. p. 291–305.
[26] Jefferson B, Burgess JE, Pichon A, Harkness J, Judd SJ. Nutrient addition to enhance biological treatment of greywater. Water Res. 2001;35:2702–2710.
[27] Sobieszuk P, Szewczyk KW. Estimation of (C/N) Ratio for microbial denitrification. Environ Technol. 2006;27:103–108.
[28] Kuntke P, Śmiech KM, Bruning H, Zeeman G, Saakes M, Sleutels THJA, Hamelers HVM, Buisman CJN. Ammonium recovery and energy production from urine by a microbial fuel cell. Water Res. 2012;46:2627–2636.
[29] Keskitalo J, Jansen JL, Leiviska K. Calibration and validation of a modified ASM1 using long-term simulation of a full-scale pulp mill wastewater treatment plant. Environ Technol. 2010;31:555–566.
[30] Gujer W, Henze M, Mino T, van Loosdrecht M. Activated sludge model no. 3. Water Sci Technol. 1999;39:183–193.
[31] Jonsson H, Vinneras B. Closing the loop: recycling nutrients to agriculture. London: Iwa Publishing; 2013.
[32] Eriksson E, Auffarth K, Henze M, Ledin A. Characteristics of grey wastewater. Urban Water. 2002;4:85–104.
[33] Palmquist H, Hanæus J. Hazardous substances in separately collected grey- and blackwater from ordinary Swedish households. Sci Total Environ. 2005;348:151–163.
[34] Oldenburg M, Albold A, Wendland C, Otterpohl R. Erfahrungen aus dem Betrieb eines neuen Sanitärkonzepts über einen Zeitraum von acht Jahren. KA Korrespondenz Abwasser, Abfall. 2008;10:1100–1105.
[35] Vinneras B, Palmquist H, Balmer P, Jonsson H. The characteristics of household wastewater and biodegradable solid waste-A proposal for new Swedish design values. Urban Water J. 2006;3:3–11.
[36] Jefferson B, Palmer A, Jeffrey P, Stuetz R, Judd S. Grey water characterisation and its impact on the selectilon and operation of technologies for urban reuse. Water Sci Technol. 2004;50:157–164.
[37] Elmitwalli TA, Otterpohl R. Anaerobic biodegradability and treatment of grey water in upflow anaerobic sludge blanket (UASB) reactor. Water Res. 2007;41:1379–1387.
[38] Hernandez Leal L, Zeeman G, Buisman CJN. Grey water treatment concept integrating water and carbon recovery and removal of micropollutants. Water Practice Technol. 2011;6:1–9.
[39] NSF/ANSI-350/2012; Onsite Residential and Commercial Water Reuse Treatment Systems; NSF international; Ann Arbor, Michigan [cited 2015 Jan 2]. Available from: http://www.nsf.org/consumer-resources/green-living/wastewater-treatment-system-alternatives/onsite-water-reuse-treatment-systems
[40] Zeeman G, Kujawa K, de Mes T, Hernandez L, de Graaff M, Abu-Ghunmi L, Mels A, Meulman B, Temmink H, Buisman C, van Lier J, Lettinga G. Anaerobic treatment as a core technology for energy, nutrients and water recovery from source-separated domestic waste(water). Water Sci Technol. 2008;57:1207–1212.
[41] Knerr H, Rechenburg A, Kistemann T, Schmitt TG. Performance of a MBR for the treatment of blackwater. Water Sci Technol. 2011;63:1247–1254.
[42] Palmquist H. Hazardous substances in wastewater systems a delicate issue for wastewater management. Luleå: Luleå Tekniska Universitet; 2001.

PAPER 2

Removal of particles in organic filters in experimental treatment systems for domestic wastewater and black water

Daniel Todt, Petter D. Jenssen, Aleksandra K. Klemenčič,
Tjaša G. Bulc, Andreea Oarga

2014

Journal of Environmental Science and Health, Part A
Toxic/Hazardous Substances and Environmental Engineering
Vol. 49, No. 8, 948–954

Journal of Environmental Science and Health, Part A (2014) **49**, 948–954

ISSN: 1093-4529 (Print); 1532-4117 (Online)
DOI: 10.1080/10934529.2014.893795

Removal of particles in organic filters in experimental treatment systems for domestic wastewater and black water

DANIEL TODT[1], PETTER D. JENSSEN[1], ALEKSANDRA KRIVOGRAD KLEMENČIČ[2], ANDREEA OARGA[3] and TJAŠA GRIESSLER BULC[4]

[1]*Department for Plant and Environmental Sciences, Norwegian University of Life Sciences, Ås, Norway*
[2]*Institute of Sanitary Engineering, Faculty of Civil and Geodetic Engineering, University of Ljubljana, Ljubljana, Slovenia*
[3]*University of Nova Gorica, Nova Gorica, Slovenia*
[4]*Department of Sanitary Engineering, Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia*

This study assesses the total suspended solids (TSS) retention capacity of different organic filter media for two potential applications: (i) a polishing unit for package treatment plants and (ii) a pretreatment for blackwater from low-flushing toilets. The results showed that the peat filter media used can be significantly improved in terms of structural stability and TSS removal capacity by mixing it with sawdust. Most of the TSS accumulated in the upper part of the filter material, and filter thickness exceeding 15 cm had no statistically significant effect ($P < 0.1$) on the TSS treatment performance. The experimental system reached a TSS reduction of 60–70% for blackwater and 80–90% for simulated effluent peaks from a package treatment plant. The main challenge of a full-scale application of an organic filter is the issue of clogging, especially when treating concentrated blackwater. However, this work indicates that a clogged filter media can be regenerated by mixing the uppermost filter layer without significant loss of filter performance regarding TSS. More research is needed to develop an appropriate mechanical unit for automatic filter media regeneration.

Keywords: Blackwater, filtration, municipal wastewater, organic filter media, peat; sawdust.

Introduction

Organic filter media such as bark, sawdust and peat, have shown good filtration and adsorption effects for particles.[1,2] In particular, peat, a cheap light-weight medium, has been widely applied due to its high retention capacity for particulate organic material [3] and sorption ability for organic micro pollutants.[4] Many studies have been conducted regarding organic filters for the treatment of domestic wastewater,[2,5–8] but its application has thus far been restricted due to hydraulic limitations.[2,5,9] Space requirements and the need for renewal of the filter media have also been indicated as potential critical issues,[9] as well as limited sorption capacity for dissolved nutrients, such as phosphorous, compared to mineral filter media.[1] However, in recent years, novel niche applications for removal of particulate matter have emerged with which the above-mentioned limitations are less critical or easier to overcome.[10–13] The goal of this study was to assess the performance of vertical flow organic filters using sphagnum peat and a peat-sawdust mixture for two different applications regarding particle retention, hydraulic service life and space requirement as basis for suggesting novel filter designs.

The first experiment was testing organic filters as an effluent polishing option for a sequencing batch reactor (SBR). Such systems have shown unstable sludge removal resulting in peaks of TSS up to 500 mg/L in the effluent.[14] Effluent polishing in natural systems (soil infiltration or wetlands) has therefore been recommended in Scandinavia when discharging to sensitive areas.[15] In such effluent peaks, the particles have a high degree of mineralization and readily biodegradable organic matter is usually absent.[14] Under the above conditions, biofilm development within the filter pores, which is the main factor for clogging,[16,17] will be substrate limited. A filter applied to effluent polishing may therefore expected to reach a significantly longer hydraulic service life than in primary or secondary treatment that have been tested by earlier studies.[2,5,9] In the first part of this experiment, simulated effluent peaks were applied to peat and peat bark

Address correspondence to Daniel Todt, Department for Plant and Environmental Sciences, Norwegian University for Life Sciences, P. O. Box 5003, NO-1432 Ås, Norway; E-mail: daniel.todt@umb.no or tjasa.bulc@zf.uni-lj.si
Received September 27, 2013.
Color versions of one or more of the figures in this article can be found online at www.tandfonline.com/lesa.

mixtures and particle retention and hydraulic service life were assessed.

In the second part of this experiment the peat and peat/sawdust mixtures were used to filter settled blackwater (BW) as part of a treatment train rendering both retentate (particles and organic matter retained in the filter) and filter media for reuse in agriculture.[5,10,13] The experiment was part of a R&D work to develop a novel compact treatment unit for BW at tourist facilities in environmentally vulnerable areas where extremely low emissions are required.[12,18] The function of the organic filter in this sequence of treatment processes is to retain the main fraction of particulate matter and to simultaneously obtain a ratio between retentate and filter media suited for the subsequent composting and hygienization process by reaching the thermophilic phase.[11,19,20] Since filter media is reused as a bulking material for composting a short filter renewal interval is not necessarily a problem.

A recent study [8] indicates that filter thickness could be halved without a critical reduction of filter performance. Such thinner filters will substantially increase the accumulated particulate matter per unit volume filter media, since the main accumulation occurs in the upper part of the filter. The second experiment was therefore assessing the effect of different filter thickness in columns treating BW. The assessment focused on TSS removal and the clogging process. Within the same experiment, a novel mechanical support enhancing the hydraulic capacity was tested in order to attempt to reach a significant footprint reduction of organic filter systems, which is necessary for a more widespread application in domestic environments.[10,13]

Material and methods

Two lab-scale experiments were performed. For both experiments four columns were used, each having a cross-sectional area of 0.0314 m^2 (Fig.1). The columns were made of acrylate and equipped with 1-mm woven textile nets (Sefar PETEX® 07–1000/45, Heiden, Switzerland) at the bottom.

The first experiment was conducted at the University of Ljubljana for testing two different, locally available organic filter media: sphagnum peat (pH 3–4, humification grade: H2-H5) and a peat-sawdust mixture (80% of sphagnum peat H2-H5, pH 3–4 and 20% of wood shavings). Each filter column was filled with 30 cm filter media, i.e., the filter height that has been applied in earlier similar experiments.[2] The columns were loaded with a diluted secondary sludge from a pilot-scale sequential batch reactor (SBR) treating the wastewater from a university building.

The second experiment was conducted at the Norwegian University for Life Sciences in Ås, Norway to assess particulate matter retention capacity for settled BW. This experiment used a peat-sawdust mixture that was found to be the most efficient filter material in the first experiment.

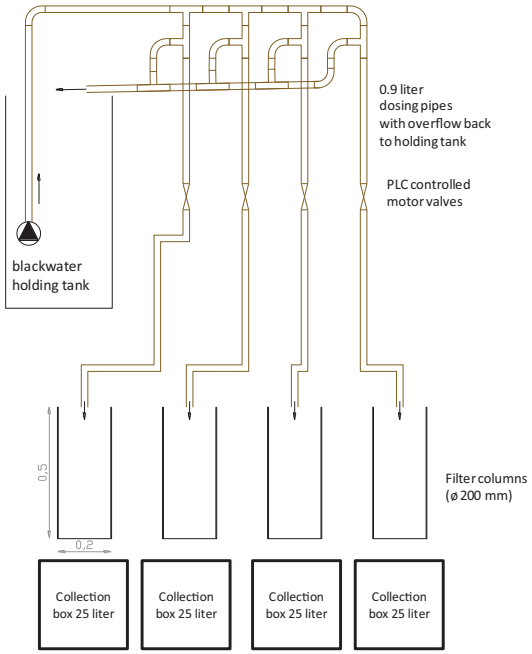


Fig. 1. The laboratory setup for testing selected organic filters and loading control with programmable logic controller (PLC).

The settled BW originated a student dormitory housing 40 residents; the building was connected to a vacuum toilet source separating sanitary system.[21] Before entering the settling tank, the BW passed a macerator pump (Jets vacuum sewer system, Jets Vacuum As, Hareid, Norway). To assess the effect of filter depth, two columns were filled with 30 cm and two columns with 15 cm of filter media.

In order to facilitate a comparison, the intermittent loading regime was the same for filters receiving the SBR mixture and the settled BW. A hydraulic loading rate of 71 cm d^{-1} was selected in for the BW experiment because the goal was to treat BW from 100 persons in 1 m^2. The BW produced by a vacuum sanitation system connected to 100 persons, is estimated to 710 liter d^{-1}. Each column of diameter 200 mm received a daily hydraulic load of 22.5 L divided into 25 doses d^{-1} each of 0.9 L. The doses were divided into three batches of 10, 5, and 10 doses to simulate a diurnal distribution pattern of toilet usage.

In both experiments a composite sample from the inflow and outflow of the filter columns were taken daily; TSS was measured according to SIST ISO 11923. For determining the suspended solids (SS) > 10 μm, a woven textile net with 10-μm mesh size was used (Sefar PETEX® 07–10/2).

The first experiment was stopped when clogging caused the columns to pond, i.e., caused standing water to

accumulate. The columns of the second experiment were regenerated after ponding started. The regeneration took place by disturbing the top of the filter media with the help of a metal stick (5 mm diameter), which resulted in the mixing of the uppermost 5 cm of the filter column.

At the end of each experiment, the accumulation of mineral material in the filter media was assessed by determining the content of volatile solids (VS) according to ASTM D7348 - 08. Due to the small number of samples and slight deviation from normality in the effluent samples (checked with Lilliefors' test[22]) a Wilcoxon signed rank test [23] was chosen for comparison of samples. A significant difference has been defined on a probability level of 0.1.

Results and discussion

Quality and characteristics of two different inlet applications

The TSS from the SBR mixture varied from 90–845 mg/L (Fig. 2a). Usually the main fraction of the particulate matter in activated sludge is medium-sized bacteria flocs in a range of 10–100 μm, while the amount of small (<10 μm), freely dispersed particles is relatively small.[24]

The TSS in settled BW varied in a range of 403–1590 mg/L (Fig. 2b), which is significantly higher than the SBR mixture (Fig. 2a). A substantial amount (45–87%) of the TSS in the BW samples refers to small particulate material (<10 μm). The two types of wastewaters that were applied to the peat filters, therefore, differed substantially in terms of both particle content and particle size distribution.

Performance of two different types of filter media applied as a post treatment for package treatment plants

Figure 3a shows the results from the two different filter media that have been loaded with the SBR mixture. The peat-sawdust mixture reached significantly ($P < 0.1$) higher reduction of TSS than peat; 89% versus 74%, respectively. Studies in unsaturated natural peat soil systems showed that a more heterogeneous media structure promotes more tortuous flow pathways and longer retention times.[25] Higher tortuosity could therefore be one explanation for this result. The adding of sawdust that consisted mostly of wood shavings should, due to a larger and different size and shape compared to the peat particles, increase the tortuosity, which again results in a better filtration effect. The data in Figure 3a show that the peat-sawdust mixture not only improves the filtration effect but also tends to give a lower variance in the effluent. Earlier studies found that a mixture of two filter media with different structure tends to result in a better performance than use of a single media.[2] These results also indicate that at least 20% of peat can be replaced with sawdust, the more environmentally friendly and cheaper material. Further reduction of peat consumption might be possible by increasing the sawdust proportion in the mixture.

Performance of peat-sawdust applied to settled blackwater and impact of filter size

Based on the results of the first experiment the peat-sawdust mixture was selected for the second experiment with the settled BW. The peat-sawdust mixture showed a slightly lower TSS reduction (in a range of 60–75%) when loaded with settled BW than in the trial with the SBR

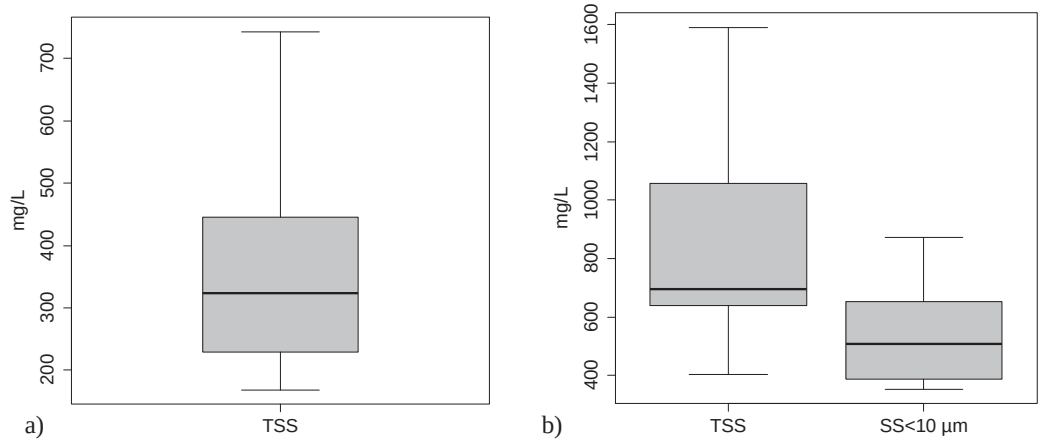


Fig. 2. Concentration of suspended solids used in the experiments: (a) in the sequential batch reactor mixture ($n = 25$), (b) in the blackwater ($n = 10$), total suspended solids (TSS) and suspended solids (SS) < 10 μm.

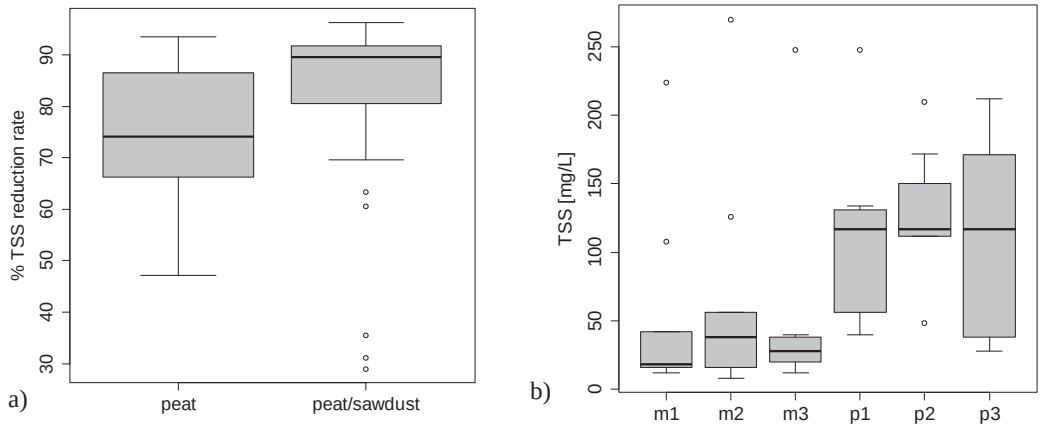


Fig. 3. (a) Total suspended solids (TSS) in outflow of 30 cm peat versus 30 cm of peat-sawdust mixture loaded with sequential batch reactor (SBR) mixture ($n = 24$). (b) TSS reduction of 30 cm peat-sawdust mixture (m1–m3) mixture versus 30 cm peat-(p1–p3) loaded with SBR mixture ($n = 30$).

mixture. This is remarkable with regards to the high fraction of small particles identified in the settled BW, which are in general more difficult to remove (Fig. 2b). However, the TSS concentration in the outflow of the filter columns was still high and ranged from 200 to 300 mg/L (Fig. 4b), requiring further treatment.

Investigations of the retention process

A Wilcoxon test showed that the difference in the TSS reduction between 15-cm and 30-cm- thick layers of peat-sawdust mixture (Fig. 4) was not statistically significant ($P < 0.1$). These observations indicate that most of the filtration process took place in the uppermost part of the filter which is supported by the data in Figure 5.

Based on 20 samples it could be determined that the VS content in the particulate fraction of BW is 800 ($\pm$120) g/kg and significantly ($P < 0.1$) lower than the VS content of 980 ($\pm$5) g/kg in the virgin filter media. The deviation between the VS content in media samples taken in particular layers of the filter columns and VS content of virgin filter media was therefore used as an indicator to determine the layers in which particulate matter of the wastewater was retained (Fig. 5).

The difference in VS content compared to the virgin media is most distinct in the first 10 cm of the filter

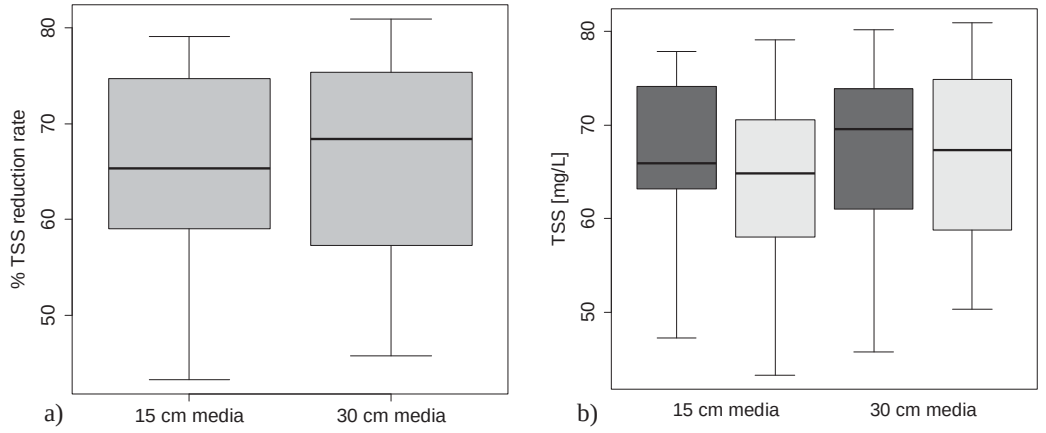


Fig. 4. Total suspended solids (TSS) in outflow of 15 cm versus 30 cm of peat-sawdust mixture loaded with blackwater, (a) reduction ($n = 20$), (b) concentration ($n = 10$).

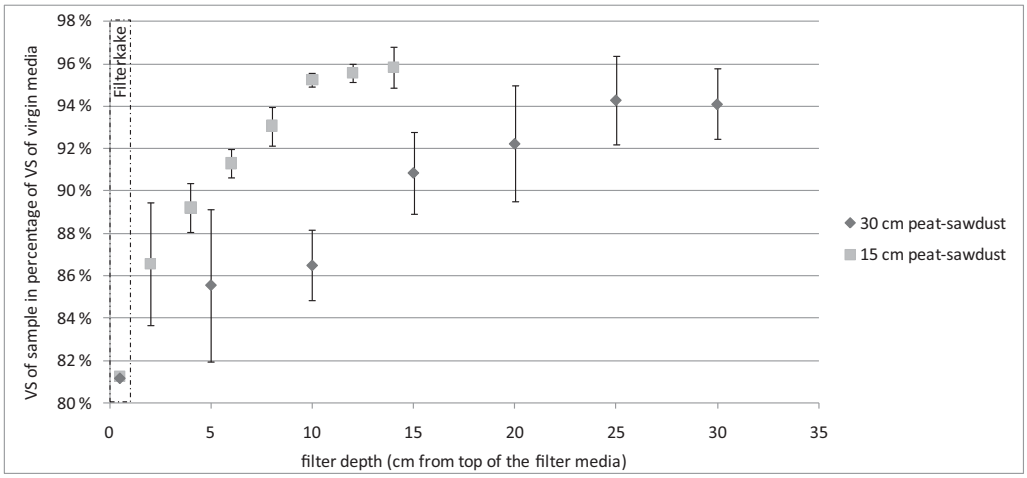


Fig. 5. Volatile solids content in percentage of volatile solids content of the virgin media from samples taken in different depths of two filter columns filled a peat-sawdust mixture. Each data point represents the average and standard deviation of $n = 6$ samples taken from two parallel filter columns for each of the filter depth; 100% refers to the VS content of 980 (±5) g/kg of the virgin filter media.

column. This indicates that TSS retention takes place in the uppermost layers, an effect that has been also reported by other authors.[26] The gradient in VS content over the filter depth seems to be more distinct in the columns with only 15-cm filters. This may possibly be due to a slightly deeper mixing of the 30-cm columns during the regeneration treatment.

These findings indicate that filter thickness and amount of filter media can be halved without having a significant effect on the filter performance. The smaller filter size saves filter media and simplifies its replacement after becoming saturated.

Investigation of the clogging mechanism and filter regeneration

Columns loaded with SBR mixture started to clog after 200 loadings of 0.9 L (8 days) for peat and after 250 loadings for the peat-sawdust mixture. All peat-sawdust columns loaded with settled BW started to pond after 65 loadings (3 days) and a first regeneration treatment took place. After the first regeneration, ponding started to occur again after 15–20 loadings with BW. The subsequent regeneration treatments therefore took place once a day. The TSS reduction in the period after the daily filter regeneration started ($n = 14$) did not differ significantly ($P < 0.1$) from the performance obtained during the first three days without filter regeneration ($n = 6$).

Clogging in vertical flow filters, such as the experimental columns used in our study, is a complex interaction between particle accumulation and biofilm development.[16,17] These processes seem to be strongly dependent on the content of biodegradable organic compounds [17] and particle size distribution [27] of the influent.

The readily biodegradable organic content in settled BW is estimated to be in a range of 1500–2000 mg COD L^{-1} and results most probably in high biofilm growth on the top of the filter columns, which is assumed to be the main clogging factor in the BW columns. The regenerated filter media started to clog significantly faster than the virgin media. This can be also explained by biofilm development. In the start of the filter operation, the biofilm will be established, which takes 2–3 days.[17] After filter regeneration (stirring of the top layer), the biomass is dispersed, but ready to grow without a lag phase of 2–3 days, resulting in a more rapid clogging development.

The effluent from the SBR usually does not contain high amounts of readily biodegradable organics, since this type of wastewater has passed an intensive biological treatment. Organic substrate to a potential biomass growth is only available in small quantities that are released by exogenous hydrolyzation of particulate material and endogenous decomposition of dead biomass.[28] Despite the fact that secondary sludge contains active biomass, biofilm development may take more time than in the columns loaded with BW due to lack of substrate for biofilm growth.

Both peat and sawdust are organic materials that start to degrade after a certain time. Wastewater such as BW that initiates a high biological activity in the filter media (e.g., biofilm growth) will probably cause more rapid degeneration of the filter media. The time for decomposition will depend on the type of wastewater and the temperature.[17] Experiences from wood chip filters loaded with

high-concentrated porcine and dairy waste indicate that such filters may keep its structure for up to two years if not clogged.[10,13] However, those systems were agricultural applications, running on 10 times lower hydraulic loading rates than in the experiments reported herein. At a loading of 30 L m^{-2} d^{-1}, TSS concentrations of 600 mg/L could be applied over a period of more than one year without the occurrence of critical clogging.[13] On such low loading rates, accumulation (particle retention, biofilm growth) and degradation (hydrolisation, endogenous decay) may remain in a balance so that excessive clogging does not occur.

Extensive systems with low loading rates as in the above-used agricultural application [10,13] are difficult to implement in domestic wastewater treatment due to the large space requirements. However, the mechanical regeneration of the hydraulic capacity applied in this study can extend the renewal interval significantly and will help to increase the TSS accumulation per unit volume media. For a practical application a device is needed that a) regenerates the clogged filter through the mixing of the filter media, and b) gradually replaces filter media. A counter-stream filter in which filter media become turned and transported into the opposite direction of the wastewater flow with the help of a helical screw may be an appropriate device for performing this task. A prototype was developed and an additional experiment is planned to test this novel compact organic filter system.

Conclusion

The results show that the both the hydraulic and treatment performance of peat filters can be significantly improved by mixing with sawdust. Such a mixture increases the tortuosity of the filter matrix and at least 20% of peat can be replaced with the more environmentally friendly and economically viable sawdust. The thickness of traditional organic filters can be halved from 30 to 15 cm without a significant performance loss in terms of particle (TSS) removal.

This study shows that mechanical regeneration increases filter life-time from 3 to 10 days and retention capacity for particulate matter without having a negative impact on the filter performance. The high accumulation of particulate matter per unit volume media facilitates composting of the media and subsequent reuse. Short renewal intervals of filter media due to concentrated blackwater can be bridged using an automatic mechanical device.

Funding

The work has been supported by the EU 7th Framework Program.

References

[1] Kõiv, M.; Vohla, C.; Mõtlep, R.; Liira, M.; Kirsimäe, K.; Mander, Ü. The performance of peat-filled subsurface flow filters treating landfill leachate and municipal wastewater. Ecol. Eng. **2009**, *35*, 204–212.

[2] Lens, P.N.; Vochten, P.M.; Speleers, L.; Verstraete, W.H. Direct treatment of domestic wastewater by percolation over peat, bark and woodchips. Water Res. **1994**, *28*, 17–26.

[3] Corley, M.; Rodgers, M.; Mulqueen, J.; Clifford, E. The performance of fibrous peat biofilters in treating domestic strength wastewater. J. Environ. Sci. Health A **2006**, *41*, 811–824.

[4] Novoselova, L.Y.; Sirotkina, E.E. Peat-based sorbents for the purification of contaminated environments: A review. Solid Fuel Chem. **2008**, *42*, 251–262.

[5] Gajurel, D.R.; Benn, O.; Li, Z.; Behrendt, J.; Otterpohl, R. Pre-treatment of domestic wastewater with pre-composting tanks: evaluation of existing systems. Water Sci. Technol. **2003**, *48*, 133–138.

[6] Hu, Z.; Gagnon, G.A. Factors affecting recirculating biofilters (RBFs) for treating municipal wastewater. J. Environ. Eng. Sci. **2006**, *5*, 349–357.

[7] Perez, J.I.; Hontoria, E.; Zamorano, M.; Gomez, M.A. Wastewater treatment using fibrist and saprist peat: A comparative study. J. Environ. Sci. Health A **2005**, *40*, 1021–1032.

[8] Tao, J.; Mancl, K.M.; Tuovinen, O.H. A potential sanitary sewer overflow treatment technology: Fixed-media bioreactors. Water Environ. Res. **2011**, *83*, 714–721.

[9] Couillard, D. The use of peat in wastewater treatment. Water Res. **1994**, *28*, 1261–1274.

[10] Buelna, G.; Dube, R.; Turgeon, N. Pig manure treatment by organic bed biofiltration. Desalination **2008**, *231*, 297–304.

[11] Gajurel, D.R.; Li, Z.; Otterpohl, R. Investigation of the effectiveness of source control sanitation concepts including pretreatment with Rottebehaelter. Water Sci. Technol. **2003**, *48*, 111–118.

[12] Masi, F.; El Hamouri, B.; Shafi, H.A.; Baban, A.; Ghrabi, A.; Regelsberger, M. Treatment of segregated black/grey domestic wastewater using constructed wetlands in the Mediterranean basin: the zer0-m experience. Water Sci. Technol. **2010**, *61*, 97–105.

[13] Ruane, E.M.; Murphy, P.N.C.; Healy, M.G.; French, P.; Rodgers, M. On-farm treatment of dairy soiled water using aerobic woodchip filters. Water Res. **2011**, *45*, 6668–6676.

[14] Moelants, N.; Janssen, G.; Smets, I.; Van Impe, J. Field performance assessment of onsite individual wastewater treatment systems. Water Sci. Technol. **2008**, *58*, 1–6.

[15] Jenssen, P.D.; Vråle, L.; Kirkerud, J.; S., J., Forprosjekt–Vann og avlùp Bunnefjordsområdet, alternativ 3, lokale lùsninger. ITF report 108/2000. Agricultural University of Norway: Ås, Norway, 2000.

[16] Hua, G.F.; Li, L.; Zhao, Y.Q.; Zhu, W.; Shen, J.Q. An integrated model of substrate clogging in vertical flow constructed wetlands. J. Environ. Manage. **2013**, *119*, 67–75.

[17] Zhao, L.F.; Zhu, W.; Tong, W. Clogging processes caused by biofilm growth and organic particle accumulation in lab-scale vertical flow constructed wetlands. J. Environ. Sci.-China **2009**, *21*, 750–757.

[18] Todt, D.; Jenssen, P.D.; Bulc, T.G. Greenhouse wetland technology for blackwater treatment in high alpine locations. In *12th IWA International Conference on Wetland Systems for Water Pollution Control*, Oct 4–8, 2010, Venice, Italy. International Water Association: London, 2010; 983–984.

[19] Masi, F. Water reuse and resources recovery: the role of constructed wetlands in the Ecosan approach. Desalination **2009**, *246*, 27–34.

[20] Otterpohl, R. Options for alternative types of sewerage and treatment systems directed to improvement of the overall performance. Water Sci. Technol. **2002**, *45*, 149–158.
[21] Jenssen, P.D.; Heyerdahl, P.H.; Warner, W.S.; Greatorex, J.M. Local recycling of wastewater and wet organic waste—A step towards zero emission community. In *8th International Conference on Environmental Science and Technology*, Sept 8–10, 2003, Lemnos, Greece. Curran Associates, Inc.: Red Hook, NY, 2003; 660–667.
[22] Lilliefors, H.W. On the Kolmogorov-Smirnov test for normality with mean and variance unknown. J. Am. Stat. Assoc. **1967**, *62*, 399–402.
[23] Wilcoxon, F. Individual comparisons by ranking methods. Biometrics Bull. **1945**, *1*, 80–83.
[24] Govoreanu, R.; Seghers, D.; Nopens, I.; De Clercq, B.; Saveyn, H.; Capalozza, C.; Van derMeeren, P.; Verstraete, W.; Top, E.; Vanrolleghem, P.A. Linking floc structure and settling properties to activated sludge population dynamics in an SBR. Water Sci. Technol. **2003**, *47*, 9–18.
[25] Rezanezhad, F.; Quinton, W.L.; Price, J.S.; Elliot, T.R.; Elrick, D.; Shook, K.R. Influence of pore size and geometry on peat unsaturated hydraulic conductivity computed from 3D computed tomography image analysis. Hydrol. Process. **2010**, *24*, 2983–2994.
[26] Taylor, M.; Clarke, W.P.; Greenfield, P.F.; Swain, G.J. Characterizing the physical and chemical properties of a vermicompost filter bed. Compost Sci. Util. **2004**, *12*, 383–391.
[27] Hua, G.F.; Zhu, W.; Zhao, L.F.; Huang, J.Y. Clogging pattern in vertical-flow constructed wetlands: Insight from a laboratory study. J. Hazard. Mater. **2010**, *180*, 668–674.
[28] Gujer, W.; Henze, M.; Mino, T.; van Loosdrecht, M. Activated Sludge Model No. 3. Water Sci. Technol. **1999**, *39*, 183–193.

PAPER 3

Particle removal in a novel sequential mechanical filter system loaded with blackwater

Daniel Todt, Petter D. Jenssen

2015

Water Science & Technology

Vol. 71, No. 9, 1407–1413

Particle removal in a novel sequential mechanical filter system loaded with blackwater

Daniel Todt and Petter D. Jenssen

ABSTRACT

A novel sequential mechanical filter system was developed as an alternative primary treatment method for onsite wastewater treatment. The filter combines traditional screening with a novel type of counter-flow filter using wood-shavings as a biodegradable filter matrix. This study tested the system in a batch loading regime simulating high frequency toilet flushing using blackwater from a student dormitory. The filter removed 78–85% of suspended solids, 60–80% of chemical oxygen demand, and 42–57% of total-P in blackwater, giving a retentate with a dry matter content of 13–20%. Data analysis clearly indicated a tendency towards higher removal performance with high inlet concentrations, hence, the system seems to be most applicable to blackwater or other types of wastewater with a high content of suspended solids.

Key words | blackwater, filtration, screening, organic filter

Daniel Todt (corresponding author)
Petter D. Jenssen
Department of Environmental Sciences,
Norwegian University of Life Sciences,
Frognerbakken 3,
N-1432 Aas,
Norway
E-mail: *danitodt@gmail.com*

INTRODUCTION

Filtration has gained increasing attention as an alternative primary treatment method. For municipal treatment plants, advanced mechanical filter systems were developed (Tchobanoglous *et al.* 2002; Rusten & Odegaard 2006) while for smaller onsite applications, simpler organic percolation filters are proposed, which are easier to operate (Lens *et al.* 1994; Taylor *et al.* 2003; Todt *et al.* 2014b). Filtration is an interesting option for blackwater treatment in source separating sanitary systems (Todt *et al.* 2014b) since blackwater contains 60% of total suspended solids (TSS) in a small volume fraction (Meinzinger & Oldenburg 2009). However, simple organic percolation filters were shown to be vulnerable to clogging, which limits practical implementation (Lens *et al.* 1994; Todt *et al.* 2014b). To overcome these problems, a novel mechanical filter device was developed as a primary treatment step for the blackwater discharge from mountain cabins with 20–40 accommodation places.

The filter system is combining traditional mechanical screening with a new type of counter-flow filter using an organic media as biodegradable filter matrix. For the coarse filtration in the first filtration step, a downscaled standard drum screen (Tchobanoglous *et al.* 2002) was chosen. The design of the counter-flow filter was based on the outcomes from a previous study on blackwater treatment with static filter columns (Todt *et al.* 2014b). The objectives of this study were to assess the system performance regarding treatment of blackwater directly connected to a vacuum toilet system and to gain knowledge on the filtration mechanisms.

doi: 10.2166/wst.2015.114

METHODS

Counter-flow matrix filter and initial test setup

The prototype of the sequential mechanical filter unit consists of a pre-screening unit that removes coarse particulate material followed by a counter-flow filter removing finer particles (Figure 1). The pre-screening unit was based on a drum screen with an opening size of 3 mm. The counter-flow filter uses an organic filter matrix that is transported upwards with the help of a conveyor. The sewage passes the filter matrix where particulate matter is retained and the liquid fraction leaves the system via a 3 mm screen at the bottom of the filter tube. The conveyor tubes had an inner diameter of 150 mm. For this experiment, wood-shavings with a grain size of 10–30 mm were used. The rotating intervals of the conveyors were controlled by the number of loading batches. At a speed of 0.5 RPM, the pre-screening conveyor was run for 3 s in each of the three loading batches and the counter-flow conveyor was run for

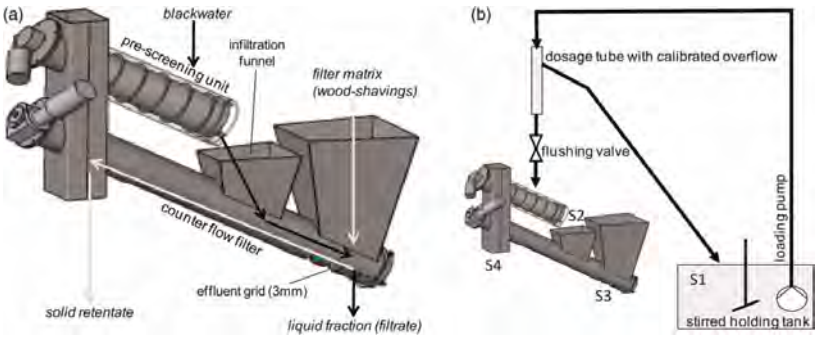


Figure 1 | (a) The two-step mechanical filtration system consisting of a pre-screening unit and counter-flow filtration through a wood-shavings matrix. (b) The test setup simulating toilet flushes using a dosage tube with calibrated overflow set at a volume of 1.1 L. Sampling points for raw blackwater (S1), effluent pre-screening unit (S2), effluent liquid fraction (S3), and solid retentate (S4).

10 s in each 10 loading batches, giving a transportation distance per feeding interval of 6 and 20 mm, respectively.

The experiment used blackwater from student dormitories that are equipped with vacuum toilets with a 1.2 L flushing volume (Todt *et al*. 2014a). For the experiment, a dosing arrangement simulating discharges of a vacuum sewer system was established (Figure 1(b)). The filter system was loaded for 33 days, interrupted by weekends with no loading. Loading took place in one daily sequence of 1.1 L batches (simulated toilet flushes) at intervals of 90 s. The hydraulic load per day was determined by the blackwater availability from the student dormitories and ranged from 60 to 312 batches.

Sampling and statistical analysis

Daily composite liquid samples were collected from the stirred blackwater holding tank (S1) and counter-flow filter (S3). The liquid effluent from the pre-screening unit (S2) was sampled using grab samples. All liquid samples were analysed for TSS, which was determined with 1.2 μm glass-fiber filters (GF-C, 47 mm, Whatman, Little Chalfont, UK). A selected number of samples were also analyzed for chemical oxygen demand (COD) and phosphorus using spectrophotometric test kits (Hach-Lange, Berlin, Germany). The raw blackwater was additionally analyzed for the content of large-sized (>1 mm) suspended solids by filtration over a calibrated woven textile filter with 1 mm mesh size (PETEX^{TM} 07-1000/45, Sefar AG, Heiden, Switzerland). To minimize filter-cake formation and potential retention of smaller particles, the filtration volume was limited to 5 mL in the TSS determination and in the determination of solids >1,000 μm. These were the smallest volumes that resulted in a good repeatability of the filtration procedure of a well-mixed sample (<20% variance between parallels). Solid retentate samples were collected in the discharge chamber (S4) and analyzed for dry matter by drying at 105 °C and loss of ignition (LOI) at 550 °C.

Due to the high natural variation in wastewater samples, as well as the limited number of data points produced by the experiments, a descriptive, quartile-based statistics method was chosen to present the data, and non-parametric Wilcoxon signed rank tests with a significance level of $p < 0.01$ for sample comparisons. The data are presented using box-plots showing 1st and 3rd quartiles, supplemented with error bars showing the minimum and maximum values. Variation ranges expressed in the text refer to 1st and 3rd quartile. To explore the dependency of TSS reduction versus the TSS concentration, simple linear regression plots were established with Microsoft-Excel.

RESULTS AND DISCUSSION

Treatment performance and filter media consumption

The filtration with the pre-screening unit resulted in a TSS reduction of 57–72%, which was further reduced to 49–64% by the counter-flow filter (Figure 2(b)), giving a final effluent TSS concentration ranging from 1,150 to 1,650 mg L^{-1} (Figure 2(a)). The whole system obtained a TSS reduction of 78–85% (Figure 2(b)), 60–80% for COD (Figures 2(d) and 2(e)), and 42–57% for total-P (Figures 2(f) and 2(g)). No significant ($p = 0.13$) reduction was found

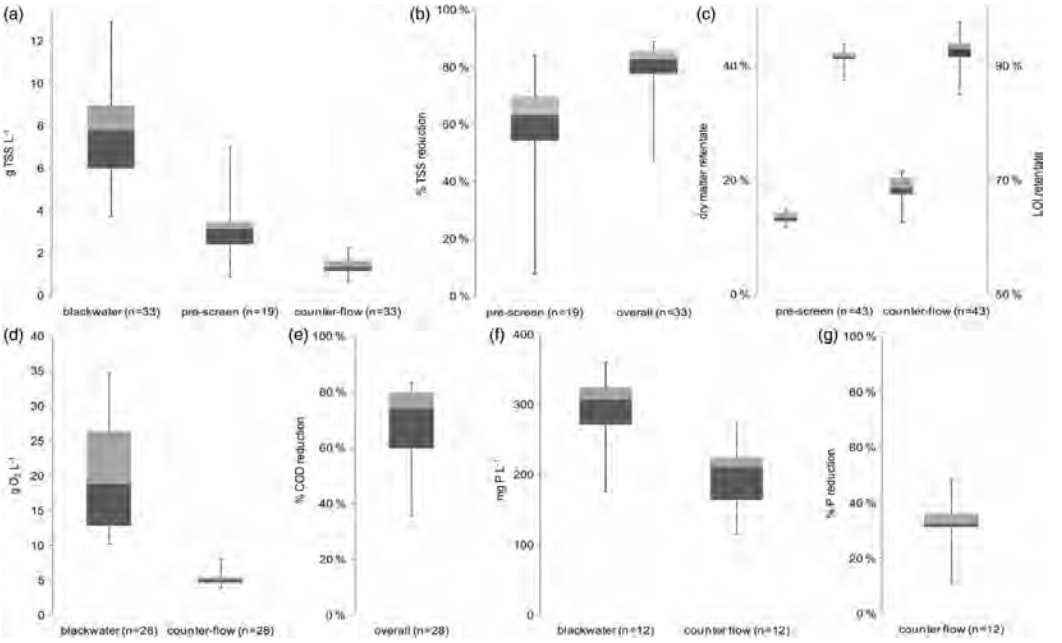


Figure 2 | (a) TSS in the blackwater, after the pre-screening unit and after the counter-flow filter; (b) TSS reduction after the pre-screening unit and the overall system; (c) dry matter and loss of ignition (LOI) of the retentate from each filtration step; (d) COD in blackwater and effluent counter-flow filter; (e) overall COD reduction; (f) total phosphorus in blackwater and effluent counter-flow filter; (g) overall reduction of total phosphorus.

for dissolved orthophosphate (not shown), hence, the majority of the retained P was particle-bound. The obtained particle (TSS) reduction was close to reported values (90%) from rotating belt sieves with significant smaller mesh sizes (350 μm) (Rusten & Odegaard 2006) or static percolator filters (Lens *et al.* 1994; Todt *et al.* 2014b). However, the inlet TSS concentration range in the blackwater used by our study (Figure 2(a)) was substantially higher than the settled blackwater (0.4–1.5 g TSS L^{-1}) used by Todt *et al.* (2014b) or municipal wastewater used by the other above-cited studies. Considering the dependency of filter efficiency on inlet concentration as indicated by Figure 3(a), a direct comparison of our results to the currently available literature data needs to be done with care.

The retained solid material (retentate) discharged from the filtration steps showed a dry matter content of 13–14% for the pre-screening retentate and 18–20% for the counter-flow retentate (Figure 2(c)). The higher dry matter content in the retentate of the counter-flow filter was most likely a result of the wood-shavings amendment. The obtained dry matter content was considerably higher than the dry matter content of 0.7–10%, which is typical for gravity sedimentation in a septic tank (Henze & Comeau 2008). The high dry matter content might be advantageous for a further processing of the retentate by composting or drying with solar heat. The pre-screening retentate showed a high LOI of 91–92% (Figure 2(c)) indicating that mainly organic matter is retained in the first filtration step. This is in accordance with the findings from other experiments with screening systems (Ruiken *et al.* 2013). The retentate of the counter-flow showed an LOI of 92–94% (Figure 2(c)), which is surprisingly low, considering its high content of wood-shavings having an LOI of 98%. This indicates that the retained solids in the second filtration step have an lower LOI than the solids retained in the pre-screening unit. Hence, the counter-flow filter has also retained a certain quantity of the inorganic compounds from the wastewater. This is supported by the phosphorus reduction (Figures 2(f) and 2(g)).

The wood-shavings consumption was in the range of 1–1.2 L per 100 simulated toilet flushes. The measurements of the wood-shavings load were notably lower than a theoretical

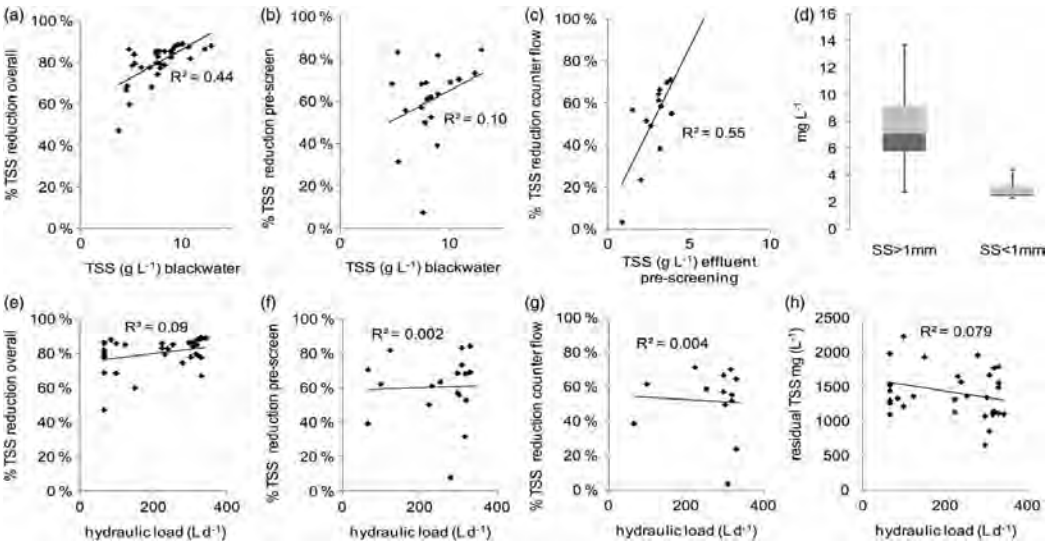


Figure 3 | Upper panel: dependency of filter performance on the loaded TSS concentration for (a) overall system, (b) pre-screening unit and (c) counter-flow filter; (d) concentration range of large-sized (>1 mm) and small-sized (<1 mm) suspended solids in ($n = 7$) blackwater samples taken within the experimental period. Lower panel: dependency of filter performance on the hydraulic load for (e) overall system, (f) pre-screening unit and (g) counter flow filter; (h) dependency of effluent residual TSS in filtrate on hydraulic load.

filter-matrix consumption, which was calculated on 1.76 L per 100 simulated flushes based on the tube cross-section and conveyor speed. These can be explained if only 60–70% of the tube cross-sectional area was filled with wood-shavings, as was confirmed by visual observations. In the first preliminary experiments, the peat-sawdust mixture used in our previous study (Todt *et al.* 2014b) was used as a filter matrix. Its hydraulic capacity, as well as structural stability, was shown to be insufficient so that wood-shavings were selected for the experiment. Other filter media with high hydraulic capacity, for example bark, may be an alternative to wood-shavings. To avoid washing out filter media or clogging the effluent grid, as well as ensuring sufficient hydraulic capacity, the grain size of the filter media should be 10 mm or larger.

Dependency of suspended solids retention on inlet concentration and hydraulic load

The dependency of the filter performance on the inlet concentration for suspended solids is elucidated by the regression plots in Figure 3. The regression plots in Figures 3(a)–3(c) indicate a tendency towards greater relative TSS retention at higher TSS concentration loads for both filtration steps. This tendency was further pronounced for the last filtration step, as indicated by the inclination of the regression lines and R-squared values (Figures 3(b) and 3(c)), which points towards different filtration mechanisms in the two filtration steps.

Comparing the effluent TSS concentration obtained via the pre-screening unit (Figure 2(a)) with the concentration determined for suspended solids >1 mm (Figure 3(d)) indicates that a majority of large-sized particles were retained in the pre-screening unit. The observed weak tendency towards greater relative particle removal with higher TSS concentration loads (Figure 3(b)) is in accordance with observations in other drum-screen systems with mechanical retentate removal (Rusten & Odegaard 2006; Ali 2013). In our experiment, this tendency is probably influenced by the high-fraction large-sized particles in the blackwater (Figure 3(d)) and filter-cake dynamics. Without the rotating conveyor, the mechanism behind the filter cake dynamics in the filter drum may be comparable to static textile filters. Experiments with textile filters showed that larger-sized solids accumulate into a filter cake, which enhanced the filtration effect by retaining a greater number of particles smaller than the apparent opening size of the textile, but at the same time substantially decreased the hydraulic capacity of the filter (Faure *et al.* 2006). The main function of the rotating conveyor inside our filter drum is to sustain hydraulic capacity and prevent clogging by antagonizing filter-cake formation. However, a

qualitative optical investigation of the filter drum showed that slight filter-cake formation still occurred, especially on days with a high TSS concentration load, which might increase the retention of small-sized particles. The magnitude of such an additional filtration effect due to filter-cake formation within the screen drum is most likely determined by the TSS load and the movement of the conveyor. Hence, it might be possible to improve the TSS removal capacity by adjusting the rotation speed and intervals of the conveyor. To determine this relationship, further experiments are needed.

The mechanisms in the wood-shaving filter matrix in the counter-flow filter are comparable to smaller-sized vertical-flow percolators or compost filter systems. In such systems, the tortuous filter matrix was shown to facilitate both filter-cake formation and biofilm growth (Zhao *et al*. 2009; Hua *et al*. 2013), which again enhances the retention of smaller-sized particles. These processes may be behind the observed greater dependency of the TSS reduction on the TSS concentration load in the counter-flow filter (Figure 3(c)) compared to the first filtration step (Figure 3(b)). As shown with our previous experiment (Todt *et al*. 2014b) and others (Lens *et al*. 1994; Taylor *et al*. 2004), in a static vertical-flow percolator, a majority of the removed particles tend to be retained in a distinct layer on the top of the filter matrix. In an earlier experiment, we obtained a 72% TSS reduction in vertical-flow percolator columns filled with a wood-shavings mixture and could not find a significant difference between 150 and 300 mm filter height (Todt *et al*. 2014b). The transverse filtration across the 150 mm filter tube, which is applied to the second filtration step in this study (Figure 1(a)), should theoretically have reached a comparable efficiency. However, the TSS reduction obtained in the second filtration step was, at 49–64% (Figure 2(b)), notably lower, which points to a significant impact of the mechanical conveyor system on the retention process. The rotation of the filter matrix (wood-shavings) likely reduce the performance of the filter system by breaking up developed filter-cakes as well as mixing accumulated particles into deeper layers from where a re-suspension and outwash into the effluent can take place. As indicated by the measured wood-shavings consumption ('Treatment performance and filter media consumption' section), the feeding mechanism of the wood-shavings was not perfect, which resulted in an incomplete filling of the tube. The retention time of the wood-shavings matrix of 5–6 days in cross-flow section of the filter tube may also be too short to facilitate a greater biofilm development.

The above findings indicate that filter efficiency of the second filtration step can be increased by improving the wood-shavings feeding and transport mechanism. The present conveying length was selected to obtain a complete renewal of the infiltration surface by turning the wood-shavings matrix 120 degrees at each conveying interval. The conveying interval was determined from trial runs aimed to keep ponding in the infiltration funnel below 10 cm. Periodical ponding of 3–5 cm was observed, which corresponds to a volume of 1.2–1.9 L. After a conveying event, this ponded volume percolated into the wood-shavings matrix within 30–60 s, which means three to four times higher infiltration velocity than the average at the applied load of 1.1 L every 90 s. A higher infiltration rate may reduce filter performance. Hence, the conveying regime can influence both capacity and treatment. Additional research is needed to optimize rotation versus these key parameters.

No significant correlation was found between the daily hydraulic load and the TSS reduction for the overall system (Figure 3(e)), the two filtration steps (Figures 3(f) and 3(g)), and the residual TSS concentration in the filtrate (Figure 3(h)). The filtration in the pre-screening unit is mainly a physical process, while biological processes are unlikely to take place. Hence, the filter performance of the first filtration step is mainly dependent on the physical parameters of the screen and the flow rate of 0.73 L min^{-1}, which was determined by the flushing interval. With the 90 s flushing interval, it should therefore be theoretically possible to increase the daily hydraulic load up to 1,056 L d^{-1} without significantly impacting the filter performance. In contrast to the pre-screening unit, the counter-flow filter facilitates biological processes that may impact the filter performance. These biological processes can be more easily influenced by the applied diurnal pattern of loading and non-loading periods. Hence, an extrapolation of the regression line in Figure 3(g), and subsequently also in Figures 3(e) and 3(h), to hydraulic loading rates exceeding the maximum load of 343 L d^{-1} would need to be verified.

An increase in peak loading (number of flushes per unit time) may have a greater impact on the filter performance than the daily hydraulic load at constant flushing intervals. This could cause washing out of retained particles in both filtration steps; however, this has not been investigated yet. In static filter columns, an inverse correlation of filter performance to the percolation velocity was shown (Lens *et al*. 1994). Considering the infiltration area of 3.1 dm^2 provided by the infiltration funnel, an average percolation velocity of 430 cm h^{-1} was calculated for the applied loading rate. This is three orders of magnitude higher than the highest percolation velocity of 0.42 cm h^{-1} applied by Lens *et al*.

(1994), but as pointed out above, the treatment performance of our filter is comparable.

Onsite test at Britannia Hut

In addition to the laboratory experiments, a pilot-scale test of the mechanical filter system was performed at Britannia Hut, a mountain in Switzerland, over two seasons. The system treated the blackwater discharge from two vacuum toilets with a 0.7–0.8 L flushing volume. These toilets were used daily by 10–60 guests, The TSS in the raw blackwater was 7–11 g L^{-1} (not shown) and slightly higher than the inlet in the laboratory experiment (Figure 2(a)). The hydraulic load ranged from 50 to 430 flushes, which translates into 40–340 L blackwater per day. The system obtained a TSS reduction of 70–90% and an effluent TSS in the range of 1.2–2 g L^{-1} (not shown). The flushing data logged during the onsite test at Britannia Hut showed diurnal peaks ranging from 30 to 70 toilet flushes per hour, translating to a peak flow rate of 0.38–0.88 L min^{-1}. These figures indicate that the peak flow rate of 73 L min^{-1} applied in the laboratory experiment is reasonable for a system treating blackwater from a mountain hut with up to 50 guests. The daily energy consumption per 100 toilet flushes was 0.003–0.004 kWh for the mechanical filtration system only, and 0.4–0.5 kWh including the vacuum sewer system (vacuum-on-demand VOD™, Jets Vacuum AS, Hareid, Norway). For solar-powered operation of the system at Britannia Hut, approximately 2 m^2 photovoltaic cells would be required to treat a daily load of 100 toilet flushes.

Design evaluation and practical application

The design of the tested filter is an outcome of our previous study where we concluded that organic percolation filters can be significantly improved by implementing mechanical filter media renewal (Todt *et al*. 2014b). The presented two-step filtration system was developed with the help of preliminary trials based on the idea to integrate an organic percolation filter into a simple drum-scree as typically used in municipal treatment plants (Tchobanoglous *et al*. 2002). In its present configuration, and with the applied conveying velocity, the system worked satisfactorily hydraulically both during the laboratory experiment and in the onsite test at Britannia Hut. An issue that needs to be improved for practical application is the filter media transport into the filter tube. Based on the results presented in the study, the maximum capacity of the system is estimated to be 20–40 PE, considering a peak loading rate that does not exceed the tested 40-toilet flushes per hour. On higher peak loading rates, a preceding equalizing tank may be needed. However, such a tank will raise the energy consumption due to the need for an additional feed pump.

CONCLUSION

A compact novel filter system was developed as a primary treatment system for the blackwater fraction of source-separated sanitary systems, or other types of wastewater with a high TSS concentration. The filtration unit showed a removal capacity of 78–85% for TSS, 60–80% for COD and 42–57% for phosphorus when treating blackwater from a vacuum toilet system. This reduction is comparable to organic filter systems that have a much lower hydraulic capacity per filter area. With a dry matter content of 13–20% the retentate is suitable for further processing by composting or drying with solar heat. The results show a positive correlation between filter performance and inlet concentration. The data on the impact of hydraulic load indicate that the system is feasible for applications up to 40 PE.

ACKNOWLEDGEMENTS

This study was greatly supported by the European Commission (FP7), and the companies: Jets Vacuum AS, Norway; Seecon GmbH Switzerland; Gysi-Berglas AG, Switzerland; Bioazul, Spai; SiA Norplast, Latvia; the Swiss Alpine Club, and special thanks go to the staff at Britannia Lodge. Grateful thanks also to Risch Tratschin (Seecon GmbH) for helping with the data collection at Britannia Hut.

REFERENCES

Ali, S. A. 2013 Design and evaluate a drum screen filter driven by undershot waterwheel for aquaculture recirculating systems. *Aquacultural Engineering* **54**, 38–44.

Faure, Y. H., Baudoin, A., Pierson, P. & Ple, O. 2006 A contribution for predicting geotextile clogging during filtration of suspended solids. *Geotextiles and Geomembranes* **24**, 11–20.

Henze, M. & Comeau, Y. 2008 Wastewater characterization. In: *Biological Wastewater Treatment* (M. Henze, M. C. M. Van Loosdrecht, G. A. Ekama & D. Brdjanovic, eds). IWA Publishing, London.

Hua, G. F., Zhu, W., Shen, J. Q., Zhang, Y. H. & Zeng, Y. T. 2013 The role of biofilm in clogging process in vertical flow

constructed wetland. *Applied Engineering in Agriculture* **29**, 61–66.

Lens, P. N., Vochten, P. M., Speleers, L. & Verstraete, W. H. 1994 Direct treatment of domestic wastewater by percolation over peat, bark and woodchips. *Water Research* **28**, 17–26.

Meinzinger, F. & Oldenburg, M. 2009 Characteristics of source-separated household wastewater flows: a statistical assessment. *Water Science and Technology* **59**, 1785–1791.

Ruiken, C. J., Breuer, G., Klaversma, E., Santiago, T. & Van Loosdrecht, M. C. M. 2013 Sieving wastewater – Cellulose recovery, economic and energy evaluation. *Water Research* **47**, 43–48.

Rusten, B. & Odegaard, H. 2006 Evaluation and testing of fine mesh sieve technologies for primary treatment of municipal wastewater. *Water Science and Technology* **54**, 31–38.

Taylor, M., Clarke, W. P. & Greenfield, P. F. 2003 The treatment of domestic wastewater using small-scale vermicompost filter beds. *Ecological Engineering* **21**, 197–203.

Taylor, M., Clarke, W. P., Greenfield, P. F. & Swain, G. J. 2004 Characterizing the physical and chemical properties of a vermicompost filter bed. *Compost Science & Utilization* **12**, 383–391.

Tchobanoglous, G., Burton, F. L., Stensel, H. D. & Metcalf & Eddy 2002 *Wastewater Engineering: Treatment and Reuse*. McGraw-Hill Professional, New York.

Todt, D., Heistad, A. & Jenssen, P. D. 2014a Load and distribution of organic matter and nutrients in a separated household wastewater stream. *Environmental Technology* 1–28.

Todt, D., Jenssen, P. D., Klemencic, A. K., Oarga, A. & Bulc, T. G. 2014b Removal of particles in organic filters in experimental treatment systems for domestic wastewater and black water. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering* **49**, 948–954.

Zhao, L. F., Zhu, W. & Tong, W. 2009 Clogging processes caused by biofilm growth and organic particle accumulation in lab-scale vertical flow constructed wetlands. *Journal of Environmental Sciences-China* **21**, 750–757.

First received 6 December 2014; accepted in revised form 25 February 2015. Available online 6 March 2015

PAPER 4

Mechanism leading to N_2O production in waste water treating biofilm systems – a review

Daniel Todt, Peter Dörsch

in preparation

Mechanism leading to N_2O production in waste water treating biofilm systems – a review

Daniel Todt* and Peter Dörsch

Norwegian University of Life Sciences, Institute for Environmental Sciences, Fougnerbakken 3, 1432 Ås

*danitodt@gmail.com

Abstract

The main groups of microorganism that mediate N removal in wastewater treatment were reviewed with focus on potential mechanism for N_2O production. A biofilm typically mediates a coexistence of aerobic and anoxic zones as well as close interactions between different groups of nitrogen converting organisms. Ammonia oxidizing bacteria play an important role for N_2O production under aerobic conditions, while heterotrophic denitrifers dominate N_2O production under anoxic conditions. In ammonia oxidizers, N_2O production can be coupled to chemical reactions of short-lived N-species released during periods of intense ammonia oxidation, to nitrifier denitrification during periods of limited O_2 availability and to nitrosative stress caused by accumulation of nitrite. Heterotrophic denitrifiers, by contrast, produce N_2O as a true intermediate during anoxic respiration. The proportion of N_2O among gaseous denitrification products (NO, N_2O, N_2) in waste water treatment systems increases during transient oxygen intrusion, when NO or HNO_2 accumulate or when electron donors become limiting. Potential N_2O emitting pathways in nitrite oxidizing bacteria and Anammox are little investigated so far. In biofilm systems, accumulation of nitrite seems to play a key role in the production of N_2O in both aerobic and anoxic zones. N_2O production tends to be low in purely autotrophic biofilms, most likely because Anammox bacteria, that do not have any known N_2O producing metabolism, dominate anoxic zones and reduce nitrite accumulated in oxic zones. Heterogeneous biofilm systems, supporting autotrophic and heterotrophic organisms, seem to be particularly conductive to high N_2O production when loaded with both organic and ammonia substrates. This can be attributed to frequent shifts in biofilm oxygenation, but also to incomplete heterotrophic dentrification by temporary electron donor limitation in anoxic biofilm zones. Current models of mass transfer and biomass interactions in biofilms are discussed in an attempt to elucidate the complex regulation of N_2O production in waste water treating biofilms.

Keywords

N_2O, NO, NO_2^-, biofilm, AOB, NOB, Anammox, hetertrophic denitrification

Contents

1 INTRODUCTION

In the last decades, wastewater treatment plants have received increasing scientific interest as important point sources for N_2O emissions in the anthropogenic nitrogen cycle (Kampschreur et al., 2009b, Schreiber et al., 2012). N_2O is a strong greenhouse gas contributing to global warming and stratospheric ozone depletion (Ravishankara et al., 2009). Even though wastewater treatment only accounts for an estimated 1.3% of the total anthropogenic N_2O production, its contribution to the total greenhouse gas production associated with the human water supply and sanitation chain may be as high as 26% (Kampschreur et al., 2009b). This includes production of drinking water as well as treatment of wastewater.

Numerous studies and review articles about N_2O emissions from wastewater treatment systems have been published (e.g. Kampschreur et al., 2009b, Wunderlin et al., 2012, Tallec et al., 2006). However most of these studies focus on suspended biomass cultures (activated sludge), while biofilm systems have received little attention so far. At the same time, biofilm systems enjoy increasing popularity owing to their robustness and efficiency, and their ability to combine several microbial processes within one system (Morgenroth, 2008a, Khan et al., 2013). However, the evaluation of N_2O producing processes in biofilm systems is notably more complex than in suspended biomass cultures, since diffusion resistance and biomass competition in space and time need to be considered (Morgenroth, 2008b, Wanner and Gujer, 1985). Therefore, knowledge about the critical factors and biomass interactions leading to N_2O production in biofilms is limited (Schreiber et al., 2012).

This review links known biomass interactions in wastewater treating biofilm systems to nature and rate of microbial N transforming processes to assess their possible role in N_2O production. The first chapter introduces the biochemistry of N_2O producing reaction chains found among the main functional groups of organism relevant to wastewater treatment systems. The second chapter deals with the regulation of N_2O production in the various metabolic reactions of microbial N transformations. Using current models of mass transfer and biomass interactions in biofilms, the third chapter explores potential N_2O producing mechanism in a biofilm and tries to link them to environmental factors that are usually measured and controlled in wastewater treatment systems.

2 KEY ORGANISMS AND ENZYMATIC PROCESSES INVOLVED IN BIOLOGICAL NITROGEN CONVERSION

2.1 Ammonia oxidizing bacteria (AOB)

Ammonia oxidizing bacteria (AOB) are obligate chemolitoautotrophic, sometimes mixotrophic bacteria of the phylum Proteobacteria, which generate energy for growth and maintenance from the oxidation of ammonia (NH_3) to nitrite (NO_2^-). This group comprises several dozen species, which are adapted to different environments (e.g. Bothe et al., 2000). AOB are the key species mediating nitrification, i.e. the microbial conversion of NH_4^+ to NO_2^- in wastewater treatment systems and have therefore been at the core of interest as potential sources for N_2O emissions (Kampschreur et al., 2009b). Here, we focus on *Nitrosomonas sp.*, which is the most studied and most important AOB genus in wastewater treatment systems (Law et al., 2012, Schreiber et al., 2009) and review metabolic pathways of N_2O production associated with NH_3 and NO_2^- oxidation.

2.1.1 AOB metabolism

AOB oxidizes ammonia (NH_3) to hydroxylamine (NH_2OH) and further to nitrite (NO_2^-). This two-step reaction is performed by the membrane bound enzyme ammonia monooxygenase (AMO) and the periplasmic enzyme hydroxylamine dehydrogenase (formerly hydroxylamine oxidoreductase) (HAO), respectively (Fig. 1). The HAO reaction releases 1 mole of NO_2^-, 4 moles of electrons and five moles of H^+ per mole NH_2OH oxidized (Wood, 1986). Two of the electrons are shuttled back to AMO where they aid the oxidation of NH_3 to NH_2OH. The electron transport chain is mediated by the hemeproteins cytrochrome C554, cytochrome C552 and a quionol pool (Whittaker et al., 2000, Arp et al., 2002). The other 2 electrons are used to generate a proton motive force through reverse electron transport (Schmidt and Bock, 1998, Whittaker et al., 2000, Wood, 1986). Together, the nitrification of 1 mole of NH_3 releases 3 moles of protons resulting in the overall reaction 1.

$NH_3 + 1.5\ O_2 \Rightarrow NO_2^- + H^+ + H_2O$ (1)

The design of wastewater treatment processes is usually based on total ammonia nitrogen (TAN) which can easily be measured (e.g. Ekama and Wentzel, 2008, Gujer et al., 1999, Kaelin et al., 2009). However, the true substrate for AMO is free NH_3 which is in equilibrium with undissociated NH_4^+ (Hyman and Arp, 1995, Suzuki et al., 1974). Owing to the low acid dissociation of NH_4^+ in water (*p*Ka NH_3/NH_4^+ 9.24) and its dependency on temperature and pH, the acid-base equilibrium between ammonium (NH_4^+) and the AOB substrate NH_3 is an important factor, which needs to be taken into account when modeling nitrification based on TAN, especially in systems operating at low pH (Quinlan, 1984, Kaelin et al., 2009). In wastewater treatment systems, highest nitrification usually occurs around pH 8.5, which was also shown to be the optimum pH for N_2O derived from nitrification intermediates (Law et al., 2012).

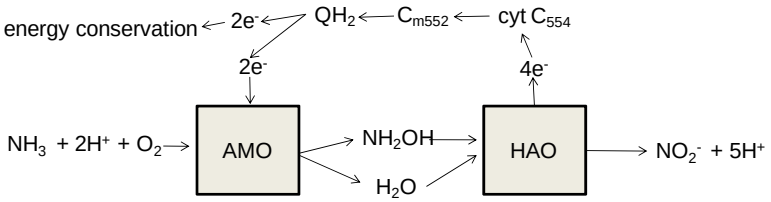


Figure 1: Electron flow during oxidation of ammonia (NH3) to nitrite (NO2-) in Nitrosomonas sp. driven by a two-step reaction involving the enzymes ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO). Hydroxylamine oxidation generates 4 electrons which are channeled via the hemeproteins cytochrome C554 and cytochrome Cm552 to a quinine pool (QH2) where two of the four electrons are transferred to an electron chain for energy conservation (adapted from Arp et al., 2002)

The oxidation of ammonia by AMO is mediated along a complex reaction chain, which is not yet completely unraveled. A hypothesis put forward by Schmidt et al. (2001a) proposes a two-step reaction based on an internal recirculation of the oxidant dinitrogen tetroxide (N_2O_4) (Fig. 2). In this so-called NO_x cycle, NH_3 is oxidized with N_2O_4 releasing NH_2OH and nitric oxide (NO). The latter is reoxidized with O_2 at another active site of the enzyme to nitrogen dioxide (NO_2) which is in equilibrium with its dimeric form N_2O_4 (Schmidt et al., 2001a). Under aerobic conditions, the presence of NO or NO_2 was shown to significantly enhance the NH_3 oxidation rate (Schmidt et al., 2001a, Zhang et al., 2010, Beyer et al., 2009). However, in another study, the NO scavenger PTIO had little effect on the ammonia oxidation rate of *Nitrosospira multiformis* (Shen et al., 2013). These findings question a significant role of NO and the proposed NO_x cycle for ammonia oxidation in *Nitrosospira multiformis*. An alternative hypothesis proposes that NO_2 or N_2O_4 act as direct oxidants in addition to O_2 (Zhang et al., 2010). PTIO was found to significantly reduce NH_3 oxidation in the ammonia-oxidizing archaea *Nitrososphaera viennensis* which suggests that different groups of ammonia oxidizing organism perform the same function (NH_3 oxidation) by different metobolism.

Studies with *N. eutropha* showed that NH_3 oxidation can take place in the absence of O_2, when N_2O_4 or NO_2 are supplied (Schmidt and Bock, 1997, Schmidt et al., 2001a). This so-called anaerobic ammonia oxidation was shown to release NO, since the hypothesized re-oxidation to NO_2^- occurs only in the presence of O_2 (Schmidt et al., 2001a). However, the relevance of such a putative anaerobic AMO activity in nature is questionable, since the atmospheric concentration of the mandatory oxidant NO_2 is far too low to allow for significant reaction rates and NO_2 is rarely produced in absence of O_2 (Schmidt, 2008, Zart et al., 2000). In biofilm systems, NO_2 can be produced from autoxidation of NO in outer, aerobic layers and transported to deeper, anaerobic layers by diffusion, which may enhance the overall NH_3 oxidation rate and NH_3 flux in the biofilm (Schmidt, 2008).

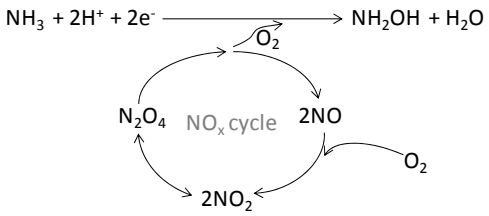


Figure 2: AMO reaction chain driven by a putative NO_x cycle (adapted from Schmidt et al., 2001a)

The conversion of NH_2OH to NO_2^- by the HAO enzyme is a complex reaction involving several intermediates (Fig. 3). NH_2OH binds to an active site P_{460}, an iron containing multi-heme, where it is oxidized via the intermediates nitroxyl (HNO) and nitric oxid (NO) to nitrite. In this process, the Fe center in P_{460} changes its oxidation state from Fe^{III+} to Fe^{II+} and back to the initial state Fe^{III+} (Kostera et al., 2008). Both intermediates HNO and NO are strong radicals with destructive impact to the cell metabolism if released under certain circumstances (see section 2.1.3)

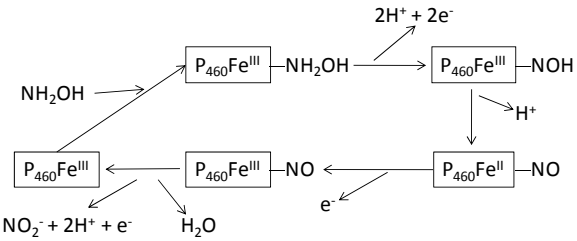


Figure 3: Oxidation of NH_2OH to NO_2^- catalyzed by hydroxylamine dehydrogenase involving the reduction and re-oxidation of iron in the multi-heme P_{460} (adapted from Kostera et al., 2008)

2.1.2 Nitrifier denitrification

N. europaea and other AOB species have been shown to be equipped with a respiratory metabolism which reduces NO_2^- to NO, N_2O or N_2 (Schmidt and Bock, 1997, Poth and Focht, 1985, Hooper, 1968). Main components of this so-called nitrifier denitrification are the enzymes nitrite reductase (NIR) (Casciotti and Ward, 2001, Hooper, 1968) and nitric oxide reductase (NOR) (Beaumont et al., 2004b). NIR performs the first reduction step from NO_2^- to NO and NOR the second step from NO to N_2O (Kostera et al., 2008, Schmidt et al., 2004a). Both NIR and NOR seem to have unidentified homologues. *N. europea* mutants lacking NirK (one of the known NIR) were shown to reduce NO_2^- via an unidentified NIR at rates comparable to the wild type. Hence, contrary to earlier assumptions, NirK seems to play little role for nitrifier denitrification or anoxic growth but is essential for efficient NH_2OH oxidation under oxic conditions (Kozlowski et al. 2014). In contrast, *norB*, the gene coding for NOR was shown to be essential for AOB's ability to reduce NO_2^-, suggesting that NOR is the sole NO reductase in the nitrifier denitrification pathway (Kozlowski et al., 2014). Notwithstanding, *norB*-deficient *N.europaea* strains have been shown to reduce added NO to N_2O (Beaumont et al., 2004b). These findings point to two alternative NO reduction pathways: NO that is produced via reduction of NO_2^- is reduced by NOR in the course of energy conversation (Schreiber et al., 2009, Kozlowski et al., 2014) and NO that is released by ammonia oxidation (NO_x cycle, AMO intermediates, Fig. 2) is reduced by an alternative NO-reductase, likely connected to Cytochrome c_{554} in course of detoxification (Upadhyay et al., 2006). *N. europaea* (Schmidt et al., 2004b), *N. eutropha* (Schmidt and Bock, 1997, Schmidt and Bock, 1998) and likely also other AOB species (Beyer et al., 2009) are able to perform complete reduction of NO_2^- to N_2 (via NO and N_2O), even though no homologue of N_2O reductase (N_2OR) has been found in AOB (Chain et al., 2003). However, in AOB, Nitrosocyanin (nsc), a protein similar to N_2OR in structure, could overtake this function, thus making AOBs fully fledged denitrifiers (Arciero et al., 2002, Schmidt, 2009, Whittaker et al., 2000, Beyer et al., 2009).

The physiological role of the denitrification pathway in the AOB metabolism is not completely understood. Early studies on *N. europaea* showed that the denitrification enzymes can sustain respiratory chemoorganotrophic growth under anoxic conditions using hydrogen or organic compounds as electron donors and NO_2^-, NO and N_2O as electron acceptors (Bock et al., 1995, Schmidt et al., 2001b, Schmidt, 2009). However, denitrification rates of AOB are low compared to rates of heterotrophic denitrifiers. Therefore, chemoorganotrophic growth seems mainly to be connected to survival metabolism rather than being a truly alternative growth mode during anoxia (Schmidt, 2009). AOB need NH_3 for biomass synthesis and growth regardless the growth mode (Abeliovich and Vonshak, 1992). Later studies showed that nitrifier denitrification also takes place under fully aerobic conditions with NH_3 as electron donor (Beaumont et al., 2004a, Schmidt et al., 2004b, Lawton et al., 2013). In this reaction, the electron transfer from NH_3 to NO_2^- is assumed to involve the nitrification enzymes (AMO, HAO) and the denitrification enzyme NIR (Jason et al., 2007, Whittaker et al., 2000). Hence, NIR may act as an additional electron sink during periods of vigorous NH_3 oxidation, by increasing the NH_2OH oxidation rate to avoid accumulation of toxic intermediates (NH_2OH, NOH) and thus enhance energy conservation and cell growth (Beyer et al., 2009, Schmidt et al., 2004b, Jason et al., 2007). The denitrification metabolism may further provide AMO with the co-substrate NO for the putative NO_x cycle and thereby enhance NH_3 oxidation activity (Laanbroek et al., 2002, Schmidt et al., 2004b).

2.1.3 Potential N_2O emitting pathways in the AOB metabolism

Recent studies found a positive correlation between the rates of NH_3 oxidation and N_2O emission in aerobic reactor systems (Law et al., 2011, Law et al., 2012, Yu et al., 2010). Several studies point at the release of the intermediates NH_2OH, NOH or NO during imbalanced NH_3 oxidation at high reaction rates as the main source for N_2O under aerobic conditions (Law et al., 2012, Wunderlin et al., 2013). The exact reactions are not yet clarified, but a recent study identified two main biotic pathways for N_2O production, both connected to the release of HAO intermediates (Wunderlin et al., 2013): a) release of NOH and subsequent chemical decomposition to N_2O (Law et al., 2012, Poughon et al., 2001b) or b) release of NO and subsequent enzymatic reduction by cytochrome c_{554} to N_2O (Hooper and Terry, 1979, Kostera et al., 2010, Kostera et al., 2008, Upadhyay et al., 2006).

Nitroxyl (NOH) is an early intermediate of the HAO reaction (Figure 3) that can be released during imbalanced NH_3 oxidation and subsequently decomposes to N_2O (Poughon et al., 2001b, Law et al., 2012, Stuven and Bock, 2001). The NH_2OH uptake rate of HAO is assumed to be significantly faster than the electron transfer to the cytochrome complex (Kostera et al., 2010). Hence, with high NH_2OH oxidation rates, the electron transfer may become a bottleneck. Stuven and Bock (2001) proposed that HAO can transfer only two electrons to the cytochrome complex under high NH_2OH oxidation rates while releasing HNO. Several studies pointed out that regardless the lowered electron release, the electron transfer rate to the cytrochrome complex may still exceed the reducing potential available for energy conservation. Under conditions of continuous electron excess, AMO activity and subsequent N_2O production via NHO release by HAO can remain high or even increase as a self-accelerating process (Law et al., 2011, Law et al., 2012, Wunderlin et al., 2013). This hypothesis would explain the observed exponential relationship between NH_3 oxidation rates and N_2O emissions (Law et al., 2012).

NO can be released from the $P_{460}Fe^{III}$-NO complex, during the final step of HAO metabolism (Kostera et al., 2008, see Figure 3). Its further reduction to N_2O is likely performed by the protein cytochrome c_{554} (Upadhyay et al., 2006), which is believed to be the immediate acceptor for electrons transferred by the HAO enzyme (Upadhyay et al., 2006, Yamanaka and Shinra, 1974). Therefore, the putative NO reducing activity of cytochrome c_{554} is likely driven by electrons supplied by the HAO process. In presence of O_2, NO is oxidized abiotically to NO_2, which again may fuel the NO_x cycle and AMO activity (Schmidt et al., 2001b). However, this chemical reaction does probably not reach significant rates under normal growth conditions (Schmidt et al., 2001b, Udert et al., 2005). Upahadyay et al. (2006) proposed that the release of NO is a consequence of a decreased electron transfer rate from HAO to the cytochrome complex, which may be provoked when O_2 depletes to a level exceeding the capacity of AMO for receiving electrons from NH_2OH oxidation (Kostera et al., 2008, Upadhyay et al., 2006). Acidity was shown to be another potential factor provoking incomplete HAO reaction (Jiang and Bakken, 1999). Under strongly acidic conditions, abiotic N_2O formation by N-nitrosation of NH_2OH and NO_2^- has been proposed (Spott et al., 2011, Freeman, 1973).

Chemical decomposition of the intermediate NH_2OH as a source of N_2O from NH_3 oxidation was proposed in the early literature (Yoshinari, 1990, Bremner et al., 1980). NH_2OH may accumulate when the NH_2OH oxidation becomes slower than the NH_3 oxidation, for example in the case of limited availability of reducing equivalents (Wunderlin et al., 2013). Recent *in vitro* experiments showed that NH_2OH decomposition does not reach significant rates under normal growing conditions (Jason et al., 2007, Wunderlin et al., 2012, Harper et al., 2009). However, since this abiotic reaction likely includes the mandatory oxidation of NH_2OH to NOH, which requires NO_2^- as electron acceptor, the chemical decomposition of NH_2OH to N_2O may be of particular relevance when NO_2^- is present at high concentrations (Tallec et al., 2006).

It is still under debate if and to what extend nitrifier denitrification contributes to N_2O emissions. Some studies found a positive (Cua and Stein, 2011, Kim et al., 2010), while others found no (Beaumont et al., 2002) or even a negative correlation (Jason et al., 2007) between the expression of NIR related genes and N_2O appearance. *In vitro* studies showed that NIR deficient strains of *N. europaea* released four times more N_2O than the wild type (Beaumont et al., 2002), while NOR deficiency did not significantly affect N_2O production (Beaumont et al., 2004b). These findings would indicate that nitrifier denitrification is not the major source of N_2O under normal growth conditions and support the hypothesis that NIR may have a supportive function for the nitrification activity by providing an additional electron sink for the HAO enzyme (cf. 2.1.2). A recent study proposed the existence of an unknown NIR homologue (Kozlowski et al., 2014), which challenges the above conclusions. Especially under conditions favoring accumulation of NO_2^- (Cua and Stein, 2011) or low O_2 tension (Kozlowski et al., 2014), nitrifier denitrification or the reduction of NO_2^- may become the major contributor to N_2O.

Table 1 gives an overview over findings from pure culture studies with AOB in which the proportion of N_2O originating from NO_2^- was determined with help of ^{15}N labeling. The study from Shaw et al. (2006) indicates that this proportion may vary among different AOB strains. Comparing the figures of Poth and Focht (1985) with those of Schmidt et al. (2004b) indicates that high NO_2^- increases the total amount of N_2O produced relative to the amount of NH_3 oxidized, the proportion of N_2O having NO_2^- as N source and the N_2O/N_2 ratio. The higher N_2O/N_2 ratio might be related to a hypothesized incomplete nitrifier denitrification with high NO_2^- (Cua and Stein, 2011, Poth and Focht, 1985). This hypothesis is supported by a pure culture study with *N. europaea* in which N_2O production substantially increased when NO_2^- concentrations exceeded 5 mM (Yu and Chandran, 2010). NO_2^- was also shown to be inhibitory (at >5 mM) or even toxic (at 20 mM) to AMO, especially at low pH, where it forms nitrous acid (HNO_2) (Yu and Chandran, 2010, Stein and Arp, 1998). The increased N_2O production at high NO_2^- may therefore be a detoxification process via nitrifier denitrification to countervail nitrosative stress. (Yu and Chandran, 2010, Stein and Arp, 1998). Accordingly, many studies attribute nitrifier denitrification to detoxification of NO_2^- (Beaumont et al., 2002, Beaumont et al., 2005, Cua and Stein, 2011), which however is questioned by others owing its relatively low reaction rates (Schmidt, 2008). The popular hypothesis of enhanced N_2O production due to NO_2^- detoxification is also challenged by a recent study in which NO_2^- increased N_2O production up to a level of 1mM while a further increase of NO_2^- up to 10 mM decreased the N_2O production rate (Law et al., 2013). The authors attributed their findings to substrate inhibition of NIR above a certain threshold in NO_2^- concentration (Law et al., 2013). However, these findings stem from a mixed AOB culture in a highly loaded partial nitrification reactor, which may suggest that continued high concentration of NO_2^- alters the AOB community towards species that are less sensitive to NO_2^- and produce less N_2O.

Comparing the studies on *N. europaea* by Poth and Focht (1985) and Shaw et al. (2006) (Table 1), DO seems to have little impact on N_2O production under aerobic conditions (DO > 1 mg O_2 L^{-1}). These findings are consistent with Kester et al. (1997b) who showed that the N_2O emission factor (relative to transformed NH_3) of *N. europaea* does not differ significantly at steady state in a DO range of 0.5 to 6.7 mg O_2 L^{-1}. However, decreased DO was shown to result in temporary peaks of both N_2O and NO, which were most pronounced in the low DO range of 0.1 - 1 mg O_2 L^{-1} (Kester et al., 1997a). Under micro-aerobic conditions, usually referring to a DO of 0.1- 0.5 mg O_2 L^{-1}, N_2O emissions of AOB were shown to be markedly greater than under aerobic conditions (Kester et al., 1997b, Jia et al., 2013). Enhanced production of N_2O (and NO) under limited O_2 availability is often attributed to incomplete nitrifier denitrification (Jia et al., 2013). This might again be a result of the above discussed reduced electron supply from AMO under limited O_2 availability.

Table 1: Results from ^{15}N labelling experiments that investigated the N-source of N_2O emission in AOB. The ratios are calculated based on N equivalents.

Study	Species	DO level mg O_2 L^{-1}	NO_2^- mg N L^{-1}	NH_4^+ mg N L^{-1}	N_2O % oxidized NH_3	% N_2O having NO_2^- as N source	Ratio N_2 / N_2O
Poth and Focht (1985)	*N. europaea*	1	5-24	21	0.6-0.65	25	7-7.5
Schmidt et al. (2004b)	*N. europaea*	5	70-200	140	0.7	94	20
Shaw et al. (2006)	*N. europaea*	4-6	14-16	49	0.45	31	
	N. europaea	4-6	14-17	49	0.19	22	
	Nitrosospira briensis	4-6	14-18	49	0.03	14	
	Nitrosospira multiformis	4-6	14-19	49	0.08	12	
	Nitrosospira tenuis	4-6	14-20	49	0.7	50	

Even though AOB belong to the best studied groups of nitrogen transforming bacteria, the biochemical reactions of their metabolism producing N_2O are far from understood. Several potential N_2O producing pathways exist, but it is not clear what the critical factors are that determine *in situ* N_2O production. In summary, N_2O can be produced by at least two metabolic pathways: ammonia oxidation involving the enzymes AMO and HAO and NO_2^- reduction involving the dissimilatory enzymes NIR, NOR and the final reduction of N_2O to N_2, which is putatively mediated by the nsc protein. N_2O released during ammonia oxidation is likely the result of an imbalanced electron flow with subsequent release of intermediates that occurs at high NH_3 oxidation rates or acid stress. Likewise, N_2O produced by incomplete nitrifier denitrification seems to be the result of an electron imbalance that may occur in the presence of high NO_2^- concentrations or limited oxygen availability (micro aerobic conditions).

2.2 Nitrite oxidizing bacteria (NOB)

Nitrite oxidizing bacteria, NOB, refer to a group of gram-negative bacteria gaining their energy from oxidizing NO_2^- to NO_3^- (Spieck and Lipski, 2011). Wastewater treatment systems are usually dominated by the two genera, *Nitrobacter sp*. and *Nitrospira sp.* (Blackburne et al., 2007). Which of these species dominates depends on the total ammonia (NH_3; NH_4^+) load, but also on O_2 availability and other environmental conditions (Okabe et al., 1999). The oxidation is catalyzed by a complex multi-enzyme reaction chain (Sundermeyer-Klinger et al., 1984, Spieck and Lipski, 2011). In brief, the membrane bound nitrite oxidoreductase (NXR) oxidizes NO_2^- to NO_3^-. This enzymatic reaction derives the oxygen needed from dissociation of H_2O and releases 2 protons and 2 electrons. The latter are transferred to the hemeprotein cytochrome-c oxidase, which reduces the final electron acceptor O_2 to O^{2-}, which again associates with the protons released by NXR to H_2O (Sundermeyer-Klinger et al., 1984), resulting in the overall equation 2, which is also used for parameterizing O_2 consumption during NO_2^- oxidation (Kaelin et al., 2009) as well as for studying NOB kinetics (Ciudad et al., 2006).

$$NO_2^- + 0.5\ O_2 \Rightarrow NO_3^- \qquad (2)$$

NOB are little studied owing to their poor growth in pure cultures (Spieck and Lipski, 2011). Recent studies support the notion that NO_2^- rather than its protonated form nitrous acid (HNO_2) (*p*Ka NO_2^-/HNO_2 3.39 in water) is the true substrate for NXR (Park and Bae, 2009, Poughon et al., 2001a, Udert and Jenni, 2013). However, *NOB* seem to be able to deprotonate HNO_2 prior to its oxidation (Park and Bae, 2009). NOB have received little attention in the literature so far with respect to N_2O production. *Nitrobacter sp.* and *Nitrospira sp.* were shown to have an anoxic growth mode reducing NO_3^- with pyruvate or H_2 as electron donor (Colliver

and Stephenson, 2000, Sundermeyer-Klinger et al., 1984, Maia and Moura, 2014). This metabolism is little studied but some authors assume that the terminal products are NO or N_2O (Starkenburg et al., 2008). In contrast to nitrifier denitrification by AOB, which was observed at variable O_2 availability (Beaumont et al., 2004a, Schmidt et al., 2004b), NO_3^- respiration by NOB is only active under anoxic conditions (Colliver and Stephenson, 2000). A potential contribution of NOB to N_2O emissions seems therefore only relevant under oxygen limiting or anoxic conditions.

It has been frequently stated that high NH_3 inhibits NOB (Anthonisen et al., 1976, Park and Bae, 2009). However, NOB can adapt to elevated NH_3 concentrations (Yoo et al., 1999). Inhibition is species dependent and reported threshold concentrations vary from 0.04-0.08 mg NH_3-N L^{-1} for *Nitrospira sp.* to 30-50 mg NH_3-N L^{-1} for *Nitrobacter sp.* (Blackburne et al., 2007). HNO_2, the protonated form of NO_2^- is another frequently cited inhibitor of NOB (Hellinga et al., 1999, Park and Bae, 2009, Zhou et al., 2011). In general, critical HNO_2 levels can be expected when NO_2^- accumulates at low pH. This typically occurs in weakly buffered systems with high nitrification rates owing to the proton release during AOB metabolism (Eq. 1) (Udert et al., 2005).

2.3 Heterotrophic denitrifiers

In wastewater treatment, heterotrophic denitrifiers refer to a large number of taxonomically unrelated chemoorganotrophic bacteria, which use NO_3^- or NO_2^- as terminal electron acceptors in the absence of oxygen and reduce them to gaseous NO, N_2O and N_2. Canonical denitrification comprises four consecutive reduction steps with the intermediates NO_2^-, NO and N_2O as substrates, involving the reductase enzymes nitrate reductase (NAR) nitrite reductase (NIR), nitric oxide reductase (NOR) and N_2O reductase N_2OR (Zumft, 1997). The overall chemical reaction is shown in equation 3 for the reduction of NO_3^- and reaction 4 for the reduction of NO_2^-.

$NO_3^- + 6H^+ \Rightarrow 0.5\ N_2 + 3H_2O$ (3)

$NO_2^- + 4H^+ \Rightarrow 0.5\ N_2 + 2H_2O$ (4)

Denitrification in a facultative metabolic trait, i.e. O_2 represses denitrification in favor of oxic respiration. O_2 tension also regulates the relative activity of the denitrification enzymes, and may thus determine the composition of the gaseous end products (Körner and Zumft, 1989). In microaerobic zones having a DO in the range of 0.2-0.5 mg L^{-1}, the expression of N_2OR, the enzyme reducing N_2O to N_2, may be repressed, resulting in high N_2O emissions (Bergaust et al., 2012, Bonin and Raymond, 1990). A second factor affecting the balance of the reduction steps is the pH. It was shown that pH below 6.8 impairs N_2OR functioning post-transcriptionally, i.e. no functional enzyme is synthesized at low pH. This was shown both in pure culture studies (Bergaust et al., 2010) and in soil bacteria (Liu et al., 2014).

Recent studies suggest that the reduction of N_2O to N_2 also depends on the availability of electron donors (Pan et al., 2013, Richardson et al., 2009). For instance, denitrification activity fuelled by endogenous electron donors in a biofilm was reported to produce substantial N_2O emissions accounting for up to 20% of biologically reduced N (Itokawa et al., 2001). This high N_2O yield was attributed to the low degradability of endogenous substrates as compared to readily degradable organic load, resulting in limited electron availability for the reduction of N_2O to N_2 (Pan et al., 2013).

Similarly to its role in nitrification, accumulation of NO_2^- in anoxic wastewater reactor systems has been pointed out to be an important factor for increased N_2O production by heterotrophic denitrification (e.g. Schulthess et al., 1995, Schreiber et al., 2012, Zhou et al., 2011). Campos et al. (2009) reported N_2O emissions accounting for up to 55% of totally reduced N when heterotrophic denitrification was fed with NO_2^- as electron acceptor as compared to 0.8% with NO_3^-. The exact mechanisms behind the enhanced N_2O production by heterotrophic denitrification in the presence of NO_2^- are not known, but nitrosative stress reactions triggered by HNO_2 or NO (both of which can be formed chemically from NO_2^-) leading to enzymatic or abiotic reduction have been proposed. HNO_2 is in acid/base equilibrium to NO_2^- (pKa NO_2^- /HNO_2 3.39 in water). NO may be formed via chemical (abiotic) reduction of HNO_2 (Stein and Arp, 1998). NO may also accumulate during denitrification of NO_2^- NOR is expression is delayed relative to NIR expression (Schulthess et al., 1995). HNO_2 is suspected to inhibit N_2OR which would lead to incomplete denitrification and release of N_2O instead of N_2 (Schreiber et al., 2012, Zhou et al., 2008b). In an anoxic activated sludge culture, an inhibition of N_2O reduction by HNO_2 was observed at extreme low

concentrations of 0-0.2 µg N L^{-1}. At HNO_2 concentration ranging from 0.7 to 1.0 µg N L^{-1}, a 50% inhibition of N_2O reduction was observed whereas 4 µg HNO_2 - N L^{-1} inhibited N_2O reduction completely. Based on these data, and the acid-base equilibrium constant for NO_2^-, a 50% inhibition at pH 7 would take place with NO_2^- concentrations of 3-4 mg N L^{-1} and at total inhibition with 17 mg N L^{-1} or higher (Zhou et al., 2008b). The presence of free NO was shown to have an immediate, irreversible inhibitory effect on the N_2OR enzyme, while leading to enhanced NIR activity (Frunzke and Zumft, 1986). Hence, regardless whether NO is formed biotically or abiotically during periods of high NO_2^-, it might result in a suppression of N_2O reduction in heterotrophic denitrification (Schulthess et al., 1995). Given the proposed irreversibility of the N_2OR inhibition, high N_2O production may be sustained over longer periods even if free NO only occurred as a temporary phenomenon.

2.4 Anaerobic ammonia oxidizing bacteria (Anammox)

A recently discovered group of obligate anaerobic, chemolitoautotrophic organisms gains energy for metabolism and growth from the oxidation of NH_4^+ to N_2 with NO_2^- as electron acceptor (Jetten et al., 1998, Mulder et al., 1995, Kuenen, 2008, van de Graaf et al., 1995). So far, only few Anammox species have been descried, all of them belonging to the class of *Planctomycetes*. The most studied ones are *Candidatus B. anammoxidans* (Strous et al., 1999a) and *Candidatus K. stuttgartiensis* (Schmid et al., 2000), which are also the main species in practical applications of wastewater treatment (Kuenen, 2008). Anammox-bacteria were shown to grow in extreme dense colonies, preferably biofilms, at extremely low growth rates and are therefore difficult to purify or isolate (Strous et al., 1999a). The Anammox metabolism converts NH_4^+, NO_2^- and protons into N_2, NO_3^- and H_2O as shown in the overall equation 5 (Kartal et al., 2013, Strous et al., 1999b).

$$1\ NH_4^+ + 1.32\ NO_2^- + 0{,}066\ HCO_3^- + 0.13\ H^+ \Rightarrow 0{,}066\ CH_2O_{0,5}N_{0,15} + 1.02\ N_2 + 0.26\ NO_3^- + 2.03\ H_2O \quad (5)$$

The coefficients for equation 5 where experimentally derived by Strous et al. (1999b) and should be verified and justified by further pure culture experiments (Lotti et al., 2014). NO_2^- is reduced to NO in a complex reaction chain (reaction 6) which again reacts with NH_4^+ to N_2H_4 (reaction 7). In a final step, N_2H_4 is oxidized to N_2 (reaction 8). This synproportionation of NH_4^+ with NO_2^- results in an energy gain of -357 kJ mol^{-1} (reaction 9) (Kartal et al., 2011).

$$NO_2^- + 2H^+ + e^- \Rightarrow NO + H_2O \ (E° = +0.38V) \quad (6)$$

$$NO + NH_4^+ + 2H^+ + 3e^- \Rightarrow N_2H_4 + H_2O \ (E° = +0.06V) \quad (7)$$

$$N_2H_4 \Rightarrow N_2 + 4H^+ + 4e^- \ (E° = -0.75V) \quad (8)$$

$$NH_4^+ + NO_2^- \Rightarrow N_2 + 2H_2O \ (\Delta G° = -357\ kJ\ mol^{-1}) \quad (9)$$

This reaction chain, performed by a series of recently discovered enzymes, is not yet fully clarified (Kartal et al., 2013). The concurrent oxidation of NO_2^- to NO_3^- is assumed to generate reducing potentials for CO_2 fixation (Lotti et al., 2014). Anammox bacteria have a little investigated optional organotrophic metabolism, which can reduce organic acids (propionate, acetate) to CO_2 with NO_3^- as electron acceptor (Güven et al., 2005). ^{15}N labelling experiments indicated that these electron acceptors are reduced to NH_4^+ with NO_2^- as intermediate (Kartal et al., 2007a). The purpose of this organotrophic metabolism may be energy conversation (Güven et al., 2005, Kartal et al., 2007b) or internal production of substrates (NH_4^+, NO_2^-) for the main metabolism (Kartal et al., 2007a). An assimilation of organic substrates into biomass was not observed (Güven et al., 2005, Kartal et al., 2007b). The organotrophic metabolism seems to be highly selective for volatile fatty acids (VFA). Other typical electron donors (e.g. glucose) had no significant effect or even inhibited (alcohols) Anammox metabolism (Güven et al., 2005).

Anammox uses NH_4^+ as a substrate (Kartal et al., 2013, Kartal et al., 2011). This distinguishes them from AOB which have NH_3 as a substrate. However, these substrates are in an acid-base equilibrium (*p*Ka NH_3/NH_4^+ 9.24 in water), hence the substrate availability and substrate competition between the organisms depends on pH and may be impacted by proton release (AOB) or proton uptake (Anammox). The Anammox metabolism is inhibited in the presence of O_2, but the inhibition was shown to be reversible even after exposure to a DO as high as 8 mg O_2 L^{-1} (Hu et al., 2013). Also NO_2^- has a reversible inhibitory effect on the organism at higher concentrations (IC_{50} 400 mg L^{-1}) (Lotti et al., 2012). Anammox shows at the same time

an extremely high tolerance to NO and was able to reduce it to N_2, most likely via the intermediate N_2H_4 with NH_4^+ as electron donor (Kartal et al., 2010).

According to present knowledge, Anammox bacteria do not produce N_2O (Hu et al., 2013). However, an earlier study showed that the heterotrophic organism *Pseudomonas aeruginosa*, can perform a biotic nitrosation of the Anammox intermediate N_2H_4 with HNO_2 to N_2O (Kim and Hollocher, 1984). It is possible that also Anammox bacteria perform a similar reaction under particular conditions. A recent pure culture study (Lotti et al., 2014) detected small emissions of N_2O (0.2% of removed N), which might have been produced by this pathway. Considerable N_2O emissions were reported from Anammox reactor systems, amounting to 0.4 – 1.3 % of the biologically transformed N (Hu et al., 2013). It was shown that emissions rates for NO and N_2O increase with increasing O_2 availability in the bulk liquid (Kampschreur et al., 2009a). However these data were obtained from systems with mixed biomass cultures of both AOB and Anammox organisms and hence N_2O may have been produced by AOB. This would be consistent with a study showing a tendency for decreased N_2O and NO production with increasing Anammox activity (Ni et al., 2013). More research is needed to clarify N_2O production potential by Anammox.

3 FACTORS CONTROLLING N_2O PRODUCTION IN BIOLOGICAL WASTEWATER TREATMENT SYSTEMS

Table 2 summarizes the key factors for N_2O production by AOB and heterotrophic denitrifiers as well as for abiotic N_2O production in biological wastewater treatment systems. In aerobic reactor systems, AOB reactions should dominate N_2O production (Kampschreur et al., 2009b, Wunderlin et al., 2012) and release of potentially toxic nitrification intermediates (H_2NOH, HOH) at high reaction rates has been shown to be the overwhelming source for N_2O, especially in wastewater treatment systems with high nitrogen load (Law et al., 2012). Most of these intermediates are toxic at higher concentrations and lead to stress reactions in nitrifying organism (2.1.3). These N_2O producing pathways are susceptible to pH, which affects the substrate (NH_3) availability (cf. 2.1.1) and thereby the NH_3 oxidation rate (Law et al., 2011).

Another frequently pointed at factor for N_2O production in aerobic wastewater treatment systems is accumulation of NO_2^- (Kampschreur et al., 2009b, Tallec et al., 2006, Wunderlin et al., 2012, Campos et al., 2009). High NO_2^- accumulation results in nitrosative stress and detoxification processes in AOB may ensue, leading to enhanced N_2O production, most likely via nitrifier denitrification (cf. 2.1.3). The study of Law et al. (2013) suggested that AOB communities may adapt to higher NO_2^-concentrations, hence critical threshold concentrations for enhance N_2O production by AOB vary from 5 to 10 mM, depending on reactor conditions.

HNO_2, the protonated form of NO_2^- seems to have some inhibitory effect on N_2OR, the enzyme performing the final step in heterotrophic denitrification (cf. 2.3). Hence, especially at lower pH, the accumulation of NO_2^- (or HNO_2) triggers N_2O emission in both aerobic (AOB dominated) and anoxic (dominated by heterotrophic denitrifiers) reactor zones. Accumulation of NO_2^- typically occurs when NOB activity is inhibited in aerated reactor zones, leading in partial nitrification. Classical NOB inhibition factors are high NH_3 and high HNO_2 concentrations (cf. 2.2) as well as high NH_2OH (Harper et al., 2009). NH_2OH may be released by AOB when reaction rates are high (cf. 2.1.3). In environments sustaining concurrent nitrification and denitrification as it is often the case in biofilms, HNO_2 may act self-enhancing by inhibiting both oxidation of NO_2^- by NOB and the reduction of NO_2^- and consumption of protons by heterotrophic denitrification (cf. 2.3). Under these conditions, the pH may overall decrease, moving the acid/base equilibrium of $HNO_2 \Leftrightarrow NO_2^-$ further towards HNO_2.

Transiently micro-aerobic conditions are a third factor that may result in high N_2O production. If small amounts of oxygen (0.1-0.5 mg O_2 L^{-1}) are present, AOB metabolism as well as denitrification of heterotrophic organism may still be active but imbalanced (AOB, see 2.1.3) or incomplete (heterotrophs, see 2.3). In systems with low nitrogen load and low nitrification rates, transient zones or stages between aerobic and anoxic environments are likely important for N_2O production (Tallec et al., 2006). At low DO levels (<1 mg O_2 L^{-1}), AOB diversity was shown to be reduced in favor of *N.europeae* (Hu et al., 2011, Purkhold et al., 2000), which might show an inherently higher N_2O production than other AOB species as indicated by the findings in the previous section (2.1.3).

Two more important factors for increased N_2O production are electron donor limitation for heterotrophic denitrifiers (cf. 2.3) and strongly acidic conditions that may occur in little buffered nitrification systems (Table 2). Latter leads to stress reactions and release of highly reactive intermediates from AOB metabolism degrading biotically or abiotically to N_2O (cf. 2.1.3) as well as enhancing the concentration of HNO_2, which is suspected to increase N_2O emissions by heterotrophic denitrifiers (cf. 2.3).

In complex environments such as in biological wastewater treatment systems, two or more critical factors may be present simultaneously and interact with each other. For instance, oxygen limitation and NO_2^- accumulation may coincide, thus leading to elevated N_2O emissions in multiple N transformation processes (e.g. Hu et al., 2011, Kampschreur et al., 2009b, Kishida et al., 2004, Rassamee et al., 2011). Hence, micro-aerobic conditions may support high N_2O production both directly and indirectly by inducing imbalanced electron flow in AOB and heterotrophic denitrifiers and by suppressing NOB activity leading to partial nitrification and accumulation of NO_2^- , respectively. The latter mechanism is a result of unilateral biomass competition and is discussed more in detail in the following chapter (section 4.3.1).

Table 2: Overview over factors known to affect N_2O production by AOB and denitrifiers in wastewater treatment systems. *Anammox* organism and NOB are omitted, since potential N_2O producing pathways are understudied

Factor	AOB	Heterotrophic denitrifiers	Abiotic reactions
High reaction rates (substrate availability)	Release of intermediates (H_2NOH, HNO, NO) conductive to oxidative N_2O formation	-	Release and chemical decomposition of NH_2OH
NO_2^- accumulation	Nitrosative stress	Incomplete denitrification to N_2O (at low pH)	Dismutation of HNO_2 to NO and N_2O under acid conditions
Transiently microaerobic conditions	Incomplete nitrifier denitrification to N_2O	Incomplete denitrification to N_2O	-
Electron donor limitation	-	Incomplete denitrification to N_2O	-
Acidity	Release of intermediates and nitrosation	Potential nitrosation of intermediates	Dismutation of HNO_2 to NO and N_2O

4 MECHANISM FOR N_2O PRODUCTION IN BIOFILM SYSTEMS

4.1 Biofilms in wastewater treatment application

This section summarizes the current understanding of biofilm functioning by using a mass balance model as a framework for discussion of N_2O producing mechanism. A biofilm is defined as densely aggregated biomass which is typically attached to a solid surface, the so called substratum (e.g. gravel, plastic media) (Morgenroth, 2008b). The mass flow of a particular compound in a biofilm can be approached with help of a diffusive/reactive mass balance equation (Eq. 10, Morgenroth, 2008b, Wanner and Gujer, 1986). It describes the balance between accumulation, diffusive transport and reaction over the biofilm cross section where C_F is concentration of a particular compound, D_F the diffusion coefficient for a particular compound, x the distance from the biofilm surface and r_F reaction rate for a particular compound in the biofilm biomass.

$$\underbrace{\frac{\partial C_F}{\partial t}}_{accumulation} = \underbrace{D_F \frac{\partial^2 C_F}{\partial x^2}}_{diffusion} - \underbrace{r_F}_{reaction} \qquad (10)$$

In addition to classical biofilms attached to a substratum, sludge granules have gained increasing attention in wastewater treatment (Khan et al., 2013). Sludge granules or granular sludge are larger biomass aggregates with a density similar to that of attached biofilm biomass (Beun et al., 1999, Morgenroth et al., 1997). Sludge granules are made of an unattached, spherical biofilm, in which the mass flow can be approached with the same diffusive-reactive mass balance equation as in attached biofilms (Khan et al., 2013).

The simplest types of biofilms consist of only one functional group of organisms, inert biofilm mass and interstitial void spaces. However, wastewater usually provides a range of different substrates (e.g. organics, ammonia) which supports multi-species biofilms (Wanner and Gujer, 1985, Wanner et al., 2006). Most of the present models describe the biofilm as a rigid, stratified biomass with fast-growing organism in the outermost layers which are in contact the bulk liquid where substrate availability is highest and slow-growing organism in deeper biofilm layers where substrate availability is limited due to diffusion constraints (Wanner and Reichert, 1996, Wanner and Gujer, 1985, Morgenroth, 2008b). However, the one-dimensional, rigid biomass distribution only partially reflects true spatial biomass dynamics. Especially biomass detachment, which typically takes place during periodical events may impact the 3-dimensional biofilm structure as well as biomass competition and need to be better characterized in future biofilm studies (Elenter et al., 2007, Derlon et al., 2013, Horn et al., 2003, Morgenroth and Wilderer, 2000).

In systems loaded with organic substrate and ammonia, as typical for domestic wastewater, the outermost biofilm layer is usually dominated by fast growing heterotrophic organisms. In the presence of abundant organic substrate, all O_2 is consumed by heterotrophs and little nitrifying biomass establishes (Wanner and Gujer, 1985). If the availability of organic substrate limits heterotrophic activity, the biofilm is substrate limited and oxygen is not completely consumed by heterotrophic activity. Under these conditions, O_2 is still present in deeper layers where slow growing nitrifying organism (AOB, NOB) can establish. Biofilms accommodating both heterotrophic and autotrophic organism are often called heterogeneous biofilms. In systems receiving little organic substrate, heterotrophic organisms are not able to compensate biomass decay by growth and will therefore only represent a minor fraction of the biofilm biomass. This results in so-called autotrophic or nitrifying biofilms which are dominated by nitrifiers in surface near zones (Wanner and Gujer, 1985, Morgenroth, 2008b).

Common for both heterogeneous (Schreiber et al., 2009) and nitrifying biofilms (Schramm et al., 1999, Okabe et al., 1999) is the rapid decline of O_2 over the cross-section. Hence most of aerobic biofilm systems accommodate anoxic zones in deeper layers where denitrification may takes place if both electron acceptors (e.g. NO_2^- NO_3^-, NO, N_2O) and electron donors (organic matter; NH_4^+) are present (Okabe et al., 1999). In anoxic biofilm zones, heterotrophic denitrifiers as well as Anammox can be found depending on the availability of organic substrates. Anammox bacteria were shown to have extremely low growth rates and are therefore outcompeted by heterotrophic denitrifiers if organic substrates are ample. When organic substrate is limiting, Anammox bacteria outcompete heterotrophic denitrifiers (Jin et al., 2012, Wang et al., 2010, Lackner and Horn, 2012).

Heterogeneous and autotrophic biofilms differ significantly in their structures. A heterogeneous biofilm, loaded with domestic wastewater, has a porous structure with a rough surface and large interstitial voids. Autotrophic biofilms, on the other hand, were shown to have a densely packed structure, a relatively smooth surface and only a few, small interstitial voids (Okabe et al., 1999). This has been attributed to the observed tendency of AOB to grow in dense clusters (Okabe et al., 1999, Schramm et al., 1996, Kindaichi et al., 2004b, Kindaichi et al., 2006). Due to their high biomass density, AOB clusters also have a greater resistance against detachment than heterotrophic dominated biomass. Especially under high external shear forces, AOB clusters may have an advantage relative to the faster growing heterotrophs, and sustain nitrification higher rates than predicted by traditional biofilm models (Elenter et al., 2007).

Also release of nitrification intermediates (2.1.3) may affect the biomass competition and stratification in a biofilm. For instance, NO was shown to impose a sessile growth mode of AOB (Schmidt et al., 2004a), while NO induced motile growth in heterotrophic pulmonary pathogens (Barraud et al., 2009). It is not known whether NO shows a similar impact on heterotrophic bacteria in wastewater treatment systems, but if so, this might have a significant impact on the biomass competition and distribution in heterogeneous biofilms. By releasing NO, AOB may increase their competitiveness relative to heterotrophs in surface near biofilm zones. More research is needed to understand the impact of N transformation intermediates on species competition in biofilms.

4.2 Reported N_2O emissions from biofilm systems

To give an overview of published N_2O emission data in biofilm systems, a search was performed with the keywords "N_2O" and "biofilm" in Web of Science (Thomson Reuters, New York). In January 2015, the search yielded a total of 59 hits comprising studies of both natural and artificial biofilm systems. The results were screened for studies on wastewater treating biofilm systems that provide data on N_2O emissions under different operational conditions (Tab. 3). The N_2O emission factor (N_2O-N in % of biologically transformed N) was calculated if not provided. Studies for which a calculation of the emission factor was not possible were omitted.

The results of the literature survey are summarized in Table 3 and show a wide range of N_2O emission factors varying from 0.2 to 57% (N_2O-N emitted per biologically transformed N). The literature review indicates that: A) both aerobic and anoxic reactor conditions allow for high N_2O emission factors; B) heterogeneous biofilm systems fed with both organics and ammonia substrates tend to have higher N_2O emission factors than autotrophic biofilms fed with ammonia only. C) Under anoxic conditions, high NO_2^- clearly indices high N_2O emissions, which is not the case for aerobic conditions. No clear tendency was found for different aeration regimes and DO levels, but the literature data give a weak indication that intermitted aeration or batch operation result in greater N_2O emission factors than operation at constant DO levels. In the following, we discuss these findings in more detail in light of the biofilm models outlined in the previous section (4.1).

Table 3: Reported greenhouse gas emission from biofilm systems under different loading conditions and system parameters. The N_2O emission factor refers to the emitted N_2O in % of biologically transformed N (in N equivalents). Some studies provide the emissions factor in % of inlet load, or removed N (which is not considering transformed N which is remaining in the reactor liquid, e.g. NO_2^-). For these studies the emission factor in % of transformed N was calculated or estimated based on the provided data.

Reference	Type of system	Dominating organism	Aeration and DO on aerated phases	Nitrite (mg N L^{-1})	N_2O emission factor
(Todt and Dörsch, 2015)	MBBR	AOB, NOB, heterotrophs	continuous (3-5 mg O_2 L^{-1})	0.1-220	0.7-8.5% (N_2O peaks coincided with NO_2^- accumulation peaks)
(Ma et al., 2015)	MBBR	AOB, Anammox	continuous (1.7 mg O_2 L^{-1})	1.5	0.5-1.5%
(Eldyasti et al., 2014)	fluidized bed	heterotrophs	none (anoxic reactor)	0.1-0.4 (with COD/N 5)	0.6-1.1% (with COD/N 5)
				0.7 with COD/N 3.5	1.9% (with COD/N 3.5)
Lochmatter et al. (2013)	granular sludge	AOB, NOB, heterotrophs	intermitted (short cycles) (5.5 mg O_2 L^{-1})	1-4	1-9% (inverse relationship to COD load)
Yang et al. (2013)	MBBR	AOB Anammox	intermitted (1.5 O_2 L^{-1})	6-7	0.5-1.3 %
			continuous (1.5 mg O_2 L^{-1})	4-7	0.7-1.3%
(Kong et al., 2013)	MBBR	AOB	intermitted (1-2 mg O_2 L^{-1})	200-250	2.7 %
Christensson et al. (2013)	MBBR	AOB Anammox	continuous (0.5-1.5 mg O_2 L^{-1})	0-5	0.1-0.8 % at DO < 1 mg O_2 L^{-1} 0.8-2.2 % at DO > 1 mg O_2 L^{-1}
Lo et al. (2010)	IFAS (MBBR)	AOB, NOB, heterotrophs	intermitted (2-5 mg O_2 L^{-1})	<1	27% (21% of N load)
Campos et al. (2009)	BAS	AOB, NOB, heterotrophs	anoxic batch incubation of biofilm from aerated reactor	0 (e.a. NO_3^-)	0.3-1.2 % (with NO_3^- as e.a.)
				100	52-57 % (with NO_2^- as e.a.)
Zhou et al. (2008a)	granular sludge	AOB, NOB, heterotrophs (PAO)	anoxic batch incubation from aerated reactor	0.5 (low) 30 (high)	1.2-1.4% at low NO_2^- 40-55% at high NO_2^-
Rezić et al. (2007)	rotating tube	heterotrophs (pure culture study with *P. denitrificans*)	rotation on different speeds (RPM) (DO n.a.)	n.a.	3-25% with inverse relationship to RPM
Bougard et al. (2006)	Fluidized bed	AOB, NOB	continuous (DO n.a.)	1600 0-500	6% (on high NO_2^-) 9% (on low NO_2^-)
Park et al. (2000)	aerated biofilm filter	AOB, NOB, heterotrophs	intermitted DO during aerobic phase 0.2 mg O_2 L^{-1}	<0.1	3% without addition of e.d. substrate
					0.2% with addition of e.d substrate

4.3 N_2O producing processes in different biofilm zones

4.3.1 N_2O production in aerobic biofilm zones

Under fully aerobic conditions, two major N_2O producing mechanisms are suggested, both of them attributed to AOB: reduction of accumulated NO_2^- to N_2O by nitrifier denitrification or chemical and enzymatic formation of N_2O from intermediates released during NH_3 oxidation (2.1.3). Given the commonly accepted diffusive/reactive mass balance equation (Wanner and Gujer, 1986) and the ammonia concentrations typically found in domestic wastewater, O_2 is usually the limiting factor for nitrification activity in a biofilm (Hem et al., 1994, Cema et al., 2011). A higher DO in the bulk liquid supports higher NH_3 oxidation rates across the biofilm and thereby enhances N_2O production if AOB releases intermediates as shown for suspended biomass cultures by Law et al. (2012) and Wunderlin et al. (2012). Campos et al. (2009) studied N_2O production in a nitrifying biofilm at different DO levels and found highest N_2O production at low DO (1 mg O_2 L^{-1}) together with high accumulation of NO_2^-, but not at high DO (5 mg O_2 L^{-1}) which would have been expected if N_2O was primarily produced from AOB intermediates. At a DO of 5 mg O_2 L^{-1}, NH_3 was almost quantitatively converted to NO_3^- and little N_2O was produced. Similar findings were reported from another study (Lochmatter et al., 2013), suggesting that N_2O production in aerobic biofilm reactors proceeds from intermediates released due to nitrosative stress caused by accumulated NO_2^- rather than from intermediates released due to nitrification rates. A lower contribution of AOB intermediates to N_2O in biofilm systems as compared with suspended biomass cultures might be related to the steep O_2 gradient in biofilms which strongly limits AOB reaction rates over the biofilm cross section (Schreiber et al., 2009). The reported higher nitrification rates per reactor volume unit for high-performing biofilm systems (e.g. MBBR) compared to traditional activated sludge systems (e.g. Hem et al., 1994) are likely the result of the significantly greater biomass density in the former (Morgenroth, 2008a) and do therefore not contradict the lower NH_3 oxidation rate per AOB mass unit in biofilm systems.

In addition to the earlier discussed effect of NO on biofilm structure (4.1), NH_2OH may change the aggregation pattern of AOB in a biofilm. Under normal conditions, AOB are growing in dense spherical clusters (Okabe et al., 1999, Schramm et al., 1996, Kindaichi et al., 2006). In presence of extracellular NH_2OH, AOB where observed to disperse into loosely scattered cells in biofilms (Kindaichi et al., 2004b) as well as in suspended biomass cultures, in which AOB usually are aggregated in flocs (Harper et al. 2009). If AOB located in the outermost biofilm layers reach high nitrification rates, release of NH_2OH may lead to self-detachment and washout of surface-near, high-performing AOB clusters. Due to this self-inhibiting mechanism, the NH_3 oxidation in a biofilm system will probably never reach reaction rates sufficiently high for release of notable amounts of AOB intermediates. However, some studies found a positive correlation between N_2O emissions and pH in biofilm systems (Yang et al., 2013), which may be due to higher AMO reaction rates with greater NH_3 availability at higher pH (Law et al., 2011, Yang et al., 2013). More research is needed to assess the combined impact of O_2 (DO) and NH_3 (TAN, ph) on AOB reaction rates and their relationship to N_2O emissions in biofilm systems.

Nitrifying biofilm systems have a tendency to accumulate NO_2^-, especially at lower DO (<2 mg O_2 L^{-1}) in the bulk liquid (Fux et al., 2004a, Sliekers et al., 2005, Brockmann et al., 2008), which was identified as one of the main factors for high N_2O production (cf. section 3). NO_2^- accumulation is commonly attributed to the higher affinity of AMO for O_2 compared to NXR (Ciudad et al., 2006, Sliekers et al., 2005, Hellinga et al., 1998). Some studies tried to utilize the greater oxygen sensibility of NOB to achieve partial nitrification by controlling the DO levels to feed the Anammox process with NO_2^- (Fux et al., 2004a, Yoo et al., 1999). However, it is difficult to maintain long-term process stability since NOB have the ability to adapt to low O_2 levels with the result that a larger fraction of biologically transformed NH_3 is nitrified to NO_3^- (Fux et al., 2004a). Schramm et al. (2000) analyzed and quantified the biomass distribution over the cross section of a nitrifying biofilm. The outermost zones accommodated both AOB and NOB, but the cell numbers of AOB exceeded NOB by almost fourfold. In deeper zones with less O_2 availability, the cell number of AOB and NOB became more equal. This is consistent with the observation of Okabe et al. (1999) whose nitrifying biofilm was almost completely dominated by AOB in the outermost layer, while deeper layers showed equal abundance of both groups. These findings may be somewhat surprising given the reportedly higher O_2 sensitivity of NOB than AOB. However, the study of Okabe et al. (1999) showed that the NOB clusters in the deeper biofilm layers were dominated by *Nitrospira sp.*, while the faster growing NOB species *Nitrobacter sp.* was almost absent. Hence, *Nitrospira sp.* seems to be better adapted to oxygen limiting

conditions than *Nitrobacter sp*. Different O_2 affinities for different NOB species may also explain the observed adaptation of NOB to low DO levels (Fux et al., 2004a). On the other hand, *Nitrospiria sp*. seems to be more sensitive to NH_3 inhibition which again would counteract an adaptation of the NOB community to niches with limited O_2 availability in systems with high NH_3 concentrations.

4.3.2 N_2O production in transient environments between aerobic conditions and anoxia

In anoxic reactor compartments, transient, micro-aerobic zones may occur along the reactor surface area (interface liquid/air) or in zones close to inlet pipes from aerobic compartments where O_2 intrusion may occur. Micro-aerobic conditions in a DO range of 0-0.5 mg O_2 L^{-1} were shown to lead to incomplete heterotrophic denitrification due to O_2 inhibition of the N_2OR enzyme (section 2.3). In addition to heterotrophic denitrifiers, also AOB may produce N_2O on micro-aerobic conditions (cf. 2.1.3) if present in the anoxic reactor compartments or biofilm zones.

Due to mass transport limitations, micro-aerobic conditions will also occur in aerated reactor compartment inside the biofilm. Most of the aerated biofilms listed in Table 3 were likely O_2 limited, accommodating transiently micro-aerobic conditions. As shown by microprobe profiles, transient micro-aerobic biofilm zones are usually relatively small compared to the aerobic and anoxic biofilm fractions (Schramm et al., 1999, Okabe et al., 1999). Hence, under stable aeration conditions, only a minor fraction of the total biomass will be exposed to micro-aerobic conditions so that basically only small amounts of N_2O are produced. However, in certain systems where no other critical factors (e.g. NO_2^- accumulation) are present, incomplete denitrifcation in microaerobic zones may be the major source of N_2O. In heterogeneous biofilm systems, natural variations in the composition of the wastewater, but also batch operating regimes may induce frequent changes in O_2 availability in deeper biofilm layers, facilitating N_2O production by AOB (cf. 2.1.3) or heterotrophic denitrifiers (cf. 2.3). Rezić et al. (2007) reported an inverse relationship of N_2O production and RPM in a rotating disk reactor. This may reflect transient shifts in O_2 availability in deeper layers of the biofilm.

4.3.3 N_2O production in anoxic zones

The results of the two research groups who studied N_2O emissions from a biofilm under anoxic conditions (Campos et al., 2009, Zhou et al., 2011, see also Tab.3) clearly indicate that NO_2^- plays a key role for N_2O production under anoxic conditions. Under complete anoxic conditions, heterotrophic denitrifiers and NOB are the potential sources for N_2O (section 3), while AOB do not play a significant role (Schreiber et al., 2009). According to the present knowledge, NOB and Anammox bacteria do not contribute significantly to N_2O (2.24), leaving heterotrophic denitrifiers the sole organisms producing N_2O in anoxic biofilm zones.

As outlined in section 2.3 , heterotrophic denitrification requires the presence of organic substrates as electron donors. However, heterotrophic denitrification activity in deeper biofilm zones may also be sustained by endogenous residuals or soluble organic exudates from AOB residing in aerobic biofilm zones (Kindaichi et al., 2004a, Rittmann et al., 1994). The reaction rates of endogenously fuelled denitrification are low compared to denitrification rates in N-removal systems and the reaction chain is often incomplete and may therefore contribute significantly to N_2O emissions (Itokawa et al., 2001). In anoxic (none-aerated) compartments of biofilm based N-removal systems supplied with readily degradable organic substrate, electron donors are unlikely to limit N_2O reduction to N_2. Yet, mass transport limitation may occur, confining N_2O production to deeper anoxic zones. Also, the produced N_2O may be consumed as electron acceptor by biomass residing in surface-near layers where readily degradable substrates are present in ample amounts so that the net N_2O production of the biofilm does not reach significant levels. Heterotrophic degradation of organic matter produces CO_2 regardless whether O_2 or NO_x^- is used as electron acceptor. In a biofilm, CO_2 may accumulate, which again can result in a measurable pH drop as shown by a recent micro sensor study (Xiao et al., 2013). If the pH in inner, anoxic biofilm layers falls below 6.8, heterotrophic denitrification may become incomplete (Xiao et al., 2013). Heterotrophic denitrification reaches higher reaction rates with NO_2^- than with NO_3^- (Campos et al., 2009). Hence, in presence of NO_2^-, CO_2 production and related pH decrease and increase of HNO_2 with subsequent N_2O production via incomplete denitrification may be significantly greater than with NO_3^- (Campos et al., 2009).

4.4 N_2O production in aerated complex heterogeneous biofilms with concurrent load of organic and ammonia substrates

Table 3 suggests that N_2O emissions are greater with heterogeneous biofilms receiving both organic and ammonia substrates than in nitrifying biofilms receiving ammonia substrates alone. This notion is supported by two studies which explored the effect of organic load in heterogeneous biofilms in more detail and showed that the addition of organic substrate increased N_2O emission especially at higher DO levels (>1mg L^{-1}) (Campos et al., 2009, Lochmatter et al., 2013, Todt and Dörsch, 2015). So far, N_2O producing mechanisms within the complex framework of interacting heterotrophic and autotrophic biomass in aerated heterogeneous biofilms have been little investigated. In stratified biomass, readily degradable organics control the O_2 availability in deeper biofilm zones, were nitrifiers are located (for details see 4.1) through consuming oxygen by heterotrophic degradation. An increase of organic load will therefore decrease O_2 supply to the nitrifiers and reduce the nitrification activity. Nitrifiers were observed to have a spatial distribution, in which NOB are located below AOB (cf. 4.3.1). Hence if less O_2 is present to the nitrifying biomass, it is likely that the proportion of NO_2^- among the total oxidized N-compounds (NO_2^-, NO_3^-) increases and NO_2^- accumulates, which again was shown to facilitate N_2O production via different pathways (Todt and Dörsch, 2015). Also transient shifts in O_2 availability across the biofilm due to varying substrate load (section 4.3.3) may be more frequent in systems loaded with organic substrate than in nitrifying biofilm systems.
Another factor affecting N_2O producing mechanisms in a biofilm may be related to the different penetration depths and degradability in a mixture of substrates of a given size distribution. A typical wastewater contains both slowly and readily degradable organics (Dulekgurgen et al., 2006, Henze and Comeau, 2008). Readily degradable substrates usually refer to low molecular organic matter, while slow- degradable substrates refer to high molecular, colloidal or particulate organic matter. However in some types of wastewater, slow-degradable organic substrates may also be represented by a larger fraction of low molecular compounds, such as humic acids (Dulekgurgen et al., 2006). With these slow-degradable substrates, the growth rate of heterotrophic organism is too low to outcompete nitrifiers (Jin et al., 2012, Wang et al., 2010, Lackner and Horn, 2012), hence the co-occurrence of substrate limitation and nitrification depends mainly on the presence of readily degradable compounds. If present at high concentrations, slow-degradable, low molecular substrates may reach deeper, anoxic biofilm zones where they fuel incomplete heterotrophic denitrification and produce N_2O (Todt and Dörsch, 2015). Heterogeneous biofilms are little studied for N_2O producing processes and more research is needed to assess the effect of molecule size and distribution of organic substrates as a controlling factor for N_2O production in biofilms.

4.5 N_2O production in autotrophic biofilms loaded with ammonia substrates

A combination of partial nitrification (also called nitritation) with anaerobic ammonia oxidation by Anammox bacteria has gained popularity for biological N-removal, especially for treatment of wastewater with high ammonia content (e.g. Fux et al., 2004b, Jetten et al., 1998, Kampschreur et al., 2006, Yang et al., 2013). In biofilm systems, nitritation and Anammox can be run in a single reactor by combining AOB activity in aerobic zones with Anammox bacteria in anoxic zones (Yang et al., 2013, Fux et al., 2004b, Christensson et al., 2013, Ma et al., 2015). A crucial issue for operating these so-called one-stage partial-nitrification-Anammox reactors is to minimize NOB activity. This is usually achieved by controlling the DO level, temperature and wash out of NOB, the latter of which is difficult to achieve in a biofilm system (Fux et al., 2004a). It was shown that NOB not only compete with AOB for O_2, but also with Anammox for NO_2^-. The competition between Anammox and NOB seems to be controlled by the availability of inorganic carbon, most likely reflecting metabolic differences in CO_2 fixation that make Anammox more sensitive to inorganic carbon limitation than NOB (Ma et al., 2015).

N removal by Anammox systems produces less N_2O than traditional nitrification/denitrification-based N-removal (Tab. 3). This might be due to a lower sensitivity of AOB and Anammox to NO_2^- accumulation compared to heterotrophic denitrifiers. In one-stage partial-nitrification-Anammox systems as usually implemented in biofilm systems, the NO_2^- accumulation can further be minimized via controlling the DO to keep the NO_2^- production of AOB below the NO_2^- (via O_2 limitation to AOB) reduction rate of *Anammox* (Cema et al., 2011, Christensson et al., 2013). A recent study indicated that minimizing NO_2^- accumulation in the reactor bulk liquid also reduces N_2O production in the system (Christensson et al., 2013).

A critical factor for N_2O emissions in autotrophic biofilms may be the temporal presence of organic substrates fuelling heterotrophic denitrifiers. Organic substrates can originate from the inlet, but also from endogenous

biomass decay (Ma et al., 2015). Table 3 suggests that the occurrence of heterotrophic denitrifiers in anoxic biofilm zones increases N_2O production, especially in the presence of high NO_2^- and/or slowly degradable substrates (cf. 4.4). The competition between heterotrophs and Anammox in anoxic biofilm zones is determined by the concentration of organic substrate relative to the substrate affinity constant of heterotrophs, but also by the ratio of volatile fatty acids (VFA) and N. Anammox use VFAs as electron donor for the organotrophic metabolism (Güven et al., 2005, Winkler et al., 2012a) and it is assumed that Anammox are able to outcompete heterotrophs for this particular substrate group as long as NH_4^+ is present in ample amounts (Kartal et al., 2007b). With VFA/N (expressed as COD/N) lower than 0.5 g O_2 g^{-1} N, Anammox bacteria were shown to outcompete heterotrophic bacteria (Winkler et al., 2012b). The extent of N_2O production by heterotrophic denitrifiers in Anammox systems may therefore not only depend on the availability, but also on the type of organic substrates present. In autotrophic biofilms, VFAs likely occur only temporary, since a continuous presence of these readily degradable substrates would support heterotrophic organisms in the aerobic surface-near biofilm layers and transform the biofilm into a heterogeneous biofilm.

5 SUMMARY

In the first chapter, the metabolism of the main groups of microorganism (heterotrophs, AOB, NOB, *Anammox*) that mediate N removal in wastewater treatment was reviewed with focus on potential mechanism for N_2O production. The literature points towards an important role of AOB for N_2O production under aerobic conditions, while heterotrophic denitrifers dominate N_2O production under anoxic conditions. In the AOB metabolism, the main N_2O producing pathways are coupled to release and abiotic reactions of NH_3 oxidation intermediates, nitrifier denitrification of NO_2^- and biochemical reactions averting nitrosative stress. According to the present knowledge, imbalanced reactions in the AOB metabolism seem mainly to occur during high nitrification rates and transient changes in O_2 availability while nitrosative stress occurs when NO_2^- accumulates. Heterotrophic denitrification was shown to become incomplete during transient O_2 penetration into anoxic environments and in presence of HNO_2 which is in pH-dependent acid/base equilibrium to NO_2^-. Potential N_2O emitting pathways in NOB and Anammox are so far little investigated, but unlikely to be a major contributor to the total N_2O emission in biofilm based wastewater treatment systems.

In the subsequent chapters, potential N_2O generating metabolic processes were compared to N_2O emissions reported from various biofilm systems. In anoxic biofilms zones, HNO_2 , the protonized form of NO_2^-, seems to be the most critical factor for N_2O emissions. In aerobic biofilm zones, NO_2^- likely leads to nitrosative stress and subsequent release of N_2O. NO_2^- seems therefore to be a key factor in both aerobic and anoxic biofilm zones. Its accumulation can be a result of classical unilateral inhibition of NOB above a critical concentration of NH_3 and HNO_2, and/or unbalanced competition between AOB and NOB under O_2 limiting conditions, especially at enhanced ambient temperatures. Biofilms typically provide anoxic zones in which NO_2^- is reduced by heterotrophic denitrifiers or Anammox.

Autotrophic biofilms that are dominated by AOB and Anammox bacteria seem to be less conductive to N_2O production than heterogeneous biofilms that consists of AOB, NOB and heterotrophic bacteria. This is likely because heterotrophic denitrifiers are more sensitive to HNO_2 (NO_2^-) accumulation than Anammox bacteri. O_2 repression of N_2OR in denitrifiers under microaerobic conditions and electron donor limitations may further increase the relative N_2O yield in denitrification. Anammox bacteria on the other hand do, according to present knowledge, not produce notable amounts of N_2O and countervail at the same time the accumulation of HNO_2 and NO_2^- in the biofilm.

Heterogeneous biofilm systems loaded with both organic and ammonia substrates seem to be particularly conductive to N_2O production. Frequent shifts of the anoxic biofilm fraction due to varying O_2 depletion by hetertrophic activity may lead to transient oxygen stress for both AOB and heterotrophic denitrifiers located in deeper biofilm zones, resulting in enhanced N_2O production. In systems with high load of slowly degradable organics penetrating into anoxic biofilm zones, additional incomplete heterotrophic dentrification activity may occur. The literature indicates that not only classical environmental parameters in the bulk liquid such as DO level and pH affect N_2O production in heterogeneous biofilms, but also the molecule size and degradability of the organic substrates.

None of the N_2O producing mechanism in biofilms is completely clarified and more research is needed to account for complex interactions of different enzymatic reaction associated with different organism located in different zones in a biofilm. There are knowledge gaps about several enzymes and their biochemistry involved in biofilm N transformations, especially for *NOB* and Anammox. Even the metabolism of the relatively well studied AOB species *N. europaea* is not fully clarified. Better mathematical models are needed to understand the far more complex biomass interactions in a biofilm relative to suspended biomass. Finally, proposed reaction rates and stoichiometries under different environmental factors/conditions have to be validated with N_2O emission data in well-designed case studies.

6 ACKNOWLEDGEMENTS

We are thankful to Linda Hink (University of Aberdeen) for reviewing the chapter on enzymatic processes.

7 REFERENCES

ABELIOVICH, A. & VONSHAK, A. 1992. ANAEROBIC METABOLISM OF NITROSOMONAS-EUROPAEA. *Archives of Microbiology,* 158**,** 267-270.

ANTHONISEN, A. C., LOEHR, R. C., PRAKASAM, T. B. S. & SRINATH, E. G. 1976. Inhibition of nitrification by ammonia and nitrous-acid. *Journal Water Pollution Control Federation,* 48**,** 835-852.

ARCIERO, D. M., PIERCE, B. S., HENDRICH, M. P. & HOOPER, A. B. 2002. Nitrosocyanin, a red cupredoxin-like protein from Nitrosomonas europaea. *Biochemistry,* 41**,** 1703-1709.

ARP, D. J., SAYAVEDRA-SOTO, L. A. & HOMMES, N. G. 2002. Molecular biology and biochemistry of ammonia oxidation by Nitrosomonas europaea. *Archives of Microbiology,* 178**,** 250-255.

BARRAUD, N., STOREY, M. V., MOORE, Z. P., WEBB, J. S., RICE, S. A. & KJELLEBERG, S. 2009. Nitric oxide-mediated dispersal in single- and multi-species biofilms of clinically and industrially relevant microorganisms. *Microbial Biotechnology,* 2**,** 370-378.

BEAUMONT, H. J. E., HOMMES, N. G., SAYAVEDRA-SOTO, L. A., ARP, D. J., ARCIERO, D. M., HOOPER, A. B., WESTERHOFF, H. V. & VAN SPANNING, R. J. M. 2002. Nitrite reductase of *Nitrosomonas europaea* is not essential for production of gaseous nitrogen oxides and confers tolerance to nitrite. *J. Bacteriol.,* 184**,** 2557-2560.

BEAUMONT, H. J. E., LENS, S. I., REIJNDERS, W. N. M., WESTERHOFF, H. V. & VAN SPANNING, R. J. M. 2004a. Expression of nitrite reductase in Nitrosomonas europaea involves NsrR, a novel nitrite-sensitive transcription repressor. *Molecular Microbiology,* 54**,** 148-158.

BEAUMONT, H. J. E., LENS, S. I., WESTERHOFF, H. V. & VAN SPANNING, R. J. A. 2005. Novel nirK cluster genes in Nitrosomonas europaea are required for NirK-dependent tolerance to nitrite. *Journal of Bacteriology,* 187**,** 6849-6851.

BEAUMONT, H. J. E., VAN SCHOOTEN, B., LENS, S. I., WESTERHOFF, H. V. & VAN SPANNING, R. J. M. 2004b. Nitrosomonas europaea expresses a nitric oxide reductase during nitrification. *Journal of Bacteriology,* 186**,** 4417-4421.

BERGAUST, L., MAO, Y., BAKKEN, L. R. & FROSTEGÅRD, Å. 2010. Denitrification Response Patterns during the Transition to Anoxic Respiration and Posttranscriptional Effects of Suboptimal pH on Nitrogen Oxide Reductase in Paracoccus denitrificans. *Applied and Environmental Microbiology,* 76**,** 6387-6396.

BERGAUST, L., VAN SPANNING, R. J. M., FROSTEGARD, A. & BAKKEN, L. R. 2012. Expression of nitrous oxide reductase in Paracoccus denitrificans is regulated by oxygen and nitric oxide through FnrP and NNR. *Microbiology-Sgm,* 158**,** 826-834.

BEUN, J. J., HENDRIKS, A., VAN LOOSDRECHT, M. C. M., MORGENROTH, E., WILDERER, P. A. & HEIJNEN, J. J. 1999. Aerobic granulation in a sequencing batch reactor. *Water Research,* 33**,** 2283-2290.

BEYER, S., GILCH, S., MEYER, O. & SCHMIDT, I. 2009. Transcription of Genes Coding for Metabolic Key Functions in Nitrosomonas europaea during Aerobic and Anaerobic Growth. *Journal of Molecular Microbiology and Biotechnology,* 16**,** 187-197.

BLACKBURNE, R., VADIVELU, V. M., YUAN, Z. & KELLER, J. 2007. Kinetic characterisation of an enriched Nitrospira culture with comparison to Nitrobacter. *Water Research,* 41**,** 3033-3042.

BOCK, E., SCHMIDT, I., STUVEN, R. & ZART, D. 1995. NITROGEN LOSS CAUSED BY DENITRIFYING NITROSOMONAS CELLS USING AMMONIUM OR HYDROGEN AS ELECTRON-DONORS AND NITRITE AS ELECTRON-ACCEPTOR. *Archives of Microbiology,* 163**,** 16-20.

BONIN, P. & RAYMOND, N. 1990. Effects of oxygen on denitrification in marine sediments. *Hydrobiologia,* 207**,** 115-122.

BOTHE, H., JOST, G., SCHLOTER, M., WARD, B. B. & WITZEL, K. P. 2000. Molecular analysis of ammonia oxidation and denitrification in natural environments. *Fems Microbiology Reviews,* 24**,** 673-690.

BOUGARD, D., BERNET, N., CHÈNEBY, D. & DELGENÈS, J. P. 2006. Nitrification of a high-strength wastewater in an inverse turbulent bed reactor: Effect of temperature on nitrite accumulation. *Process Biochemistry,* 41**,** 106-113.

BREMNER, J. M., BLACKMER, A. M. & WARING, S. A. 1980. Formation of nitrous oxide and dinitrogen by chemical decomposition of hydroxylamine in soils. *Soil Biology and Biochemistry,* 12**,** 263-269.

BROCKMANN, D., ROSENWINKEL, K. H. & MORGENROTH, E. 2008. Practical identifiability of biokinetic parameters of a model describing two-step nitrification in biofilms. *Biotechnology and Bioengineering,* 101**,** 497-514.

CAMPOS, J. L., ARROJO, B., VÁZQUEZ-PADÍN, J. R., MOSQUERA-CORRAL, A. & MÉNDEZ, R. 2009. N2O Production by Nitrifying Biomass Under Anoxic and Aerobic Conditions. *Applied Biochemistry and Biotechnology,* 152**,** 189-198.

CASCIOTTI, K. L. & WARD, B. B. 2001. Dissimilatory Nitrite Reductase Genes from Autotrophic Ammonia-Oxidizing Bacteria. *Applied and Environmental Microbiology,* 67**,** 2213-2221.

CEMA, G., PLAZA, E., TRELA, J. & SURMACZ-GORSKA, J. 2011. Dissolved oxygen as a factor influencing nitrogen removal rates in a one-stage system with partial nitritation and Anammox process. *Water Science and Technology,* 64**,** 1009-1015.

CHAIN, P., LAMERDIN, J., LARIMER, F., REGALA, W., LAO, V., LAND, M., HAUSER, L., HOOPER, A., KLOTZ, M., NORTON, J., SAYAVEDRA-SOTO, L., ARCIERO, D., HOMMES, N., WHITTAKER, M. & ARP, D. 2003. Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph Nitrosomonas europaea. *Journal of Bacteriology,* 185**,** 2759-2773.

CHRISTENSSON, M., EKSTROM, S., CHAN, A. A., LE VAILLANT, E. & LEMAIRE, R. 2013. Experience from start-ups of the first ANITA Mox Plants. *Water Science and Technology,* 67**,** 2677-2684.

CIUDAD, G., WERNER, A., BORNHARDT, C., MUÑOZ, C. & ANTILEO, C. 2006. Differential kinetics of ammonia- and nitrite-oxidizing bacteria: A simple kinetic study based on oxygen affinity and proton release during nitrification. *Process Biochemistry,* 41**,** 1764-1772.

COLLIVER, B. B. & STEPHENSON, T. 2000. Production of nitrogen oxide and dinitrogen oxide by autotrophic nitrifiers. *Biotechnology Advances,* 18**,** 219-232.

CUA, L. S. & STEIN, L. Y. 2011. Effects of nitrite on ammonia-oxidizing activity and gene regulation in three ammonia-oxidizing bacteria. *Fems Microbiology Letters,* 319**,** 169-175.

DERLON, N., COUFORT-SAUDEJAUD, C., QUEINNEC, I. & PAUL, E. 2013. Growth limiting conditions and denitrification govern extent and frequency of volume detachment of biofilms. *Chemical Engineering Journal,* 218**,** 368-375.

DULEKGURGEN, E., DOGRUEL, S., KARAHAN, Ö. & ORHON, D. 2006. Size distribution of wastewater COD fractions as an index for biodegradability. *Water Research,* 40**,** 273-282.

EKAMA, G. A. & WENTZEL, M. C. 2008. Nitrogen Removal. *In:* HENZE, M., VAN LOOSDRECHT, M. C. M., EKAMA, G. A. & BRDJANOVIC, D. (eds.) *Biological Wastewater Treatment.* London: IWA Publishing.

ELDYASTI, A., NAKHLA, G. & ZHU, J. 2014. Influence of biofilm thickness on nitrous oxide (N2O) emissions from denitrifying fluidized bed bioreactors (DFBBRs). *Journal of Biotechnology,* 192, Part A**,** 281-290.

ELENTER, D., MILFERSTEDT, K., ZHANG, W., HAUSNER, M. & MORGENROTH, E. 2007. Influence of detachment on substrate removal and microbial ecology in a heterotrophic/autotrophic biofilm. *Water Research,* 41**,** 4657-4671.

FREEMAN, J. P. 1973. Less familiar reactions of oximes. *Chemical Reviews,* 73**,** 283-292.

FRUNZKE, K. & ZUMFT, W. G. 1986. Inhibition of nitrous-oxide respiration by nitric oxide in the denitrifying bacterium Pseudomonas perfectomarina. *Biochimica et Biophysica Acta (BBA) - Bioenergetics,* 852**,** 119-125.

FUX, C., HUANG, D., MONTI, A. & SIEGRIST, H. 2004a. Difficulties in maintaining long-term partial nitritation of ammonium-rich sludge digester liquids in a moving-bed biofilm reactor (MBBR). *Water Science and Technology,* 49**,** 53-60.

FUX, C., MARCHESI, V., BRUNNER, I. & SIEGRIST, H. 2004b. Anaerobic ammonium oxidation of ammonium-rich waste streams in fixed-bed reactors. *Water Science and Technology,* 49**,** 77-82.

GUJER, W., HENZE, M., MINO, T. & VAN LOOSDRECHT, M. 1999. Activated Sludge Model No. 3. *Water Science and Technology,* 39**,** 183-193.

GÜVEN, D., DAPENA, A., KARTAL, B., SCHMID, M. C., MAAS, B., VAN DE PAS-SCHOONEN, K., SOZEN, S., MENDEZ, R., OP DEN CAMP, H. J. M., JETTEN, M. S. M., STROUS, M. & SCHMIDT, I. 2005. Propionate Oxidation by and Methanol Inhibition of Anaerobic Ammonium-Oxidizing Bacteria. *Applied and Environmental Microbiology,* 71**,** 1066-1071.

HARPER, W. F., TERADA, A., POLY, F., LE ROUX, X., KRISTENSEN, K., MAZHER, M. & SMETS, B. F. 2009. The effect of hydroxylamine on the activity and aggregate structure of autotrophic nitrifying bioreactor cultures. *Biotechnology and Bioengineering,* 102**,** 714-724.

HELLINGA, C., SCHELLEN, A., MULDER, J. W., VAN LOOSDRECHT, M. C. M. & HEIJNEN, J. J. 1998. The SHARON process: An innovative method for nitrogen removal from ammonium-rich waste water. *Water Science and Technology,* 37**,** 135-142.

HELLINGA, C., VAN LOOSDRECHT, M. C. M. & HEIJNEN, J. J. 1999. Model based design of a novel process for nitrogen removal from concentrated flows. *Mathematical and Computer Modelling of Dynamical Systems,* 5**,** 351-371.

HEM, L. J., RUSTEN, B. & ØDEGAARD, H. 1994. Nitrification in a moving bed biofilm reactor. *Water Research,* 28**,** 1425-1433.

HENZE, M. & COMEAU, Y. 2008. Wastewater Characterization. *In:* HENZE, M., VAN LOOSDRECHT, M. C. M., EKAMA, G. A. & BRDJANOVIC, D. (eds.) *Biological Wastewater Treatment.* London: IWA Publishing.

HOOPER, A. B. 1968. A NITRITE-REDUCING ENZYME FROM NITROSOMONAS EUROPAEA - PRELIMINARY CHARACTERIZATION WITH HYDROXYLAMINE AS ELECTRON DONOR. *Biochimica Et Biophysica Acta,* 162**,** 49-&.

HOOPER, A. B. & TERRY, K. R. 1979. HYDROXYLAMINE OXIDOREDUCTASE OF NITROSOMONAS PRODUCTION OF NITRIC-OXIDE FROM HYDROXYLAMINE. *Biochimica Et Biophysica Acta,* 571**,** 12-20.

HORN, H., REIFF, H. & MORGENROTH, E. 2003. Simulation of growth and detachment in biofilm systems under defined hydrodynamic conditions. *Biotechnology and Bioengineering,* 81**,** 607-617.

HU, Z., ZHANG, J., XIE, H. J., LI, S. P., ZHANG, T. T. & WANG, J. H. 2011. Identifying sources of nitrous oxide emission in anoxic/aerobic sequencing batch reactors (A/O SBRs) acclimated in different aeration rates. *Enzyme and Microbial Technology,* 49**,** 237-245.

HU, Z. Y., LOTTI, T., VAN LOOSDRECHT, M. & KARTAL, B. 2013. Nitrogen removal with the anaerobic ammonium oxidation process. *Biotechnology Letters,* 35**,** 1145-1154.

HYMAN, M. R. & ARP, D. J. 1995. Effects of ammonia on the de novo synthesis of polypeptides in cells of Nitrosomonas europaea denied ammonia as an energy source. *Journal of Bacteriology,* 177**,** 4974-9.

ITOKAWA, H., HANAKI, K. & MATSUO, T. 2001. Nitrous oxide production in high-loading biological nitrogen removal process under low COD/N ratio condition. *Water Research,* 35**,** 657-664.

JASON, J., CANTERA, L. & STEIN, L. Y. 2007. Role of nitrite reductase in the ammonia-oxidizing pathway of Nitrosomonas europaea. *Archives of Microbiology,* 188**,** 349-354.

JETTEN, M. S. M., STROUS, M., VAN DE PAS-SCHOONEN, K. T., SCHALK, J., VAN DONGEN, U. G. J. M., VAN DE GRAAF, A. A., LOGEMANN, S., MUYZER, G., VAN LOOSDRECHT, M. C. M. & KUENEN, J. G. 1998. The anaerobic oxidation of ammonium. *FEMS Microbiology Reviews,* 22**,** 421-437.

JIA, W. L., LIANG, S., ZHANG, J., NGO, H. H., GUO, W. S., YAN, Y. J. & ZOU, Y. N. 2013. Nitrous oxide emission in low-oxygen simultaneous nitrification and denitrification process: Sources and mechanisms. *Bioresource Technology,* 136**,** 444-451.

JIANG, Q. Q. & BAKKEN, L. R. 1999. Nitrous oxide production and methane oxidation by different ammonia-oxidizing bacteria. *Applied and Environmental Microbiology,* 65**,** 2679-2684.

JIN, R. C., YANG, G. F., YU, J. J. & ZHENG, P. 2012. The inhibition of the Anammox process: A review. *Chemical Engineering Journal,* 197**,** 67-79.

KAELIN, D., MANSER, R., RIEGER, L., EUGSTER, J., ROTTERMANN, K. & SIEGRIST, H. 2009. Extension of ASM3 for two-step nitrification and denitrification and its calibration and validation with batch tests and pilot scale data. *Water Research,* 43**,** 1680-1692.

KAMPSCHREUR, M. J., POLDERMANS, R., KLEEREBEZEM, R., VAN DER STAR, W. R. L., HAARHUIS, R., ABMA, W. R., JETTEN, M. S. M. & VAN LOOSDRECHT, M. C. M. 2009a. Emission of nitrous oxide and nitric oxide from a full-scale single-stage nitritation-anammox reactor. *Water Science and Technology,* 60**,** 3211-3217.

KAMPSCHREUR, M. J., TAN, N. C. G., PICIOREANU, C., JETTEN, M. S. M., SCHMIDT, I. & VAN LOOSDRECHT, M. C. M. 2006. Role of nitrogen oxides in the metabolism of ammonia-oxidizing bacteria. *Biochemical Society Transactions,* 34**,** 179-181.

KAMPSCHREUR, M. J., TEMMINK, H., KLEEREBEZEM, R., JETTEN, M. S. M. & VAN LOOSDRECHT, M. C. M. 2009b. Nitrous oxide emission during wastewater treatment. *Water Research,* 43**,** 4093-4103.

KARTAL, B., DE ALMEIDA, N. M., MAALCKE, W. J., OP DEN CAMP, H. J. M., JETTEN, M. S. M. & KELTJENS, J. T. 2013. How to make a living from anaerobic ammonium oxidation. *Fems Microbiology Reviews,* 37**,** 428-461.

KARTAL, B., KUYPERS, M. M. M., LAVIK, G., SCHALK, J., OP DEN CAMP, H. J. M., JETTEN, M. S. M. & STROUS, M. 2007a. Anammox bacteria disguised as denitrifiers: nitrate reduction to dinitrogen gas via nitrite and ammonium. *Environmental Microbiology,* 9**,** 635-642.

KARTAL, B., MAALCKE, W. J., DE ALMEIDA, N. M., CIRPUS, I., GLOERICH, J., GEERTS, W., DEN CAMP, H., HARHANGI, H. R., JANSSEN-MEGENS, E. M., FRANCOIJS, K. J., STUNNENBERG, H. G., KELTJENS, J. T., JETTEN, M. S. M. & STROUS, M. 2011. Molecular mechanism of anaerobic ammonium oxidation. *Nature,* 479**,** 127-U159.

KARTAL, B., RATTRAY, J., VAN NIFTRIK, L. A., VAN DE VOSSENBERG, J., SCHMID, M. C., WEBB, R. I., SCHOUTEN, S., FUERST, J. A., DAMSTÉ, J. S., JETTEN, M. S. M. & STROUS, M. 2007b. Candidatus "Anammoxoglobus propionicus" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria. *Systematic and Applied Microbiology,* 30**,** 39-49.

KARTAL, B., TAN, N. C. G., VAN DE BIEZEN, E., KAMPSCHREUR, M. J., VAN LOOSDRECHT, M. C. M. & JETTEN, M. S. M. 2010. Effect of Nitric Oxide on Anammox Bacteria. *Applied and Environmental Microbiology,* 76**,** 6304-6306.

KESTER, R. A., DE BOER, W. & LAANBROEK, H. J. 1997a. Production of NO and N(inf2)O by Pure Cultures of Nitrifying and Denitrifying Bacteria during Changes in Aeration. *Applied and Environmental Microbiology,* 63**,** 3872-7.

KESTER, R. A., DEBOER, W. & LAANBROEK, H. J. 1997b. Production of NO and N2O by pure cultures of nitrifying and denitrifying bacteria during changes in aeration. *Applied and Environmental Microbiology,* 63**,** 3872-3877.

KHAN, M. Z., MONDAL, P. K. & SABIR, S. 2013. Aerobic granulation for wastewater bioremediation: A review. *The Canadian Journal of Chemical Engineering,* 91**,** 1045-1058.

KIM, C. H. & HOLLOCHER, T. C. 1984. Catalysis of nitrosyl transfer reactions by a dissimilatory nitrite reductase (cytochrome c,d1). *Journal of Biological Chemistry,* 259**,** 2092-9.

KIM, S. W., MIYAHARA, M., FUSHINOBU, S., WAKAGI, T. & SHOUN, H. 2010. Nitrous oxide emission from nitrifying activated sludge dependent on denitrification by ammonia-oxidizing bacteria. *Bioresource Technology,* 101**,** 3958-3963.

KINDAICHI, T., ITO, T. & OKABE, S. 2004a. Ecophysiological interaction between nitrifying bacteria and heterotrophic bacteria in autotrophic nitrifying biofilms as determined by microautoradiography-fluorescence in situ hybridization. *Applied and Environmental Microbiology,* 70**,** 1641-1650.

KINDAICHI, T., KAWANO, Y., ITO, T., SATOH, H. & OKABE, S. 2006. Population dynamics and in situ kinetics of nitrifying bacteria in autotrophic nitrifying biofilms as determined by real-time quantitative PCR. *Biotechnology and Bioengineering,* 94**,** 1111-1121.

KINDAICHI, T., OKABE, S., SATOH, H. & WATANABE, Y. 2004b. Effects of hydroxylamine on microbial community structure and function of autotrophic nitrifying biofilms determined by in situ hybridization and the use of microelectrodes. *Water Science and Technology,* 49**,** 61-68.

KISHIDA, N., KIM, J. H., KIMOCHI, Y., NISHIMURA, O., SASAKI, H. & SUDO, R. 2004. Effect of C/N ratio on nitrous oxide emission from swine wastewater treatment process. *Water Science and Technology,* 49**,** 359-365.

KONG, Q., ZHANG, J., MIAO, M., TIAN, L., GUO, N. & LIANG, S. 2013. Partial nitrification and nitrous oxide emission in an intermittently aerated sequencing batch biofilm reactor. *Chemical Engineering Journal,* 217**,** 435-441.

KOSTERA, J., MCGARRY, J. & PACHECO, A. A. 2010. Enzymatic Interconversion of Ammonia and Nitrite: The Right Tool for the Job. *Biochemistry,* 49**,** 8546-8553.

KOSTERA, J., YOUNGBLUT, M. D., SLOSARCZYK, J. M. & PACHECO, A. A. 2008. Kinetic and product distribution analysis of NO center dot reductase activity in Nitrosomonas europaea hydroxylamine oxidoreductase. *Journal of Biological Inorganic Chemistry,* 13**,** 1073-1083.

KOZLOWSKI, J. A., PRICE, J. & STEIN, L. Y. 2014. Revision of N2O-Producing Pathways in the Ammonia-Oxidizing Bacterium Nitrosomonas europaea ATCC 19718. *Applied and Environmental Microbiology,* 80**,** 4930-4935.

KUENEN, J. G. 2008. Anammox bacteria: from discovery to application. *Nat Rev Micro,* 6**,** 320-326.

KÖRNER, H. & ZUMFT, W. G. 1989. Expression of denitrification enzymes in response to the dissolved oxygen level and respiratory substrate in continuous culture of Pseudomonas stutzeri. *Applied and Environmental Microbiology,* 55**,** 1670-1676.

LAANBROEK, H. J., BAR-GILISSEN, M. J. & HOOGVELD, H. L. 2002. Nitrite as a stimulus for ammonia-starved Nitrosomonas europaea. *Applied and Environmental Microbiology,* 68**,** 1454-1457.

LACKNER, S. & HORN, H. 2012. Comparing the performance and operation stability of an SBR and MBBR for single-stage nitritation-anammox treating wastewater with high organic load. *Environmental Technology,* 34**,** 1319-1328.

LAW, Y., LANT, P. & YUAN, Z. 2013. The Confounding Effect of Nitrite on N2O Production by an Enriched Ammonia-Oxidizing Culture. *Environmental Science & Technology,* 47**,** 7186-7194.

LAW, Y., LANT, P. & YUAN, Z. G. 2011. The effect of pH on N2O production under aerobic conditions in a partial nitritation system. *Water Research,* 45**,** 5934-5944.

LAW, Y., NI, B. J., LANT, P. & YUAN, Z. G. 2012. N_2O production rate of an enriched ammonia-oxidising bacteria culture exponentially correlates to its ammonia oxidation rate. *Water Research,* 46**,** 3409-3419.

LAWTON, T. J., BOWEN, K. E., SAYAVEDRA-SOTO, L. A., ARP, D. J. & ROSENZWEIG, A. C. 2013. Characterization of a Nitrite Reductase Involved in Nitrifier Denitrification. *Journal of Biological Chemistry,* 288**,** 25575-25583.

LIU, B., FROSTEGÅRD, Å. & BAKKEN, L. R. 2014. Impaired Reduction of N2O to N2 in Acid Soils Is Due to a Posttranscriptional Interference with the Expression of nosZ. *mBio,* 5.

LO, I. W., LO, K. V., MAVINIC, D. S., SHISKOWSKI, D. & RAMEY, W. 2010. Contributions of biofilm and suspended sludge to nitrogen transformation and nitrous oxide emission in hybrid sequencing batch system. *Journal of Environmental Sciences,* 22**,** 953-960.

LOCHMATTER, S., GONZALEZ-GIL, G. & HOLLIGER, C. 2013. Optimized aeration strategies for nitrogen and phosphorus removal with aerobic granular sludge. *Water Research,* 47**,** 6187-6197.

LOTTI, T., KLEEREBEZEM, R., LUBELLO, C. & VAN LOOSDRECHT, M. C. M. 2014. Physiological and kinetic characterization of a suspended cell anammox culture. *Water Research,* 60**,** 1-14.

LOTTI, T., VAN DER STAR, W. R. L., KLEEREBEZEM, R., LUBELLO, C. & VAN LOOSDRECHT, M. C. M. 2012. The effect of nitrite inhibition on the anammox process. *Water Research,* 46**,** 2559-2569.

MA, Y., SUNDAR, S., PARK, H. & CHANDRAN, K. 2015. The effect of inorganic carbon on microbial interactions in a biofilm nitritation–anammox process. *Water Research,* 70**,** 246-254.

MAIA, L. B. & MOURA, J. J. G. 2014. How Biology Handles Nitrite. *Chemical Reviews,* 114**,** 5273-5357.

MORGENROTH, E. 2008a. Biofilm Reactors. *In:* HENZE, M., VAN LOOSDRECHT, M. C. M., EKAMA, G. A. & BRDJANOVIC, D. (eds.) *Biological Wastewater Treatment.* London: IWA Publishing.

MORGENROTH, E. 2008b. Modelling Biofilm. *In:* HENZE, M., VAN LOOSDRECHT, M. C. M., EKAMA, G. A. & BRDJANOVIC, D. (eds.) *Biological Wastewater Treatment.* London: IWA Publishing.

MORGENROTH, E., SHERDEN, T., VAN LOOSDRECHT, M. C. M., HEIJNEN, J. J. & WILDERER, P. A. 1997. Aerobic granular sludge in a sequencing batch reactor. *Water Research,* 31**,** 3191-3194.

MORGENROTH, E. & WILDERER, P. A. 2000. Influence of detachment mechanisms on competition in biofilms. *Water Research,* 34**,** 417-426.

MULDER, A., VANDEGRAAF, A. A., ROBERTSON, L. A. & KUENEN, J. G. 1995. Anaerobic ammonium oxidation discovered in a denitrifying fluidized-bed reactor. *Fems Microbiology Ecology,* 16**,** 177-183.

NI, B.-J., SMETS, B. F., YUAN, Z. & PELLICER-NÀCHER, C. 2013. Model-based evaluation of the role of Anammox on nitric oxide and nitrous oxide productions in membrane aerated biofilm reactor. *Journal of Membrane Science,* 446**,** 332-340.

OKABE, S., SATOH, H. & WATANABE, Y. 1999. In situ analysis of nitrifying biofilms as determined by in situ hybridization and the use of microelectrodes. *Applied and Environmental Microbiology,* 65**,** 3182-3191.

PAN, Y. T., NI, B. J. & YUAN, Z. G. 2013. Modeling electron competition among nitrogen oxides reduction and N_2O accumulation in denitrification. *Environmental Science & Technology,* 47**,** 11083-11091.

PARK, K. Y., INAMORI, Y., MIZUOCHI, M. & AHN, K. H. 2000. Emission and control of nitrous oxide from a biological wastewater treatment system with intermittent aeration. *Journal of Bioscience and Bioengineering,* 90**,** 247-252.

PARK, S. & BAE, W. 2009. Modeling kinetics of ammonium oxidation and nitrite oxidation under simultaneous inhibition by free ammonia and free nitrous acid. *Process Biochemistry,* 44**,** 631-640.

POTH, M. & FOCHT, D. D. 1985. N-15 kinetic-analysis of N_2O production by *Nitrosomonas europaea* - an examination of nitrifier dentrification. *Applied and Environmental Microbiology,* 49**,** 1134-1141.

POUGHON, L., DUSSAP, C.-G. & GROS, J.-B. 2001a. Energy model and metabolic flux analysis for autotrophic nitrifiers. *Biotechnology and Bioengineering,* 72**,** 416-433.

POUGHON, L., DUSSAP, C. G. & GROS, J. B. 2001b. Energy model and metabolic flux analysis for autotrophic nitrifiers. *Biotechnology and Bioengineering,* 72**,** 416-433.

PURKHOLD, U., POMMERENING-ROSER, A., JURETSCHKO, S., SCHMID, M. C., KOOPS, H. P. & WAGNER, M. 2000. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoA sequence analysis: Implications for molecular diversity surveys. *Applied and Environmental Microbiology,* 66**,** 5368-5382.

QUINLAN, A. V. 1984. Prediction of the optimum pH for ammonia-n oxidation by nitrosomonas Europaea in well-aerated natural and domestic-waste waters. *Water Research,* 18**,** 561-566.

RASSAMEE, V., SATTAYATEWA, C., PAGILLA, K. & CHANDRAN, K. 2011. Effect of Oxic and Anoxic Conditions on Nitrous Oxide Emissions from Nitrification and Denitrification Processes. *Biotechnology and Bioengineering,* 108**,** 2036-2045.

RAVISHANKARA, A. R., DANIEL, J. S. & PORTMANN, R. W. 2009. Nitrous Oxide (N2O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science,* 326**,** 123-125.

REZIĆ, T., ŠANTEK, B., NOVAK, S. & MARIĆ, V. 2007. Heterotrophic cultivation of Paracoccus denitrificans in a horizontal rotating tubular bioreactor. *World Journal of Microbiology and Biotechnology,* 23**,** 987-996.

RICHARDSON, D., FELGATE, H., WATMOUGH, N., THOMSON, A. & BAGGS, E. 2009. Mitigating release of the potent greenhouse gas N_2O from the nitrogen cycle - could enzymic regulation hold the key? *Trends in Biotechnology,* 27**,** 388-397.

RITTMANN, B. E., REGAN, J. M. & STAHL, D. A. 1994. Nitrification as a source of soluble organic substrate in biological treatment. *Water Science and Technology,* 30**,** 1-8.

SCHMID, M., TWACHTMANN, U., KLEIN, M., STROUS, M., JURETSCHKO, S., JETTEN, M., METZGER, J. W., SCHLEIFER, K.-H. & WAGNER, M. 2000. Molecular Evidence for Genus Level Diversity of Bacteria Capable of Catalyzing Anaerobic Ammonium Oxidation. *Systematic and Applied Microbiology,* 23**,** 93-106.

SCHMIDT, I. 2008. Nitric oxide: Interaction with the ammonia monooxygenase and regulation of metabolic activities in ammonia oxidizers. *In:* CADENAS, E. & PACKER, L. (eds.) *Nitric Oxide, Part F: Oxidative and Nitrosative Stress in Redox Regulation of Cell Signaling.* San Diego: Elsevier Academic Press Inc.

SCHMIDT, I. 2009. Chemoorganoheterotrophic Growth of *Nitrosomonas europaea* and *Nitrosomonas eutropha*. *Current Microbiology,* 59**,** 130-138.

SCHMIDT, I. & BOCK, E. 1997. Anaerobic ammonia oxidation with nitrogen dioxide by Nitrosomonas eutropha. *Archives of Microbiology,* 167**,** 106-111.

SCHMIDT, I. & BOCK, E. 1998. Anaerobic ammonia oxidation by cell-free extracts of Nitrosomonas eutropha. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology,* 73**,** 271-278.

SCHMIDT, I., BOCK, E. & JETTEN, M. S. M. 2001a. Ammonia oxidation by Nitrosomonas eutropha with NO2 as oxidant is not inhibited by acetylene. *Microbiology-Sgm,* 147**,** 2247-2253.

SCHMIDT, I., STEENBAKKERS, P. J. M., OP DEN CAMP, H. J. M., SCHMIDT, K. & JETTEN, M. S. M. 2004a. Physiologic and proteomic evidence for a role of nitric oxide in biofilm formation by Nitrosomonas europaea and other ammonia oxidizers. *Journal of Bacteriology,* 186**,** 2781-2788.

SCHMIDT, I., VAN SPANNING, R. J. M. & JETTEN, M. S. M. 2004b. Denitrification and ammonia oxidation by Nitrosomonas europaea wild-type, and NirK- and NorB-deficient mutants. *Microbiology-Sgm,* 150**,** 4107-4114.

SCHMIDT, I., ZART, D. & BOCK, E. 2001b. Effects of gaseous NO2 on cells of Nitrosomonas eutropha previously incapable of using ammonia as an energy source. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology,* 79**,** 39-47.

SCHRAMM, A., DE BEER, D., GIESEKE, A. & AMANN, R. 2000. Microenvironments and distribution of nitrifying bacteria in a membrane-bound biofilm. *Environmental Microbiology,* 2**,** 680-686.

SCHRAMM, A., DE BEER, D., VAN DEN HEUVEL, J. C., OTTENGRAF, S. & AMANN, R. 1999. Microscale Distribution of Populations and Activities ofNitrosospira and Nitrospira spp. along a Macroscale Gradient in a Nitrifying Bioreactor: Quantification by In Situ Hybridization and the Use of Microsensors. *Applied and Environmental Microbiology,* 65**,** 3690-3696.

SCHRAMM, A., LARSEN, L. H., REVSBECH, N. P., RAMSING, N. B., AMANN, R. & SCHLEIFER, K. H. 1996. Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes. *Applied and Environmental Microbiology,* 62**,** 4641-4647.

SCHREIBER, F., LOEFFLER, B., POLERECKY, L., KUYPERS, M. M. M. & DE BEER, D. 2009. Mechanisms of transient nitric oxide and nitrous oxide production in a complex biofilm. *Isme Journal,* 3**,** 1301-1313.

SCHREIBER, F., WUNDERLIN, P., UDERT, K. M. & WELLS, G. F. 2012. Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: biological pathways, chemical reactions, and novel technologies. *Frontiers in Microbiology,* 3**,** 24.

SCHULTHESS, R. V., KÜHNI, M. & GUJER, W. 1995. Release of nitric and nitrous oxides from denitrifying activated sludge. *Water Research,* 29**,** 215-226.

SHAW, L. J., NICOL, G. W., SMITH, Z., FEAR, J., PROSSER, J. I. & BAGGS, E. M. 2006. Nitrosospira spp. can produce nitrous oxide via a nitrifier denitrification pathway. *Environmental Microbiology,* 8**,** 214-222.

SHEN, T., STIEGLMEIER, M., DAI, J., URICH, T. & SCHLEPER, C. 2013. *Responses of the terrestrial ammonia-oxidizing archaeon Ca. Nitrososphaera viennensis and the ammonia-oxidizing bacterium Nitrosospira multiformis to nitrification inhibitors*.

SLIEKERS, A. O., HAAIJER, S. C. M., STAFSNES, M. H., KUENEN, J. G. & JETTEN, M. S. M. 2005. Competition and coexistence of aerobic ammonium- and nitrite-oxidizing bacteria at low oxygen concentrations. *Applied Microbiology and Biotechnology,* 68**,** 808-817.

SPIECK, E. & LIPSKI, A. 2011. Chapter five - Cultivation, Growth Physiology, and Chemotaxonomy of Nitrite-Oxidizing Bacteria. *In:* MARTIN, G. K. (ed.) *Methods in Enzymology.* Academic Press.

SPOTT, O., RUSSOW, R. & STANGE, C. F. 2011. Formation of hybrid N2O and hybrid N2 due to codenitrification: First review of a barely considered process of microbially mediated N-nitrosation. *Soil Biology and Biochemistry,* 43**,** 1995-2011.

STARKENBURG, S. R., ARP, D. J. & BOTTOMLEY, P. J. 2008. Expression of a putative nitrite reductase and the reversible inhibition of nitrite-dependent respiration by nitric oxide in Nitrobacter winogradskyi Nb-255. *Environmental Microbiology,* 10**,** 3036-3042.

STEIN, L. Y. & ARP, D. J. 1998. Loss of Ammonia Monooxygenase Activity in Nitrosomonas europaea upon Exposure to Nitrite. *Applied and Environmental Microbiology,* 64**,** 4098-4102.

STROUS, M., FUERST, J. A., KRAMER, E. H. M., LOGEMANN, S., MUYZER, G., VAN DE PAS-SCHOONEN, K. T., WEBB, R., KUENEN, J. G. & JETTEN, M. S. M. 1999a. Missing lithotroph identified as new planctomycete. *Nature,* 400**,** 446-449.

STROUS, M., KUENEN, J. G. & JETTEN, M. S. M. 1999b. Key physiology of anaerobic ammonium oxidation. *Applied and Environmental Microbiology,* 65**,** 3248-3250.

STUVEN, R. & BOCK, E. 2001. Nitrification and denitrification as a source for NO and NO2 production in high-strength wastewater. *Water Research,* 35**,** 1905-1914.

SUNDERMEYER-KLINGER, H., MEYER, W., WARNINGHOFF, B. & BOCK, E. 1984. Membrane-bound nitrite oxidoreductase of Nitrobacter: evidence for a nitrate reductase system. *Archives of Microbiology,* 140**,** 153-158.

SUZUKI, I., DULAR, U. & KWOK, S. C. 1974. Ammonia or Ammonium Ion as Substrate for Oxidation by Nitrosomonas europaea Cells and Extracts. *Journal of Bacteriology,* 120**,** 556-558.

TALLEC, G., GARNIER, J. & GOUSAILLES, M. 2006. Nitrogen removal in a wastewater treatment plant through biofilters: nitrous oxide emissions during nitrification and denitrification. *Bioprocess and Biosystems Engineering,* 29**,** 323-333.

TODT, D. & DÖRSCH, P. 2015. Nitrous oxide emissions in a biofilm loaded with different mixtures of concentrated household wastewater. *International Journal of Environmental Science and Technology***,** 1-12.

UDERT, K. M. & JENNI, S. 2013. Biological nitrogen conversion processes. *In:* LARSEN, T. A., UDERT, K. M. & LIENERT, J. (eds.) *Source separation and decentralization for wastewater management.* London: IWA Publishing.

UDERT, K. M., LARSEN, T. A. & GUJER, W. 2005. Chemical nitrite oxidation in acid solutions as a consequence of microbial ammonium oxidation. *Environmental Science & Technology,* 39**,** 4066-4075.

UPADHYAY, A. K., HOOPER, A. B. & HENDRICH, M. P. 2006. NO reductase activity of the tetraheme cytochrome c(554) of *Nitrosomonas europaea*. *Journal of the American Chemical Society,* 128**,** 4330-4337.

VAN DE GRAAF, A. A., MULDER, A., DE BRUIJN, P., JETTEN, M. S., ROBERTSON, L. A. & KUENEN, J. G. 1995. Anaerobic oxidation of ammonium is a biologically mediated process. *Applied and Environmental Microbiology,* 61**,** 1246-51.

WANG, C. C., LEE, P. H., KUMAR, M., HUANG, Y. T., SUNG, S. W. & LIN, J. G. 2010. Simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD) in a full-scale landfill-leachate treatment plant. *Journal of Hazardous Materials,* 175**,** 622-628.

WANNER, O., EBERL, H. J., MORGENROTH, E., NOGUERA, D. R., PICIOREANU, C., RITTMANN, B. E. & VAN LOOSDRECHT, M. C. M. 2006. Mathematical Modeling of Biofilms. London, UK: IWA Publishing.

WANNER, O. & GUJER, W. 1985. Competition in Biofilms. *Water Science and Technology,* 17**,** 27-44.

WANNER, O. & GUJER, W. 1986. A multispecies biofilm model. *Biotechnology and Bioengineering,* 28**,** 314-328.

WANNER, O. & REICHERT, P. 1996. Mathematical modeling of mixed-culture biofilms. *Biotechnology and Bioengineering,* 49**,** 172-184.

WHITTAKER, M., BERGMANN, D., ARCIERO, D. & HOOPER, A. B. 2000. Electron transfer during the oxidation of ammonia by the chemolithotrophic bacterium Nitrosomonas europaea. *Biochimica Et Biophysica Acta-Bioenergetics,* 1459**,** 346-355.

WINKLER, M. K. H., KLEEREBEZEM, R. & VAN LOOSDRECHT, M. C. M. 2012a. Integration of anammox into the aerobic granular sludge process for main stream wastewater treatment at ambient temperatures. *Water Research,* 46**,** 136-144.

WINKLER, M. K. H., YANG, J., KLEEREBEZEM, R., PLAZA, E., TRELA, J., HULTMAN, B. & VAN LOOSDRECHT, M. C. M. 2012b. Nitrate reduction by organotrophic Anammox bacteria in a nitritation/anammox granular sludge and a moving bed biofilm reactor. *Bioresource Technology,* 114**,** 217-223.

WOOD, P. M. 1986. Nitrification as a bacterial energy source. *In:* PROSSER, J. I. (ed.) *Nitrification.* Oxford: Society for General Microbiology, IRL press.

WUNDERLIN, P., LEHMANN, M. F., SIEGRIST, H., TUZSON, B., JOSS, A., EMMENEGGER, L. & MOHN, J. 2013. Isotope Signatures of N2O in a Mixed Microbial Population System: Constraints on N2O Producing Pathways in Wastewater Treatment. *Environmental Science & Technology,* 47**,** 1339-1348.

WUNDERLIN, P., MOHN, J., JOSS, A., EMMENEGGER, L. & SIEGRIST, H. 2012. Mechanisms of N_2O production in biological wastewater treatment under nitrifying and denitrifying conditions. *Water Research,* 46**,** 1027-1037.

XIAO, Y., WU, S., YANG, Z.-H., WANG, Z.-J., YAN, C.-Z. & ZHAO, F. 2013. In situ probing the effect of potentials on the microenvironment of heterotrophic denitrification biofilm with microelectrodes. *Chemosphere,* 93**,** 1295-1300.

YAMANAKA, T. & SHINRA, M. 1974. CYTOCHROME C-552 AND CYTOCHROME C-554 DERIVED FROM NITROSOMONAS-EUROPAES - PURIFICATION, PROPERTIES, AND THEIR FUNCTION IN HYDROXYLAMINE OXIDATION. *Journal of Biochemistry,* 75**,** 1265-1273.

YANG, J. J., TRELA, J., PLAZA, E. & TJUS, K. 2013. N_2O emissions from a one stage partial nitrification/anammox process in moving bed biofilm reactors. *Water Science and Technology,* 68**,** 144-152.

YOO, H., AHN, K.-H., LEE, H.-J., LEE, K.-H., KWAK, Y.-J. & SONG, K.-G. 1999. Nitrogen removal from synthetic wastewater by simultaneous nitrification and denitrification (SND) via nitrite in an intermittently-aerated reactor. *Water Research,* 33**,** 145-154.

YOSHINARI, T. 1990. Emissions of N2O from Various Environments — The Use of Stable Isotope Composition of N2O as Tracer for the Studies of N2O Biogeochemical Cycling. *In:* REVSBECH, N. & SØRENSEN, J. (eds.) *Denitrification in Soil and Sediment.* Springer US.

YU, R. & CHANDRAN, K. 2010. Strategies of Nitrosomonas europaea 19718 to counter low dissolved oxygen and high nitrite concentrations. *Bmc Microbiology,* 10**,** 11.

YU, R., KAMPSCHREUR, M. J., VAN LOOSDRECHT, M. C. M. & CHANDRAN, K. 2010. Mechanisms and Specific Directionality of Autotrophic Nitrous Oxide and Nitric Oxide Generation during Transient Anoxia. *Environmental Science & Technology,* 44**,** 1313-1319.

ZART, D., SCHMIDT, I. & BOCK, E. 2000. Significance of gaseous NO for ammonia oxidation by Nitrosomonas eutropha. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology,* 77**,** 49-55.

ZHANG, D. J., CAI, Q., ZU, B., BAI, C. & ZHANG, P. 2010. The influence of trace NO(2) on the kinetics of ammonia oxidation and the characteristics of nitrogen removal from wastewater. *Water Science and Technology,* 62**,** 1037-1044.

ZHOU, Y., OEHMEN, A., LIM, M., VADIVELU, V. & NG, W. J. 2011. The role of nitrite and free nitrous acid (FNA) in wastewater treatment plants. *Water Research,* 45**,** 4672-4682.

ZHOU, Y., PIJUAN, M. & YUAN, Z. 2008a. Development of a 2-sludge, 3-stage system for nitrogen and phosphorous removal from nutrient-rich wastewater using granular sludge and biofilms. *Water Research,* 42**,** 3207-3217.

ZHOU, Y., PIJUAN, M., ZENG, R. J. & YUAN, Z. 2008b. Free Nitrous Acid Inhibition on Nitrous Oxide Reduction by a Denitrifying-Enhanced Biological Phosphorus Removal Sludge. *Environmental Science & Technology,* 42**,** 8260-8265.

ZUMFT, W. G. 1997. Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Reviews,* 61**,** 533-+.

PAPER 5

Nitrous oxide emissions in a biofilm loaded with different mixtures of concentrated household wastewater

Daniel Todt, Peter Dörsch

2015

International Journal of Environmental Science and Technology
DOI 10.1007/s13762-015-0778-1

Int. J. Environ. Sci. Technol.
DOI 10.1007/s13762-015-0778-1

ORIGINAL PAPER

Nitrous oxide emissions in a biofilm loaded with different mixtures of concentrated household wastewater

D. Todt · P. Dörsch

Received: 23 October 2014 / Revised: 7 January 2015 / Accepted: 14 February 2015

Abstract Aerobic biofilm systems are increasingly used for household wastewater treatment, but little is known about their potential to emit nitrous oxide in response to different loading conditions. We studied nitrification and nitrous oxide production in a biofilm reactor that was continuously fed with three different mixtures of household wastewater. Higher proportions of blackwater increased nitrification activity, which resulted in enhanced nitrite accumulation and nitrous oxide emissions. Applying a conceptual biofilm model together with the results of ancillary batch incubations suggested that this was caused by a higher proportion of slowly degradable compounds in blackwater. Increasing amounts of blackwater would result in less oxygen depletion by heterotrophic degradation in outer biofilm layers, leading to nitrite accumulation by enhanced ammonia oxidation as well as electron limitation of denitrification in anoxic biofilm layers. Under such conditions, nitrifier denitrification and incomplete heterotrophic denitrification would be the prevailing sources of nitrous oxide emission. These assumptions are supported by an exponential increase in the determined emission factor (nitrous oxide relative to oxidized ammonia), which accounted for 0.7 with 20 % blackwater, 1.1 with 50 % blackwater, and 8.5 with 100 % blackwater in the wastewater load.

Keywords Biofilm · Nitrous oxide · Nitric oxide · Household wastewater · Blackwater · Greywater

D. Todt (✉) · P. Dörsch
Department of Environmental Sciences, Norwegian University of Life Sciences, Frognerbakken 3, 1432 Ås, Norway
e-mail: danitodt@gmail.com

Introduction

Due to their robustness, aerobic biofilm systems are gaining increasing popularity for onsite treatment of household wastewater (Azizi et al. 2013). Household wastewater has usually higher concentrations of nitrogen and organic compounds and a significantly lower C/N ratio than municipal wastewater, but the composition varies with source depending on both infrastructure and user habits. In regions with water shortage, separation and reuse of particular wastewater fractions is gaining importance which can result in highly concentrated sewage (Penn et al. 2012). During biological treatment, highly concentrated household wastewaters, especially pure blackwater, pose an environmental risk by potentially being a strong source for nitrous oxide (N_2O; de Graaff et al. 2010; Itokawa et al. 2001). Nitrous oxide is a strong greenhouse gas (ICCP 2001) and involved in the depletion of the stratospheric ozone layer (Ravishankara et al. 2009). In the last decade, N_2O emissions have gained increasing awareness in design and operation of wastewater treatment plants (Kampschreur et al. 2009; Schreiber et al. 2012). Hence, more knowledge is needed to understand how process dynamics of C and N turnover in highly charged biofilms control N_2O formation and emission.

Most of the present knowledge on NO and N_2O emissions in wastewater treatment processes originates from pure culture studies (e.g. Beaumont et al. 2002; Poth and Focht 1985; Schmidt 2009) or systems with suspended biomass (activated sludge; (Kampschreur et al. 2009). Only few studies have investigated N_2O in biofilm systems that support complex biomass interactions driven by diffusion limited substrate fluxes. Most of these studies dealt with pure nitrifying biofilm systems dominated by autotrophic communities (de Graaff et al. 2010; Yang et al. 2013). So

Published online: 04 March 2015

far, little research has been dedicated to N_2O-emitting processes in more complex heterogeneous communities of interacting heterotrophic and autotrophic organisms typical for biofilm systems loaded with domestic wastewater, with the notable exception of a recent bench-scale study using synthetic substrates (Schreiber et al. 2009). However, synthetic substrates may not be fully representative for real household wastewaters, which constitute a complex mixture of mineral and organic molecules (Dulekgurgen et al. 2006; Hocaoglu et al. 2010).

The objective of the present study was to assess N gas production (NO, N_2O) in a biofilm system loaded with different mixtures of concentrated household wastewater, simulating inlet conditions typical for onsite applications in systems with source separation and reuse of greywater. For this purpose, a long-term experiment with three mixtures of source separated sewage from a student dormitory was set up as a moving bed biofilm reactor (MBBR). MBBR systems are simple to operate and monitor at steady state and therefore often used in applied biofilm experiments (e.g. de Graaff et al. 2010; Fux et al. 2004). Moreover, simple MBBR systems are typically used in decentralized applications, in which nitrification is not necessarily the main purpose but often occurs as a side effect (Daude and Stephenson 2003). Our study used an experimental MBBR which was operated as heterotrophic biofilm system. To explore the impact of wastewater compositions and system conditions on N gas production (NO, N_2O) in more detail, additional bench-scale incubations of biofilm material were carried out. The research of this study was carried out in the period from March to June 2013 at the Norwegian University for Life Sciences in Ås, Norway.

Materials and methods

Experimental setup of the MBBR reactor system

A continuous flow MBBR system with a well-established biofilm was loaded with three different wastewater mixtures according to Table 1. The MBBR system comprised two sequential reactor tanks (R1 and R2) of 200 L volume each, filled to 60 % with a 600 m^2 m^{-3} carrier material (BWTX, Biowater, Tønsberg, Norway). The tanks were aerated via coarse diffusers (3 mm orifice) from the bottom of the reactor tanks. R1 was equipped with 16 diffusers and R2 with 12 diffusers. The air flow in the reactor tanks, Q_{airR}, was estimated based on the number of diffusers and the total air flow measured with a flow meter (SS 30.300, SCHMIDT, St. Georgen, Germany) at the inlet of the tanks. Based on the measured total air flow of 8840 ± 100 L h^{-1}, a Q_{air} of 5050 L h^{-1} was estimated for R1 and 3790 L h^{-1} for R2. Prior to the experiment, the reactor was operated for 6 months on a loading regime equal to the first loading period. The aeration ensured a DO in the range of 3–5 mg L^{-1} in both reactor tanks during the entire experimental period.

The wastewater was taken from a nearby student dormitory equipped with source separating sanitation (Todt et al. 2014). The inlet mixtures (Table 1) were established once per day with help of peristaltic pumps (Bredel SPX, Whatson Marlos, Falmouth, UK) in a third stirred tank from which the first reactor tank was fed. Mixing and feeding was controlled by a PLC. The inlet flow was measured with a flow meter (Optiflux2000, Krohne, Duisburg, Germany).

Liquid analysis and calculation of fluxes

For the liquid analysis, grab samples were taken from the inlet tank and from the reactor tanks R1 and R2. Soluble COD and nitrogen species (total ammonia nitrogen (TAN), NO_2^-, NO_3^-) were analyzed by spectrophotometric test kits from Hach-Lange, Berlin, Germany (LCK314, LCK114, LCK304, LCK341, LCK339). The samples were filtered through 1.2-µm glassfiber filters (Whatman™ GF-C) prior to analysis. Fluxes of dissolved N compounds and COD in each tank were calculated as the difference in measured concentrations between inlet and R1 or R1 and R2, respectively. To account for differences in short-term inlet variations, the fluxes where calculated as weekly averages of 3–5 measurements. Statistical variation was estimated with help of random variable algebra, assuming normal distribution for samples taken within the same week.

Table 1 Loading periods P1–P3 in 2013 with different mixtures of household wastewater, hydraulic loading rate (Q), and hydraulic retention time (HRT) in the MBBR system

	Blackwater fraction	Greywater fraction	Q	HRT
First period P1 (February 5–March 12)	20 % (324 L day^{-1})	80 % (1167 L day^{-1})	1487 L day^{-1}	0.26 day
Second period P2 (April 5–April 19)	50 % (309 L day^{-1})	50 % (318 L day^{-1})	627 L day^{-1}	0.64 day
Third period P3 (April 25–May 16)	100 % (320 L day^{-1})	–	320 L day^{-1}	1.26 day

Gas sampling and analysis

In each of the reactor tanks, gas samples were taken from two different sampling points. For the first sampling point, a 500-mm-long and 75-mm-wide cylinder was immersed 5 cm into the reactor to collect the air leaving the bulk liquid. Gas samples were taken with an injection needle from the center of the cylinder 250 mm above the water level. The second sampling point was established in the ventilation outlet pipe of each of the reactor tanks. The funnel method ensured collection of air that had passed the reactor with no dilution from ambient air, while the small area covered by the funnel was probably not representative for the average gas flow across the reactor. The sample drawn from the ventilation system, on the other hand, can be assumed to be representative for the whole reactor surface, but is sensitive to dilution by ambient air leaking into the ventilation pipes. Average values were calculated from both sampling points, unless the deviation in gas concentration was >20 %, in which case the measurements were discarded. In addition to the reactor samples, a third sampling point was established at the inlet of the aeration pump. The N_2O flux from a particular reactor tank, J_{N_2o-Ri} (mg N m^{-2} biofilm h^{-1}), was calculated based on the concentration difference between the airstream leaving ($ppmv_{SjRi}$) and the airstream entering a reactor tank ($ppmv_{in}$), the air flow rate in the reactor tank Q_{airRi} (m^3 h^{-1}), the air temperature in the head space above the reactor tanks T_{Ri} (K), the gas constant R (J K^{-1} mol^{-1}), and the active surface area A (m^2) (Eq. 1).

$$J_{N_2o-Ri} = \left(ppmv_{SjRi} - ppmv_{in}\right) \times Q_{airRi} \times \frac{1}{(R \times T_{Ri})} \times 28 \times \frac{1}{A} \quad (1)$$

The gas samples were stored and transported in helium washed, pre-evacuated septum vials (Model 10-CV-Crimp, Chromacol, Herts). N_2O was analyzed on a gas chromatograph (Model 7890A, Agilent, Santa Clara, US) using an electron capture detector (ECD) for low (<4 ppmv) and a thermal conductivity detector for high N_2O concentrations. For separation of N_2O from air, a 20-m-wide bore Poraplot Q capillary (0.53 mm) column at 38 °C with backflushing and He as carrier gas was used. The ECD conditions were 375 °C with 17 mL min^{-1} $ArCH_4$ (90/10 vol%) as makeup gas.

Ex-situ studies to explore biofilm processes

Additional short-term (<5 h) batch incubations in air with different C and N additions were performed in constantly stirred 120-mL serum bottles loaded with three biofilm media grits and 20-mL incubation liquid, mimicking a filling degree comparable to that of the reactor tanks. For each incubation experiment, fresh biofilm media grits were taken from the pilot-scale system. The bottles were crimp-sealed with butyl rubber septa and incubated under constant magnetic stirring (300 rpm) at 15 °C in a water bath. Headspace was sampled automatically every 50 min by an autosampler and analyzed by a gas chromatograph and a connected chemoluminescence NO analyzer for N_2O and NO, respectively (Molstad et al. 2007).

To obtain more information about the kinetics of organic C and N depletion, a 1.4-L open bench-scale MBBR was set up. The cylindrical reactor of 75 mm diameter was aerated from the bottom with a coarse diffuser and an aeration pump having a capacity of 0.2 N m^3 h^{-1}. For each run, the reactor was filled with 800 mL fresh biofilm media taken from the second reactor tank corresponding to a filling degree of 60 %.

Table 2 gives an overview over the treatments used in the incubations experiments. The mixtures A1 and B1 simulated loading conditions in the second reactor tank during period 1 and 2, respectively. The inlet mixtures C1 and C2 simulated a higher organic loading rate, while C2 was amended with KNO_2 to test the effect NO_2^- accumulation on N gas production. To explore the biofilm processes with a more defined substrate, a sterile basis medium (synthetic mixtures D1) was prepared by filtering

Table 2 Incubation mixtures used in the incubation trials

	Period	Incubation mixture	Addition	$COD_{soluble}$ (mg O_2 L^{-1})	TAN (mg N L^{-1})	NO_2^- (mg N L^{-1})	NO_3^- (mg N L^{-1})
A1	P1	50 % R1 50 % R2	None	205	275	1.4	0.75
B1	P2	50 % R1 50 % R2	None	332	560	30	7
C1	P2	Sewage P2 1:2 diluted	None	407	223	–	1
C2			NO_2^-	407	223	27	1
D1	P2	Synthetic	NH_4^+	464	332	–	2

and diluting (1:20) a thermally treated (90 °C for 20 min) sewage mixture of P2, to which acetate and NH_4Cl was added to reach TAN and COD values similar to the inlet mixtures (C1, C2).

Ammonia fluxes related to biological N transformation (J_{NH_4oxi})

To determine the net TAN flux related to biological oxidation (J_{NH_4oxi}), the TAN fraction lost by stripping of NH_3 (J_{NH_3g}) and by immobilization into the growing biomass (J_{NH_4immob}) were estimated and subtracted from the gross flux of TAN (J_{NH_4gross}) which was observed between inlet and R1 or R2 and R1 (Eq. 2). For batch incubation in closed bottles, J_{NH_3g} was assumed to be negligible, and only, J_{NH_4immob} was considered.

$$J_{NH_4oxi} = J_{NH_4gross} - J_{NH_3g} - J_{NH_4immob} \quad (2)$$

Heterotrophic growth J_{NH_4immob} (Eq. 3) was estimated based on a yield coefficient Y_{het} of 0.54 g COD g^{-1} COD and a nitrogen content $N_{biomass}$ of 0.07 g N g^{-1} COD biomass (Gujer et al. 1999).

$$J_{NH_4immob} = J_{COD} \times Y_{het} \times N_{biomass} \quad (3)$$

The flux of stripped NH_3 (J_{NH_3g}) was approached by measured NH_4^+ and by the aeration volume and its mass transfer rate from the water phase to the air (Zhang et al. 1994) according to Eq. 4:

$$J_{NH_3g} = [NH_3]_{liquid} \times \frac{1}{kH_{CC}} \times Q_{airRi} \quad (4)$$

with kH_{cc} being the temperature corrected Henry constant of NH_3 (Aarnink and Elzing 1998) and $[NH_3]_{liquid}$ the dissolved concentration of free ammonia calculated according to Anthonisen et al. (1976).

Results and discussion

Inlet mixtures and loadings

The loading of the tanks was governed by the natural variation in composition with raw wastewater from the student dormitories. In general, soluble organic compounds (expressed as COD) showed a higher variation than NH_4^+, and its variability increased from P1 to P3 (Fig. 1). P1 and P3 showed a comparable COD/TAN ratio ranging from 2.2 to 2.7, while the ratio was significantly lower in P2 ranging from 1.4 to 2.1 (Fig. 1). The average loads based on the measured TAN and COD concentrations shown in Fig. 1 and the hydrological loading rate Q given in Table 1 were 1021 ± 118 g O_2 day^{-1} and 411 ± 26 g N day^{-1} for P1, 700 ± 168 g O_2 day^{-1} and 400 ± 36 g N day^{-1} for P2 and 860 ± 68 g O_2 day^{-1} and 358 ± 25 g N day^{-1} for P3 for R1 and R2, respectively. During the first half of P3, soluble COD was relatively stable around 2500 mg O_2 L^{-1} before it increased to 3000 mg O_2 L^{-1} (Fig. 1c), likely because unusually high ambient temperatures during this period supported hydrolysis of particulate matter in the sewer system and the mixing tank.

Nitrification activity and controlling factors

Both reactor tanks showed considerable ammonia oxidation activity as indicated by the accumulation of NO_2^-. Also NO_2^- oxidation to NO_3^- took place but at a lower rate than ammonia oxidation, especially during P3 (Fig. 2). With a reactor pH of 8.0–8.5, the oxidation of NO_2^- was most likely inhibited by free NH_3 as reported by earlier studies (e.g. Fux et al. 2004). Applying a classical inhibition model (Anthonisen et al. 1976) to our reactor tanks resulted in a 20–30 % inhibition of NO_2^- oxidation for P1,

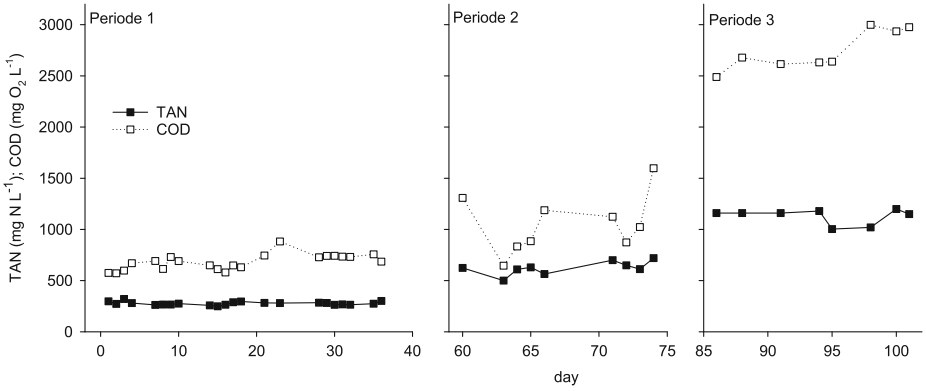


Fig. 1 Concentration of soluble COD and TAN in the inlet mixtures during the loading periods P1 (day 0–40), P2 (day 60–75) and P3 (day 85–101)

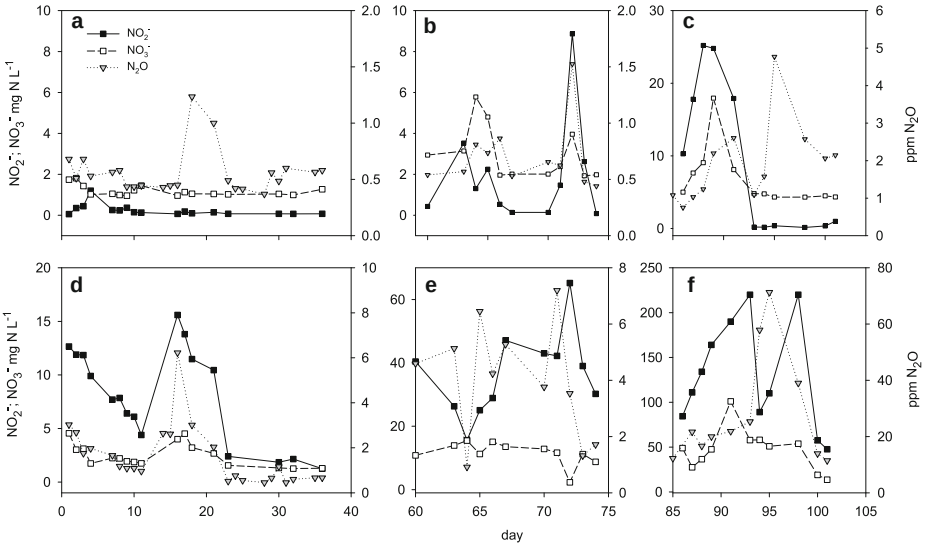


Fig. 2 Dynamics of NO_2^-, NO_3^-, and N_2O concentrations in the first (**a**–**c**) and second (**d**–**f**) reactor tank during loading periods P1 (**a**, **d**), P2 (**b**, **e**), and P3 (**c**, **f**), Note different scaling for *y*-axes

50–60 % for P2 and 60–70 % for P3, supporting the assumption that NH_3 inhibited nitrite oxidation.

The estimated nitrification and denitrification activity was evaluated with help of a simplified biofilm modeling approach based on Wanner et al. (2006). The model predicts oxygen limitation of heterotrophic activity when the coefficient Y_{S,O_2} exceeds 1 (Eq. 5). Under such conditions, no oxygen is available for ammonia oxidation by nitrifying organism in deeper biofilm layers.

$$Y_{S,O_2} = (1 - Y_{het,O_2}) \frac{D_S}{D_{O_2}} \frac{S_{org}}{S_{O_2}} \quad (5)$$

The bulk liquid O_2 concentration (S_{O_2}) was parameterized with measured values in R1 and the diffusion coefficient for O_2 (D_{O_2}) with 2.1×10^{-4} m^2 day^{-1} (Wanner and Reichert 1996). D_S was parameterized with 0.43×10^{-4} m^2 day^{-1} (Logan et al. 1987) which refers to organic molecules of a size of 100–500 kDa, approximately reflecting the average molecule size found for soluble compounds of a household wastewater (Dulekgurgen et al. 2006). The substrate concentration (S_{org}) was calculated as the difference in soluble COD between R1 and R2 with the assumption that all readily degradable soluble organics were depleted in R2.

Following the stratified biofilm model (Wanner et al. 2006; Wanner and Reichert 1996), competition for O_2 with heterotrophs in the biofilm was likely the major controlling factor for the N turnover in R1, as supported by the inverse relationship between J_{HN4oxi} and the oxygen limitation coefficient Y_{S,O_2} (Fig. 3). Elenter et al. (2007) reported an ammonia oxidation rate corresponding to a J_{NH_4oxi} of 60 mg N m^{-2} h^{-1} for a Y_{S,O_2} of 0.3 and 8 mg N m^{-2} h^{-1} for Y_{S,O_2} of 1.1 in a MBBR with mixed load. In our study, estimated Y_{S,O_2} values ranged from 1.0 to 2.9 (first and third quartile of graph in Fig. 3) and supported a J_{NH_4oxi} ranging from 2 to 18 mg N m^{-2} h^{-1} (Fig. 3), which is in the same order of magnitude. This suggests that our approach to estimate J_{NH_4oxi} as decrease in TAN corrected for NH_3 volatilization and microbial immobilization is reasonable.

In R1, we observed a marked imbalance between J_{NH_4oxi} and $J_{NO_2+NO_3}$ throughout the entire experiment (Fig. 3), suggesting that a major fraction accounting for 60–95 % of J_{NH_4oxi} was transformed into gaseous N compounds by denitrification in deeper, anoxic biofilm zones. However, the occurrence of denitrification may be questioned during periods of $Y_{S,O_2} < 1$, because this value predicts that all substrates (S_{org}) are depleted in aerobic biofilm zones, and no electron donor is left for denitrification in anoxic zones. Under such conditions, endogenous residues as well as soluble organic exudates from AOB have been pointed out as a potential energy source for denitrification (Kindaichi et al. 2004; Rittmann et al. 1994). However, if the

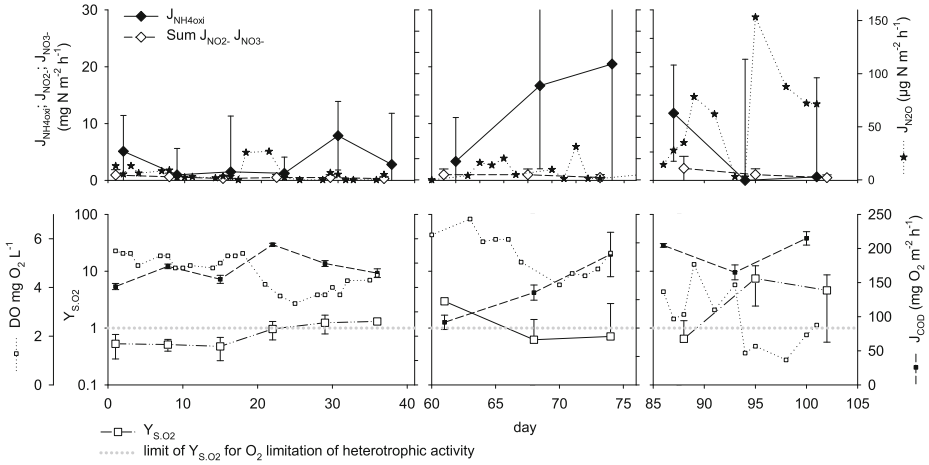


Fig. 3 *Upper panel* calculated N fluxes related to ammonia oxidation (J_{NH_4oxi}) and release of NO_2^- and NO_3^- into bulk liquid and measured N_2O flux in the second reactor tank (periods 1–3). The *lower panel* shows the calculated oxygen limitation coefficient Y_{S,O_2} for nitrification activity and measured DO. The calculated values are shown as weakly means with *error bars* denoting the variation (SD). For the directly measured parameters (J_{N_2O}, DO), individual measure points are displayed

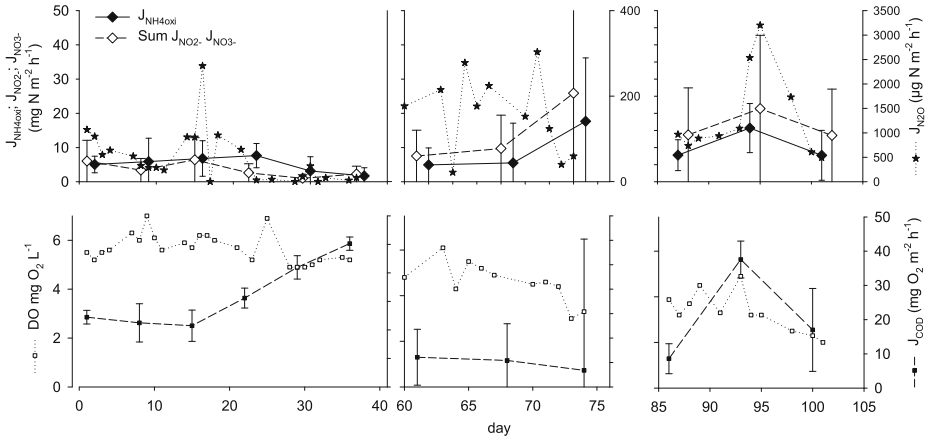


Fig. 4 *Upper panel* calculated N fluxes related to ammonia oxidation (J_{NH_4oxi}) and release of NO_2^- and NO_3^- into bulk liquid and measured N_2O flux in the second reactor tank (periods 1–3). The *lower panel* shows weekly means of the calculated COD flux and measured DO values. The *calculated values* are shown as weakly means with error bars denoting the variation (SD). For the directly measured parameters (J_{N_2O}, DO), individual measure points are displayed

contribution of those internal sources should have been significant, a substantially higher denitrification activity would have been expected also in R2 in which no significant imbalance between J_{NH_4oxi} and the sum of J_{NO_2} and J_{NO_3} was found (Fig. 4). We therefore believe that anoxic biofilm zones in R1 were supplied with electron donors during transient shifts in organic load (Fig. 1) which resulted in Y_{S,O_2} values fluctuating around one (Fig. 3).

In R2, heterotrophic activity was likely permanently substrate limited, resulting in Y_{S,O_2} values $\ll 1$, as indicated by the low J_{COD} (Fig. 4), which was one order of magnitude lower than in R1 (Fig. 3), as well as by the

absence of any relationship between J_{COD} and DO (Fig. 4). Considering the low heterotrophic activity, it is surprising that J_{NH_4oxi} in R2 was not significantly higher than in R1 as a $Y_{S,O_2} \ll 1$ would suggest. This phenomenon might be a result of different biofilm structures and detachment rates. A qualitative optical analysis of the media grits showed that the biofilm in R1 was relatively thin, likely reflecting the high turbulence and shear forces in R1. In R2, the turbulence was lower, and the biofilm looked fluffier and markedly thicker. This difference is also supported by the measured biomass dry weight which was 4–9 g m^{-2} for R1 and 13–27 g m^{-2} for R2. Due to their higher resistance against biomass detachment, AOB clusters may persist in surface-near biofilm layers and thus decrease the degree of biomass stratification if frequent detachment events take place (Elenter et al. 2007). This may have facilitated the interaction of particulate organics with the biofilm in R1, thus resulting in additional O_2 depletion in outer biofilm zones by hydrolysis and degradation of particulate COD, which was not captured in our study.

Regardless of the magnitude of J_{NH_4oxi}, the presence of detectable NO_2^- and NO_3^- in both reactor tanks (Fig. 2) suggests that nitrification activity is unavertable in aerobic biofilm systems loaded with mixtures of household wastewaters with frequent variations in organic and nitrogen load. Even though the estimated reaction rates (J_{NH_4oxi}) were small relative to N removal, they are significant in terms of N_2O emissions as indicated by the high N_2O emissions of the reactor tanks (Fig. 2).

N_2O fluxes and emission factor

In both reactor tanks, high N_2O concentrations were detected in the exhaust air, which were fivefold to tenfold higher in R2 than in R1 (Fig. 2). In R1, the calculated J_{N_2O} accounted for 0.2–0.7 % of J_{NH_4oxi} in average, while this emission factor was substantially higher in R2 (0.4–12 %). These fractions do not account for dissolved N_2O lost via the effluent, which is usually negligible in highly aerated reactors (Kampschreur et al. 2009). In R1, the emission factor remained unchanged over the first two periods (0.1–0.6 % in P1 and 0.1–0.4 % in P2) but increased dramatically in P3 (0.2–12 %; Fig. 3). In R2, the emission factor increased with increasing proportions of blackwater in the inlet mixture (0.4–1.8 % in P1; 2.5–3.7 % in P2 and 8.4–12.4 % in P3; Fig. 4). Overall, the emission factor of both reactor tanks was 0.7 % in P1 which is in the range of emission factors reported for biological wastewater treatment systems (Kampschreur et al. 2009), while it increased to values of 1.1 % in P2 and 8.5 % in P3, indicating an exponential increase of N_2O emissions with increasing blackwater content in wastewater.

Ex-situ studies

Batch trials with bulk liquid mixtures in closed incubation bottles and open bench-scale reactor

The interaction between decompostion of organics, biological N transformations, and N gas emissions in R2 was investigated in more detail in closed batch incubations and in an open bench-scale reactor run in batch modus. For both systems, biofilm grits from the first two loading periods (P1, P2) were used together with reactor bulk liquid mixtures representing typical loading conditions for R2 (Table 2).

J_{NH_4oxi} in bulk liquid incubations was two to three times higher than the maximum J_{NH_4oxi} observed in the second reactor tank during P1 and P2 (compare Figs. 4, 5a,). The higher J_{NH_4oxi} rates can be attributed to the higher DO in the stirred incubations which was estimated to be 6–8 mg L^{-1} based on observed O_2 depletion in the headspace (data not shown) and the O_2 transfer rate to the stirred medium provided by Molstad et al. (2007). Open batch trials run in parallel showed a comparable J_{NH_4oxi} when the DO was adjusted to the DO level estimated for the incubation bottles (data not shown). The open batch trials carried out in parallel to the bottle incubations showed that readily biodegradable soluble organics were depleted within 2 h in P1 (80 mg O_2 L^{-1}) and within 1 h in P2 (50 mg O_2 L^{-1}) (Fig. 5d, e). Additional samples taken after 24 h showed that a further COD depletion of 40 mg O_2 L^{-1} in P1 and 70 mg O_2 L^{-1} in P2 took place at low but relatively constant rates of 6–7 mg O_2 m^{-2} h^{-1} and 10–11 mg O_2 m^{-2} h^{-1}, respectively (not shown). These data suggest that J_{COD} measured in the bulk liquid incubations represented both readily and slowly degradable organics, and that, a markedly higher proportion of these organic substrates (70 %) was readily degradable in P1 (mixture of 80 % greywater; 20 % blackwater) as compared to only 40 % in P2 (mixture of 50 % greywater; 50 % blackwater). The COD degradation data from the open batch trials can also be used to approach the residual S_{org} in R2 which was <5 mg O_2 L^{-1} in P1 and around 10 mg O_2 L^{-1} in P2, supporting the assumption of a strong substrate limitation of the heterotrophic acitivity in the second reactor tank (see "Nitrification activity and controlling factors" section).

Assuming that the observed imbalance between disappearing TAN and appering NO_2^-, NO_3^-, NO, and N_2O was due to denitrification, the total gaseous N loss in the closed incubations bottles was higher in P2 (B1, 25–50 %

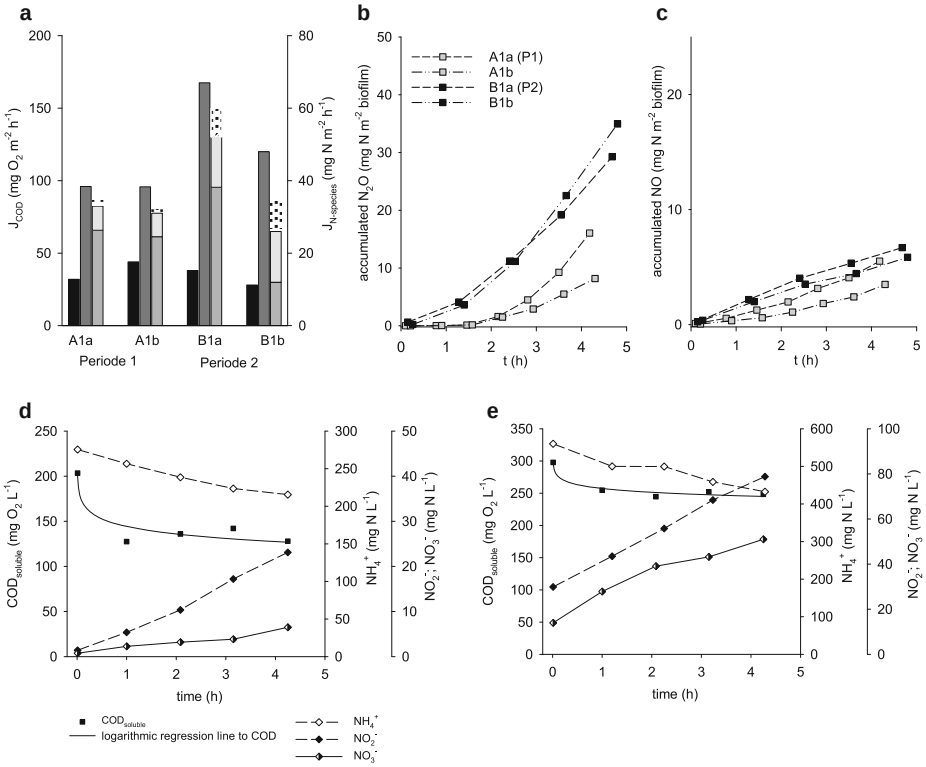


Fig. 5 *Upper panel* **a** average flux of COD, ■, TAN ■, NO_2^- ■ NO_3^- ■, NO and ▨ N_2O ▦ throughout 5-h incubation in closed stirred bottles; the *left column* refers to COD (secondary *y*-axis); the *middle column* refers to disappearing and the *right column* to appearing N compounds (primary *y*-axis). Shown are two replicate incubations per treatment (*a, b*), **b** accumulation of N_2O and **c** NO expressed in mg N per m^2 biofilm. *Lower panel* time course of bulk liquid concentrations of soluble organics (COD), NH_4^+, NO_2^-, and NO_3^- throughout a 4-h batch incubation in an open bench-scale MBBR reactor loaded with **d** bulk liquid mixture from the first loading period and **e** bulk liquid mixture from the second loading period. The COD data points were fitted to a logarithmic regression line

of J_{NH_4oxi}) than in P1 (A1, 17–23 %; Fig. 5a). The accumulation of N_2O in the batch incubations followed exponential kinetics (Fig. 5b), while the accumulation of NO followed a linear pattern, especially in P2 (Fig. 5c), indicating that the two gases may have originated from different processes. In P1 (A1), N_2O production accounted for 2–3 % of J_{NH_4oxi} and was almost negligible during the first 2 h before it increased exponentially (Fig. 5b). The incubation of P2 (B1) showed similar kinetics, but a markedly higher fraction of 9–16 % of J_{NH_4oxi} was converted to N_2O (Fig. 5b). The increasing J_{N_2O} was likely paralleled by a decreasing COD flux as shown in open batch experiments in both periods and increasing NO_2^- concentrations (Fig. 5d, e). In summary, the presence of NO_2^- seemed to stimulate N_2O production, while the presence of readily degradable organics reduced N_2O production.

Batch incubations with enhanced organic load and added NO_2^-

Additional incubations were carried out with material from P2 to investigate the impact of NO_2^- and two different types of high organic load; an inlet mixture with real wastwater (inlet incubation) and a synthetic mixture enriched with acetate (acetate incubation; Table 2).

In these experiments (C1,2: inlet mixture and D1: acetate in Fig. 6), J_{COD} was tenfold higher than in the previous bulk liquid incubations (Fig. 5a), while J_{NH_4oxi} was markedly reduced (compare Figs. 6a, 5a). The addition of

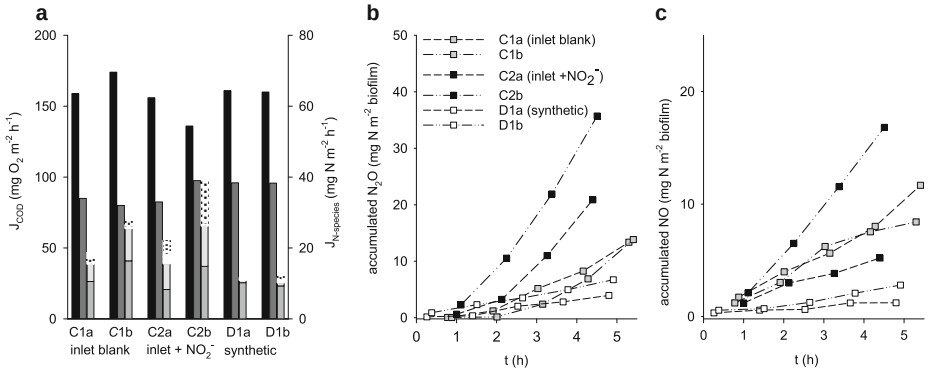


Fig. 6 Average flux of COD, ■, TAN ■, NO_2^- ■, NO_3^- ■, NO and ▨ N_2O ▦ throughout 5-h incubation in closed stirred bottles; the *left column* refers to COD (secondary *y*-axis); the *middle column* refers to disappearing and the *right column* to appearing N compounds (primary *y*-axis). Shown are two replicate incubations per treatment (**a**, **b**), **b** accumulation of N_2O and **c** NO expressed in mg N per m^2 biofilm

NO_2^- did neither affect J_{COD} nor J_{NH_4oxi}. The inlet incubations (C1, C2; Fig. 6a) showed a total gasesous N loss comparable to the bulk liquid incubation of P2 (B1, Fig. 5a), regardless whether NO_2^- was added or not. Conversely, the acetate incubation (D1) showed a substantialy higher total gaseous N loss accounting for ~80 % of J_{NH_4oxi} (Fig. 6c). At the same time, the acetate incubation showed the greatest apparent denitrification among all treatments. This suggests that more electron donors were provided to anoxic biofilm zones with the acetate medium, likely because of the smaller molecule size and higher diffusity of acetate. Moreover, the wastewater mixtures used in our study may have contained slowly degradable substrates as shown by the open batch trial (see "Batch trials with bulk liquid mixtures in closed incubation bottles and open bench-scale reactor" section), which would reduce the denitrification rates compared to acetate.

N_2O accumulation in incubations with inlet and synthetic mixture without added NO_2^- (C1, D1) was negligible during the first 2 h (Fig. 6b), and the lag phase was clearly longer than in incubations with bulk liquid and biofilm from the same loading period (B1; Fig. 5b). In contrast, the loading regime did not seem to have any effect on the kinetics of NO accumulation (compare Figs. 5c, 6c). Addition of NO_2^- clearly stimulated N_2O accumulation (Fig. 6b) but showed no consistent effect on NO accumulation. On the other hand, NO accumulation in the incubation with acetate was significantly smaller (Fig. 6c), suggesting that also NO production might depend on the type of organic substrate load. J_{N_2O} accounted for 6–7 % of J_{NH_4oxi} in incubations with inlet mixture and 2–3 % with acteate (C1, Fig. 6a), which is markedly lower than the 9–16 % observed in incubations with bulk liquid taken from the same period (B2, Fig. 5a). These results reaffirm the finding from the bulk liquid incubations that elevated NO_2^- increases, while readily degradable organics decrease N_2O production in the biofilm.

Potential mechanism for N_2O emissions

N_2O production in municipal wastewater treatment plants has been reported to be greatest at high NH_4^+ oxidation rates (Law et al. 2012; Upadhyay et al. 2006; Wunderlin et al. 2012) and has been attributed to the oxidation of intermediates released during NH_4^+ oxidation (e.g. NOH, NO). J_{NH_4oxi} in our reactors was five to ten times lower than rates reported from biofilm systems designed for nitrification (e.g. Elenter et al. 2007; Fux et al. 2004). Therefore, it is unlikely that oxidative processes during nitrification were the main source for N_2O production. In our system, the emission of N_2O was linked to NO_2^- accumulation (Fig. 2), and this was confirmed in incubation experiments with inlet mixture amended with NO_2^-, which accumulated significantly more N_2O than without added NO_2^- (Fig. 6b). N_2O emissions observed at high NO_2^- concentrations are commonly attributed to nitrifier denitrification (Beaumont et al. 2002; Schreiber et al. 2009). Schreiber et al. (2009) determined a NO_2^- threshold of 2.8 mg N L^{-1} (200 µm) for a biofilm, above which nitrifier denitrification started to reduce NO_2^-–N_2O. In R1, NO_2^-

passed this threshold only during three distinct peaks in P2 and P3 which fell together with elevated N_2O emissions. Also R2 showed N_2O emission peaks coinciding with high NO_2^- (Fig. 2). However, in R2, NO_2^- was above the putative threshold concentration throughout the entire experiment, which does not preclude that increasing NO_2^- concentrations above a threshold triggered more N_2O emission. As shown by the open batch trials (Fig. 6), the proportion of readily degradable organics (S_{org}) in the inlet decreased with an increasing fraction of blackwater in the wastewater, which is in accordance with earlier findings (Itokawa et al. 2001). The open batch trial also showed that the availability of readily degradable organics decreased from P1 to P3 (Fig. 5d, e) which explains the increasing NO_2^- accumulation and N_2O emissions (Fig. 3, 4) by increased substrate limitation of heterotrophic activity and higher oxygen availability to nitrifying organism.

The presence of NO_2^- may also have triggered heterotrophic denitrification in anoxic biofilm zones, which likely took place in both reactor tanks as indicated by the N mass balance in the bulk liquid incubations. Obviously, this denitrification activity was fueled by small amounts of readily degradable organics that reached anoxic zones during the first 1–2 h of incubation (Fig. 5a, d, e). With increasing incubation time, heterotrophic denitrification became likely increasingly fueled by organics with low degradability that might stem from the inlet or be produced by endogenous biomass decay. Itokawa et al. (2001) and Pan et al. (2013) showed that N_2O emissions may be increased by the presence of slowly degradable substrates, since the reduction of N_2O to N_2 is kinetically less favorable than the reduction of NO_3^- (or NO_2^-) to N_2O when electrons are a limiting (Richardson et al. 2009). Later during incubation, these slowly degradable organics were likely the sole electron donors present in anoxic biofilm layers and might have sustained denitrification with a high N_2O to N_2 product ratio. Similar conditions were present in the biofilm of R2 where substrate limited denitrification might have been an important additional source of N_2O when NO_2^- accumulated under enhanced blackwater proportions in the load of the reactor tanks (Fig. 2).

N_2O emissions from wastewater treatment are estimated to account for as much as 26 % of the total greenhouse gas emissions of the anthropogenic water cycle (Kampschreur et al. 2009). Accordingly, there is an increasing focus on reducing N_2O emissions when planning and operating municipal wastewater treatment plants (Kampschreur et al. 2009; Schreiber et al. 2012). Our study indicates that onsite wastewater treatment systems may represent substantial point sources for N_2O, especially on locations where an enhanced proportion of blackwater can be expected as for example on mountain refugees and other decentralized tourist facilities (Andreottola et al. 2003). Hence, not only municipal wastewater treatment plants but also decentralized sewer systems need to be critically assessed in terms of greenhouse gas emissions. More effort is needed to develop climate smart source separating sanitation, which has gained popularity due to its high efficiency in water and nutrient reuse (Larsen et al. 2009). However, the substantial N loss in form of N_2O in our biofilm system when loaded with pure blackwater may outweigh such beneficial effects. A careful evaluation and selection of technologies is therefore needed for those novel sanitation approaches to avoid adverse greenhouse gas emissions.

Conclusion

In view of natural loading variations, biological nitrogen conversion seems inevitable in biofilm systems loaded with household wastewater regardless whether the system is designed for nitrogen removal or not. In our study, nitrogen fluxes increased with increasing blackwater content, dominated by partial nitrification leading to NO_2^- accumulation. N_2O emission peaks coincided with NO_2^- accumulation, pointing at nitrifier denitrification as the main process of N_2O production. However, N mass balances in closed batch experiments indicated that also denitrification may have contributed to the observed N_2O production. To reconcile these observations, we interpreted our data with a simple conceptual biofilm model predicting oxygen limitation as a function of substrate availability. According to the model, higher proportion of slowly degradable organics contained in blackwater would allow for enhanced nitrification in outer biofilm layers while leading to incomplete denitrification to N_2O in deeper biofilm layers. With 20 % blackwater in the sewage, the emission factor for N_2O was within the commonly reported range for biological wastewater treatment, but increased exponentially with increasing proportions of blackwater. A careful evaluation of suitable technologies is therefore needed for source separating sanitation approaches to avoid that adverse greenhouse gas emissions outweigh the benefits of water and nutrient reuse.

Acknowledgments This study was supported by the Norwegian Research Council (Grant No skattefunn-226774). A special thanks also to Associate Prof. Arve Heistad for the support to the pilot-scale experiment setup and Olga Popovic for helping with analysis work.

References

Aarnink AJA, Elzing A (1998) Dynamic model for ammonia volatilization in housing with partially slatted floors, for fattening pigs. Livest Prod Sci 53:153–169. doi:10.1016/s0301-6226(97)00153-x

Andreottola G, Damiani E, Foladori P, Nardelli P, Ragazzi M (2003) Treatment of mountain refuge wastewater by fixed and moving bed biofilm systems. Water Sci Technol 48:169–177

Anthonisen AC, Loehr RC, Prakasam TBS, Srinath EG (1976) Inhibition of nitrification by ammonia and nitrous acid. J Water Pollut Control Fed 48:835–852

Azizi S, Valipour A, Sithebe T (2013) Evaluation of different wastewater treatment processes and development of a modified attached growth bioreactor as a decentralized approach for small communities. Sci World J. doi:10.1155/2013/156870

Beaumont HJE et al (2002) Nitrite reductase of *Nitrosomonas europaea* is not essential for production of gaseous nitrogen oxides and confers tolerance to nitrite. J Bacteriol 184:2557–2560. doi:10.1128/jb.184.9.2557-2560.2002

Daude D, Stephenson T (2003) Moving bed biofilm reactors: a small-scale treatment solution. Water Sci Technol 48:251–257

de Graaff MS, Zeeman G, Temmink H, van Loosdrecht MCM, Buisman CJN (2010) Long term partial nitritation of anaerobically treated black water and the emission of nitrous oxide. Water Res 44:2171–2178

Dulekgurgen E, Dogruel S, Karahan Ö, Orhon D (2006) Size distribution of wastewater COD fractions as an index for biodegradability. Water Res 40:273–282

Elenter D, Milferstedt K, Zhang W, Hausner M, Morgenroth E (2007) Influence of detachment on substrate removal and microbial ecology in a heterotrophic/autotrophic biofilm. Water Res 41:4657–4671. doi:10.1016/j.watres.2007.06.050

Fux C, Huang D, Monti A, Siegrist H (2004) Difficulties in maintaining long-term partial nitritation of ammonium-rich sludge digester liquids in a moving-bed biofilm reactor (MBBR). Water Sci Technol 49:53–60

Gujer W, Henze M, Mino T, van Loosdrecht M (1999) Activated sludge model no. 3. Water Sci Technol 39:183–193. doi:10.1016/s0273-1223(98)00785-9

Hocaoglu SM, Insel G, Cokgor EU, Baban A, Orhon D (2010) COD fractionation and biodegradation kinetics of segregated domestic wastewater: black and grey water fractions. J Chem Technol Biotechnol 85:1241–1249. doi:10.1002/jctb.2423

ICCP (2001) Climate change 2001: the scientific basis. Cambridge University Press, New York

Itokawa H, Hanaki K, Matsuo T (2001) Nitrous oxide production in high-loading biological nitrogen removal process under low COD/N ratio condition. Water Res 35:657–664. doi:10.1016/s0043-1354(00)00309-2

Kampschreur MJ, Temmink H, Kleerebezem R, Jetten MSM, van Loosdrecht MCM (2009) Nitrous oxide emission during wastewater treatment. Water Res 43:4093–4103. doi:10.1016/j.watres.2009.03.001

Kindaichi T, Ito T, Okabe S (2004) Ecophysiological interaction between nitrifying bacteria and heterotrophic bacteria in autotrophic nitrifying biofilms as determined by microautoradiography-fluorescence in situ hybridization. Appl Environ Microbiol 70:1641–1650. doi:10.1128/aem.70.3.1641-1650.2004

Larsen TA, Alder AC, Eggen RIL, Maurer M, Lienert J (2009) Source separation: will we see a paradigm shift in wastewater handling. Environ Sci Technol 43:6121–6125. doi:10.1021/es803001r

Law Y, Ni BJ, Lant P, Yuan ZG (2012) N_2O production rate of an enriched ammonia-oxidising bacteria culture exponentially correlates to its ammonia oxidation rate. Water Res 46:3409–3419. doi:10.1016/j.watres.2012.03.043

Logan BE, Hermanowicz SW, Parker DS (1987) A fundamental model for trickling filter process design. J Water Pollut Control Fed 59:1029–1042

Molstad L, Dörsch P, Bakken LR (2007) Robotized incubation system for monitoring gases (O_2, NO, N_2O, N_2) in denitrifying cultures. J Microbiol Methods 71:202–211. doi:10.1016/j.mimet.2007.08.011

Pan YT, Ni BJ, Yuan ZG (2013) Modeling electron competition among nitrogen oxides reduction and N_2O accumulation in denitrification. Environ Sci Technol 47:11083–11091. doi:10.1021/es402348n

Penn R, Hadari M, Friedler E (2012) Evaluation of the effects of greywater reuse on domestic wastewater quality and quantity. Urban Water J 9:137–148. doi:10.1080/1573062x.2011.652132

Poth M, Focht DD (1985) N-15 kinetic-analysis of N_2O production by *Nitrosomonas europaea*—an examination of nitrifier dentrification. Appl Environ Microbiol 49:1134–1141

Ravishankara AR, Daniel JS, Portmann RW (2009) Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. Science 326:123–125. doi:10.1126/science.1176985

Richardson D, Felgate H, Watmough N, Thomson A, Baggs E (2009) Mitigating release of the potent greenhouse gas N_2O from the nitrogen cycle—could enzymic regulation hold the key? Trends Biotechnol 27:388–397. doi:10.1016/j.tibtech.2009.03.009

Rittmann BE, Regan JM, Stahl DA (1994) Nitrification as a source of soluble organic substrate in biological treatment. Water Sci Technol 30:1–8

Schmidt I (2009) Chemoorganoheterotrophic growth of *Nitrosomonas europaea* and *Nitrosomonas eutropha*. Curr Microbiol 59:130–138. doi:10.1007/s00284-009-9409-8

Schreiber F, Loeffler B, Polerecky L, Kuypers MMM, de Beer D (2009) Mechanisms of transient nitric oxide and nitrous oxide production in a complex biofilm. ISME J 3:1301–1313. doi:10.1038/ismej.2009.55

Schreiber F, Wunderlin P, Udert KM, Wells GF (2012) Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: biological pathways, chemical reactions, and novel technologies. Front Microbiol 3:24. doi:10.3389/fmicb.2012.00372

Todt D, Heistad A, Jenssen PD (2014) Load and distribution of organic matter and nutrients in a separated household wastewater stream. Environ Technol. doi:10.1080/09593330.2014.997300

Upadhyay AK, Hooper AB, Hendrich MP (2006) NO reductase activity of the tetraheme cytochrome c (554) of *Nitrosomonas europaea*. J Am Chem Soc 128:4330–4337. doi:10.1021/ja055183+

Wanner O, Reichert P (1996) Mathematical modeling of mixed-culture biofilms. Biotechnol Bioeng 49:172–184

Wanner O, Eberl HJ, Morgenroth E, Noguera DR, Picioreanu C, Rittmann BE, van Loosdrecht MCM (2006) Mathematical modeling of biofilms. IWA Publishing, London

Wunderlin P, Mohn J, Joss A, Emmenegger L, Siegrist H (2012) Mechanisms of N_2O production in biological wastewater

treatment under nitrifying and denitrifying conditions. Water Res 46:1027–1037. doi:10.1016/j.watres.2011.11.080

Yang JJ, Trela J, Plaza E, Tjus K (2013) N_2O emissions from a one stage partial nitrification/anammox process in moving bed biofilm reactors. Water Sci Technol 68:144–152. doi:10.2166/wst.2013.232

Zhang RH, Day DL, Christianson LL, Jepson WP (1994) A computer-model for predicting ammonia release rates from swine manure pits. J Agric Eng Res 58:223–229. doi:10.1006/jaer.1994.1052