Resource recovery from black water

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Thesis committee

Thesis supervisor

Prof. dr. ir. C.J.N. Buisman
Professor of Biological Recycling Technology
Wageningen University

Thesis co-supervisors

Dr. ir. G. Zeeman
Assistant professor at the sub-department of Environmental Technology,
Wageningen University
Dr. ir. H. Temmink
Assistant professor at the sub-department of Environmental Technology,
Wageningen University

Other members

Prof. dr. ir. W. Verstraete	University of Ghent, Belgium
Prof. dr. ir. G. Spaargaren	Wageningen University
Prof. dr. ir. M.S.M. Jetten	Radboud University Nijmegen
Prof. dr. A.A. Koelmans	Wageningen University

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Resource recovery from black water

Marthe Sophie de Graaff

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Abstract

New sanitation concepts are based on separation at source of household wastewater streams, e.g. into grey water and black water (faeces and urine), and have a large potential to recover the important resources energy, nutrients and water. A promising treatment system for highly concentrated black water, collected with vacuum toilets, was designed, consisting of anaerobic treatment followed by struvite precipitation for phosphorus recovery and autotrophic nitrogen removal. A UASB (Upflow Anaerobic Sludge Blanket) reactor removed on average 78% of the influent organic load at a relatively short hydraulic retention time (HRT) of 8.7 days. The methane production was 1.8 m^3 CH_4 per m^3 of black water, which can be converted to 56 MJ/p/y as electricity and 84 MJ/p/y as heat by combined heat and power (CHP). Autotrophic nitrogen removal was selected to remove the nitrogen from black water in a two reactor nitritation-anammox process. Partial nitritation of the ammonium in the UASB effluent was successfully achieved in a continuous system operated at 34 °C and at 25 °C. The main fraction, 85 to 89%, of total nitrogen could be removed in the anammox reactor at 35 °C and 25 °C, however only after the calcium concentration of the black water of 42 mg/L was increased with an additional 39 mg/L for better granulation and biomass retention. The strong global warming gas nitrous oxide (N_2O) was emitted in the two reactor nitritation-anammox process and more research is needed to elucidate the mechanisms of N_2O emission. Phosphorus was conserved for 61% in the UASB effluent and precipitation of phosphate as struvite can result in a recovery of 0.22 kgP/p/y, representing 10% of the global artificial phosphorus fertilizer production. The remaining part of the phosphorus ended up in the UASB sludge, mainly due to precipitation (39%) because of a low dilution and a high pH in the black water. This sludge may be used as a phosphorus-enriched organic fertilizer, provided it is safe with respect to heavy metals, pathogens and micro-pollutants. Although black water is very complex matrix, it was possible to detect the presence of a selection of hormones and pharmaceuticals. The presence of persistent micro-pollutants, which are not susceptible for biodegradation, makes the application of advanced physical and chemical treatment unavoidable. More research is needed with respect to the presence and removal of heavy metals, pathogens and micro-pollutants in new sanitation concepts.

Keywords: new sanitation, separation at source, resource recovery, black water, anaerobic treatment, UASB reactor, partial nitritation, anammox, nitrous oxide, phosphorus, struvite, hormones, pharmaceuticals, LC MS/MS

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Treatment options for black water: review and scope of this thesis

Abstract

New sanitation systems based on separation at source offer a large potential for resource recovery from wastewater, e.g. energy and nutrients from black water and irrigation water from grey water. This review focuses on the components in source separated black water. The treatment options for the key components are reviewed, focussing on recovery of organic compounds, nitrogen and phosphorus and removal of hormones, pharmaceutical residues and pathogens. A feasible treatment system for black water (faeces and urine), collected with vacuum toilets, would consist of anaerobic treatment followed by struvite precipitation for phosphorus recovery and autotrophic nitrogen removal. By applying these techniques, it is possible to produce 56 MJ/p/y of electricity, representing 40% of the energy that now is required for conventional WWTPs. Furthermore, the production of global artificial phosphorus fertilizer can be reduced by maximum 21%. As an alternative a higher degree of separation could be applied by separating the urine from the faeces, but this only would be feasible if the urine can be directly reused as a fertilizer. The issue of hormones, pharmaceuticals and pathogens in wastewater and their entry into the environment requires more research to determine to which extent additional treatment is necessary. Finally, the scope of this thesis is explained and the research questions which were addressed are presented.

M.S. de Graaff, G. Zeeman, H. Temmink and C.J.N. Buisman

1.1 Introduction

1.1.1 *Resource recovery from wastewater*

Water is essential for all organisms, but only about 2.5% of the total amount of water on Earth consists of fresh water resources. About 1 % of the total amount of water is easily accessible, because fresh water is also stored in glaciers and as deep groundwater. Sustainable water management therefore, is crucial to protect world water resources. Another concern is the increasing amount of people living under water stress. More than 1.1 billion people lack access to safe drinking water and 2.4 billion do not have adequate sanitation services (WHO, 2000). Around 2.2 million people, mostly children in developing countries, die because of diarrhoea caused by polluted drinking water (WHO, 2000). Millennium development goals (MDGs) were set to establish quantitative benchmarks to reduce extreme poverty in all its forms by half in 2015. One of these MDGs is to reduce by 2015 the number of people without sustainable access to safe drinking water and basic sanitation by 50%. Despite a growing awareness of problems related to sanitation, the environment and water scarcity, a lot of effort still is required to realise the MDGs (UN, 2009).

From a hygienic point of view high standards of sanitation and water supply have been achieved in the industrialized world from the 19th century onwards by application of centralized sewerage and wastewater treatment plants (Mays et al., 2007). Centralized wastewater treatment plants (conventional WWTPs) are applied widely, and are designed to remove macropollutants, such as organic material, nitrogen and phosphorus. The main problems associated with these conventional WWTPs are a large production of polluted sludge, emissions of greenhouse gases (e.g. methane and nitrous oxide) to the atmosphere and the discharge of pharmaceuticals and other emerging contaminants to receiving waters. Furthermore, a lot of energy is needed to remove organic compounds and nutrients, while at the same time these organic compounds represent a potential energy source and the nutrients may be recovered as valuable fertilizers (Larsen and Gujer, 1997; Otterpohl et al., 1997; Zeeman and Lettinga, 1999; Hao and van Loosdrecht, 2003; Wilsenach et al., 2003; Guest et al., 2009; Verstraete et al., 2009).

Recently, planetary boundaries were defined to ensure a safe operating space for humanity (Rockström et al., 2009). These boundaries include boundaries for climate change and for the nitrogen and phosphorus cycle. They include a maximum atmospheric CO_2 concentration, a maximum amount of atmospheric N_2 transformed by humans into reactive forms, and a maximum amount of phosphorus flowing into the oceans. The boundaries for climate change and the nitrogen cycle have already been exceeded and the boundary for the phosphorus cycle is approaching its proposed maximum. Carbon, nitrogen and phosphorus are important elements in the food and energy chain and an efficient recycling helps to reduce their emissions to the environment, such that the boundaries are not overstepped.

Resource recycling from human wastes was practiced in many cultures in the past. Human excreta were used in several countries as a valuable resource in agriculture. For example the Celts and Romans used urine to clean their clothes (Bracken et al., 2007). In the 19th century, a pneumatic faeces drainage system was developed by Liernur (1828-1893) and was applied in cities in the Netherlands (Leiden, Dordrecht, Amsterdam), but also in Prague and in St. Petersburg, in the period between 1870 and 1915. This system was developed to produce products for agriculture, like ammonia and a poudrette (dried black water), which made the system lucrative. However, the industrial production of ammonia and other fertilizers, and the application of the water closet made the system less popular and it finally disappeared (Anonymous, 1908; van Zon, 1986). The collection of night soil, i.e. human waste collected during the night, is still adopted in countries such as China and Japan (Matsui, 1997). Successful codigestion of night soil sludge and kitchen waste was applied to produce energy and compost in a full scale plant (Yoneyama and Takeno, 2002). Conventional WWTPs are designed as end-of-pipe systems and generally do not reuse nutrients or energy. Although sewage sludge is applied on agricultural fields, because it contains nutrients and valuable organic matter, this practice is prohibited in more and more countries due to the high levels of heavy metals in the sludge. Currently the European Commission is reviewing its Directive (86/278/EEC) for the application of sewage sludge in agriculture[1]. The Netherlands for example already adopted a much more stringent guideline with respect to heavy metals, resulting in the incineration of all the sewage sludge[2].

Careful control of the urban water cycle can contribute to water conservation and food production. In developing countries facing water scarcity, farmers move to the neighbourhood of cities where wastewater is produced, in order to have access to free water and nutrients for crop growth (Raschid-Sally et al., 2005). This water usually is

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[1] http://ec.europa.eu/environment/waste/sludge/index.htm, visited on 26-10-2009

[2] http://www.eu-milieubeleid.nl/ch05s10.html, visited on 25-09-2009; Manual of Environmental Policy, in Dutch

(partly) untreated and presents a threat to human health. Safe use of wastewater or effluents from treatment plants could lead to cost optimization on two sides of the chain: less complicated treatment systems and a reduced need for artificial fertilizers (Huibers and van Lier, 2005). Recovery of nitrogen and phosphorus from wastewater will become increasingly interesting from an energy point of view, because agriculture is currently strongly dependent on fossil fuels to produce fertilizers (Gajdos, 1998; Verstraete et al., 2005). As will be described in the next paragraphs, a different method for collection, transport and treatment of domestic wastewater has the potential to save 200 MJ/p/d compared to conventional WWTPs (Zeeman et al., 2008). Furthermore, 30% of artificial nitrogen fertilizer and 21% of artificial phosphorus fertilizer produced worldwide ends up in domestic wastewater (Mulder, 2003; Cordell et al., 2009). It can therefore be concluded that domestic wastewater presents a potential resource for energy, nutrients and water.

1.1.2 Separation at source

A logical approach to achieve efficient resource recovery from wastewater is by separating household wastewater streams at the source, and treating them separately to recover bio-energy, nutrients and clean water (Otterpohl et al., 1999; Zeeman and Lettinga, 1999).

By applying separation at source domestic waste(water) streams can be divided into: black water (toilet water: faeces and urine), solid kitchen waste, grey water (originating from showers, bath, laundry, etc.) and rain water (figure 1.1). The characteristics of these domestic resource waste(water) streams are extensively described by Kujawa-Roeleveld and Zeeman (2006). Because these streams differ in quantity and in quality, they should be treated accordingly. Grey water has a high potential for reuse, for example as irrigation water, because it not only is the major volume fraction (70%) of domestic wastewater, but also relatively low in pollution (Hernandez Leal et al., 2007). Black water does not only contain half the load of organic material in domestic wastewater and the major fraction of the nutrients nitrogen and phosphorus, but also most of the pathogens, hormones and pharmaceutical residues.

Several concepts based on separation at source have been designed and several names were proposed: e.g. Decentralized Sanitation and Reuse (DESAR)[3], Ecological

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[3] http://www.ete.wur.nl/UK/Projects/DESAR/, visited on 7th December 2009

Sanitation (EcoSan)[4] or new sanitation[5]. In this review the term ‘new sanitation’ is used, referring to new concepts based on separation at source compared to ‘old’ conventional sewerage and wastewater treatment. Other research focuses on urine separation only, to investigate the reuse potential of urine and to reduce the nutrient and micro-pollutant load to conventional WWTPs, e.g. in Sweden, Switzerland and the Netherlands (Wilsenach and Van Loosdrecht, 2003; Berndtsson, 2006; Larsen and Lienert, 2007). Treatment options for urine were extensively reviewed by Maurer et al. (2006).

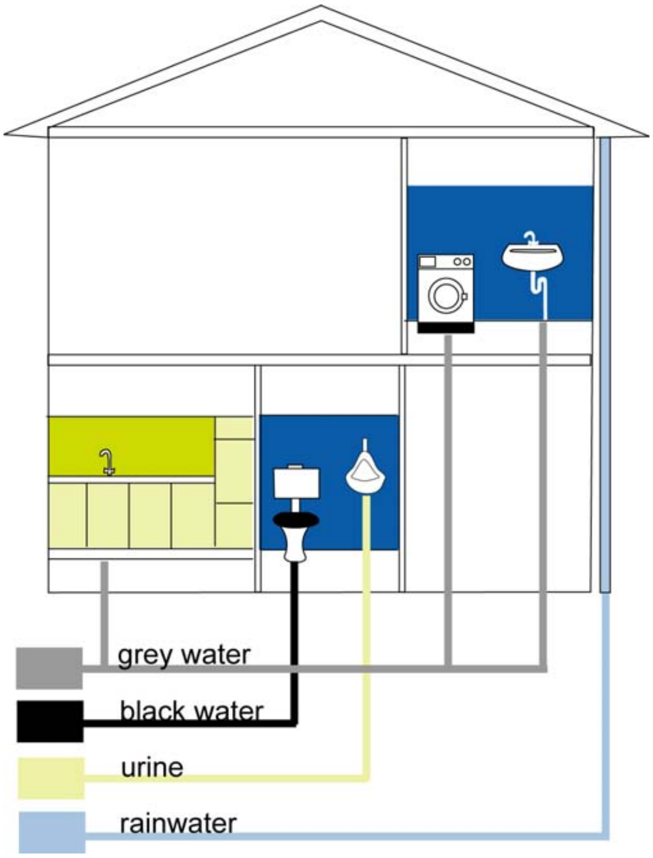

Figure 1.1 Separation at source in a household.

1.1.3 Black water and its collection

Black water is the mixture of faeces and urine, usually collected with a certain amount of flushing water. Of the total loads in domestic wastewater including kitchen waste, 38% of COD, 82% of nitrogen and 68% of phosphorus is present in the black water. Together urine and faeces occupy only 1% of the total volume in a household

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[4] www.ecosan.nl; http://www.gtz.de/en/themen/8524.htm, visited on 7th December 2009

[5] http://www.stowa.nl/Thema_s/Nieuwe_Sanitatie/index.aspx

(Kujawa-Roeleveld and Zeeman, 2006), and therefore black water represents a relatively concentrated stream with a high potential for resource recovery. Apart from organic compounds and nutrients, black water also contains almost all the hormones, pharmaceutical residues and pathogens. Details about black water and its composition are given by Kujawa-Roeleveld and Zeeman (2006).

For optimal recovery of resources from black waste(water), collection, toilet and transport systems are needed, that ideally use as little flushing water as possible to limit the dilution. Dry sanitation systems like composting toilets offer a way to directly recycle nutrients and organic matter from the faeces and urine. However, their success depends on proper installation and management (EPA, 1999). Composting systems are vulnerable if they contain too much water. Separate urine collection and faeces composting is a more promising alternative (Vinnerås et al., 2003). According to Otterpohl (2008) improvements are needed to make composting toilets applicable on a large scale.

In developing countries pit latrines are widely used, in which the faeces and urine are collected together. Pit latrines are however, open systems to soil, resulting in that nutrients and pathogens penetrate into the soil, which can result in groundwater and surface water pollution. When properly managed stabilized faecal sludge can be used as a slow release fertilizer. A lot needs to be improved to apply dry sanitation technologies like composting on a large scale (Bhagwan et al., 2008; Otterpohl, 2008).

Conventional flush toilets use up to 12 litres of drinking water per flush, although more and more modern houses are equipped with low-flush toilets only using 4-6 litres per flush (Kujawa-Roeleveld and Zeeman, 2006). Vacuum toilets, already applied in for example planes and trains, only use 1 litre of water per flush (figure 1.2). With these vacuum toilets up to 24% of drinking water consumption in a Dutch household can be saved (Kanne, 2005)[6]. The application of vacuum toilets is therefore a promising alternative for conventional toilets. Vacuum toilets produce a different and higher level of sound than conventional toilets, but various options exist to reduce the sound. Generally vacuum toilets are appreciated by the user, because of its water saving aspect (Telkamp et al., 2008).

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[6] In a Dutch household on average 35.6 L/p/d of drinking water was used to flush the toilet. The total drinking water consumption was 123.8 L/p/d. A vacuum toilet would need 6 L/p/d, reducing the consumption of drinking water with 24%. (Kanne, 2005)

Figure 1.2 Vacuum toilet.

This review focuses on the resources available in source separated black water. The treatment options for the key components are reviewed, focussing on recovery of organic compounds, nitrogen and phosphorus and removal of hormones, pharmaceutical residues and pathogens. Finally, new sanitation concepts based on separation at source are discussed.

1.2 Organic compounds

1.2.1 Background on the carbon cycle and bio-energy

Although there is a lot of debate about global warming in relation to the increasing carbon dioxide (CO_2) concentrations in the atmosphere, it is clear that by combustion of fossil fuels we convert more carbon into CO_2 than is naturally fixed (Normile, 2009). The boundary for the atmospheric CO_2 concentration in the atmosphere, which was defined to prevent possible climate change, is already transgressed and the concentration of CO_2 should be reduced (Rockström et al., 2009).

Sanitation concepts based on separation at source have the potential to contribute to the production of (bio-)energy instead of requiring energy generated from fossil fuels for the conventional treatment of wastewater. Conventional sewer systems and WWTPs consume in total 139 MJ/p/y (Frijns et al., 2008). It was estimated that a similar amount of energy can be produced from wastewater (black water, kitchen waste and grey water) in the form of biogas (131 MJ/p/y) (Zeeman et al., 2008). Furthermore, by recovering nutrients from domestic wastewater, the amount of energy needed for the production of fertilizers can be significantly reduced (Gajdos, 1998). Part of the organic material, 38% of the total household waste streams including kitchen waste (Kujawa-Roeleveld and Zeeman, 2006), is present in black water and on average 62 gCOD/p/d is produced. A possible technology is composting, where the organic material in converted into heat. A second option is to produce methane by anaerobic treatment and subsequent conversion of this methane into electricity and heat. A promising future anaerobic technology could be the microbial fuel cell, which converts dissolved organics directly into electricity using electrochemically active microorganisms. However, microbial fuel cells still need important breakthroughs to become economically competitive, and until now only soluble readily degradable organic compounds can be used (Rabaey and Verstraete, 2005; Rozendal et al., 2008). Therefore, in the next sections, only the feasibility of composting and anaerobic treatment of black water will be discussed in more detail.

1.2.2 Composting

Composting is the aerobic biological conversion of solid organic matter and organic matter is converted to carbon dioxide, water and stabilized sludge. Organic material that is properly composted, is a nuisance-free, humus like material and can be used as a soil conditioner in agriculture. Usually a bulking agent such as wood chips is added to reduce the moisture content of the raw material (Tchobanoglous et al., 2003).

Composting of human excreta is already applied in practice. However, its success depends on proper installation and management of the composting toilets (EPA, 1999). Important parameters are the biodegradability of organic matter, the amount of water/moisture and temperature to achieve sufficient stabilization and pathogen inactivation. If the human excreta are not properly aerated, anaerobic degradation can occur, which generates odour. Most nutrients, except for nitrogen, are completely retained in the compost. Usually high nitrogen losses are observed due to the formation of free ammonia, which generates odour as well. Pathogen inactivation can be achieved at temperature above 45 °C, which can be attained by proper mixing to avoid low temperature zones (Lopez Zavala and Funamizu, 2006).

One improvement could be to apply urine separation, such that only the relatively dry faeces are composted. Thermal composting of faeces at a temperature > 45 °C results in a pathogen free compost which can be safely recycled (Vinnerås et al., 2003). The separated urine can be used as a fertilizer after disinfection and removal of micro-pollutants (Höglund et al., 2002; Jönsson et al., 2004; Winker et al., 2009).

The main limitations to apply composting are a proper installation and management of the toilets. Composting toilets still need important breakthroughs before they become generally accepted and applied on a large scale (EPA, 1999; Otterpohl, 2008).

1.2.3 Anaerobic treatment

During anaerobic treatment organic material is converted to biogas and stabilized sludge. Anaerobic treatment can be applied to a wide variety of wastewater streams (Kujawa-Roeleveld and Zeeman, 2006) and is successfully applied for the treatment of industrial wastewater streams and in tropical regions also for domestic wastewater (Lettinga et al., 1993; van Haandel et al., 2006).

In conventional WWTPs anaerobic digestion produces biogas from primary and secondary sludge. In the Netherlands, where sludge digestion is common practice at large scale WWTPs, approximately 25% of the COD from the domestic wastewater is converted to methane, which is used for electricity and heat or as fuel for cars (Frijns et al., 2009). However, most of the wastewater COD is not collected as sludge, but is aerobically mineralized to CO_2 and water in the water line. Furthermore, a significant fraction of the wastewater COD is used for denitrification in order to remove nitrogen. By applying direct anaerobic treatment of the domestic wastewater much more COD may be recovered, and up to 60-80% can be converted to methane, although the nitrogen is not removed in this case (Lettinga et al., 1993).

The efficiency of anaerobic processes depends on the temperature, the characteristics and concentration of the pollutants and on the fluctuations in composition and load (Lettinga et al., 1993). At lower temperatures (< 25 °C) the anaerobic processes proceed much slower, necessitating longer retention times and larger reactor volumes. Furthermore, at lower temperatures more methane dissolves in the effluent (for diluted streams up to 50% (Lettinga et al., 1993)), resulting in an uncontrolled emission of methane, which contributes to global warming (Verstraete et al., 2009). Technological options can solve the problems of inefficient anaerobic treatment at lower temperatures, for example an integrated UASB-digester system (Mahmoud, 2002). When black water is separately collected using a minimum amount of flushing and transport water, efficient anaerobic treatment can be applied because concentrations are high and the temperature reduction by flushing with cold tap water is limited (Kujawa-Roeleveld and Zeeman, 2006). For example, with a vacuum system black water can be collected at a temperature of 20 °C (Meulman et al., 2008).

With an average load of 62 gCOD/p/d and a methanisation level of 60% (Kujawa-Roeleveld et al., 2006), 12.5 L CH_4/p/d can be produced from black water (0.35 L CH_4 /gCOD, (Standard temperature and pressure (STP))). Assuming a conversion efficiency of 35%, the produced methane can be converted to 56 MJ/p/y of electricity, representing 40% of the energy that now is required for conventional WWTPs (Frijns et al., 2008). Therefore anaerobic treatment of black water could significantly contribute in reducing the use of fossil fuels for wastewater treatment. However, it is then necessary to apply COD independent denitrification to remove the nitrogen.

Anaerobic treatment systems can be divided into systems with and without sludge retention. The choice depends on the type of wastewater and its concentration (Zeeman et al., 2001). For wastewaters such as black water, containing complex solid material the rate of hydrolysis is the rate limiting step and an efficient entrapment and long solids retention time (SRT) are needed (Zeeman and Sanders, 2001).

Due to the low biomass yield of anaerobic bacteria, the biomass production in anaerobic treatment systems also is low. Furthermore, soluble nutrients such as ammonium and phosphate are largely conserved in the effluent. Depending on the distance to agricultural fields, direct reuse of nutrient rich anaerobic effluent is possible, if it is treated to remove pathogens and micro-pollutants or if modified land application methods, to prevent contact of the wastewater with crops, are applied (Huibers and van Lier, 2005; Halalsheh et al., 2008). When direct reuse is not possible, the effluent of the anaerobic treatment requires further treatment with the potential for nutrient recovery.

1.3 Nitrogen

1.3.1 Background on nitrogen cycle

Nitrogen is an essential element for growth of micro-organisms, plants and animals. Several bacteria fix dinitrogen gas (N_2) into ammonia, which can be further used by other organisms. By the production of synthetic fertilizers, such as ammonia by the Haber-Bosch process, humans intervened in the nitrogen cycle (Erisman et al., 2008). Human activity now fixes more atmospheric N_2 into reactive forms (e.g. ammonia, nitrate and nitrous oxide) than all terrestrial natural processes combined (Galloway et al., 2008). Rockström et al. (2009) stated that the amount of fixed N_2 for human use has transgressed its boundary for a safe operating space for humanity. A large fraction of the reactive nitrogen ends up in the environment, polluting water ways and the atmosphere. Nitrous oxide (N_2O) is an important greenhouse gas, because it has a much stronger effect (300-fold) than carbon dioxide. Furthermore,

N_2O also has a large biological effect as a toxic compound on organisms and humans (IPCC, 2001). New strategies are needed to close the nitrogen cycle and to minimize the losses of reactive nitrogen to the environment (Galloway et al., 2008; Rockström et al., 2009).

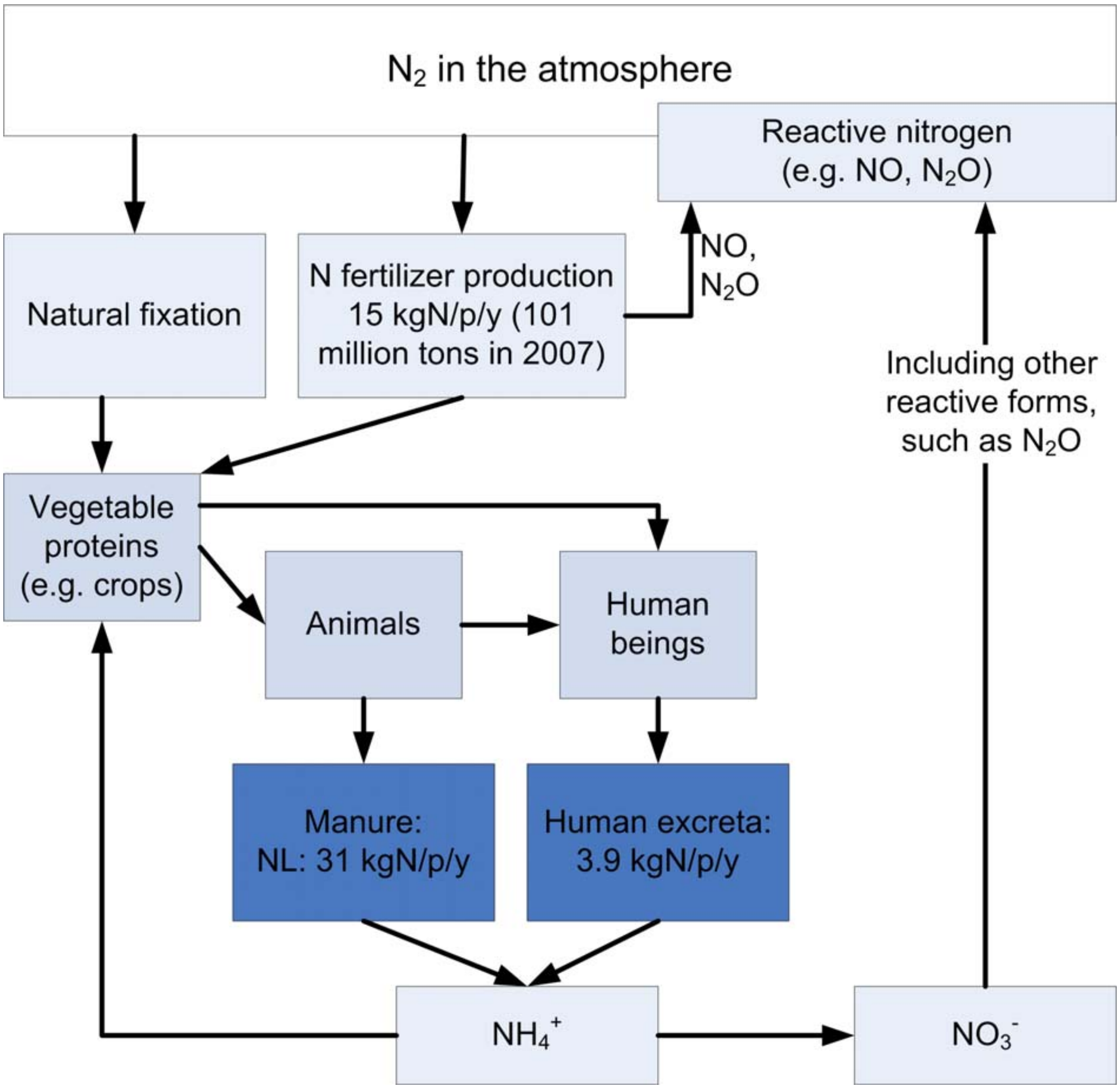

Figure 1.3 The nitrogen cycle, dominated by humans, and focussing on manure and human excreta (based on (Mulder, 2003)).

The nitrogen cycle, dominated by humans, is presented in figure 1.3. The current production of artificial nitrogen fertilizers is 101 million tons in 2007, which is about 15 kgN/p/y (FAO, 2007). Not all nitrogen in fertilizers ends up in food and a large fraction is lost to the environment, where it is partly transferred back to nitrogen gas by denitrification (40%, (Galloway et al., 2004)). Humans excrete on average 3.9

kgN/p/y (Kujawa-Roeleveld and Zeeman, 2006), which is 26% of the amount of global artificial nitrogen fertilizer that is produced. In the Netherlands, a country with an intensive agriculture, on average 31 kgN/p/y is produced in animal manure (CBS-Statline, 2008), which exceeds the global artificial nitrogen fertilizer production per person per year. Part of this manure is applied on land, but due to run-off, over-fertilisation and volatilization nitrogen can end up in surface waters causing eutrophication, and in the atmosphere causing acid rain. In countries which are nutrient-demanding, and do not have a surplus of nitrogen as manure, nitrogen recovery from sewage can be relevant (Mulder, 2003) and help to minimize the losses of nitrogen to the environment. Furthermore, recovery of nitrogen from wastewater can reduce the amount of energy needed to produce fertilizers (Gajdos, 1998; Verstraete et al., 2005).

Nitrogen removal from wastewater is common practice and mainly accomplished by activated sludge processes including nitrification and (heterotrophic) denitrification. The main disadvantages of the conventional WWTP with respect to nitrogen, are that a fraction of the nitrogen is lost to the atmosphere as reactive nitrogen (e.g. N_2O) and that nitrification requires aeration which is an energy demanding process. Furthermore, heterotrophic denitrification requires a carbon source, which is usually provided by the wastewater COD, reducing the potential of energy recovery from wastewater (Mulder, 2003; Wilsenach et al., 2003).

Because black water contains almost all the nitrogen from the household waste stream in a relatively small volume, it is a potential stream to recover ammonia or to remove the ammonia more efficiently than current WWTPs do. However, recovery of ammonia also consumes energy, and the question to remove or recover ammonia from wastewater is mainly determined by its nitrogen concentration (Siegrist, 1996; Maurer et al., 2003; Mulder, 2003; Wilsenach et al., 2003).

1.3.2 Nitrogen recovery techniques

Several authors review techniques to recover nitrogen, for example Siegrist (1996) for digester supernatants, Mulder (2003) for domestic wastewater and Maurer et al. (2003 and 2006) for urine. Proven techniques for nitrogen recovery include ion exchange, struvite precipitation and ammonia stripping. The application of natural zeolites such as clinoptilolite combined with struvite precipitation ($Mg(K;NH_4)(PO_4). 6H_2O$) can result in nitrogen removal efficiencies of 65 % – 80 % (Lind et al., 2000), but would require relatively large amounts of zeolite (Hellström, 2001). Ammonia-clinoptilolite and struvite can be applied as a soil conditioner or slowly releasing fertilizer (Bridger et al., 1962; Lind et al., 2000). In black water and urine, ammonia usually is present in excess and addition of phosphate and magnesium is required for struvite precipitation. This makes struvite precipitation a rather expensive and non-sustainable technique for nitrogen recovery (Siegrist, 1996; Di Iaconi et al., 2009). The precipitated magnesium and phosphate can be recovered by thermal treatment of the struvite, producing a ($Mg_3(PO_4)_2$) salt and ammonia gas (Stefanowicz et al., 1992). However, additional treatment is necessary to polish the effluent to remove the excess of phosphate which was needed for the struvite precipitation. Struvite precipitation for nitrogen recovery from black water or urine is therefore overall not beneficial. Air- or steam-stripping to produce an ammonia-water has been researched for concentrated streams such as urine, producing a 10% w/w ammonia product (Behrendt et al., 2002). The disadvantage of air- and steam-stripping is that it requires a large amount of energy. In general, techniques for nitrogen recovery are feasible from an energy and economical point of view only for streams which have an ammonium concentration above 5 kgN/m^3 (Siegrist, 1996; Mulder, 2003), such as urine.

1.3.3 Autotrophic nitrogen removal by nitritation-anammox

For ammonia concentrations in the range of 0.1 – 5 kgN/m^3 autotrophic nitrogen removal, consisting of partial nitritation and anaerobic ammonium oxidation (anammox), is regarded as a promising technology to remove nitrogen (Jetten et al., 1999; Jetten et al., 2005b). In the combined nitritation-anammox process ammonium first is partially oxidized to nitrite (partial nitritation). Subsequently ammonium and nitrite are converted to nitrogen gas by anaerobic ammonia oxidizers (anammox). These processes can also be combined in one reactor where anammox organisms grow in oxygen-free zones (van der Star et al., 2007).

Autotrophic nitrogen removal by nitritation-anammox is a promising low cost ammonium removal process for concentrated wastewaters, such as anaerobically treated black water (1-1.5 kgN/m^3, chapter 2). There is no need for an additional carbon source, the oxygen consumption is reduced and the sludge production is low (Strous et al., 1997). The combined nitritation-anammox process only consumes 5 MJ/kgN of electricity compared to 14 MJ/kgN of electricity needed for conventional nitrification/denitrification in WWTPs (Maurer et al., 2003). Although more and more full-scale nitritation-anammox plants are built, a remaining concern is the emission of the greenhouse gas nitrous oxide (Kampschreur et al., 2009b). Also, in view of energy-efficient treatment concepts in cold-climate countries, operation at lower temperatures than the optimum temperature of 35 °C for anammox (Strous et al., 1998) is preferable.

1.4 Phosphorus

1.4.1 Background on phosphorus cycle

Like nitrogen, phosphorus is an essential element for the growth of micro-organisms, plants and animals. Phosphorus is present in crucial compounds in life processes such as DNA and ATP. It is used in chemical compounds, such as fertilizers, explosives and pesticides. Interestingly, especially in the context of this research on black water, is that phosphorus was discovered by the distillation of urine by Hennig Brandt in 1669 (Bryant, 2004).

Naturally occurring and most common forms of phosphorus are apatites, which are hardly soluble orthophosphate minerals. The primary bulk storage of phosphorus on earth is in phosphate ore deposits, accumulated by biogeochemical processes (Valsami-Jones, 2004). The industrialization and intervention of humanity led to the development of much more soluble phosphates such as monocalcium phosphate ($Ca(H_2PO_4)_2$) and ammonium phosphates. The use of these compounds as fertilizers increases the bioavailability of phosphate in nature and can cause eutrophication. Nowadays the main fraction of the mined phosphate rock (85%, 14.9 million ton P/year) is used for the production of fertilizers to produce food (Cordell et al., 2009). Phosphates are also used as ingredients in toothpastes, flame retardants and food additives such as baking powder (Bryant, 2004).

Phosphorus is a limiting, non-renewable resource and reserves are becoming increasingly scarce (Driver et al., 1999). It is estimated that global phosphate rock reserves will be depleted in 50 – 100 years (Cordell et al., 2009). Although there are disagreements on the total global phosphate rock reserves, it is becoming a strategic resource for many countries (Gilbert, 2009). Phosphorus not only is a non-renewable resource, mining of phosphate rock also has a large negative environmental impact, because it leads to the production of gypsum that is contaminated with heavy metals and radioactive elements (Driver et al., 1999; Wilsenach et al., 2003). To prevent a phosphorus crisis, the focus should be on recycling phosphorus (Gilbert, 2009; Rockström et al., 2009).

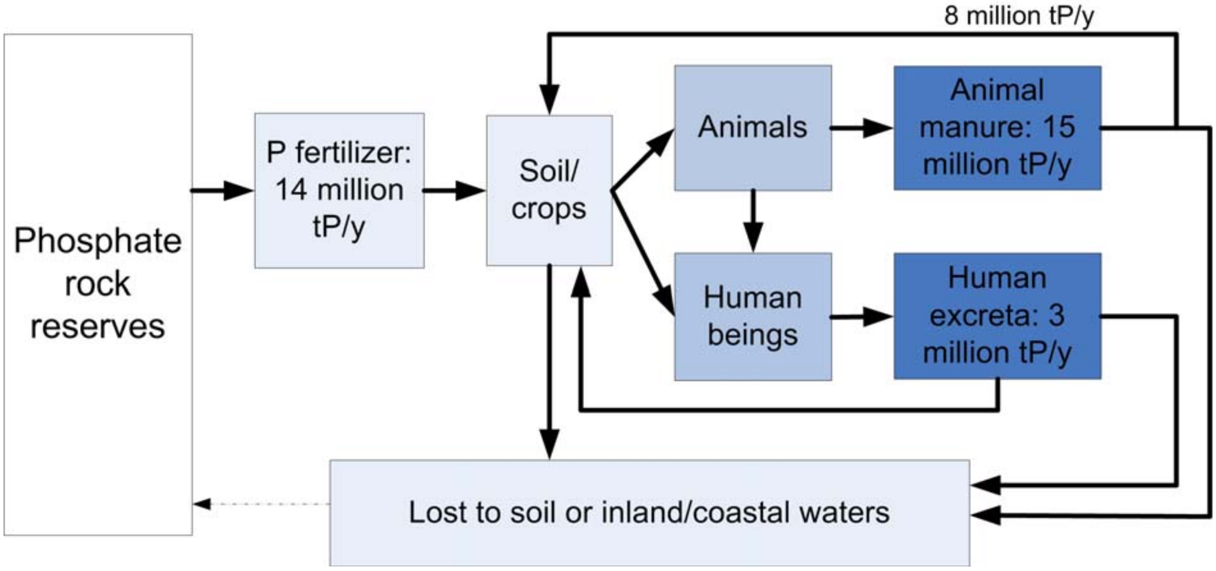

Figure 1.4 Global phosphorus cycle, simplified version based on (Cordell et al., 2009).

The global phosphorus cycle is shown in figure 1.4, which shows that significant amounts of phosphorus are lost to inland and coastal waters, from which recovery is not possible. In animal manure worldwide 15 million tons of phosphorus per year are produced, which is about the same amount that is produced as chemical fertilizer. About half of the manure is recycled to the soil (figure 1.4), but the remaining fraction is lost (Cordell et al., 2009). In the Netherlands on average 7.6 ktP/year are excreted by humans in their faeces and urine (16 million people, 1.3 gP/p/d (Kujawa-Roeleveld and Zeeman, 2006)), which is 36% of the artificial phosphorus fertilizer consumption in the Netherlands (21 ktP/year, (CBS-Statline, 2008)). Worldwide this is 3 million tons P/year (figure 1.4), which is 21% of the total amount of artificial phosphorus fertilizer produced (14 million tons P/year) (Cordell et al., 2009). Human waste therefore is a potential source for phosphorus recovery. Approximately 60% of the phosphorus in human waste is present in urine, mainly as soluble phosphates. The other 40% originates from faeces, mainly as mineral calcium phosphates and fixed in organic compounds originating from bacteria and enzymes (Vinnerås, 2002).

1.4.2 Phosphorus recovery from wastewater

In conventional WWTPs phosphorus recovery is rarely applied and phosphorus is mainly eliminated by chemical precipitation with metals salts (iron or aluminium) or by enhanced biological phosphorus removal (EBPR) (Tchobanoglous et al., 2003). Chemical precipitation with metals salts can remove the phosphorus to low levels in the effluent, but disadvantages are the large amount of chemicals that is required and the large amount of sludge that is produced. Furthermore, metal phosphate salts, like iron or aluminium, cannot be reused in agriculture because the iron or aluminium phosphates are not available for plants under normal pH conditions. With enhanced biological phosphorus removal, phosphorus accumulating organisms (PAOs) incorporate phosphorus into cell biomass and the phosphorus is removed from the process by sludge wasting (bio-P sludge) (Tchobanoglous et al., 2003). Due to the presence of iron, aluminium (which were added to precipitate phosphates) and other heavy metals, reuse of sewage sludge is restricted and for example in the Netherlands sewage sludge is incinerated. During incineration the phosphorus ends up in the ashes and this is used in asphalt or it is land filled[7].

Recently sewage ashes are regarded as a potential source for the phosphorus industry. One of the requirements is that the iron content is low (Fe/P molar ratio of < 0.2), which can be accomplished by applying biological phosphorus removal or precipitation with aluminium (Schipper et al., 2007; Reitsma et al., 2008). However, heavy metals like cupper and zinc in the ashes are still above their required maximum concentrations (500 mg Cu/mg ash and 1000 mg Zn/mg ash), and are a major concern because they tend to build up in the phosphorus production process (Schipper et al., 2007). The maximum amount that may be recycled from sewage in the Netherlands was estimated to be 12 ktP/year, based on the difference between influent and effluent phosphorus concentrations of WWTPs in the Netherlands (CBS-Statline, 2007b).

Another possible source for phosphorus recovery at WWTPs is the sludge liquor from digesters (Driver et al., 1999). For example, struvite recovery from sludge liquor already is applied at full scale in Japan (Ueno and Fujii, 2001). A few examples in the Netherlands exist for phosphorus recovery at WWTPs, mainly by precipitation of calcium phosphates or struvite (Reitsma and Bults, 2007). However, it was estimated that only 40% of the phosphorus in the wastewater could be recovered from the sludge liquor and additional phosphorus removal to meet discharge limits would be required (Reitsma and Kuipers, 2005).

Black water, containing 68% of the phosphorus in the total household wastewater stream including kitchen waste (Kujawa-Roeleveld and Zeeman, 2006) is a potential source for phosphorus recovery (7.6 ktP/year in the Netherlands). Because most heavy metals are present in industrial wastewater, relatively clean phosphorus rich products can be produced from black water that could be reused in agriculture as a fertilizer (Winker et al., 2009). Products with a high reuse potential are calcium

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[7] http://en.wikipedia.org/wiki/Fly_ash, visited 20th December 2009

phosphates and struvite ($Mg(NH_4;K)PO_4.6H_2O$) (Driver et al., 1999). The disadvantage of using calcium is that it will also precipitate with carbonate(s) and that amorphous precipitates are formed (Tchobanoglous et al., 2003). This implies that a large amount of calcium is required. The addition of magnesium to form struvite precipitates therefore is preferred.

1.4.3 Struvite precipitation

Le Corre et al. (2009) extensively reviewed phosphorus recovery from wastewater by struvite crystallization. They concluded that improvements are still needed with respect to quality and quantity to make struvite production an economically viable route. To obtain high removal of phosphate from solution, several factors like pH, degree of supersaturation, presence of other ions such as calcium and temperature are important (Doyle and Parsons, 2002). Generally, an excess of magnesium and elevated pH (higher than 8) are needed to precipitate struvite and to achieve removal efficiencies of more than 90% (Ueno and Fujii, 2001; von Münch and Barr, 2001; Doyle and Parsons, 2002).

In conventional WWTPs scaling is a problem at places where CO_2 is lost by stripping and this causes elevated pH values and undesirable struvite formation (Doyle et al., 2002). By struvite precipitation under controlled conditions, problems due to scaling can be prevented and a useful fertilizer is produced (Battistoni et al., 1997; Doyle and Parsons, 2002). It was shown that struvite recovery from sludge liquors could be economically feasible with a payback period of less than five years for a struvite plant processing 55000 m^3/d of wastewater (Shu et al., 2006).

Ammonium is usually present in excess in black water and to recover phosphate in the form of struvite only requires the addition of magnesium. Struvite precipitation for phosphorus recovery could be applied from black water that is first anaerobically treated. During this treatment, suspended solids and organic material that could interfere with the precipitation process, are removed (Schuiling and Andrade, 1999). Alternatively, if ammonium already has been removed, potassium, which is usually present in excess as well, can substitute ammonium and $MgKPO_4.6H_2O$ precipitates under the same conditions (Schuiling and Andrade, 1999) as ammonium struvite. An important aspect in applying struvite as fertilizer in agriculture is its purity. Struvite can be precipitated from urine, free from organic micro-pollutants and with low levels of heavy metals (Ronteltap et al., 2007b). Because of the high reuse potential of struvite and the high removal efficiencies that can be achieved, struvite precipitation in new sanitation concepts is an attractive option.

1.5 Hormones and pharmaceutical residues

1.5.1 Background of hormones and pharmaceuticals in the environment

Over the last 10 -15 years there is a growing concern about the emission of hormones, pharmaceuticals and personal care products to the environment. In this research the focus is on hormones (naturally excreted and synthetic estrogens) and pharmaceuticals, because these are the main micro-pollutants in black water. Personal care products usually end up in grey water and therefore were not considered in this research.

The first review on the fate of pharmaceutical residues dates from 1985 (Richardson and Bowron, 1985). Since about 10 years there has been a fast development and increasing use of new analytical tools which enable the detection of micro-pollutants at very low concentrations in all kinds of wastewaters (Ternes and Joss, 2006). The fact that several micro-pollutants were detected in drinking water, and that they can have a feminization or masculinisation effect already at ng/L levels, increased the concern (Harries et al., 1997; Ternes and Joss, 2006). News items like 'Prozac found in drinking water'[8] also highlighted the potential problem of pharmaceuticals entering the environment. Furthermore, it was found that Prozac in wastewater threatens mussel reproduction (Fong, 1998; Fong and Molnar, 2008). Hormones and pharmaceutical residues enter the environment mainly through excretion and improper disposal of unused or expired pharmaceuticals in the toilet (Fisher and Borland, 2003; Ternes et al., 2004). Because conventional WWTPs are not designed to remove these compounds, surface waters, ground waters and drinking waters become contaminated by persistent micro-pollutants and their metabolites (Jones et al., 2005; Ternes and Joss, 2006). To remove these persistent compounds, post-treatment of WWTP effluents is necessary to avoid their emission to the environment.

Separation at source offers the possibility to collect different waste streams with low dilution, offering more efficient removal of hormones and pharmaceutical residues (Larsen et al., 2004; Ternes et al., 2004). Larsen et al. (2004) concluded that urine source separation offers an elegant solution to the problem of pharmaceuticals in the environment. However, with urine separation not all ecotoxicological risks can be decreased, because still on average 30% of the pharmaceuticals are excreted with the

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[8] http://news.bbc.co.uk/2/hi/3545684.stm, visited on 27th October 2009

faeces (Lienert et al., 2007). For example ibuprofen, one of the compounds that could pose an ecotoxicological risk, is excreted for 50% with the faeces (Lienert et al., 2007). In black water hormones and pharmaceutical residues are present at relatively high concentrations (µg/L to mg/L range). Specific treatment of this small concentrated stream may minimize their discharge to surface waters (Joss et al., 2006b).

Already thousands of compounds are registered and used by humans as pharmaceuticals (Daughton and Ternes, 1999; Lienert et al., 2007). To monitor sewage and estimate the fate of micro-pollutants in the environment a selection process is needed to narrow down the number of compounds that is of interest. Selection criteria can be the amounts used or prescribed and the potential toxicity (Jones et al., 2005). Reliable and sensitive techniques such as gas chromatography (GC) or liquid chromatography (LC) combined with mass spectrometry are available to detect micro-pollutants in environmental samples at low levels (µg/L to ng/L). Analytical problems concern mainly extraction and analysis in difficult matrices such as sludge or concentrated streams (Jones et al., 2005). Another concern is the formation of metabolites in the human body such as simple conjugated compounds or structurally (un)related compounds. Conjugated compounds are usually the more soluble and inactive forms of the parent compound. In faeces enzymes are present that can hydrolyse conjugates back into their original and therefore active form (Ternes et al., 1999).

1.5.2 Removal of hormones and pharmaceuticals

Techniques for the removal of hormones and pharmaceuticals have been reviewed by a number of authors (e.g.(Larsen et al., 2004; Jones et al., 2005; Maurer et al., 2006; Cirja et al., 2008)). The removal of micro-pollutants in WWTPs depends on the biodegradability (rate) and adsorption of the compounds to solids or sludge. Several commonly used pharmaceuticals are not detected in WWTP effluents and proved to be biodegradable under aerobic conditions, such as paracetamol (Onesios et al., 2009). However, many compounds are not or only partially biodegradable, do not adsorb to sludge, and eventually end up in surface waters (Joss et al., 2006b). For example, carbamazepine was already proposed as a marker for the sewage contamination of surface waters and groundwater (Nakada et al., 2008). The fate of estrogens in biological treatment of concentrated black water was studied in detail by de Mes (2007). It was found that adsorption and biodegradation were the main processes for the removal of estrogens in biological treatment, but additional treatment is necessary to remove the remaining estrogens which were mainly present as conjugates (>70%, (de Mes, 2007)).

Membrane filtration, adsorption on activated carbon or advanced oxidation were found to be suitable techniques to remove persistent micro-pollutants (e.g. (Ternes et al., 2003; Nghiem et al., 2005; Rossner et al., 2009).

Retention by nanofiltration is mainly based on steric (size) exclusion and (near) complete removal of several compounds was observed (Nghiem et al., 2005). However, this removal is dominated by charge effects and some compounds can adsorb onto the membrane and diffuse through the membrane, decreasing their removal efficiency (Nghiem et al., 2005; Verliefde et al., 2007). Separation by membrane filtration has the disadvantage that the micro-pollutants are concentrated and not destructed. Therefore, there will always be a concentrate stream that needs to be disposed of (Verliefde et al., 2007). Another drawback is the high energy costs for nanofiltration (ca. 1 kWh/m^3, (Larsen et al., 2004)).

Activated carbon was shown to be very effective in the removal of various contaminants from water. Research on the application of activated carbon for the removal of micro-pollutants focuses on drinking water (Rossner et al., 2009; Yu et al., 2009). The presence of other organics and naturally organic matter (NOM) has a large effect on the capacity of the activated carbon. Recently, granular activated carbon (GAC) was used to remove a selection of pharmaceuticals from urine. Approximately 2 kg of GAC was needed to treat 1 L of urine (Jacobi and Kujawa-Roeleveld, 2009), which is much higher than the 25 mg GAC needed for 1 L of drinking water (40 m^3 / kg GAC, (Joss et al., 2006a)). However, several types of GAC are available and optimization may reduce the amount of GAC needed for the treatment of urine. For example, nanofiltration followed by granular activated carbon (GAC) can increase the adsorption capacity of the GAC for micro-pollutants because the main fraction of the background organic matter already is removed by nanofiltration (Verliefde et al., 2007).

To disinfect (drinking) water and to remove odour and colours, ozone is widely used in drinking water production (von Gunten, 2003b). Ozone reacts selectively with compounds that have for example electron-donating groups. $^{\cdot}OH$ radicals, formed by ozone decomposition, react unselectively with any (in)organic compound. $^{\cdot}OH$ radicals can also be formed in advanced oxidation processes where a combination of O_3/H_2O_2 or UV/ H_2O_2 is used to produce the $^{\cdot}OH$ radicals. Complete mineralization is economically not feasible and can lead to unwanted by-products (von Gunten, 2003a). Compounds that reduce the removal efficiency of micro-pollutants by (advanced) oxidation are organic compounds (COD), because these compounds will also be oxidized. Several studies showed the removal of pharmaceuticals by (advanced) oxidation in drinking water (e.g. (Huber et al., 2003)), but the main concern is the formation of oxidation products which can be more toxic than the original compounds (Joss et al., 2006a). On the other hand, destruction of micro-pollutants by ozonation into smaller fragments can increase their biodegradability and these can be removed by biologically activated carbon or in an additional sand filter (Hammes et al., 2006; Hollender et al., 2009).

Several recent studies focus on the oxidation pathways and oxidation products formed by ozonation of wastewaters containing persistent micro-pollutants (e.g. (Benner and Ternes, 2009)). Other studies focus on combination of techniques, for example nanofiltration combined with GAC as mentioned before.
Black water is a relatively small stream and therefore it may be more cost effective than treating a large diluted stream. Many compounds are slowly biodegradable and removal efficiencies can also be improved by optimizing the biological treatment, adjusting for example sludge ages and investigating different redox conditions (Jones et al., 2007). All the techniques to remove persistent micro-pollutants are relatively complicated and expensive, and a thorough risk assessment would be necessary to conclude on their necessity. Furthermore, there is a need for new advanced technologies which are more energy efficient[9].

1.6 Pathogens

1.6.1 Background on pathogens in wastewater

In wastewater several pathogens are present, such as viruses and faecal coliforms. Contact with polluted water can cause waterborne diseases, like diarrhoea. Sewerage and centralized wastewater treatment systems have achieved relatively high standards with respect to hygiene. Activated sludge processes achieve a substantial removal of indicator organisms (2 log reduction (Crocket, 2007) or 90-98% removal (Tchobanoglous et al., 2003)). However, combined sewer overflows still occur, resulting in untreated wastewater entering surface waters and endangering public

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[9] For example the use of fluidized bed electrodes for disinfection and decomposition of organics. http://www.wetsus.nl/pageid=117/Advanced_waste_water_treatment.html, visited 24th December 2009

health (Otterpohl et al., 1997). Moreover, for WWTPs discharging to recreational surface waters, a new European guideline was launched in 2006, which requires a WWTP removal of more than 4 log units (Derksen et al., 2006; EU, 2006). Another concern is that the current regulatory standard based on indicator organisms may not be sufficient to mitigate the pathogens problem in water supplies and recreational areas (Crocket, 2007; Wen et al., 2009). For example, the presence of protozoan parasites in WWTP effluent did not correlate with the presence of indicator organisms and they were not completely removed (Bonadonna et al., 2002).

Grey water is relatively unpolluted and is a potential source for reuse (Hernandez Leal et al., 2007), while black water contains most of the pathogens. Dedicated treatment of black water therefore can considerably reduce the emission of pathogens to surface waters. Such a source separated approach also avoids the risk of sewer overflows.

1.6.2 Removal of pathogens

Several techniques are available to disinfect water, for example pasteurisation or introducing pH shifts to low (<6) and high values (>9). Thermal composting was shown to be successful for the treatment of faeces, resulting in a small volume of compost that could be safely recycled (Vinnerås et al., 2003). Free ammonia at concentrations of > 60 mM proved to be a good sanitizing agent for the treatment of urine and faeces (Vinnerås, 2007; Nordin et al., 2009). Urine generally contains a low level of pathogens. Because it contains the main fraction of the nutrients as well, it is considered as a potential fertilizer (Kirchmann and Pettersson, 1995). Storage of urine for six months is recommended because due to the high pH during storage (pH of 9), pathogens are sufficiently eliminated (Höglund et al., 2002; Jönsson et al., 2004).

For reuse of anaerobically treated wastewater as irrigation water, proper agricultural practices can prevent the need for disinfection (Halalsheh et al., 2008). This was done by applying mulch to prevent direct contact of irrigation water with plants. Similar techniques might also be applied in new sanitation concepts based on separation at source, depending on the distance to agricultural fields.

For the production of drinking water UV or ozonation is used for disinfection (von Gunten, 2003a). Because (advanced) oxidation can also remove micro-pollutants (see previous paragraph), disinfection and the removal of micro-pollutants can be investigated in combination. Present research focuses on energy efficient advanced techniques for disinfection and decomposition of organics, which are promising to apply for wastewater treatment[10].

Before a wide implementation, a risk assessment is needed to evaluate the safety of new sanitation concepts.

1.7 New sanitation concepts and treatment

This review describes systems for collection, transport and treatment of source separated black water. Many alternatives are available and the selection will depend on local circumstances and requirements (Kujawa-Roeleveld and Zeeman, 2006). The design of treatment systems within new sanitation concepts is determined by the degree of separation and the degree of dilution with flushing and transport water. Additionally, depending on local scale, demand and supply of resources, and on available space, the degree of decentralization (e.g. house-on-site or community-on-site) is important. Kujawa-Roeleveld and Zeeman (2006) proposed several schemes for new sanitation concepts. An adapted version, summarizing the most feasible options, is shown in figure 1.5.

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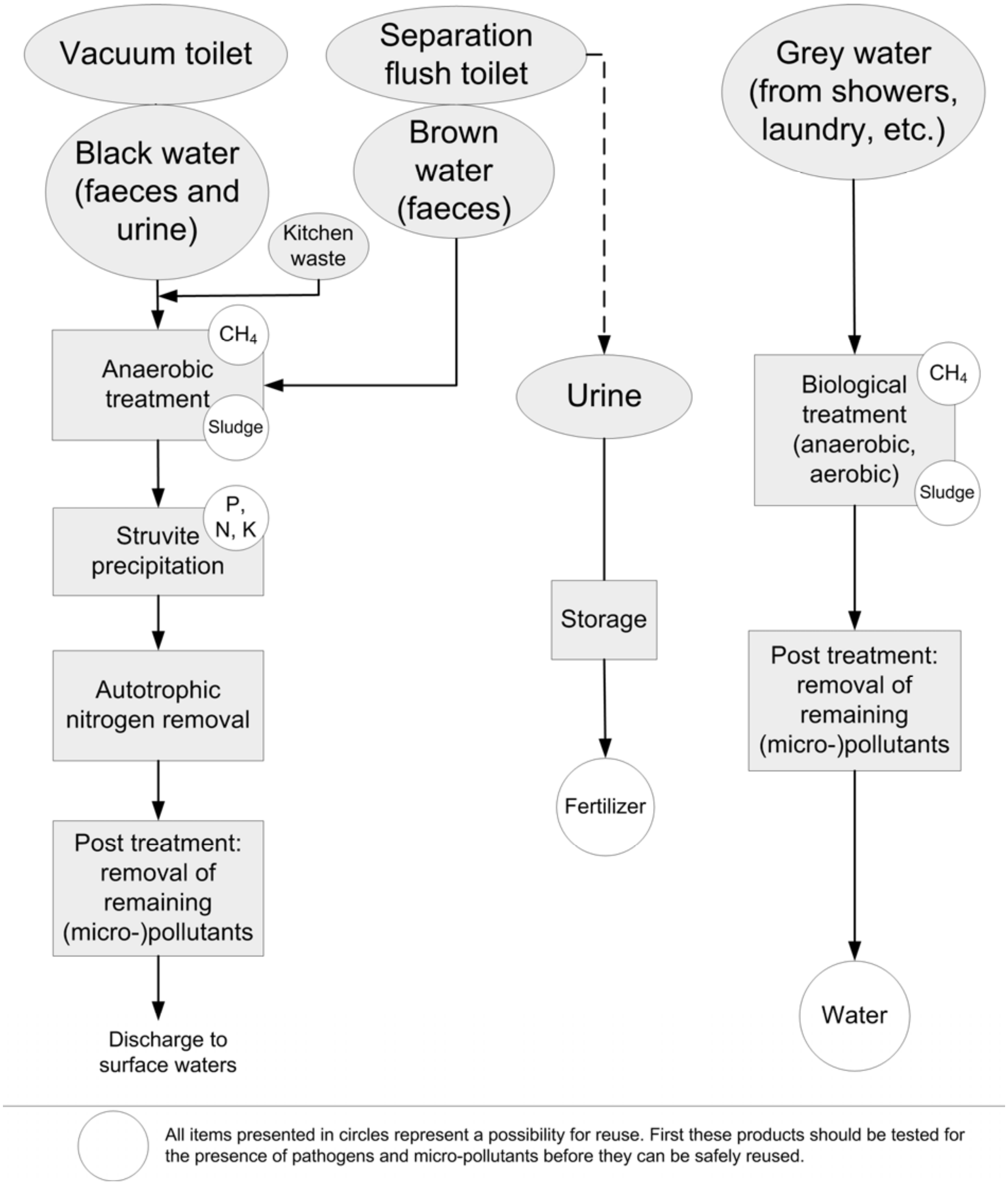
[10] http://www.wetsus.nl/pageid=117/Advanced_waste_water_treatment.html, visited 24th December 2009



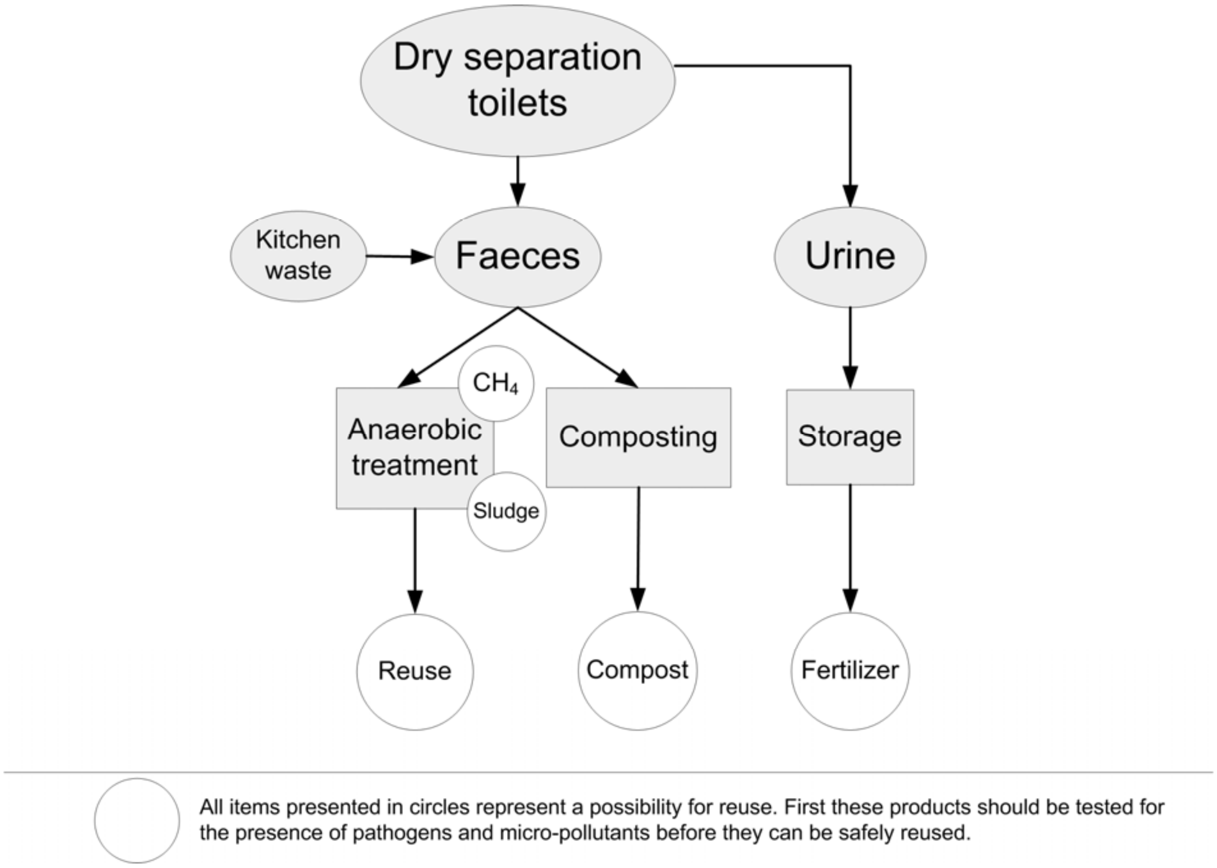
All items presented in circles represent a possibility for reuse. First these products should be tested for the presence of pathogens and micro-pollutants before they can be safely reused.

Figure 1.5 Treatment options of new sanitation concepts based on separation at source, showing the most feasible treatment options.

As mentioned in the introduction, vacuum toilets are a promising alternative for conventional toilets, collecting faeces and urine with a minimum amount of flushing water. Generally, anaerobic treatment is regarded as the core technology for energy and nutrient recovery from source separated black water (Zeeman and Lettinga, 1999; Verstraete et al., 2005; Kujawa-Roeleveld and Zeeman, 2006). Several techniques have been researched for urine (Maurer et al., 2006), but research on black water is limited to anaerobic treatment and composting (Vinnerås et al., 2003; Kujawa-Roeleveld et al., 2006; Wendland et al., 2007). Subsequently phosphorus can be recovered in the form of struvite and the ammonium can be removed by autotrophic nitrogen removal. Finally a post-treatment step could be needed before it can be safely discharged to surface waters.

Another option is to collect the urine separately, because this is a relatively clean stream, containing the main fraction of the nutrients. However, because urine still contains a large amount of water, transport of urine over long distances is not feasible. Separate urine collection seems to be attractive within new sanitation concepts when the urine can be reused directly, because otherwise three different treatment concepts (for urine, faeces (brown water) and grey water) would be necessary to recover all resources. When urine is separately collected, two primary treatment options are available for the faeces. When collected in dry toilets, composting of the faeces can result in pathogen free compost which can be safely recycled (Vinnerås et al., 2003). Composting toilets however are not suitable to apply at large scale yet (EPA, 1999; Otterpohl, 2008). Faeces collected in dry toilets or with a low amount of flushing water (often called brown water) can also be treated anaerobically, producing methane. Because solid kitchen waste contains a lot of biodegradable material, combined anaerobic treatment is feasible, doubling the biogas production (Kujawa-Roeleveld et al., 2006). The options for the treatment of urine and faeces in dry toilets are presented in figure 1.6.

Grey water can be efficiently treated by combined biological and physical/chemical treatment, producing an effluent with a large potential for reuse such as irrigation water (Hernandez Leal et al., 2007).



All items presented in circles represent a possibility for reuse. First these products should be tested for the presence of pathogens and micro-pollutants before they can be safely reused.

Figure 1.6 Treatment options in new sanitation systems based on collection with dry separation toilets.

New sanitation systems with resource recovery require a different infrastructure than conventional sewerage and WWTPs, but they present a promising alternative for new housing estates and for areas where no wastewater infrastructure is available.

1.8 Conclusions

New sanitation systems based on separation at source offer a large potential for resource recovery from wastewater, e.g. energy and nutrients from black water and irrigation water from grey water. They present a promising alternative for sewerage combined with centralized aerobic wastewater treatment plants without resource recovery.
A feasible treatment system for black water (faeces and urine) collected with vacuum toilets, would consists of anaerobic treatment followed by struvite precipitation for phosphorus recovery and autotrophic nitrogen removal. Applying these techniques, it is possible to produce 56 MJ/p/y of electricity, representing 40% of the energy that is required for conventional WWTPs. Furthermore a maximum of 21% of the worldwide artificial fertilizer phosphorus production can be recovered. In addition, with the application of vacuum toilets, up to 24% of total drinking water consumption in a household can be saved. As an alternative an even higher degree of separation could be applied by separating the urine from the faeces, but this is only attractive if the urine can be directly reused. The issue of hormones, pharmaceuticals and pathogens in wastewater and their entry to the environment requires more research to determine to which degree treatment is necessary.

1.9 Scope and outline of this thesis

The objective of this project is to design a compact treatment concept for concentrated black water with the focus on energy and nutrient recovery and the removal of micro-pollutants. The fate of pathogens in black water treatment is not researched in this thesis. An overview of the research subjects in this thesis is presented in figure 1.7.

Black water collected by vacuum toilets in the DESAR (Decentralised Sanitation and Reuse) project in Sneek (the Netherlands) was used as the feed for laboratory scale treatment. Concentrated black water, produced by vacuum toilets at 5 L/p/d (Meulman et al., 2008), is about 25 times more concentrated than the total wastewater stream from Dutch households (124 L/p/d (Kanne, 2005)). Black water contains COD concentrations in the range of 7.7 up to 16 gCOD/L, nitrogen (TN) of 1.0 up to 1.8 gN/L and phosphorus (TP) of 0.15 up to 0.24 gP/L (Kujawa-Roeleveld, 2005; Wendland et al., 2007) (chapter 2). Based on the overview presented in this chapter, the following techniques were selected to investigate their suitability in black water treatment.

Anaerobic treatment is regarded as the core technology for energy and nutrient recovery from source separated black water (Zeeman and Lettinga, 1999; Verstraete et al., 2005; Kujawa-Roeleveld and Zeeman, 2006). This is because it converts organic matter to methane, which can be used to produce electricity and heat, while at the same time anaerobic treatment yields low amounts of excess sludge and the nutrients are largely conserved in the liquid phase. UASB (Upflow Anaerobic Sludge Blanket) systems offer the possibility to apply a long solids retention time (SRT) at a relatively short hydraulic retention time (HRT) and would offer an attractive alternative for large CSTR and septic tank systems. The feasibility of UASB reactors for energy recovery from black water therefore was investigated in **chapter 2**.

Autotrophic nitrogen removal was selected to remove nitrogen compounds from the anaerobic effluent and to be able to convert as much organic material as possible into methane for energy recovery. Furthermore from an energy and cost perspective autotrophic nitrogen removal is preferred over direct recovery (Mulder, 2003; Wilsenach et al., 2003). To study the two processes independently, the two reactor nitritation-anammox process was selected. Long term partial nitritation of anaerobically treated black water at 25 °C to achieve an influent suitable for the anammox process is described in **chapter 3** of this thesis. The possible emission of nitrous oxide (N_2O) was investigated and the mechanisms to achieve partial nitritation of ammonium to nitrite without nitrate formation were discussed.

Component:	Treatment:	Research subject:
Organic compounds	Anaerobic	Feasibility of applying a UASB reactor at a short HRT (chapter 2)
Nitrogen	Autotrophic Nitrogen Removal	Long term partial nitritation (chapter 3) Emission of N_2O (chapters 3 and 4) Possibility to apply anammox to remove nitrogen from black water (chapter 4)
Phosphorus	Struvite	Fate of phosphorus in black water and during anaerobic treatment Struvite precipitation (chapter 5)
Hormones and pharmaceuticals	To be determined	Development of analytical method Fate of selected compounds during biological treatment (chapter 6)
Pathogens	To be determined	Recommendation for further research (discussed in chapter 7)

Figure 1.7 Outline of this thesis.

In **chapter 4** of this thesis the anammox process is studied. Two sequencing batch reactors (SBR) were used to investigate the nitrogen removal from black water at 35 °C as the optimum temperature for anammox growth (Strous et al., 1998) and at 25 °C as the preferred temperature for an energy efficient treatment concept for black water (chapter 2). Because of its low growth rate, excellent biomass retention is essential for anammox reactors, and the formation of granules therefore is generally favourable (Strous et al., 1998). Because the calcium concentration appeared to be low in the vacuum produced black water, the effect of calcium concentration and addition of calcium on granulation of anammox biomass were studied. The possible emission of nitrous oxide (N_2O) was investigated in the anammox reactors as well.

Black water is a potential source for phosphorus recovery (7.6 ktP/year in the Netherlands) (paragraph 1.4). In **chapter 5** the fate of phosphorus during anaerobic treatment was investigated in detail and the potential to recover struvite from black water was discussed.

Vacuum produced black water is a complex new matrix, and **chapter 6** describes the method that is developed to detect hormones and pharmaceuticals in black water and in effluents from the biological treatment processes (anaerobic and aerobic). This chapter also reports the results of this method, i.e. the fate of the micro-pollutants during biological treatment of black water.

The results and conclusions are summarized and discussed in **chapter 7**, including an outlook and recommendations for further research.

2

Anaerobic treatment of concentrated black water in a UASB reactor at a short HRT

Abstract

Black water (toilet water) contains half the load of organic material and the major fraction of the nutrients nitrogen and phosphorus in domestic wastewater. This research describes the feasibility of applying a UASB reactor for the treatment of concentrated black water at 25 °C. The UASB reactor removed on average 78% of the influent load of COD at a hydraulic retention time (HRT) of 8.7 days and a loading rate of 1.0 kgCOD/m^3/d. A methane production of 1.8 m^3 CH_4 per m^3 of black water was achieved, which can be converted to 56 MJ/p/y as electricity and 84 MJ/p/y as heat by combined heat and power (CHP). The effluent of the UASB reactor needs further treatment to remove remaining COD (1.2 gCOD/L, BOD_5 is 0.48 g/L) and to recover nutrients nitrogen and phosphorus. The minimum reactor volume at full scale was calculated to be 63L per person (for black water containing 16 gCOD/L produced at 5 L/p/d) and this is more than two times smaller than other type of reactors for anaerobic treatment of concentrated black water.

M.S. de Graaff, G. Zeeman, H. Temmink and C.J.N. Buisman

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2.1 Introduction

Separation of domestic waste(water) at the source results in black water from the toilet (faeces and urine) and less polluted grey water from showers, laundry and kitchen. These source separated waste(water) streams differ in quantity and quality and should be treated separately according to their concentrations and composition. The main benefits of such an approach include the possibility of recovering energy and nutrients and the efficient removal of micro-pollutants. Grey water has a high potential of reuse because it is the major fraction (70%) of domestic wastewater and relatively low in pollution (Hernandez Leal et al., 2007). Black water contains half the load of organic material in domestic wastewater, the major fraction of the nutrients nitrogen and phosphorus (Otterpohl et al., 1999; Kujawa-Roeleveld and Zeeman, 2006) and can be collected with a small amount of water (one liter per flush) using, for example, vacuum toilets. Black water also contains most of the pathogens, hormones and pharmaceutical residues. The volume of black water depends on the type of toilet and amount of water needed to flush.

Anaerobic treatment is regarded as the core technology for energy and nutrient recovery from source separated black water (Zeeman and Lettinga, 1999; Verstraete et al., 2005; Kujawa-Roeleveld and Zeeman, 2006) because it converts organic matter to methane, which can be used to produce electricity and heat, while at the same time anaerobic treatment yields low amounts of excess sludge. The nutrients are largely conserved in the liquid phase and can be subsequently recovered with physical-chemical processes such as precipitation and ion-exchange or removed biologically (Driver et al., 1999; Maurer et al., 2006). Depending on the distance to agricultural fields, direct reuse of nutrient rich anaerobic effluent is possible if it is treated to remove pathogens and micro-pollutants (Huibers and van Lier, 2005; Halalsheh et al., 2008).

With an average load of 62 gCOD/p/d and a methanisation level of 60% (Kujawa-Roeleveld et al., 2006), 12.5 L CH_4/p/d can be produced from black water (0.35 L CH_4 /gCOD, (Standard temperature and pressure (STP))). When solid kitchen refuse is included ((60 gCOD/p/d), (Kujawa-Roeleveld et al., 2006)) the biogas production can be doubled, resulting in 25 L CH_4/p/d, which represents 335 MJ/p/y (35.6 MJ/Nm^3 CH_4). Combined heat and power (CHP) generation systems can be used to produce heat and electricity at an efficiency of 85% (of which 40% electricity and 60% heat) (van den Berg, 2008). This results in a production of 32 kWh/p/y electricity (2.1% of the electricity consumption in a household (87 PJ electricity consumption in the Netherlands in 2006 (CBS-Statline, 2006) i.e. 1487 kWh/p/y)) and 47 kWh/p/y of heat when using the methane produced from black water and solid kitchen refuse.

The use of three types of reactors for anaerobic treatment of black water collected with vacuum toilets at different temperatures is reported in literature, namely a CSTR (continuously stirred tank reactor), an Accumulation system and a UASB-septic tank (Upflow anaerobic sludge blanket).

Wendland et al. (2007) investigated anaerobic treatment of black water from vacuum toilets in a CSTR operated at mesophilic conditions (37 °C). A removal efficiency of total COD of 61% was achieved at an HRT of 20 days. Applying a CSTR for anaerobic treatment of black water (7 L/p/d) requires a volume of 140 L per person (Wendland et al., 2007).

Kujawa-Roeleveld et al. (2006) investigated anaerobic treatment of black water and kitchen refuse in an accumulation system operated at 20 °C. An accumulation system is a continuously fed reactor and combines digestion and storage in one reactor volume. Stabilization of the black water for 80% was achieved within 150 days. Due to the long storage time a relatively large volume is needed of 1.0 m^3 per person for the treatment of black water. An accumulation system therefore is only suitable for even more concentrated streams (e.g. only faeces (brown water) and kitchen waste) and less suitable for black water (Kujawa-Roeleveld et al., 2006).

The second system that Kujawa-Roeleveld et al. (2005, 2006) investigated was a UASB-septic tank operated at 15 and 25 °C. UASB reactors enable long sludge retention times (SRT) at relatively short hydraulic retention times (HRT), because biomass retention is accomplished by an internal gas/sludge/liquid separation system (Zeeman and Lettinga, 1999). A UASB-septic tank is a continuous reactor with respect to the liquid, but accumulates the solids, combining the features of a UASB reactor and a septic tank. The UASB-septic tank removed 61% of the total COD at 15 °C and 78% of the total COD at 25 °C. For sludge stabilization and total reduction of volatile fatty acids (VFA) at 25 °C a minimum volume of 200 L per person is needed, corresponding to an HRT of about 30 days (Kujawa-Roeleveld et al., 2006).

The reactors mentioned above require relatively large volumes per person (table 2.1). Unlike the UASB-septic tank, a UASB reactor without additional space for the accumulation of solids (no septic tank) would require regular sludge removal, but it will reduce the volume of the reactor. This is important for application at larger scale where space might be limited.

Table 2.1 Reactors for anaerobic treatment of concentrated black water.

	CSTR (Wendland et al., 2007)	Accumulation system (Kujawa-Roeleveld et al., 2006)	UASB-septic tank Kujawa-Roeleveld et al., 2005 and 2006)	
Temperature (˚C)	37	20	15	25
Total COD removal (%)	61	80	61	78
HRT (d)	20	150	30	30
SRT (d)	20	150	> 365	> 365
Volume required (L/p)	140	$1.0*10^3$	n.d.	200
Methanisation* (%)	60	58	39	60

n.d. = not determined

*Calculation based on obtained methane production and influent load

UASB reactors so far have not been investigated for their capability to treat concentrated wastewater streams such as black water and was only shortly discussed by (Zeeman et al., 2008). The volume of a UASB reactor will depend on the minimum SRT required to achieve methanisation and stabilization of the sludge (Zeeman and Lettinga, 1999). For the anaerobic treatment of black water hydrolysis of particulate organic substrates is the rate-limiting step (Zeeman and Sanders, 2001). With first order kinetics and a hydrolysis constant of 0.1 d^{-1} (average value at 20 – 30 ˚C (Kujawa-Roeleveld, 2005)) it can be calculated that a high percentage of hydrolysis (between 80 and 90%) can be achieved at a SRT between 40 and 90 days. Other research showed as well that the minimum SRT was estimated to be 75 days at 25 ˚C to achieve methanisation and stabilization of the sludge (Zeeman and Lettinga, 1999; Halalsheh et al., 2005). Other factors that are important for the anaerobic treatment of black water are the temperature and inhibition by free ammonia (Tchobanoglous et al., 2003). Luostarinen et al. (2007) investigated the effect of temperature on anaerobic treatment of black water in UASB-septic tanks. The temperature had no significant effect on suspended solids removal, but the removal of dissolved COD improved because sludge adapted to lower temperatures (15 ˚C) (Luostarinen et al., 2007). The black water can be produced at a temperature of about 20 ˚C (Meulman et al., 2008). A higher temperature could result in a shorter HRT, but this would require extra energy requirements for heating the black water. Therefore a temperature of 25 ˚C was selected for the treatment of black water in a UASB reactor. In concentrated black water high concentrations of ammonium (0.8 – 1.4 gNH_4-N/L) are present which can inhibit methanisation and therefore higher retention times could be needed to achieve a maximum production of methane (Koster and Koomen, 1988).

This paper describes the feasibility of applying a compact UASB reactor for the treatment of concentrated black water from vacuum toilets at these conditions. Furthermore the design of the UASB reactor will be discussed, as well as the minimum volume needed at full scale.

2.2 Materials and methods

2.2.1 Black water collection

Black water, collected in vacuum toilets, was obtained from the DESAR (Decentralized Sanitation and Reuse) demonstration site in Sneek (Friesland, NL) (Zeeman et al., 2007). Every two weeks jerry cans were filled with black water from the buffer tank at the demonstration site (hydraulic retention time of 4 h, not cooled), transported to the lab and stored at 4 °C. Black water was pumped from a stirred, cooled (6 °C) influent tank into the UASB reactor with a Masterflex L/S peristaltic pump. A course filter (4 – 5 mm holes) in the influent tank prevented clogging of the inlet tube.

2.2.2 UASB reactor

A 50 L flocculent sludge UASB (Upflow Anaerobic Sludge Blanket) reactor (figure 2.1) was operated for 951 days at 25 °C to produce biogas from black water. The reactor was made of a transparent Perspex/Plexiglas tube (height: 1.30 m and inner diameter 0.20 m) with a double wall for temperature control. Temperature was controlled with a thermo stated water bath (Haake DC10/K10). The top was made of non-transparent plastic (polypropylene) and served as a gas/solid/liquid separator (height 0.19 m and width 0.22 m). Five taps at different heights (0, 0.26, 0.52, 0.78 and 1.02 m) enabled sludge sampling. Liquid effluent and gas were collected at the top. Gas production was monitored with a gasflow meter (Ritter TG05/5). A magnetic stirrer (VarioMag Mobil) at the bottom of the UASB reactor provided an even distribution of the influent through the sludge bed. The magnetic stirrer only mixed the bottom section of the sludge bed. The reactor was inoculated with 20 L anaerobic sludge (1.1 gVSS/L) from a UASB-septic tank treating concentrated black water at a temperature of 25 °C and a loading rate of 0.42 kgCOD/m^3/d (Kujawa-Roeleveld et al., 2005).

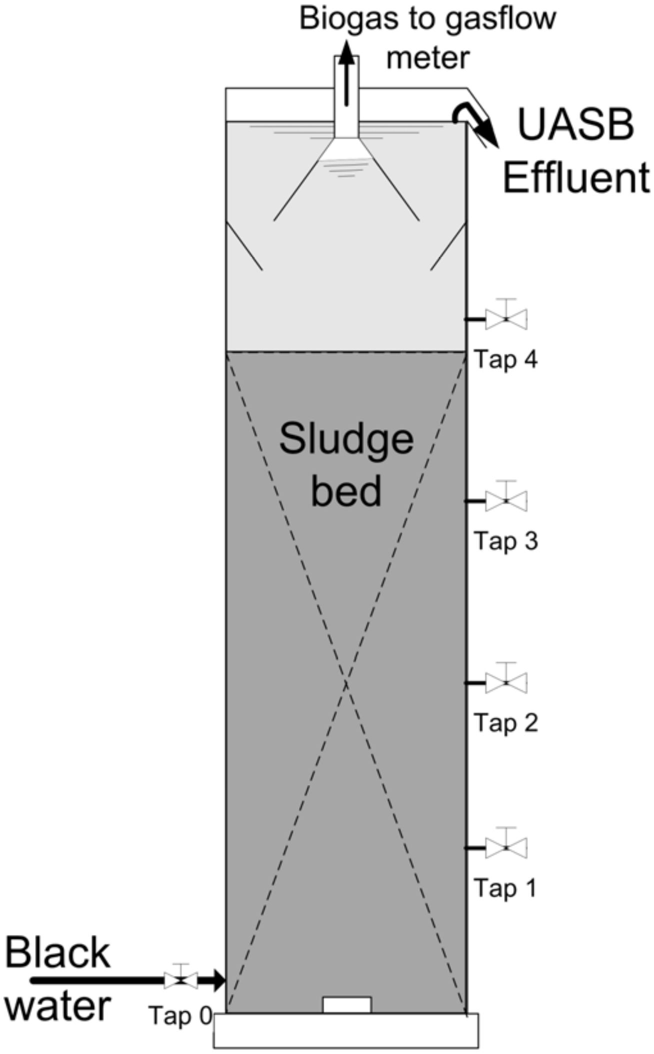

Figure 2.1 UASB (Upflow Anaerobic Sludge Blanket) reactor treating concentrated black water.

2.2.3 Design of the UASB reactor

The HRT to be applied in the UASB reactor was calculated using the following equation proposed by Zeeman and Lettinga (1999):

$$HRT = C \cdot \frac{SS}{X} \cdot R \cdot (1 - H) \cdot SRT \tag{2.1}$$

where C is the COD concentration in the influent (COD_{total}, in gCOD/L), SS is the fraction of suspended solids in the influent (COD_{SS}/COD_{total}), X is the sludge concentration in the reactor (in gCOD/L), R is the fraction of COD_{SS} removed and H is the level of hydrolysis of the removed solids.

Values of C, SS, X, R and H were taken from the research of Kujawa-Roeleveld (2005) and this resulted in a design HRT of 6.9 days (table 2.2).

Table 2.2 Initial design values for the UASB reactor.

	Unit	Remarks	Initial design of UASB reactor
SRT	d	Minimum value at 25 °C	75
C	gCOD/L	COD_{total} in the influent	12
SS	-	CODss/CODt influent	0.78
X	gCOD/L	Sludge concentration in the reactor	28
R	-	Fraction of CODss removed	0.9
H	-	Level of hydrolysis	0.7
HRT	d		6.9

Initially the reactor was operated at a longer HRT of 14 days to prevent accumulation of volatile fatty acids (VFA). The first 200 days were used as start-up period and the HRT was subsequently reduced in steps (every 5-6 weeks) when no VFA accumulation was observed. The average HRT achieved was 8.7 days, but fluctuated between 5.8 and 13 days due to silting of the influent tube. The influent tube was cleaned monthly to remove the silted solids.

Sludge was removed regularly from tap 4 at a height of 1.02m to maintain a maximum sludge bed height of 75% of the reactor volume.

2.2.4 Analyses and measurements

Every week influent and effluent composition was analyzed (125 samples in total) immediately after sample collection. Influent was collected from a tap just before the inlet of the UASB reactor (tap 0, figure 2.1) and effluent was collected during 3 hours in the morning (about 0.5 - 1 L for both samples). COD_{total} was determined from unfiltered samples, filtered COD (COD_f) was determined from paper filtered samples (black ribbon paper filter (Schleicher & Schuell)) and soluble COD ($COD_{soluble}$) was determined from membrane filtered samples (0.45 µm membrane filter (Cronus filter PTFE)) using DrLange kits (LCK514). Total Nitrogen (TN) en Total Phosphorus (TP) were determined from unfiltered samples using DrLange kits (LCK238 and LCK350). DrLange kits LCK302 and LCK303 were used to determine the total ammonia nitrogen concentration (NH_4-N) in paper filtered samples. Ion chromatography (Metrohm 761 Compact IC) was used to measure anions concentrations (Cl^-, NO_3^- NO_2^-, SO_4^{2-} and PO_4^{3-}) and volatile fatty acids (VFA: acetic acid, propionic acid and butyric acid) in membrane filtered samples. ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy) was used to measure concentrations of the element phosphorus in the membrane filtered sample. Inorganic carbon (IC) was determined with a Shimadzu TOC analyzer by difference from the measured total carbon (TC) and non-purgeable organic carbon (NPOC) in the paper filtered sample. Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) were determined according to standard methods using black ribbon ashless paper filter (Schleicher & Schuell) (APHA, 1998). Biological oxygen demand (BOD) of the UASB effluent was determined using OxiTop heads calibrated for BOD determination. Depletion of oxygen was monitored for 5 days (BOD_5) (17 samples in total). Biogas composition (sample of 5 mL, 43 samples in total) was analyzed with gas chromatography (Shimadzu GC-2010 Gas Chromatograph containing GS-Q (CO_2) and HP molsieve (O_2, N_2, H_2S and CH_4) columns). Wasted sludge (51 samples in total) was analyzed for TSS and VSS and total COD using the methods indicated above. Maximum biodegradability of the black water (2 samples in total) and stability of the UASB sludge (13 samples in total) was tested in closed bottles with Oxitop pressure measuring heads by incubation at 37°C (Kujawa-Roeleveld, 2005). The development of the sludge bed was analyzed by taking sludge samples from every tap (0.2 L) and these samples were analyzed for TSS and VSS and total COD (10 times 5 samples in total). The flow rate in the UASB was measured by weighing the collected effluent over a certain period of time and this was used to calculate the HRT.

2.2.5 Calculations

The concentration of suspended solids COD (COD_{SS}) was calculated as the difference between COD_{total} and COD_f. The concentration of colloidal COD ($COD_{colloidal}$) was calculated as the difference between COD_f and $COD_{soluble}$. The SRT in the UASB (SRT_{UASB}) was calculated using the following equation:

$$SRT_{\text{UASB}} = \frac{solids_{\text{reactor}}}{solids_{\text{washed,out}} + solids_{\text{wasted}}} \tag{2.2}$$

where $solids_{reactor}$ is the amount of solids in the reactor (gVSS), $solids_{washed,out}$ is the amount of solids that washed out with the effluent (gVSS/d) and $solids_{wasted}$ is the amount of solids that was wasted manually (gVSS/d).

The total amount of sludge in the reactor was calculated using the analyzed concentration and a volume of 1/6 of 50L as each tap is evenly distributed over the reactor (including effluent 'tap'):

$$solids_{\text{reactor}} = \sum_i \left(x_i \cdot \frac{1}{6} \cdot 50 \right) \tag{2.3}$$

where x_i is the sludge concentration in gVSS/L of each tap i and i is 0, 1, ..., 5.

The COD mass balance was calculated by adding up all measured incoming COD (influent and inoculum sludge) and measured outgoing COD (produced methane, sludge in reactor, wasted sludge and effluent) over the total period of operation including the start-up period.

$$\begin{gathered}\sum COD\,IN = \sum COD\,OUT \\ COD_{\mathrm{total,influent}} + COD_{\mathrm{inoculum,sludge}} = COD_{\mathrm{methane}} + COD_{\mathrm{sludge,reactor}} + COD_{\mathrm{sludge,wasted}} + COD_{\mathrm{total,effluent}}\end{gathered} \tag{2.4}$$

The amount of produced methane-COD was calculated from the average measured biogas composition, the average gas flow rate (L/d) and a conversion factor of 2.6 gCOD / L CH_4 at 25 °C at standard pressure.

The reported level of methanisation in the UASB reactor was calculated as the percentage of cumulative methane-COD production from the cumulative load of influent COD over the total period of operation.

The level of hydrolysis of solids was determined with the following formula (Halalsheh et al., 2005) :

$$hydrolysis = \frac{COD_{\mathrm{CH_4}} + COD_{\mathrm{soluble,effl}} - COD_{\mathrm{soluble,in}}}{COD_{\mathrm{total,in}} - COD_{\mathrm{soluble,in}}} \tag{2.5}$$

where COD_{CH4} is the methane produced, $COD_{\mathrm{soluble,effl}}$ is the soluble COD in the effluent, $COD_{\mathrm{soluble,in}}$ is the soluble COD in the influent and $COD_{\mathrm{total,in}}$ is the total COD load in the influent (all in gCOD).

The rate of hydrolysis was estimated using first order kinetics: $\frac{dF_{\mathrm{degr}}}{dt} = -k_h \cdot F_{\mathrm{degr}}$ (Zeeman and Sanders, 2001; Batstone, 2006).

Assuming the sludge bed as a CSTR, the following equation can be derived:

$$\frac{F_{\mathrm{degr}}}{F_{\mathrm{degr,0}}} = \frac{1}{1 + k_h \cdot SRT} \tag{2.6}$$

where F_{degr} is the amount of biodegradable solids in the sludge bed, $F_{\mathrm{degr,o}}$ is the amount of biodegradable solids in the influent and k_h is the hydrolysis constant. $1 - \frac{F_{\mathrm{degr}}}{F_{\mathrm{degr,0}}}$ is representative for the hydrolysis of suspended solids and therefore representative for the stabilization of the sludge.

The bicarbonate concentration was calculated from the total inorganic carbon (IC) as a function of pH and temperature (Tchobanoglous et al., 2003).

2.3 Results

2.3.1 Performance of the UASB reactor

The composition of the black water influent to the UASB reactor is shown in table 2.3. The black water was more diluted in the second period of operation because more flushing water was used in the vacuum toilets due to installation of noise reducers (table 2.3) (resulting in a black water production of 7.8 L/p/d instead of 5 L/p/d, (Gorter, 2009)).

The maximum biodegradability of the black water was 55% after 70 days of incubation at 37 °C. In figure 2.2 the influent and effluent COD concentrations and the total COD removal are shown. The UASB reactor removed an average of 74% of the influent load of COD.

Table 2.3 Composition of the black water influent.

		Day 1 – 518		Day 519 – 951	
	Unit	Influent	s.d.	Influent	s.d.
pH	-	8.8	0.22	8.6	0.53
COD_{total}	[gCOD/L]	9.8	2.6	7.7	2.5
COD_{SS}	[gCOD/L]	5.1	2.7	4.9	2.0
$COD_{colloidal}$	[gCOD/L]	1.3	0.42	0.5	0.22
$COD_{soluble}$	[gCOD/L]	3.4	0.47	2.3	0.81
VFA	[gCOD/L]	1.5	0.48	1.2	0.89
HCO_3^-	[gC/L]	1.2	0.37	0.67	0.20
TN	[gN/L]	1.9	0.19	1.2	0.18
NH_4-N	[gN/L]	1.4	0.15	0.85	0.15
TP	[gP/L]	0.22	0.067	0.15	0.064
TP soluble	[gP/L]	0.090	0.0087	0.057	0.018
PO_4-P	[gP/L]	0.079	0.0085	0.054	0.027

s.d. = standard deviation

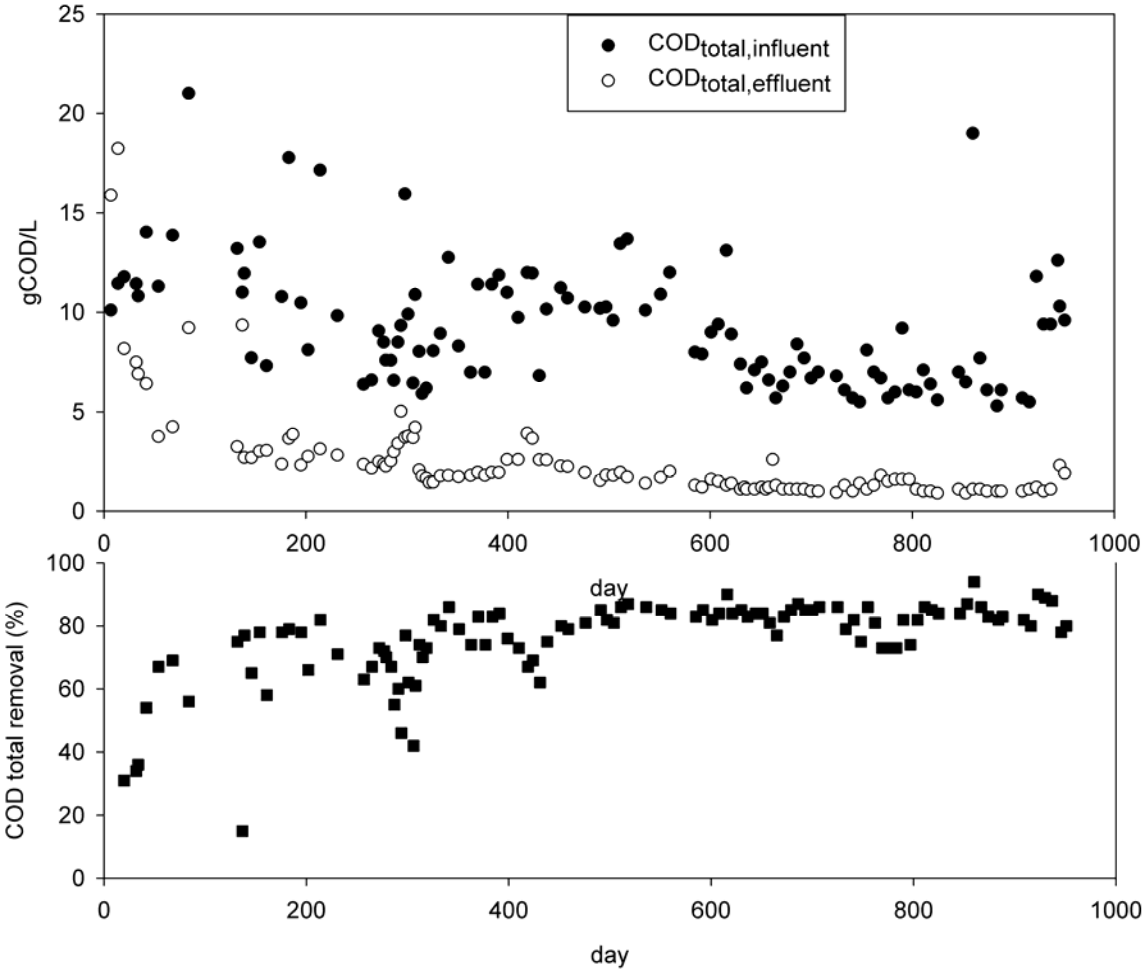

Figure 2.2 Influent and effluent COD concentrations and the COD total removal of the UASB reactor.

The removal efficiency varied between 42 and 94% as shown in figure 2.2, and became stable at a value of about 80% from day 500 onward. On average 10 L/d of biogas was produced, consisting of 78% (s.d. 5.8%) CH_4, 22% (s.d. 5.7%) of CO_2 and traces of H_2S (<0.5%).

In figure 2.3 the HRT in the UASB is plotted, together with the VFA concentration in the effluent. A few times VFA concentrations in the effluent increased, showing that anaerobic degradation was incomplete. The increase in VFA was always accompanied by a drop in COD removal, but never resulted in complete inhibition of biogas production. Furthermore a thick scum layer in the gas/solid/liquid separator was observed when the VFA concentration increased in the effluent (figure 2.3). This scum layer was transferred back to the bottom of the reactor and was no longer observed afterwards.

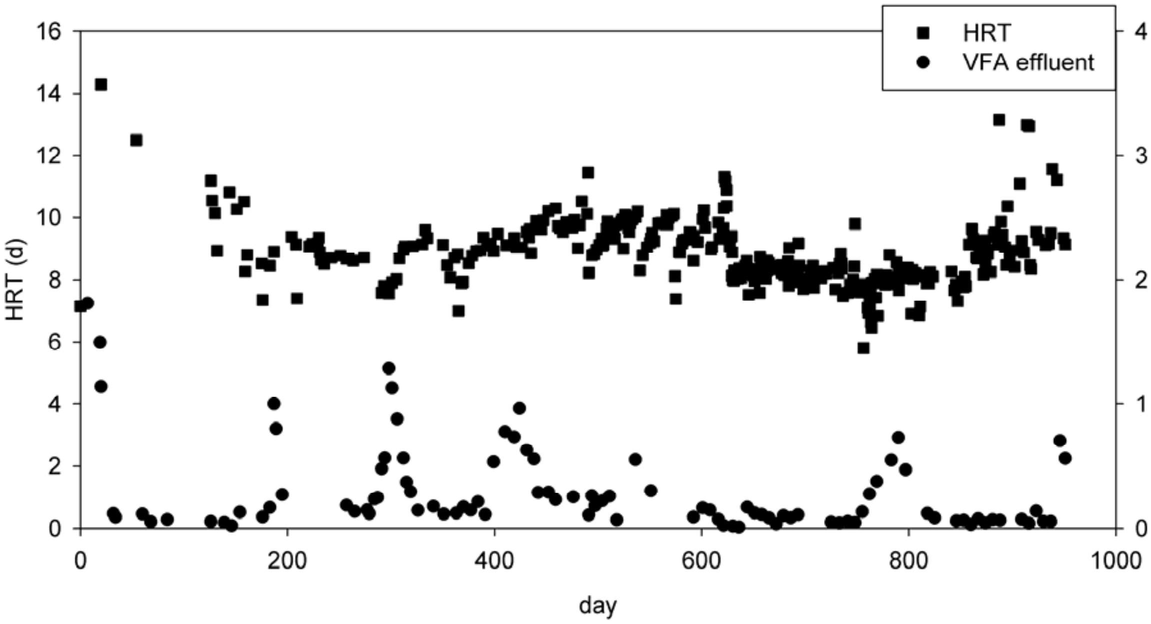

Figure 2.3 HRT and VFA in UASB effluent.

2.3.2 Sludge bed development

Figure 2.4 shows that the sludge bed developed to a compact sludge bed with concentrations of up to 45 g VSS/kg sludge. After day 800 sludge had to be wasted less frequently because the sludge concentration increased. The ratio of VSS/TSS of the wasted sludge decreased after day 800 from 0.80 to 0.68 (figure 2.4).

The SRT was estimated at 254 days and on average the reactor contained 19 gVSS/$L_{reactor}$(s.d. 4.0) and 34 gCOD/$L_{reactor}$ (s.d. 8.0). The wasted sludge from the UASB reactor showed a stability of 91% in 106 days at 37 °C, which means that only 9% of the COD in the UASB sludge could still be converted to methane. The percentage of influent suspended solids that were hydrolyzed and converted to methane was 53%.

The structure and the colour of the sludge changed from black, fine flocculant sludge in the inoculum to a brown, compact muddy sludge. This change in colour was probably due to leaching of iron, because the inoculum sludge originated from a UASB-septic tank where iron was added to precipitate phosphate (data not shown).

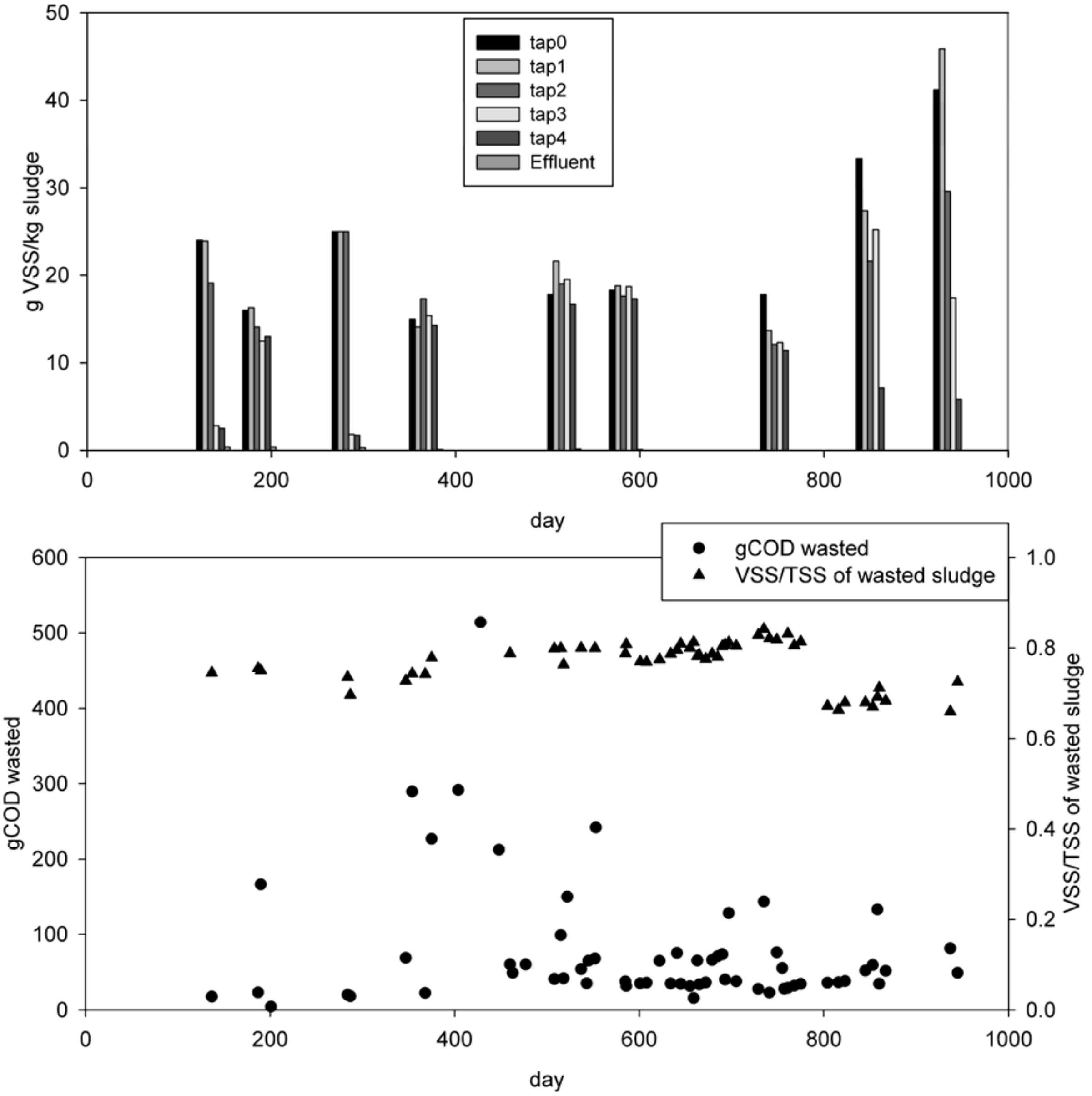

Figure 2.4 Sludge bed development (upper graph), the amount of sludge wasted (lower graph) and VSS/TSS ratio of the wasted sludge (lower graph).

2.3.3 COD mass balance

The COD mass balance over the total period of operation (951 days) showed only a small difference between total incoming COD and total outgoing COD (4%) (figure 2.5), which probably can be explained by errors introduced by sampling and analyses. Maximum 1% of total incoming COD left the reactor with effluent as dissolved methane (using a maximum solubility of 22 mg CH_4/L, (Lide, 2004)).

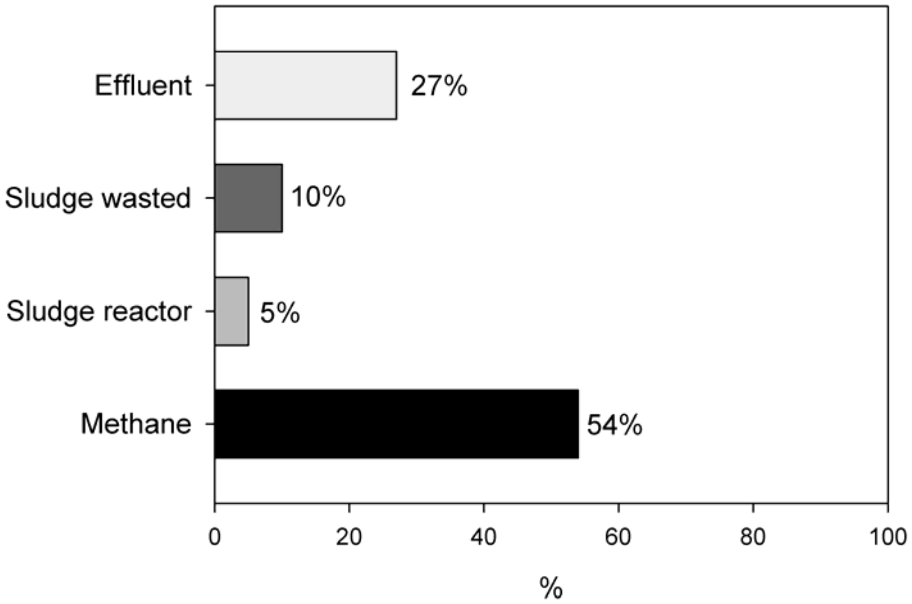

Figure 2.5 COD mass balance over the total period of operation (951 days) (The total amount of COD fed to the reactor during this period was 49 kgCOD, of which 0.1% was inoculum sludge).

2.3.4 Effluent composition

Table 2.4 shows the effluent composition of the UASB reactor. The pH dropped from 8.8 / 8.6 (table 2.3) in the influent to 7.6 / 7.4 in the effluent of the UASB during the anaerobic treatment.

A fraction of the COD in the effluent was still biodegradable, because the effluent contained a BOD_5 concentration of 0.87 g/L in the first period and 0.48 g/L in the second period.

Table 2.4 UASB effluent composition.

		Day 1 – 518		Day 519 – 951	
	Unit	UASB effluent	s.d.	UASB effluent	s.d.
pH	-	7.6	0.13	7.4	0.17
COD_{total}	[gCOD/L]	2.4	0.84	1.2	0.34
COD_{SS}	[gCOD/L]	0.43	0.43	0.10	0.08
$COD_{colloidal}$	[gCOD/L]	0.53	0.27	0.21	0.07
$COD_{soluble}$	[gCOD/L]	1.5	0.39	0.90	0.21
VFA	[gCOD/L]	0.36	0.30	0.14	0.18
BOD_5	[g/L]	0.87	0.24	0.48	0.06
HCO_3^-	[gC/L]	1.4	0.14	0.87	0.10
TN	[gN/L]	1.8	0.22	1.2	0.12
NH_4-N	[gN/L]	1.5	0.19	1.0	0.18
TP	[gP/L]	0.13	0.015	0.094	0.018
TP soluble	[gP/L]	0.103	0.010	0.070	0.011
PO_4-P	[gP/L]	0.092	0.011	0.069	0.013

s.d. = standard deviation

Nitrogen was conserved for more than 91% in the liquid effluent, mainly as ammonium, and the effluent contained 1.8 gTN/L (of which 1.5 gNH_4-N/L) in the first period and 1.2 gTN/L (of which 1.0 gNH_4-N/L) in the second period. Phosphorus was conserved for 61% in the effluent, mainly as phosphate, and the effluent contained 0.13 gTP/L (of which 0.092 gPO_4-P/L) in the first period and 0.094 gTP/L (of which 0.069 gPO_4-P/L) in the second period.

2.4 Discussion

2.4.1 Removal efficiency of the UASB reactor

This research shows that anaerobic treatment of concentrated black water can successfully be achieved in a UASB reactor. Generally the reactor exhibited a stable operation and removed more than 78% of the incoming COD. On three occasions VFA accumulated in the effluent and this was probably due to a sudden increase in load due to higher influent concentrations. In those periods scum formation was observed, which was probably caused by the reduced conversion efficiency of the anaerobic treatment process (Halalsheh et al., 2005). In the concentrated black water the free ammonia concentration was as high as 485 $mgNH_3$-N/L (25 °C, pH 8.8, 1.4 gNH_4-N/L) and reduced in the effluent to 35 $mgNH_3$-N/L (25 °C, pH 7.5, 1.5 gNH_4-N/L). It is likely that inside the UASB reactor a pH gradient exists and shock loads may have caused a temporary inhibition of the methanogenesis by free ammonia (Koster and Koomen, 1988), resulting in a reduced efficiency. The decrease in pH in the UASB reactor was probably caused by the production of CO_2 which dissolves in the liquid phase. The concentrated black water, however, contains enough alkalinity and pH control is therefore not necessary (Tchobanoglous et al., 2003).

Almost all suspended solids were retained in the reactor (93%, table 2.5) and high sludge bed concentrations were obtained. After day 800 the sludge contained more inorganic material because the ratio of VSS/TSS of the wasted sludge decreased from 0.80 to 0.68 and the sludge bed concentrations increased from about 20 gVSS/kg sludge to 30 – 45 gVSS/kg sludge (figure 2.4). This was probably because the concentrated black water contained more calcium (80 mg/L instead of 40 mg/L) and this caused more inorganic precipitation with carbonate and/or phosphate (Batstone, 2006).

Table 2.5 Operational characteristics of anaerobic treatment of concentrated black water at 25 °C.

	Unit	UASB reactor This research	UASB-septic tank Kujawa-Roeleveld et al., 2005 and 2006)	Pilot plant UASB-septic tank (Meulman et al., 2008)
Influent	-	Black water, vacuum toilets, DESAR pilot plant Sneek, Filtered with a coarse filter	Black water, vacuum toilets, Wageningen University	Black water, vacuum toilets, DESAR pilot plant Sneek
Reactor volume	L	50	200	7400
Up flow velocity	cm/h	0.76	0.23	0.42
Loading rate	$kgCOD/m^3/d$	1.0	0.42	0.36
HRT	days	8.7 (0.96)	29	30
SRT	days	254	> 365	> 365
COD_{total} removal	%	78 (9%)	78	87
COD_{SS} removal	%	93 (11%)	94	95
Methane production	$L\ CH_4/p/d$ $m^3\ CH_4/m^3$ BW	10 1.8	14 2.0	13 2.1

(standard deviation), BW = black water

In table 2.5 the operational characteristics of the UASB reactor are compared with the operation in a UASB-septic tank. In comparison with a lab scale UASB-septic tank a similar removal efficiency (78%, table 2.5) and effluent quality (not shown) were obtained, but the applied loading rate in the UASB reactor was much higher (1.0 $kgCOD/m^3/d$) than in the UASB-septic tank (0.42 $kgCOD/m^3/d$). Recently the anaerobic treatment of concentrated black water was also investigated at pilot scale in a UASB-septic tank by Meulman et al., 2008, but the applied loading rate was much lower (0.36 kgCOD/m^3/d). The removal efficiency in this UASB-septic tank was higher (87%, table 2.5), because the concentrated black water did not pass a coarse filter as within this study and therefore contained a higher amount of suspended solids (Meulman et al., 2008).

2.4.2 Design of the UASB reactor

Design values from (Lettinga and Hulshoff Pol, 1991) show that treatment in a UASB is not recommended for wastewaters with high amount of suspended solids and high concentrations of organic material. These design values do not take into account that hydrolysis is the rate-limiting process and therefore a long SRT is needed for stabilization of the solids and sludge inside the UASB. Based on the fact that the hydrolysis is the rate limiting step, Zeeman and Lettinga (1999) derived the formula for calculation of the HRT (equation 2.1).

An unexpected long SRT of 254 days was achieved in the present research and a large amount of sludge could be retained in the reactor (average of 19 gVSS/L$_{reactor}$, 34 gCOD/L). Because of this long SRT, the hydrolysis of influent suspended solids (53%) is close to the maximum biodegradability of the suspended solids in the black water (55%). Assuming that the same amount of sludge can be retained in the reactor, the design of the UASB reactor can be optimized. A shorter SRT will result in a lower percentage of hydrolysis and therefore less stabilized sludge, which is illustrated hypothetically in figure 2.6. A shorter SRT also means a shorter HRT and therefore the reactor volume can be further reduced at the expense of less stabilized sludge and less biogas production. This can be illustrated using equations 2.1 and 2.6. For example a reduction of the volume by reducing the SRT to 75 days will result in 8.2% reduction of the hydrolysis (1-F/F0 reduces from 96.4% to 88.2%), resulting in a hydrolysis of 49%. This reduction in hydrolysis means that 4% of the suspended solids are not hydrolysed and converted to methane. On the total methane production this is a reduction of 5%.

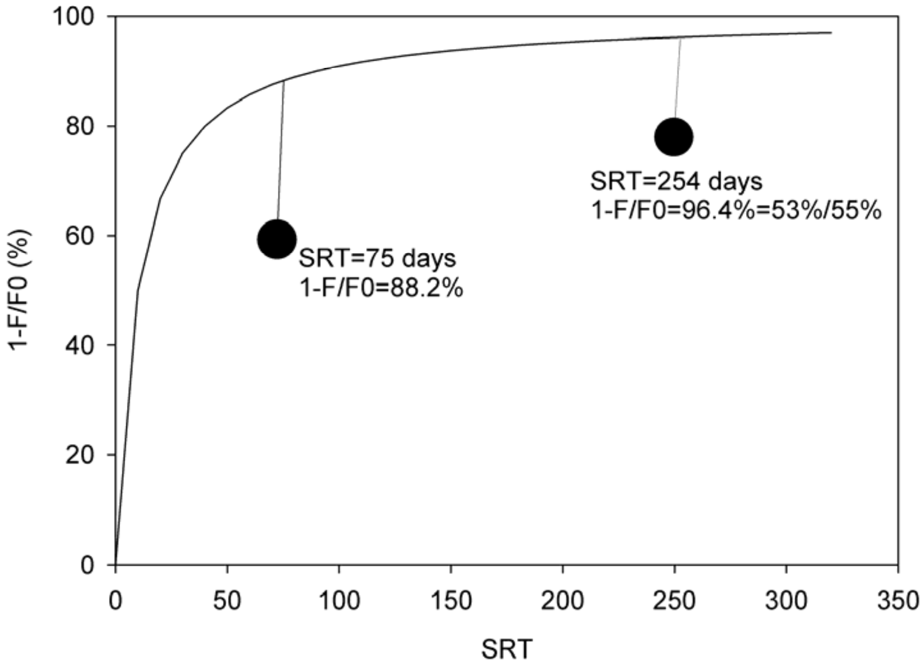

Figure 2.6 Stabilisation of the sludge represented by 1-F/F0 as a function of the SRT (equation 2.6, hypothetical, k_h is 0.1 d^{-1}).

A large reduction in volume can be achieved at the expense of a small reduction in hydrolysis of the suspended solids. Using equation 2.1 the minimum reactor volume for application at full scale can be estimated. Values for X, SS, R and H were adjusted using the results of this research and the conditions at full scale (table 2.6). The concentrated black water composition reported by Meulman et al. (2008) was used, because in this research it appeared to be impossible to feed all the suspended solids of the black water to a lab scale reactor. Furthermore Meulman et al. (2008) showed that the production of concentrated black water is only 5 L/p/d instead of the expected 7 L/p/d. Using adjusted values for the parameters of equation 2.1 the minimum reactor volume for application at full scale would be 63 L/p (based on 5 L/p/d) corresponding to an organic loading rate of 1.3 kgCOD/m^3/d (table 2.6). This is a significantly lower reactor volume and higher load than when a CSTR or UASB-septic tank is applied for the anaerobic treatment of concentrated black water (table 2.1). The non-hydrolyzed suspended solids left in the wasted sludge could be used for aerobic composting, including garden waste, to remove pathogens and to produce a soil conditioner (Vinnerås et al., 2003) provided that it is safe with respect to heavy metals and micro-pollutants (Winker et al., 2009).

Table 2.6 Adjusted design values for the UASB reactor.

		UASB reactor full scale
SRT	(d)	75
C	COD concentration in the influent (gCOD/L)	16.1[a]
SS	CODss/CODt influent (-)	0.75[a]
X	sludge concentration in the reactor (gCOD/L)	34.2
R	fraction of CODss removed (-)	0.93
H	fraction of removed solids which is hydrolyzed (-)	0.49
HRT	(d)	12.6
V	(L/p)	63
Loading rate	(kgCOD/m^3/d)	1.3

[a] using data from (Meulman et al., 2008)

2.4.3 Fate of nutrients and further treatment

When direct reuse in agriculture of the anaerobic effluent is not feasible because of long distances between cities and agriculture, treatment of the anaerobic effluent is required before the wastewater can be discharged to surface waters. The effluent of the UASB reactor still contains 1.2 - 2.4 gCOD/L, of which part could be removed by aerobic treatment. Furthermore this stream still contains pathogens and hormones and pharmaceutical residues.

Nitrogen was for more than 91% conserved in the effluent, mainly in the form of ammonium. Because nitrogen is not a limiting compound the choice to recover or remove nitrogen from wastewater will depend on the energy requirements. For the nitrogen concentration in black water (about 1.5 kgN/m^3) biological removal is preferred (Mulder, 2003). Techniques such as a one or two reactor nitritation-anammox process are available to remove ammonium from wastewater streams with a low COD/N ratio (van der Star et al., 2007) and results show that a two reactor nitritation-anammox process is suitable to treat the effluent from the UASB reactor (de Graaff et al., 2009) (chapter 3 and 4).

For phosphorus a conservation of 60% was observed. The removal of 40% is mainly due to the removal of suspended solids. Probably part of the released phosphate during degradation of the solids directly precipitated in the UASB reactor (Ohlinger et al., 1998). Detailed analysis of the sludge fraction is needed to close the phosphorus balance (chapter 5). Similar phosphorus conservation in the effluent was found by Kujawa-Roeleveld et al. (2005) in the UASB-septic tank. The degree of phosphorus conservation is most likely related to the pH and degree of dilution of the black water. In a UASB reactor on more diluted black water a much higher phosphorus conservation in the liquid effluent stream of 95% was observed (toilets used 5L of water per flush) (van Voorthuizen et al., 2008). Phosphorus is a limiting resource and the available phosphorus resources become increasingly scarce (Driver et al., 1999; Cordell et al., 2009). Depending on the degree of phosphorus conservation during the anaerobic treatment, phosphorus can be recovered from the sludge or from the effluent in the form of struvite ($MgNH_4PO_4.6H_2O$) (Ueno and Fujii, 2001).

Further research will focus on the subsequent removal and recovery of the nutrients nitrogen and phosphorus and on the removal of the remaining micro-pollutants such as hormones and pharmaceutical residues.

2.5 Conclusions

Anaerobic treatment of concentrated black water in a UASB reactor was successfully achieved at a HRT of 8.7 days and a load of 1.0 kgCOD/m^3/d. Due to high sludge concentrations (19 gVSS/L$_{reactor}$) a long SRT of 254 days was achieved and 53% of the suspended solids were hydrolyzed to methane. The effluent of the UASB reactor needs further treatment to remove remaining COD (2.4 gCOD/L, BOD_5 is 0.87 g/L) and to remove and/or recover nutrients nitrogen and phosphorus.

On the expense of a lower hydrolysis of 49% and some less stabilized sludge, the design of the UASB reactor was optimized and the minimum reactor volume at full scale was calculated to be 63 L/p for black water containing 16 gCOD/L produced at 5 L/p/d.

Acknowledgements

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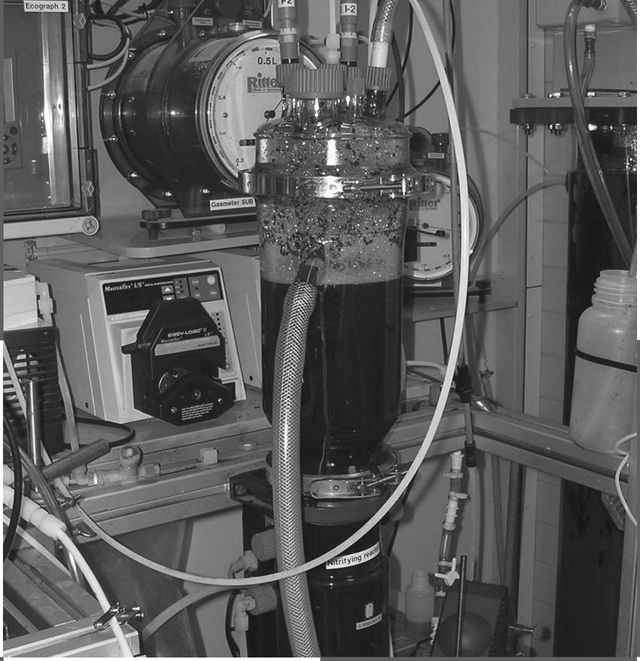

3

Long term partial nitritation of anaerobically treated black water and the emission of nitrous oxide

Abstract

Black water (toilet water) contains half the load of organic material and the major fraction of the nutrients nitrogen and phosphorus in a household and is 25 times more concentrated, when collected with a vacuum toilet, than the total wastewater stream from a Dutch household. This research focuses on the partial nitritation of anaerobically treated black water to produce an effluent suitable to feed to the anammox process. Successful partial nitritation was achieved at 34 °C and 25 °C and for a long period (almost 400 days in the second period at 25 °C) without strict process control a stable effluent at a ratio of 1.3 NO_2-N/NH_4-N was produced, which is suitable to feed to the anammox process. Nitrite oxidizers were successfully outcompeted due to inhibition by free ammonia and nitrous acid and due to fluctuating conditions in SRT (1.0 to 17 days) and pH (from 6.3 tot 7.7) in the reactor. Microbial analysis of the sludge confirmed the presence of mainly ammonium oxidizers. The emission of nitrous oxide (N_2O) is of growing concern and it corresponded to 0.6 - 2.6% (average 1.9%) of the total nitrogen load.

M.S. de Graaff, G. Zeeman, H. Temmink, M.C.M. van Loosdrecht and C.J.N. Buisman
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3.1 Introduction

Autotrophic nitrogen removal by combined partial nitritation and anammox has gained a lot of interest to treat high strength nitrogen wastewaters containing a low amount of organic carbon (e.g. (Jetten et al., 1999; Kartal et al., 2004)).

New sanitation concepts are based on separation at source of household wastewater streams (e.g. black water (faeces and urine), grey water, or urine and brown water (faeces)) and have a large potential to recover resources in the form of energy, nutrients and water (Otterpohl et al., 1999; Zeeman and Lettinga, 1999).

Previous research showed that concentrated black water, collected with vacuum toilets, can be efficiently treated in a UASB (Upflow Anaerobic Sludge Blanket) reactor at a relatively short hydraulic retention time (HRT) of 8.7 days (chapter 2), in a UASB-septic tank at a considerably longer HRT of 29 days (Kujawa-Roeleveld et al., 2006) or in a CSTR without separation of solids and liquid (Wendland et al., 2007). In this way the organic material in the black water is converted to methane which is a sustainable energy source. The nutrients are largely conserved in the effluent of the anaerobic treatment step and the effluent should be further treated before discharge to surface waters. Autotrophic nitrogen removal was selected to remove nitrogen compounds from the anaerobic effluent. The main fraction of the organic material in black water was converted to methane in the anaerobic treatment step, and therefore the anaerobic effluent has too low COD/N ratio (only 1 – 2) to achieve sufficient heterotrophic denitrification. Furthermore from an energy and costs perspective autotrophic nitrogen removal is preferred over direct recovery (Mulder, 2003; Wilsenach et al., 2003).

In the nitritation-anammox process the ammonium, the main form of nitrogen in black water, is first partially oxidized to nitrite (partial nitritation). Subsequently ammonium (NH_4-N) and nitrite (NO_2-N) are converted to nitrogen gas by anaerobic ammonia oxidizers (anammox). These processes can be combined in one reactor nitritation-anammox process where anammox organisms grow in oxygen-free zones. The second option is to separate the two processes in two reactors (van der Star et al., 2007). Partial nitritation can be achieved by selecting a short sludge retention time (SRT) at elevated temperatures (30 – 40 °C) resulting in the wash-out of nitrite oxidizers (the SHARON process, (Hellinga et al., 1998)). Other selection mechanisms have been reported as well, including inhibition by low oxygen concentrations and by high concentrations of free ammonia and nitrous acid. However, the inhibition mechanisms of free ammonia and nitrous acid and potential adaptations of nitrite oxidizers are still not fully understood (Fux et al., 2003).

Anaerobically treated source-separated black water (5 L/p/d, (Meulman et al., 2008)) is about 25 times more concentrated than the total wastewater stream from a Dutch household (124 L/p/d, (Kanne, 2005)). Furthermore it has higher concentrations (1 - 1.5 gN/L and 1.5 – 2 gCOD/L) compared to sludge liquors (0.6 – 1 gN/L and 0.1 – 0.8 gCOD/L) which has been researched for the application of nitritation-anammox process to upgrade wastewater treatment plants (Hellinga et al., 1998; Caffaz et al., 2006)).

The emission of nitrous oxide (N_2O) during wastewater treatment is of growing concern (Kampschreur et al., 2009b). Nitrous oxide is an important greenhouse gas because it has a much stronger effect (300-fold) than carbon dioxide. Furthermore, nitrous oxide also has a large biological effect on organisms and humans (IPCC, 2001). Kampschreur et al. (2008) detected N_2O emission during full-scale reject water treatment by the two reactor nitritation-anammox process. Emission of N_2O in new sanitation concepts will negatively influence the sustainability and should therefore be avoided.

The aim of this study was to investigate the long term partial nitritation of the anaerobically treated concentrated black water at 25 °C to achieve an influent suitable for the anammox process. Furthermore the possible emission of nitrous oxide (N_2O) was investigated and the mechanisms to achieve partial nitritation of ammonium to nitrite without nitrate formation are discussed.

3.2 Materials and methods

3.2.1 Anaerobically treated black water

The liquid effluent of a UASB reactor treating concentrated black water (chapter 2) was used as influent for the partial nitritation reactor (figure 3.1). The UASB effluent contained 1.5 gNH_4-N/L (s.d. 0.19) and 2.4 gCOD/L (s.d. 0.84) (chapter 2).

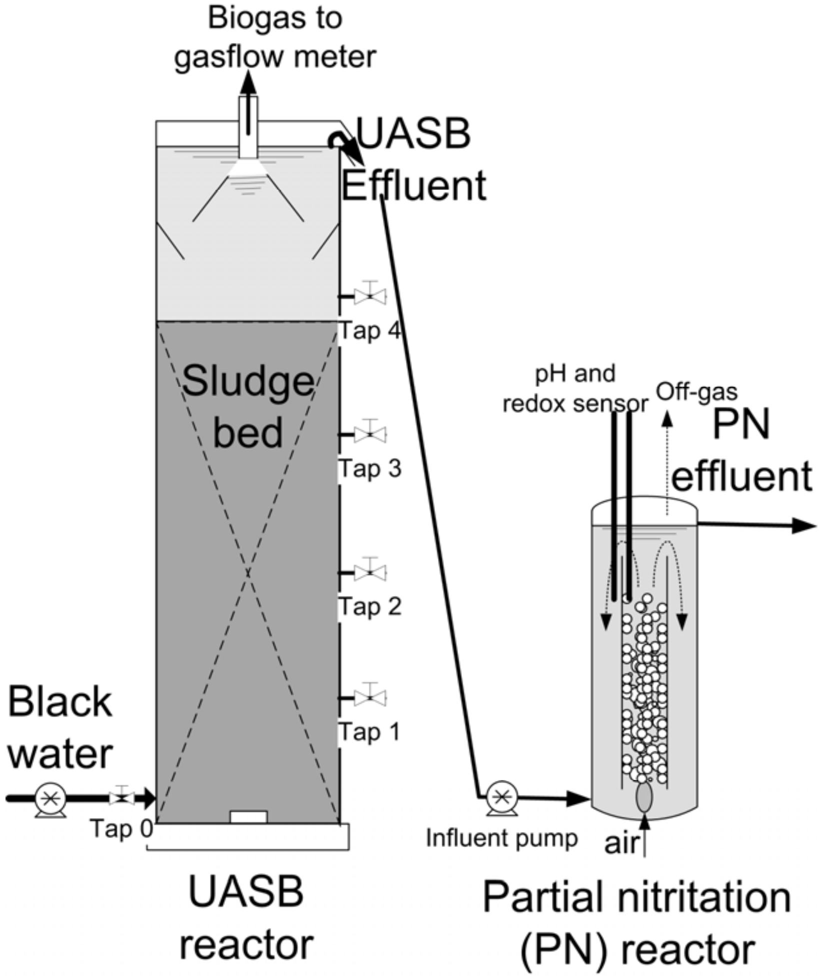

Figure 3.1 Schematic drawing of the reactors used for treatment of black water.

3.2.2 Partial nitritation reactor

The partial nitritation reactor was a continuous reactor without intentional biomass retention. The reactor had a volume of 3.2 L, a HRT of 1.3 days and was operated at 34 °C for 231 days. Efficient mixing of reactor contents was provided by an air-lift and at the top the reactor was overflowing. Air was supplied at the bottom at a flow rate of 1.3 L/min, maintaining an oxygen concentration above 2 mg/L and checked daily offline with a portable Hach LDO HQ10 DO meter. Temperature in the water jacketed reactor was controlled by means of a thermo stated water bath (Haake DC10/K10). When the pH exceeded 7.7, automatic 0.1 M HCl addition was applied until the pH reached a value of 7.5. Nitrifying sludge from the SHARON reactor at wastewater treatment plant Zwolle (NL) was used as inoculum (3 L, 0.5 gVSS/L). Because the inoculum was acclimated to lower ammonium concentrations (500 mgNH_4-N/L, personal communication wastewater treatment plant Zwolle, June

2007), the UASB effluent initially was diluted with tap water to lower the ammonium concentration. The sludge immediately started to convert ammonium to nitrite and after a stable conversion for about a week, the ammonium concentration was increased in steps by decreasing the dilution of the UASB effluent by 3, 2, 1.5 times. From day 50 onward the UASB effluent was no longer diluted. At day 231 the reactor volume was increased to 6.1 L and the temperature was reduced in steps of 1 – 2 ˚C every 7 days from 34 ˚C to 25 ˚C. This caused an increase in pH and less ammonium conversion to nitrite. When the pH increased to values higher than 7.5, the HRT was increased by reducing the flowrate in steps of 0.14 L/d (0.1 mL/min). From day 288 to 678 the reactor was operated at 25 ˚C. Overall the HRT was increased from 1.3 days to an average of 1.7 days (table 3.1).

Table 3.1 Operational characteristics.

Period	Temperature (˚C)	HRT (d)
Day 0 – 50; start up	34	1.3
Day 50 – 231	34	1.3
Day 231 – 288	34 → 25	1.3 → 1.7
Day 288 – 678	25	1.7

3.2.3 *Analytical methods*

Total COD, total nitrogen, total phosphorus and total ammonia nitrogen concentration (NH_4-N) were determined according to standard methods using commercial DrLange ® test kits (Hach-Lange) (APHA, 1998). Ion chromatography was used to measure the anions concentrations in membrane filtered samples to prevent clogging of the instrument (0.45 µm, Cronus filter PTFE) and inorganic carbon (IC) was determined with a Shimadzu TOC analyzer. Influent and effluent samples for total ammonia concentration and IC were filtered over an ashless black ribbon paper filter (Schleicher & Schuell). Total Suspended Solids (TSS) and Volatile Suspended Solids were determined according to standard methods using the ashless black ribbon paper filter (Schleicher & Schuell). Biochemical oxygen demand (BOD) was determined using OxiTop® heads (WTW, Oxitop control 110) calibrated for BOD determination and depletion of oxygen was monitored for 5 days (BOD_5).

3.2.4 *Nitrous oxide gas analysis*

Gas samples from the headspace of the reactor were analyzed off line for nitrous oxide (N_2O) on a Varian 8300 Custom Solutions gas chromatograph (Hayesep Q 80/100 Mesh 0.25m x 1/16" x 1 mm Ultimetal CP1308) in which helium gas was used as carrier and detected with an electron capture detector (ECD) at 200 ˚C. The temperature of the injector and the column were respectively 200 ˚C and 50 ˚C (Delft University of Technology). The second gas chromatograph (Wageningen University) that was used, because the first GC was not available for analysis for three samples (day 627, 628 and 641), was a CEinstruments GC8000 Top (Interscience, Breda the Netherlands) and N_2O was separated on a Haysep Q 80-100 mesh 3m x 1/8" SS column and detected with an ECD.

3.2.5 FISH

FISH (Fluorescent In Situ Hybridization) technique was used to characterize the bacteria in the sludge. Cell fixation to the gelatine-coated slides and hybridization steps were carried out according to methods previously described by (Amann et al., 1990). The 16S rRNA-targeted oligonucleotide probes used in this study are listed by (Egli et al., 2003). To detect all bacteria probes EUB338, EUB338II, and EUB338III were used in an equimolar mixture (EUB338mix). To detect ammonium oxidizers probe NEU (together with competitor CTE) was used and to detect nitrite oxidizers probe Nit3 (together with competitor CNIT3) was used. All probes were purchased as Cy3 and Fluos labelled derivatives from MWG-Biotech (Ebersberg, Germany) and were diluted to a final concentration of 50 ng/µl. Hybridization was performed at 46 °C for 1.5 h followed by washing with pre-warmed (48 °C) washing buffer and 15 min incubation at 48 °C in washing buffer containing DAPI (0.2 µg/µl). The cells were observed under an epifluorescent microscope Leica DMI 6000B (Leica, Germany) equipped with Leica DFC 350 FX camera.

3.2.6 Kinetic model calculations

To gain more insight in the results, a kinetic model described by (Jubany et al., 2009) and (Hellinga et al., 1999) was used to calculate the growth rates and necessary SRT for ammonium oxidizers ($SRT_{min,aob}$) and nitrite oxidizers ($SRT_{min,nob}$) in concentrated wastewater under the known conditions. Inhibition of nitrous acid on the growth rate of AOB and inhibition of free ammonia on the growth rate of NOB were included in the equations. The dependence of the decay rate with oxygen concentration was described with a Monod model. Equations 3.1 and 3.2 show the growth rate of AOB (μ^{aob}) and NOB (μ^{nob}) and equation 3.3 the decay rate (b) for both.

$$\mu^{nob} = \mu_{\max}^{nob} \cdot \frac{C_{HNO_2}}{K_{HNO_2}^{nob} + C_{HNO_2} + C_{HNO_2}{}^{2} / K_{I,HNO_2}^{nob}} \cdot \frac{C_{O_2}}{K_{O_2}^{nob} + C_{O_2}} \cdot \frac{K_{I,NH_3}^{nob}}{K_{I,NH_3}^{nob} + C_{NH_3}} \quad (3.1)$$

$$\mu^{aob} = \mu_{\max}^{aob} \cdot \frac{C_{NH_3}}{K_{NH_3}^{aob} + C_{NH_3} + C_{NH_3}^{2} / K_{I,NH_3}^{aob}} \cdot \frac{C_{O_2}}{K_{O_2}^{aob} + C_{O_2}} \frac{K_{I,HNO_2}^{aob}}{K_{I,HNO_2}^{aob} + C_{HNO_2}} \quad (3.2)$$

$$b = b_{\max} \frac{C_{O_2}}{K_{O_2}^{\cdots} + C_{O_2}} \quad (3.3)$$

All model parameters values were obtained from (Sin et al., 2008) and chosen in such a way that the bacteria had the highest affinity and lowest inhibition (table 3.2).

Table 3.2 Parameters used for model calculations (values were taken from Sin et al. (2008)).

Parameter	Explanation and value	Parameter	Explanation and value
$\mu_{\max}^{aob}$	maximum growth rate of ammonium oxidizers, 2.1 d^{-1}	$\mu_{\max}^{nob}$	maximum growth rate of nitrite oxidizers, 1.02 d^{-1}
$K_{NH_3}^{aob}$	affinity for free ammonia of AOB, 0.75 mgN/L	$K_{HNO_2}^{nob}$	affinity for nitrous acid of NOB, 0.0014 mgN/L
K_{I,NH_3}^{aob}	inhibition by free ammonia of AOB, 150 mgN/L	K_{I,HNO_2}^{nob}	inhibition by nitrous acid of NOB, 2.8 mgN/L
K_{I,HNO_2}^{aob}	inhibition by nitrous acid of AOB, 2.8 mgN/L	K_{I,NH_3}^{nob}	inhibition by free ammonia of NOB, 1.0 mgN/L
$K_{O_2}^{aob}$	affinity for oxygen of AOB, 0.4 mg/L	$K_{O_2}^{nob}$	affinity for oxygen of NOB, 0.5 mg/L
$b_{\max}$	maximal decay rate, 0.17 d^{-1}	C_{NH_3}	free ammonia concentration (mgN/L)
C_{HNO_2}	concentration of nitrous acid (mgN/L)	C_{O_2}	oxygen concentration (mg/L)

The minimum SRT was calculated with the equation 3.4:

$$SRT_{\min} = \frac{1}{\mu - b} \text{ (Ahn et al., 2008)} \quad (3.4)$$

3.2.7 Calculations

Because nitrite exerts a COD of 1.1 gCOD/gNO$_2$-N, the COD values were corrected correspondingly. The bicarbonate concentration was calculated from the total inorganic carbon (IC) as a function of pH and temperature (Tchobanoglous et al., 2003).

The nitrous acid and free ammonia concentrations were calculated according to (Anthonisen et al., 1976).

The nitrogen balance was calculated as the difference between the sum of NH_4-N, NO_2-N and NO_3-N in the influent and in the effluent.

3.3 Results

3.3.1 Reactor operation

Influent and effluent nitrogen concentrations (NH_4, NO_2 and NO_3) of the partial nitritation reactor operated on anaerobically treated black water are shown in figure 3.2.

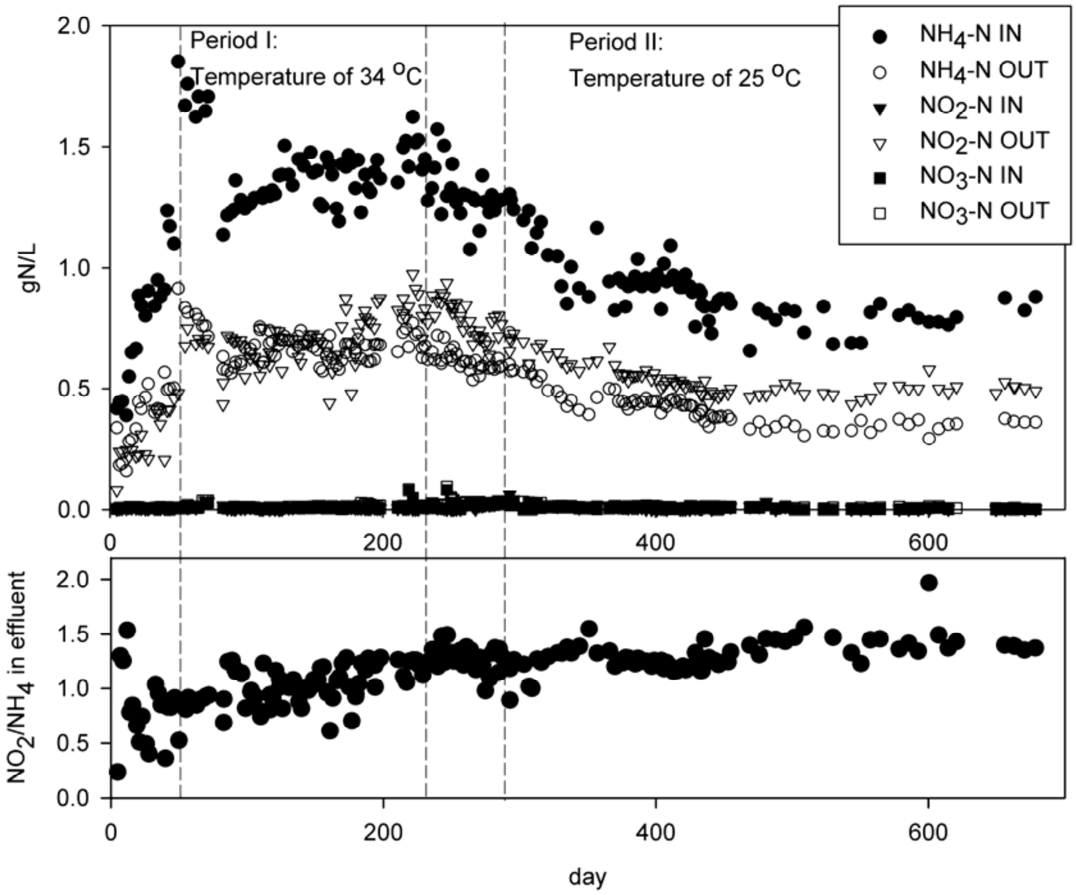

Figure 3.2 Influent and effluent concentrations of the partial nitritation reactor and NO_2-N/NH_4-N ratio in effluent.

The ammonium in the anaerobically treated black water was successfully converted to a NO_2 to NH_4 ratio of 1.0 in the first period at 34 °C and 1.3 in the second period at 25 °C (table 3.3). During the whole period of operation no significant nitrite oxidation to nitrate was observed; only 0.60% ± 0.39% of the nitrogen load was converted to nitrate. In the first period the nitrite production was 50% of influent total ammonia nitrogen and in the second period the nitrite production was 59% of influent total ammonia nitrogen. The nitrogen balance was 101% in the first period and 106% in the second period. The concentration of ammonium in the anaerobically treated black water decreased after day 300 from 1.5 gNH_4-N/L to 1.0 gNH_4-N/L, because more flushing water was used in the vacuum toilets due to installation of noise reducers resulting in more diluted black water in the second period.

Table 3.3 Characteristics of the partial nitritation reactor.

	Unit	34 ˚C	25 ˚C
Day	-	50 – 231	288 – 678
Reactor volume	L	3.2	6.1
Nitrogen load	$gN/L_{reactor}/d$	1.1	0.52
HRT	d	1.3 ± 0.073	1.7 ± 0.13
pH	-	7.3 ± 0.35	6.8 ± 0.33
DO	mgO_2/L	4.2 ± 0.75	4.1 ± 0.73
HCO_3/NH_4 influent	mol/mol	1.1 ± 0.21	1.0 ± 0.11
NO_2-N/NH_4-N effluent	mol/mol	1.0 ± 0.24	1.3 ± 0.14
Oxygen consumption by COD removal (calculated)	$gCOD/L_{reactor}/d$	0.79 ± 0.36	0.21 ± 0.11

Visual observation indicated that not all produced sludge was wasted with the effluent. Due to foam formation, sludge ended up at the top of the reactor. Furthermore sludge growth was observed on the walls and inner tube of the reactor. Regularly this sludge was removed and put back to the liquid phase. The calculated SRT, determined on 9 occasions based on the sludge concentration (gVSS/L) in the reactor and in the effluent, fluctuated between 1 day and 17 days with an average of 9.0 days for both periods.

In figure 3.3 the pH inside the reactor is shown. In the first period pH control was needed to protect the process from inhibition at high pH. A few times the pH control failed (day 145 and 160) and this resulted in less nitritation because the NO_2-N/NH_4-N ratio dropped to 0.6 (figure 3.2). In the second period pH control was not needed and apparently the process controlled itself by the amount of bicarbonate available in the influent to an average value of 6.8.

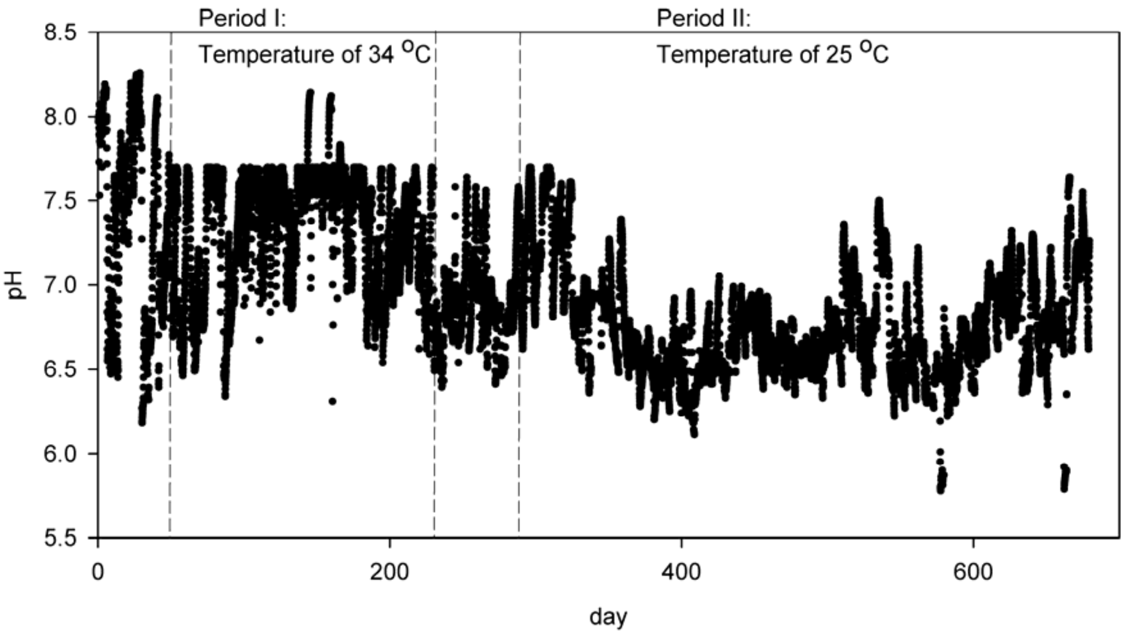

Figure 3.3 pH profile inside the partial nitritation reactor.

The quality of the anaerobically treated black water fluctuated and this affected the partial nitritation as well. As shown in figure 3.4 during the first period at 34 °C the COD concentrations in the influent were much higher than in the period of operation at 25 °C. This resulted in a higher oxygen consumption due to COD removal in the first period (0.79 ± 0.36 gCOD/L/d) compared to the second period (0.21 ± 0.11 gCOD/L/d) (table 3.3). Furthermore the sludge concentration in the first period was higher (0.71 gVSS/L) than in the second period (0.45 gVSS/L).

In both periods the partial nitritation reactor removed 35% of the COD and this corresponded to the amount of the readily biodegradable COD (BOD_5) available in the influent (0.78 $gBOD_5/L$ in the first period and 0.37 $gBOD_5/L$ in the second period).

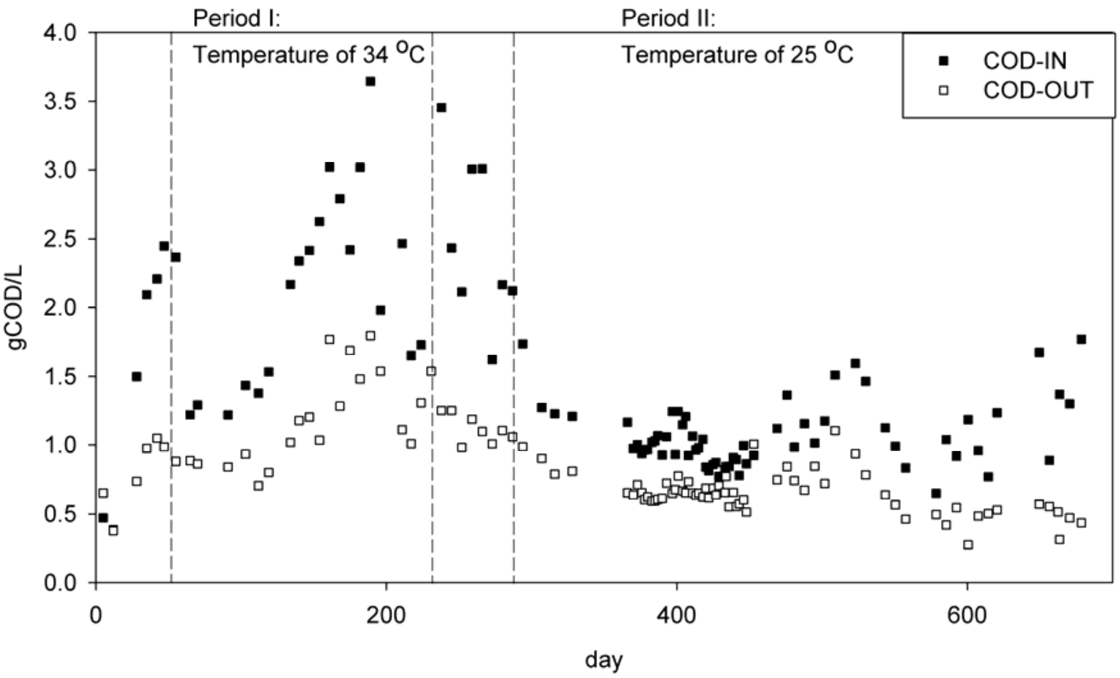

Figure 3.4 COD concentrations of influent and effluent (corrected for the presence of NO_2).

3.3.2 Nitrous oxide (N_2O) production

Nitrous oxide concentrations in the headspace varied from 15 ppm to 61 ppm measured in the period of day 529 – 678 (10 samples). At an air flowrate of 1.3 L/min this corresponded to 0.6 - 2.6% (average 1.9% ± 0.53%) nitrous oxide (N_2O) of the total nitrogen load to the reactor. In figure 3.5 the N_2O production in the period of day 529 – 678 is shown. Only once a relatively low percentage of 0.6% was measured on day 582. No clear reason was found for this lower value. In all other samples the N_2O production varied around 2%.

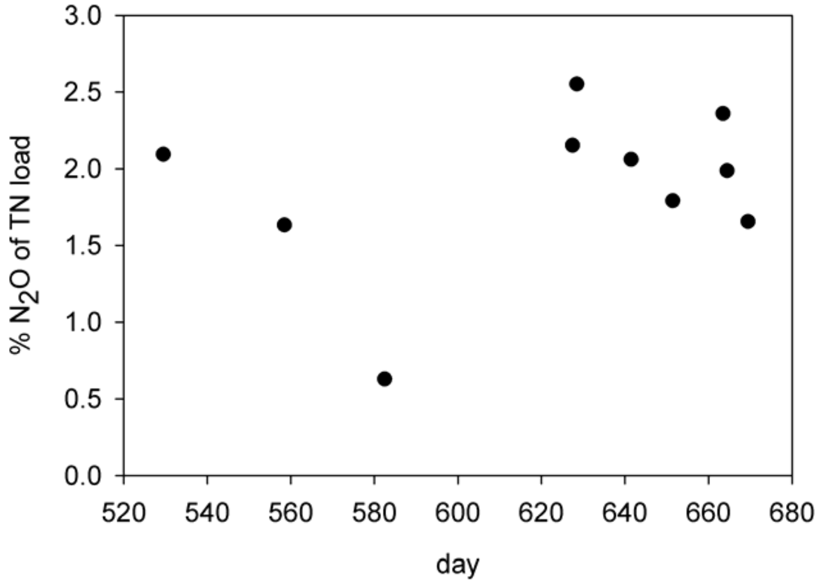

Figure 3.5 Nitrous oxide production in the partial nitritation reactor.

3.3.3 Fish results

Two biomass samples taken at day 280 and day 638 were characterized by FISH. In both samples almost all bacteria hybridized with the NEU probe which confirms the presence of mainly ammonium oxidizers. The bacteria did not hybridize with the NIT probe.

3.4 Discussion

3.4.1 Inhibition of nitrite oxidizers

During the whole period of operation (678 days) no significant nitrate production was observed which indicates that the nitrite oxidizers were successfully outcompeted. Despite high concentration of oxygen (> 2 mg/L) and the observed sludge retention (SRT varied from 1.0 to 17 days), partial nitritation to produce an influent suitable for the anammox process was easily achieved.

Model estimations were made in order to support the results and to explain why the nitrite oxidizers were outcompeted (table 3.4). The conditions in the second period at 25 °C were chosen for the model calculations because these are the conditions where nitrite oxidation is most likely to be possible. All constants were chosen in such a way that the bacteria had the highest affinity and lowest inhibition (table 3.4), to show that inhibition can be significant even at low inhibition factors. Table 3.4 shows that the SRT for nitrite oxidizers to survive ($SRT_{min,nob}$) varies a lot, from 2.8 at a low pH to infinite at high pH. Furthermore the model shows that the nitrite oxidizers are very sensitive to the free ammonia concentration and at a moderate pH of 7.2 (which was frequently observed in the partial nitritation reactor) the minimum SRT is already 32 days. It should be mentioned that if other values were chosen for the model parameters the $SRT_{min,nob}$ would be even higher, because these model parameters reported in literature show a large variability (Sin et al., 2008). Under all conditions the model shows that the SRT for ammonium oxidizers ($SRT_{min,aob}$) was between 0.66 and 2.2 days which shows that the ammonium oxidizers could easily sustain in the partial nitritation reactor.

Table 3.4 Minimum SRT for the ammonium (aob) and nitrite oxidizers (nob) under the conditions in this research.

		Minimum pH	Maximum pH	Average pH	Moderate pH
DO	mg/L	4.1	4.1	4.1	4.1
pH	-	6.3	7.7	6.8	7.2
HNO_2	mgN/L	0.61	0.024	0.19	0.08
NH_3	mgN/L	0.48	12	1.5	3.8
μ_{aob}	d^{-1}	0.61	1.7	1.2	1.5
$decay_{aob}$	d^{-1}	0.15	0.15	0.15	0.15
$SRT_{min,aob}$	**d**	**2.2**	**0.66**	**0.97**	**0.73**
μ_{nob}	d^{-1}	0.50	0.067	0.34	0.18
$decay_{nob}$	d^{-1}	0.15	0.15	0.15	0.15
$SRT_{min,nob}$	**d**	**2.8**	∞	**5.4**	**32**

For the treatment of urine Udert et al. (2003b) found a minimum SRT for the growth of nitrite oxidizers of 4.9 days, which was equal to the actual SRT in their CSTR. They concluded that nitrite oxidizers were inhibited by high concentrations of nitrous acid, salts and hydroxylamine (Udert et al., 2003b). Partial nitritation of anaerobically treated black water can therefore be easily achieved and strict control of pH and SRT at a fixed value would not be necessary.

3.4.2 Difference between the two periods

As shown in the results different ratios of NO_2-N/NH_4-N in the effluent were obtained in the two periods: 1.0 at 34 °C and 1.3 at 25 °C (table 3.3). Using the estimation by (van Hulle et al., 2005) the amount of water that evaporated was small (3.1% at 34 °C and 1.2% at 25 °C), assuming that the air leaves the reactor saturated. Water evaporation therefore did not influence the nitrogen balance significantly. In the first period the free ammonia concentration was high (38 mgNH_3-N/L (pH 7.7, 750 $mgNH_4$-N/L, 34 °C), which is in the range were Nitrosomonas (ammonia oxidizers) start to be inhibited by the free ammonia (10 – 150 mg/L, (Anthonisen et al., 1976; Sin et al., 2008) and which resulted in less nitritation. Due to the high pH part of the ammonia could be stripped with the air, but because the nitrogen balance was close to 100%, the loss of ammonia by stripping was negligible. Later in the same period more nitritation (ratio of NO_2^--N/NH_4-N increased from 0.81 at day 55 to 1.2 at day 226) resulted in a decrease in pH (figure 3.3) from day 200 onward (NH_3 concentration decreased to 12 NH_3-N/L) and pH control to protect the process from pH values higher than 7.7 was not needed anymore. Another difference is that the nitrogen load in the first period was two times higher than in the second period (1.1 gN/L/d versus 0.52 gN/L/d) due to more diluted black water in the second period. An oxygen concentration of lower than 2 mg/L was never observed and therefore oxygen could not have been limiting for the nitritation process despite the higher amount of COD removal in the first period.

3.4.3 Nitrous oxide emissions

Nitrous oxide (N_2O) emissions from the partial nitritation reactor were comparable to values reported by Kampschreur et al. (2008), i.e. 1.7% from a full scale Sharon process for reject water treatment (Kampschreur et al., 2008). The N_2O was most likely produced by ammonium oxidizers, due to the high nitrite concentrations present in the partial nitritation reactor (Kampschreur et al., 2008). This was confirmed later by Kampschreur et al. (2009b) who concluded that the main parameters that cause N_2O production are low dissolved oxygen concentration and increased nitrite concentrations like in this research. In a one reactor nitritation-anammox process high nitrite concentrations are avoided, however usually oxygen limitation is used to achieve the nitritation which is one of the main operational parameters that cause N_2O production (Kampschreur et al., 2008). The emission of N_2O in a full scale one reactor nitritation-anammox process was similar (1.2% of the nitrogen load) as the two reactor nitritation-anammox process, but could be reduced by preventing over-aeration and stripping of N_2O (Kampschreur et al., 2009a). Because in this research the aeration was more than sufficient, reduction of the aeration could lead to a reduction of the N_2O emission. However due to the high nitrite concentrations in this research, it seems that N_2O emission cannot be avoided. A recent study at full scale conventional wastewater treatment plants showed that the N_2O emission could be as low as 0.04%, but also as high as 6.1% and this depended a lot on a number of factors, such as nitrogen load and BOD/N ratio (van Voorthuizen et al., 2009). More research is needed to elucidate the relationship of these factors with the N_2O emission. The emission of N_2O is an important aspect to take into account when choosing the technique to remove or recover ammonium from concentrated black water.

3.4.4 Application in practice

For a long period of operation (almost 400 days in the second period) a stable influent at a ratio of 1.3 NO_2-N/NH_4-N was produced, which is suitable to feed an anammox reactor. With this composition high ammonium removal efficiencies can be achieved in the anammox process, because anammox needs 1.32 NO_2-N/NH_4-N at maximum growth (Strous et al., 1998).

This research also showed that partial nitritation of the anaerobically treated concentrated black water was easily achieved, despite the fluctuating conditions of pH and SRT. This is important for application of new sanitation concepts (Zeeman et al., 2008) because there is no need for a strict process control. There is only a need for a standby pH control to protect the process from inhibition at high pH due to high free ammonia concentrations.

Further research is focussing on treating the effluent of the partial nitritation reactor in an anammox reactor to remove the ammonium from the black water. Two anammox SBRs have been coupled to the effluent of the PN reactor and these results are discussed in chapter 4.

3.5 Conclusions

Successful partial nitritation of the ammonium in anaerobically treated source-separated black water was achieved at 34 °C and 25 °C. For a long period (almost 400 days in the second period at 25 °C) a stable influent at a ratio of 1.3 NO_2-N/NH_4-N was produced and this is suitable to feed an anammox process. No significant nitrate formation was observed during the full period of operation and the nitrite oxidizers were successfully outcompeted due to inhibition by free ammonia and nitrous acid and due to the fluctuating conditions in SRT (from 1.0 to 17 days) and pH (from 6.3 to 7.7) in the reactor. Microbial analysis of the sludge by FISH technique confirmed the presence of mainly ammonium oxidizers. The emission of nitrous oxide (N_2O) corresponded to 0.6 - 2.6% (average 1.9%) of the total nitrogen load to the reactor. For the application in new sanitation concepts there will be no need for strict process control and partial nitritation of anaerobically treated black water can be achieved easily.

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4

Autotrophic nitrogen removal from black water: calcium addition as a requirement for settleability

Abstract

Black (toilet) water contains half of the organic load in the domestic wastewater, as well as the major fraction of the nutrients nitrogen and phosphorus. When collected with vacuum toilets, the black water is 25 times more concentrated than the total domestic wastewater stream, i.e. including grey water produced by laundry, showers etc. A two reactor nitritation-anammox process was successfully employed and removed 85% to 89% of total nitrogen in anaerobically treated black water. The (free) calcium concentration in black water was too low (42 mg/L) to obtain sufficient granulation of anammox biomass. The granulation and retention of the biomass was improved considerably by the addition of 39 mg/L of extra calcium. This resulted in a volumetric nitrogen removal rate of 0.5 gN/L/d, irrespective of the two temperatures of 35˚C and 25 ˚C at which the anammox reactors were operated. Nitrous oxide, a very strong global warming gas, was produced in situations of an incomplete anammox conversion accompanied by elevated levels of nitrite.

M.S. de Graaff, G. Zeeman, H. Temmink, M.C.M. van Loosdrecht and C.J.N. Buisman

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4.1 Introduction

Separation at source of household wastewater results in a concentrated stream from the toilet, called black water, and a relatively diluted stream from the bathroom, kitchen and laundry, called grey water (Otterpohl et al., 1999). A new sanitation concept was proposed (Zeeman et al., 2008), in which energy and nutrients are recovered from black water and clean water is produced from grey water. Concentrated black water can be efficiently treated in a UASB (Upflow Anaerobic Sludge Blanket) reactor at a relatively short hydraulic retention time (HRT) of 8.7 days (chapter 2). Other options are treatment in a UASB-septic tank at a considerably longer HRT of 29 days (Kujawa-Roeleveld and Zeeman, 2006) or in a CSTR at an HRT of 20 days (Wendland et al., 2007). The nutrients nitrogen and phosphorus are largely conserved in the effluent of these anaerobic reactors. Both from an energy and a cost perspective biological nitrogen removal from this effluent is preferred over nitrogen recovery (Strous et al., 1997; Wilsenach et al., 2003). Because during anaerobic treatment most of the organic material is removed to produce energy from the black water, autotrophic nitrogen removal by the nitritation-anammox process is the only feasible option (Strous et al., 1997). This process consists of partial nitritation where ca. 50% of the ammonium is converted to nitrite, in combination with the anammox process where ammonium and nitrite are converted to dinitrogen gas. Nitrogen removal by nitritation-anammox already is successfully applied on full scale in reject water from municipal (van der Star et al., 2007; Wett, 2007; Joss et al., 2009) and industrial wastewater treatment plants (Abma et al., 2009).

In this study the two reactor nitritation-anammox process was applied to remove nitrogen from anaerobically treated source-separated black water, produced from vacuum toilets with a flushing volume of only 5 L/p/d (Meulman et al., 2008). This wastewater is about 25 times more concentrated, with respect to nitrogen, than the total wastewater stream from Dutch households, which includes grey water and flushing with conventional toilets (124 L/p/d (Kanne, 2005)). During anaerobic treatment, COD in this black water is reduced from 7.7 – 9.7 gCOD/L to 1.2 – 2.4 gCOD/L, but the liquid effluent still contains readily biodegradable organic material (0.48 – 0.87 g BOD_5/L) for which aerobic post-treatment is required (chapters 2 and 3). Nitrogen and COD concentrations (1 - 1.5 gN/L and 1.2 – 2.4 gCOD/L) are considerably higher compared to digested domestic sludge liquors (0.6 – 1 gN/L and 0.1 – 0.8 gCOD/L) (e.g. (Hellinga et al., 1998; Caffaz et al., 2006)). Very recently, Vlaeminck et al. (2009) demonstrated the technical feasibility of a one reactor nitritation-anammox process to treat digested black water in a rotating contactor.

The two reactor nitritation-anammox process was chosen in this study to allow the independent study of the application of the separate processes (van der Star et al., 2007). Also, a separate reactor for partial nitritation may remove biodegradable organic material that otherwise could interfere negatively with the anammox process by stimulating heterotrophic denitrification (Udert et al., 2008). The aerobic conditions in the partial nitritation reactor also may enhance (bio-)flocculation of organic and colloidal material (Wilén et al., 2004), which therefore can easily be separated from the black water before it is treated in the anammox reactor. In a sequencing batch reactor (SBR) start up and stable operation of the anammox process was evaluated at 25 °C and 35 °C.

In view of its environmental impact (Kampschreur et al., 2008), also the emission of greenhouse gas nitrous oxide (N_2O) was included in this study. Emission of N_2O from the new sanitation concept would have a negative impact on its sustainability and therefore should be avoided.

Because of its low growth rate, excellent biomass retention is essential for anammox reactors, and the formation of granules therefore is desired (Strous et al., 1998). The presence of sufficient amounts of calcium stimulates granule formation and thus biomass retention. Van der Star et al. (2008) reported growth of anammox in free cells rather than in granules at a calcium concentration of only 41 mg/L. In the anaerobically treated black water used in this research, calcium concentrations were similar to van der Star et al. (2008) (41-44 mg/L, chapter 5). The effect of calcium concentration and the addition of calcium on granulation of anammox biomass were therefore also studied in this research.

4.2 Materials and Methods

4.2.1 Combined anaerobic treatment and nitrogen removal from black water

The effluent of a UASB reactor treating concentrated black water (chapter 2) was used as the influent for a two reactor nitritation-anammox process (figure 4.1). Partial nitritation of the anaerobically treated black water took place in a continuously stirred reactor at 25 °C. Details and results of the partial nitritation reactor can be found elsewhere (chapter 3). Effluent from the partial nitritation reactor was treated in two anammox reactors. For this purpose sequencing batch reactors (SBRs) were used at two different temperatures. SBR35 was operated at 35 °C, i.e. the optimum temperature for anammox growth (Strous et al., 1998) and SBR25 at 25 °C as this is the preferred temperature for an energy efficient treatment concept for black water (chapter 2).

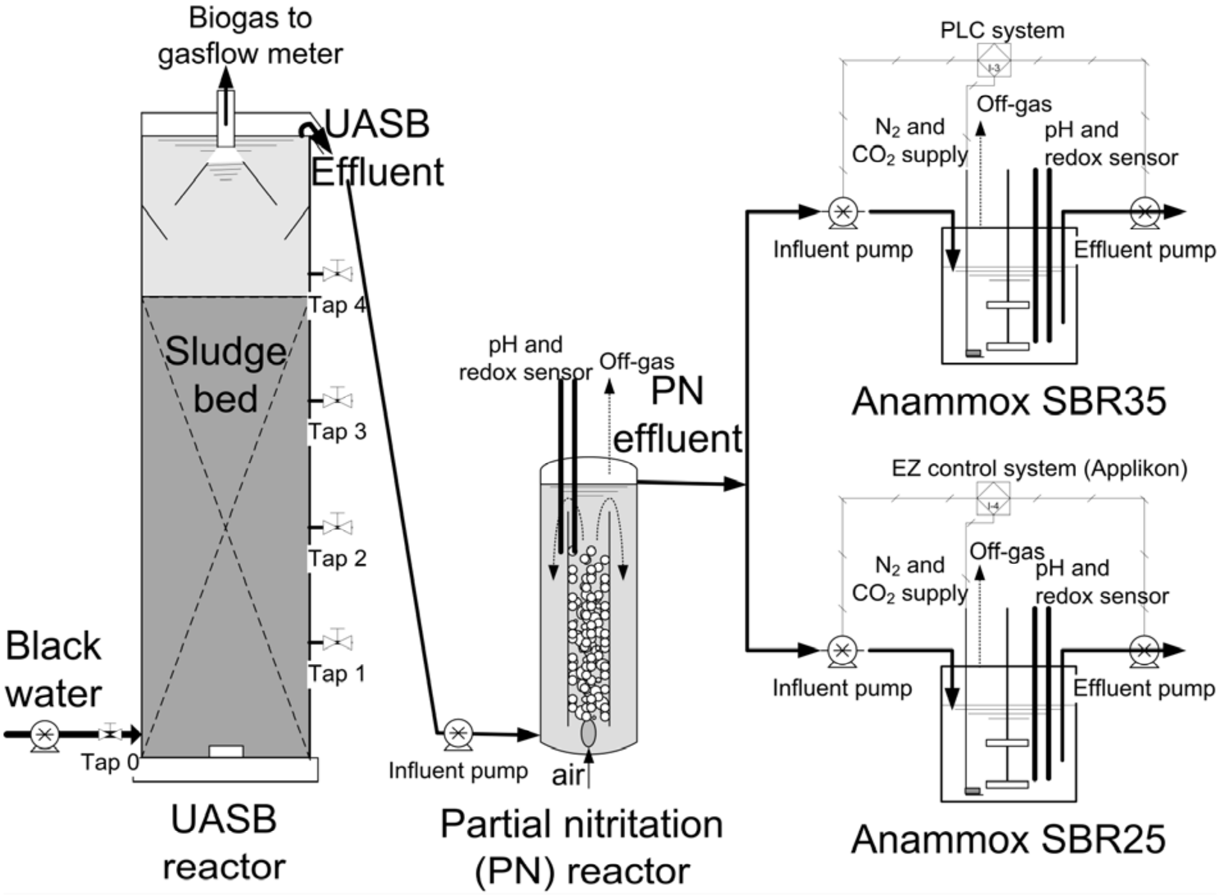

Figure 4.1 Treatment concept for black water: combined anaerobic treatment and nitrogen removal.

4.2.2 Influent to the anammox process

Before feeding it to the anammox process, the effluent from the partial nitritation reactor was filtered over a 100 µm sieve to prevent accumulation of sludge from the partial nitritation reactor in the anammox reactor. EDTA (0.5 g/L), $FeSO_4$ (0.00625 g/L) and a trace elements solution (1.25 mL/L) were added (van de Graaf et al., 1996). Minor amounts of ammonium (0.5 mL/L of 3 M $(NH_4)_2SO_4$) were added to prevent ammonium limitation in the reactor, because this may lead to accumulation of nitrite towards inhibiting levels. The composition of the influent to the anammox process is shown in table 4.1.

Calcium was added to SBR35 from day 223 at a concentration of 39 mg/L (0.6 mL/L of 240 g/L $CaCl_2.2H_2O$). SBR25 was started up later than SBR35 and additional calcium was added during the whole period of operation at a concentration of 39 mg/L (0.6 mL/L of 240 g/L $CaCl_2.2H_2O$).

Table 4.1 Characteristics of black water after anaerobic treatment, partial nitritation, sieving over 100 µm and addition of EDTA, $FeSO_4$ and trace elements solution.

	Unit	average	s.d.
NH_4^+-N	mg/L	408	49.0
NO_2^--N	mg/L	483	29.3
NO_3^--N	mg/L	6.0	0.7
COD_{total}	mg/L	459*	72.3
$COD_{soluble}$	mg/L	389*	51.4
TOC	mg/L	191	29.7

*corrected for the contribution of NO_2^- to COD (Nitrite exerts a COD of 1.1 gCOD/ gNO_2^--N)

4.2.3 SBR35, operated at 35 °C

SBR35 was operated at 35 °C for 348 days and seeded with anammox sludge from the full scale anammox reactor in Sluisjesdijk (Rotterdam, NL) (0.7 L of 26 gVSS/L, maximum load of 10 $kgN/m^3/d$ at 33 °C (van der Star et al., 2007)). The water jacketed SBR had a total liquid volume of 5 litres and was operated in cycles of 12 h. The reactor system was controlled by a PLC system (Siemens-PLC, logo 230RC). Each cycle consisted of 10 minutes settling, 10 minutes effluent discharge, 10 minutes idle time, 10.5 hours of feeding and 1 hour to complete the conversion processes. The reactor content was flushed with nitrogen gas (9.5 mL/min) and carbon dioxide (0.5 mL/min) during the feeding phase. To avoid entrance of air during effluent discharge, a gas bag (5L) containing nitrogen was connected to the headspace of the reactor. The reactor content was mixed with a stirrer at a speed of 100 rpm. The reactor was equipped with baffles to prevent concentration gradients in the reactor. Excess sludge was removed daily (60 mL/d), which corresponds with a SRT of 83 days assuming that no biomass was lost with the effluent. The effluent from the partial nitritation reactor initially was diluted by 50%, decreasing to 35%, 20% and 10% and 0% each week with synthetic medium without ammonium and nitrite (Jetten et al., 2005a). From day 33 onward the influent no longer was diluted.

4.2.4 SBR25, operated at 25 °C

SBR25 was operated at 25 °C with synthetic influent (Jetten et al., 2005a) for the first 123 days and was seeded with anammox sludge from SBR35 (0.8 L, several samples mixed, 1.4 gVSS/L). The reactor vessel was surrounded by a silicone heating blanket. The SBR had a total liquid volume of 4 liters and was operated in cycles of 12 h. The reactor system was controlled by an ez-Control system (Applicon Biotechnology, Holland). Each cycle consisted of 10 minutes settling, 10 minutes effluent discharge, 15 minutes idle time and the rest of the cycle was used for feeding until the maximum liquid level was reached. The pH was controlled at 7.7 by CO_2 supply. Nitrogen gas was continuously added at a flow rate of 10 mL/min, except during settling and effluent discharge. To avoid the entrance of air during effluent discharge, a gas bag (5L) containing nitrogen was connected to the headspace of the reactor. The reactor content was mixed with a stainless steel stirrer at a speed of 80 rpm and the reactor was not equipped with baffles. Starting at day 124 the synthetic influent was replaced with effluent from the partial nitritation reactor, increasing the percentage every week from 10%, 50%, 75%, 90% to 100%. SBR25 was operated for a period of 266 days.

4.2.5 Sludge analysis

Sludge samples were destructed to determine total concentrations of calcium and phosphorus using the Ethos 1 Advanced Microwave digestion system from Milestone. Two grams of sample were put into a special microwave vessel, 10 mL of HNO_3 (68%) was added and milliQ water was added up to a total volume of 30 mL. The vessels were put into the manifold and placed into the microwave. Samples were heated to 180 °C in 15 minutes and this temperature was maintained for another 15 minutes. After cooling down, the content of the vessels was transferred to a 100 mL flask and diluted to 1% acid for the ICP analysis.

4.2.6 Analysis

Liquid samples, taken once or twice a week, were fractionated into suspended, colloidal and soluble compounds by filtering through a black ribbon paper filter (Schleicher & Schuell) and a membrane filter (0.45 µm Cronus filter PTFE). Chemical oxygen demand was determined using DrLange® test kits according to standard methods (APHA, 1998). Because nitrite exerts a COD of 1.1 gCOD/gNO_2^--N, the COD values were corrected accordingly. Total ammonium nitrogen concentration (NH_4^+-N) was determined in membrane filtered samples (0.45 µm) using DrLange® test kits according to standard methods (APHA, 1998). Total nitrogen (TN) was determined in the unfiltered sample using DrLange® test kits according to standard methods (APHA, 1998). Samples were diluted 5 or 10 times prior to analysis to exclude the possible interfering effect of other ions.

Anions (SO_4^{2-}, PO_4^{3-}, Cl^-, NO_3^- and NO_2^-) were determined according to standard methods using ion chromatography (Metrohm 761 Compact IC). Calcium concentration was determined by ICP-OES (Perkin Elmer 5300 DV). TOC was analyzed with a Shimadzu TOC analyzer. Total Suspended Solids (TSS) and Volatile Suspended Solids were determined according to standard methods using the ashless black ribbon paper filter (Schleicher & Schuell).

Gas samples from the headspace of the reactor were analyzed off-line for N_2O on a Varian 8300 Custom Solutions gas chromatograph (Hayesep Q 80/100 Mesh 0.25m x 1/16" x 1 mm Ultimetal CP1308) in which Helium gas was used as carrier and N_2O was detected with an electron capture detector (ECD) at 200 °C. The temperature of the injector and the column were respectively 200 °C and 50 °C (at Delft University of Technology). The second gas chromatograph (at Wageningen University) that was used was a CEinstruments GC8000 Top (Interscience, Breda the Netherlands), because the first GC was not available for analysis for a few samples (SBR35 day 236 and SBR25 day 144). N_2O was separated on a Haysep Q 80-100 mesh 3m x 1/8" SS column and detected with an ECD.

4.2.7 Microbial analysis (SEM and FISH)

Granules from the two SBRs were used for scanning electron microscopical analysis (SEM). Immediately after sampling the samples were washed with phosphate buffer solution (PBS) for 10 min in Eppendorf tubes (those were used for the whole procedure). Samples were centrifuged for 2 min at 13.000 rpm and the supernatant was discarded, after each following step the centrifugation was carried out in the same way to remove the respective liquid. The fixation took place in 3.7%

glutaraldehyde solution (Sigma-Aldrich, Steinheim, Germany) at room temperature for 2h or at 4°C for 24h. Following that, the samples were washed twice in PBS and dehydrated in ascending concentrations of ethanol (30, 50, 70, 90% for 20 min each; 96% for 30 min, twice). Finally they were air dried in a drying chamber (45°C, 30-60min) and stored in a desiccator until the microscopical investigation. SEM was performed with a JEOL JSM 6480 LV microscope (JOEL Technics Ltd., Tokyo, Japan) in high vacuum mode (emission electrons detection, acceleration voltage 6-10 kV, operating distance 10 mm). The SEM Control V 7.07 software was used for control of the microscope and acquisition of the micrographs. Pictures were stored in bitmap format.

FISH (Fluorescent In Situ Hybridization) was used to characterize the bacteria in the sludge. Cell fixation to the gelatine-coated slides and hybridization steps were carried out according to methods previously described by (Amann et al. 1990). The 16S rRNA-targeting oligonucleotide probes used in this study are AMX368, to detect all anammox organisms, and EUB338, EUB338II, and EUB338III, to detect all bacteria (Schmid et al., 2003). All probes were purchased as Cy3 (AMX368 probe) or 5(6)-carboxyfluorescein- N-hydroxysuccinimide ester (FLUOS) (EUB probe) labelled derivatives from MWG-Biotech (Ebersberg, Germany) and were diluted to a final concentration of 50 ng/μl. Hybridization was performed at 46 °C for 1.5 h followed by washing with pre-warmed (48 °C) washing buffer and 15 min incubation at 48 °C in washing buffer containing DAPI (0.2 μg/μl). The cells were observed under an epifluorescent microscope Leica DMI 6000B (Leica, Germany) equipped with Leica DFC 350 FX camera.

4.2.8 Calculations

Maximum specific activities in the reactor were determined monthly by increasing the influent flow for about 1 hour to such an extent that a slow accumulation of nitrite and ammonium could be observed, up to a maximum nitrite concentration of 20 mgNO_2^--N/L. The maximum removal rate (gN(NH_4+NO_2^-)/L/d) was calculated as the difference between the loading rate and the accumulation rate. After determination of the biomass concentration in the reactor, the maximum specific activity was calculated (gN(NH_4+NO_2^-)/gVSS/d).

4.3 Results

4.3.1 SBR35, operated at 35 ˚C

SBR35 was operated for 348 days on partially nitritated anaerobic black water and figure 4.2 shows the removal of ammonium and nitrite, together with the effluent concentration of nitrite.

Because an excess of active biomass (2.2 gVSS/L) was present in the reactor at day 1 with an unknown activity (table 4.2), the load could be increased relatively fast. For about 70 days (day 33 – 100) nitrogen removal was stable, and all of the effluent of the partial nitritation reactor could be treated at a removal rate of 0.52 gN(NH_4+NO_2^-)/L/d.

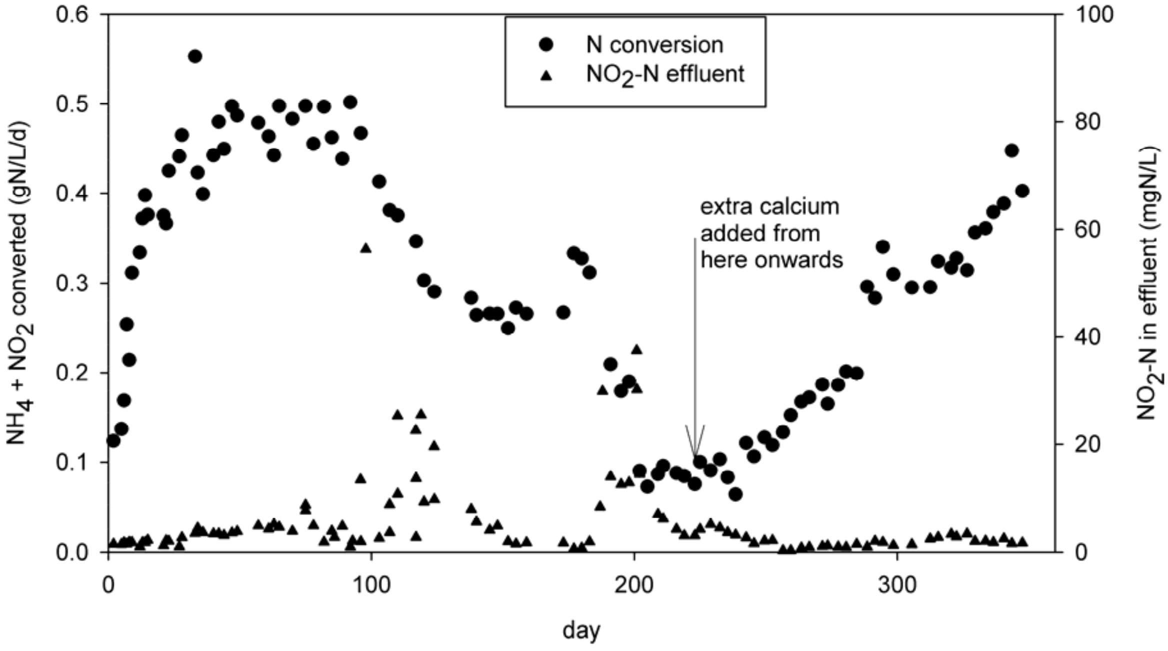

Figure 4.2 NH_4 and NO_2^- removal, and the effluent NO_2^--N concentration in SBR35 at 35 ˚C. Extra calcium was added from day 223 onwards.

Nitrite was completely removed (99%) and ammonium was removed for 85% (table 4.3). However, after day 100 nitrite started to accumulate and the load had to be decreased to 0.29 gN/L/d. On day 131 the stirrer speed was reduced to 90 rpm, decreasing the shear in the reactor, and excess sludge no longer was removed. As a result, the removal rate stabilized at a value of 0.27 gN(NH_4+NO_2^-)/L/d. Effluent VSS concentrations decreased from 18 mgVSS/L to 2.3 mgVSS/L. The maximum removal rate decreased to 0.43 gN(NH_4+NO_2^-)/L/d and the specific activity to 0.32 gN(NH_4+NO_2^-)/gVSS/d on day 140 (table 4.2). An average SRT of 55 days was estimated for the first 131 days of operation, which due to the loss of biomass with the effluent was shorter than was expected. Although an SRT of 55 days still should be long enough to avoid complete wash-out of anammox biomass, growth of other biomass, such as heterotrophs, could have reduced the specific SRT of the anammox bacteria.

Table 4.2 Maximum removal and activity in the anammox SBR35 at 35 ˚C.

Day	Maximum removal [gN (NH_4+NO_2^-)/ L/d]	Biomass concentration [gTSS/L]	[gVSS/L]	VSS/ TSS	Maximum specific activity [gN (NH_4+NO_2^-)/ gVSS/d]
1	n.d.	2.4	2.2	92%	n.d.
36	1.2	1.3	1.1	85%	1.1
49	1.3	2.1	1.7	79%	0.78
70	1.1	1.6	1.5	90%	0.77
90	0.89	2.1	1.7	81%	0.51
140	0.43	1.8	1.4	75%	0.32
154	0.32	1.8	1.3	75%	0.24
223	0.13	1.9	1.2	63%	0.11
273	0.48	1.2*	0.75*	65%	0.64*
341	0.87	2.2*	1.3*	59%	0.67*
348	Reactor stopped	6.9#	2.9#	42%	0.30

n.d. = not determined

* Due to growth on the walls, biomass concentration did not represent the total biomass concentration in the reactor.

Biomass concentration determined after opening the reactor, including biomass on the walls and bottom.

On day 190 nitrite started to accumulate once more; apparently biomass retention still was not sufficient to sustain a stable removal rate, despite the reduction in stirrer speed and the discontinuation of sludge wasting. Table 4.2 shows as well that the maximum removal in the reactor decreases from day 70 to a very low value (0.13 gN(NH_4+NO_2^-)/L/d) on day 223. From day 223 the calcium concentration in the influent was increased from 42±5.7 mg/L to 73±14 mg/L in an attempt to stimulate the granulation process and in this manner improve biomass retention. As a result, the nitrogen removal increased rapidly (see figure 4.2 and table 4.2). At the same time significant wall growth was observed, which had not been the case before, and the biomass concentration no longer could be measured without opening the whole reactor. The maximum nitrogen removal rate in the reactor increased to 0.87

$gN(NH_4{+}NO_2^-)/L/d$ (table 4.2) on day 341 and the volumetric reactor removal to 0.40 $gN(NH_4{+}NO_2^-)/L/d$ on day 348 (figure 4.2).

At day 348 the reactor was emptied and the total solids concentration and volatile suspended solids concentrations were determined to be 6.9 gTSS/L and 2.9 gVSS/L, respectively. About half of the solids were attached to the reactor walls and 58% of the solids was inorganic material. A large fraction of the additional calcium precipitated and the reactor contained an increasing amount of inorganic material; the VSS/TSS ratio of the sludge decreased from 92% to 42% (from day 1 to day 348). Apparently phosphate was removed as well with the addition of calcium, because the concentration decreased from 64 $mgPO_4$-P/L to 44 $mgPO_4$-P/L from day 223 onward. The calcium content of the sludge increased from 116 mgCa/gVSS on day 211 to 409 mgCa/gVSS on day 348 while the phosphorus content increased from 144 mgP/gVSS to 284 mgP/gVSS, strongly indicating the occurrence of calcium phosphate precipitation.

Figure 4.3 shows the microscopic images of the granules that were formed in SBR35. Granules on day 140 without additional calcium in the influent exhibited filamentous structures. The granules on day 341 with additional calcium were more dense and bigger.

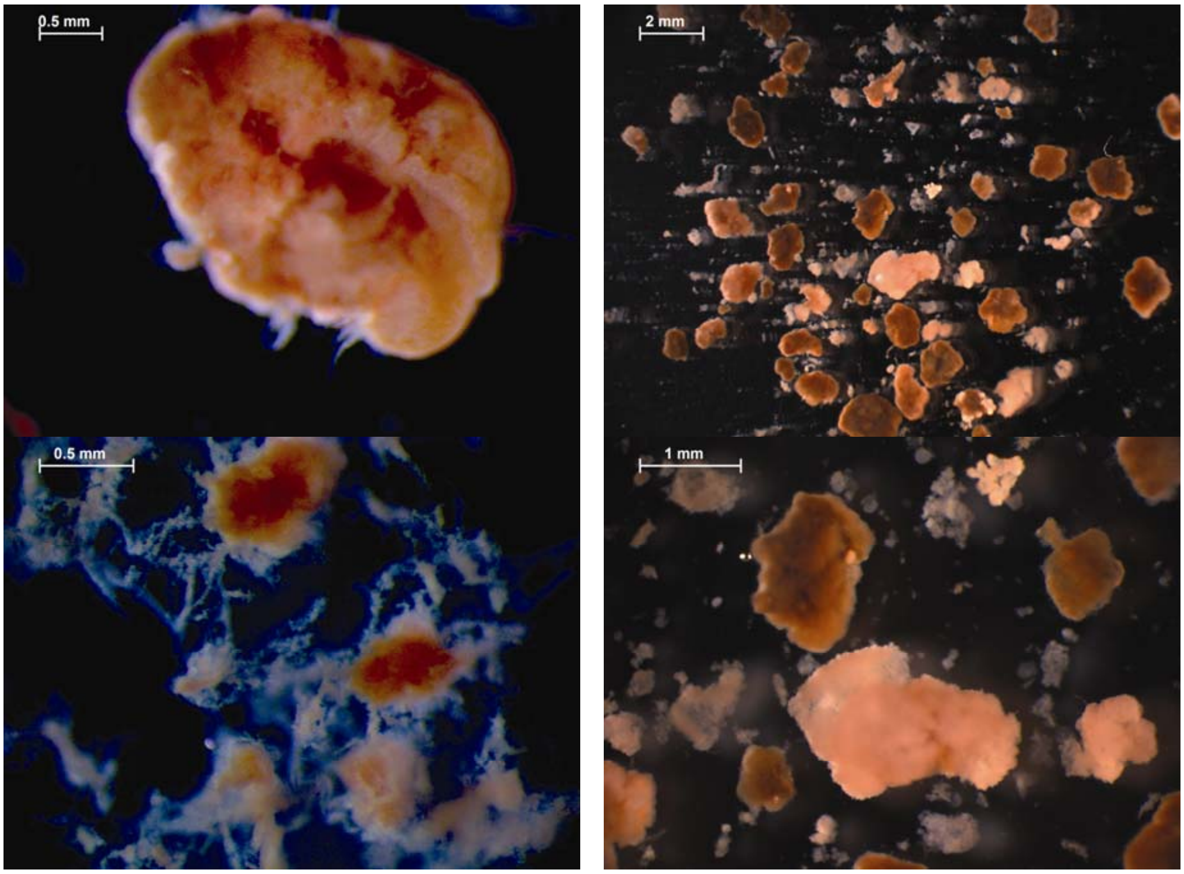

Figure 4.3 Pictures of the granules in anammox SBR35 taken at day 140 (left) and day 341 (right).

Table 4.3 gives an overview of the removal efficiencies and production of nitrate in SBR35. Nitrite was always limiting and removed for 99 – 100%. Ammonium was usually present in excess and was removed for 85 – 98%. Total nitrogen removal was 89% during the last period. Remaining nitrogen in the effluent was 102 mgN/L, mainly consisting of nitrate (50 mgNO_3^--N/L) and small amounts of ammonium (9.8 mgNH_4-N/L) and nitrite (1.8 mgNO_2^--N/L). Part of the nitrogen is soluble organically bound material and cannot be removed by the anammox process (40 mgN/L). Nitrate production always was lower (0.079 – 0.11 NO_3^--N/NH_4-N) than stoichiometrically expected at maximum anammox growth (0.26 NO_3^--N/NH_4-N (Strous et al., 1998)). Two reasons can explain this lower nitrate production, heterotrophic activity or a lower growth rate of anammox. In the first two periods (day 33 – 100, and day 100 – 200) nitrite removal was close to what was expected from anammox stoichiometry (1.32 NO_2^--N/NH_4-N) and probably nitrate was removed by heterotrophic denitrification explaining the deviation from the expected stoichiometry. In the last period after day 250 the lower nitrate production can be explained by a lower removal of nitrite (1.19 NO_2^--N/NH_4-N instead of 1.32 NO_2^--N/NH_4-N), indicating that anammox was not growing at its maximum growth rate, producing less nitrate.

Table 4.3 Overview of the performance in anammox SBR35 at 35 ˚C.

	Unit	day 33 – 100	day 100 – 200	day 250 – 348
NO_2^--N/NH_4-N removed	mol/mol	-1.26	-1.35	-1.19
NO_3^--N/NH_4-N produced	mol/mol	0.079	0.11	0.11
TN removal	%	n.d.	87%	89%
NH_4-N removal	%	85%	94%	98%
NO_2^--N removal	%	99%	99%	100%
COD_{total} removed	%	13%	11%	5.1%
$COD_{soluble}$ removed	%	8%	1.1%	3.2%

n.d. = not determined

4.3.2 SBR25, operated at 25 ˚C

SBR25 was operated at 25 ˚C for 266 days to investigate the nitrogen removal from black water at the preferred temperature for an energy efficient treatment concept for black water (chapter 2). The first 123 days synthetic medium was used as influent at a load of 0.42 gN/L/d (data not shown). Figure 4.4 shows that the removal rate did not change after changing the influent to 100% effluent from the partial nitritation reactor and with addition of extra calcium. This demonstrates that the anaerobically treated black water did not contain compounds that may inhibit the anammox process. On day 186 the pH control did not work properly and nitrite accumulated, but the removal recovered quickly and could still be increased to 0.49 $g(NH_4+NO_2^-)N/L/d$ at day 266. The maximum specific activity was determined on four occasions and was 0.34 ± 0.091 $g(NH_4+NO_2^-)N/gVSS/d$. On day 279 the reactor was stopped and emptied. The total solids concentration and volatile suspended solids concentrations were determined to be 2.7 gTSS/L and 1.7 gVSS/L, respectively. The amount of solids attached to the walls was negligible. Calcium and phosphate were removed from the influent and similarly to SBR35 precipitated in SBR25. Because of the lower temperature less calcium carbonate may have precipitated than in SBR35, which can also explain the lower amount of inorganic material that was found in SBR25 compared to SBR35.

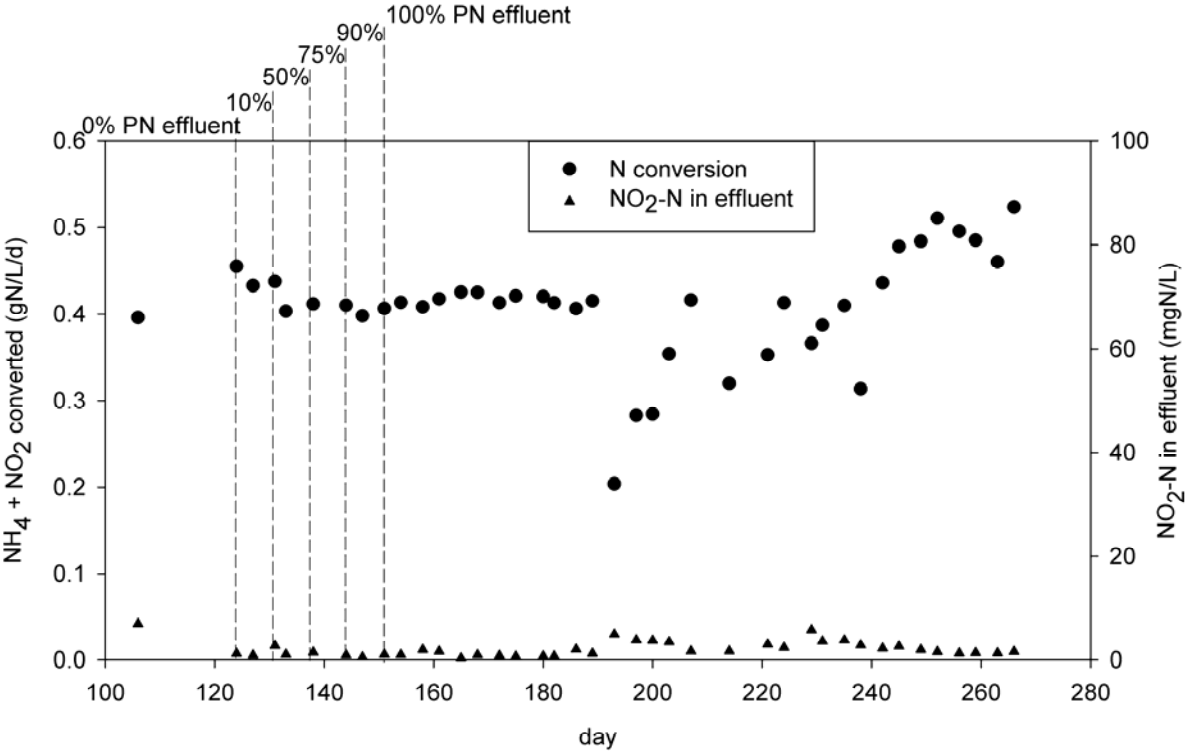

Figure 4.4 NH_4 and NO_2^- removal, and the effluent NO_2^--N concentration in SBR25 at 25 °C.

On average SBR25 achieved 85 % nitrogen removal (table 4.4), with effluent concentrations of 133 mg TN/L, 33 mgNH_4-N/L, 2.2 mgNO_2^--N/L and 65 mgNO_3^--N/L. Nitrate production was lower (0.15 NO_3^--N/NH_4-N) than stoichiometrically expected (0.26 NO_3^--N/NH_4-N), possibly due to heterotrophic denitrification (table 4.4). Heterotrophic denitrification activity was confirmed in batch tests by the addition of acetate to sludge samples taken from the reactor on day 279. Nitrate and nitrite concentrations during these batch tests showed a small decrease in nitrate concentration and an increase in nitrite concentration (results not shown).

A similar nitrogen removal of 85% was achieved in SBR25 compared to 89% in SBR35. Despite the lower temperature in SBR25 also a similar nitrogen removal rate of 0.49 g(NH_4+NO_2^-)N/L/d was achieved in SBR25 compared to 0.40 g(NH_4+NO_2^-)N/L/d in SBR35. As the maximum specific activity in SBR25 was lower than in SBR35 (0.34 gN/gVSS/d in SBR25 versus 0.78-1.1 gN/gVSS/d in SBR35), a sufficient biomass retention by addition of extra calcium seemed to be the crucial part in this research.

Table 4.4 Overview of the performance in anammox SBR25 at 25 ˚C.

	unit	Day 151 – 266
NO_2^--N/NH_4-N removed	mol/mol	-1.27
NO_3^--N/NH_4-N produced	mol/mol	0.15
TN removal	%	85%
NH_4-N removal	%	92%
NO_2^--N removal	%	100%
COD_{total} removed	%	5.0%
$COD_{soluble}$ removed	%	4.6%

4.3.3 Microbial analysis (SEM and FISH)

The SEM pictures in figure 4.5 show the difference in granules from SBR35 before and after addition of extra calcium. Granules observed on day 348 showed a more densely populated surface than granules from day 204, which was most probably due to the addition of extra calcium.

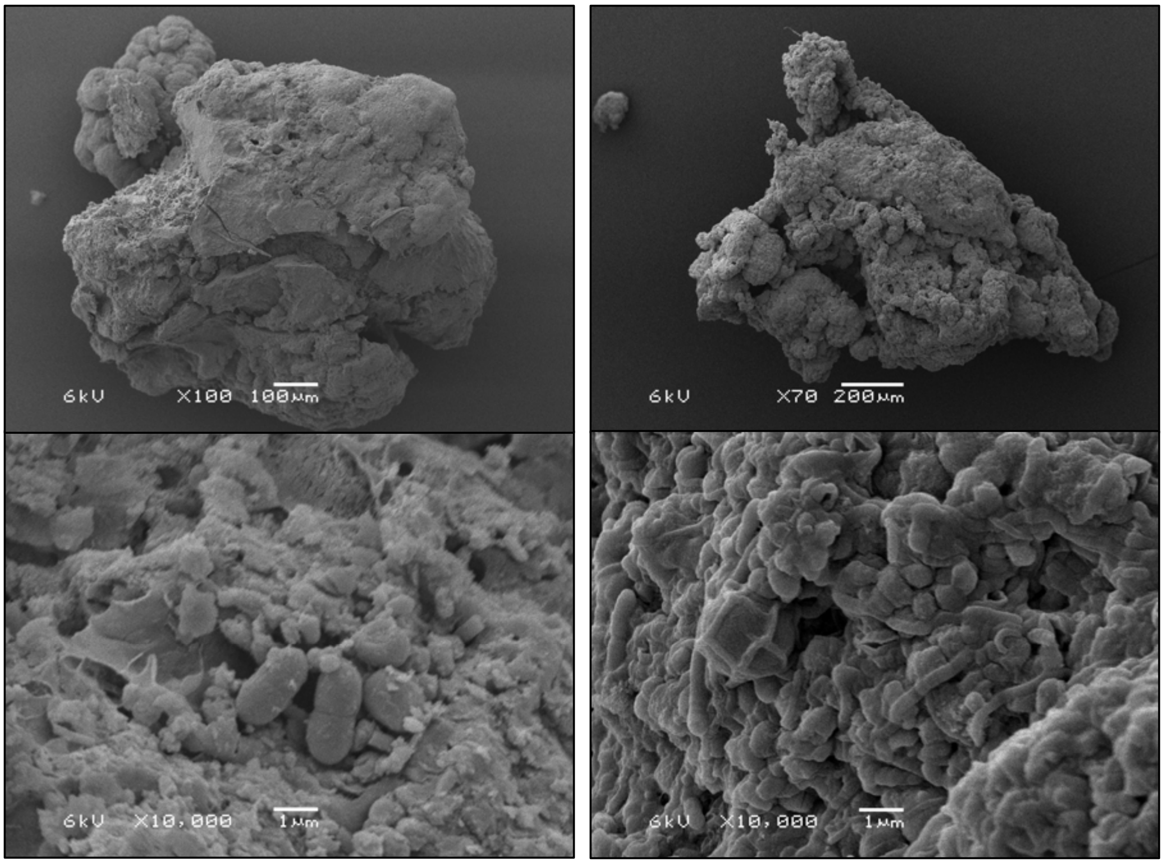

Figure 4.5 SEM observations of SBR35 granules (on the left granules in the reactor at day 204 without additional calcium and on the right granules in the reactor at day 348 with additional calcium).

Microbial analysis by FISH showed that anammox in SBR35 at day 153 was present in a large fraction, although not all bacteria detected with the EUB338 probe hybridized with the AMX368 probe for the anammox (figure 4.6A). In SBR25 anammox was present in an even larger fraction, because almost all bacteria hybridized with the AMX368 probe for the biomass at day 181 (figure 4.6B). This showed that changing the influent to SBR25 from synthetic to anaerobic UASB effluent did not have a negative effect on the anammox bacteria.

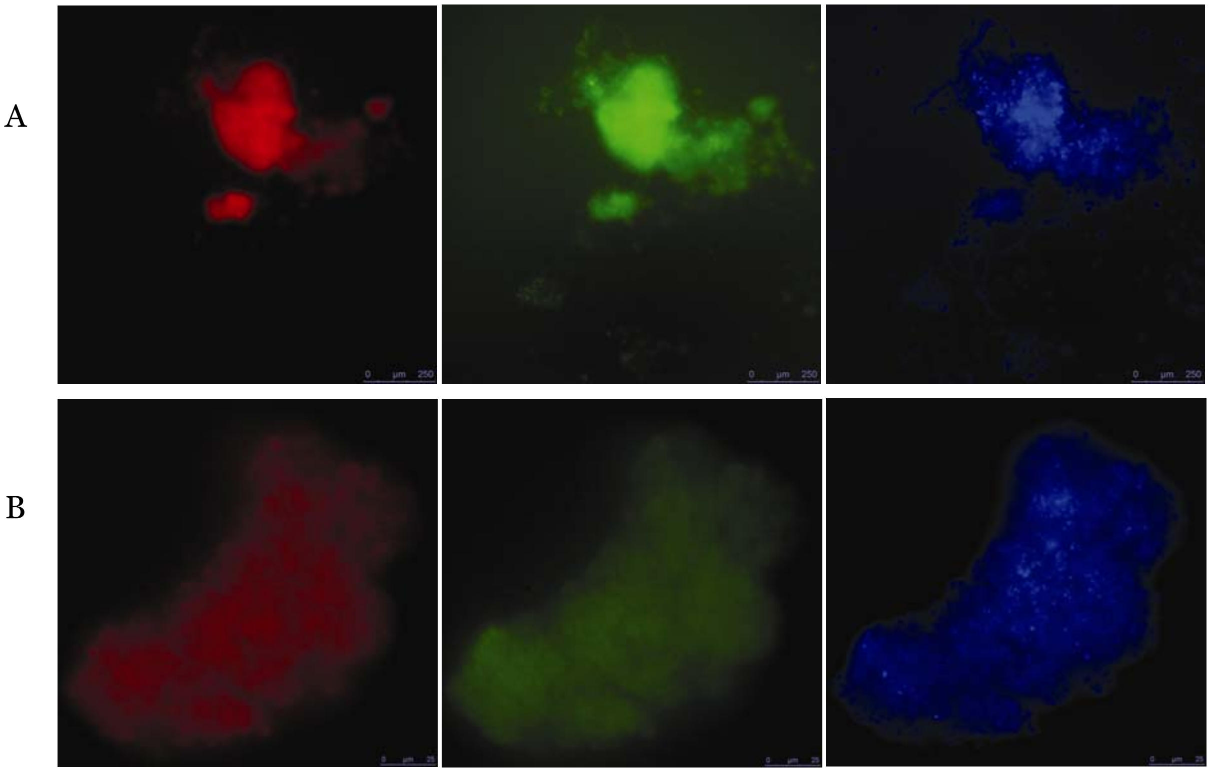

Figure 4.6 FISH pictures (left (red) AMX 368, middle (green) EUBmix and right (blue) DAPI); A: SBR35 on day 153; B: SBR25 on day 181.

4.3.4 Nitrous oxide (N_2O) emissions

N_2O concentrations in the headspace of SBR35 varied from 25 ppm to 1825 ppm, measured in between days 119 and 281 (9 samples). This corresponded to respectively 0.02% to 1.0% of the total nitrogen load to SBR35 (figure 4.7). The highest concentration of N_2O in the off gas was measured when the nitrite concentration in the reactor was higher than usual.

N_2O concentrations in the headspace of SBR25 varied from 7.1 ppm to 14 ppm measured between days 144 and 172 (5 samples). This corresponded to approximately 0.01% of the total nitrogen load. On day 189 a high concentration of N_2O was detected of 411 ppm, when the nitrite concentration in the reactor was 6.1 $mgNO_2^-$-N/L, which was much higher than the usual concentration.

Figure 4.7 clearly shows that the percentage of N_2O in the off gas increased with the nitrite concentration in the reactor.

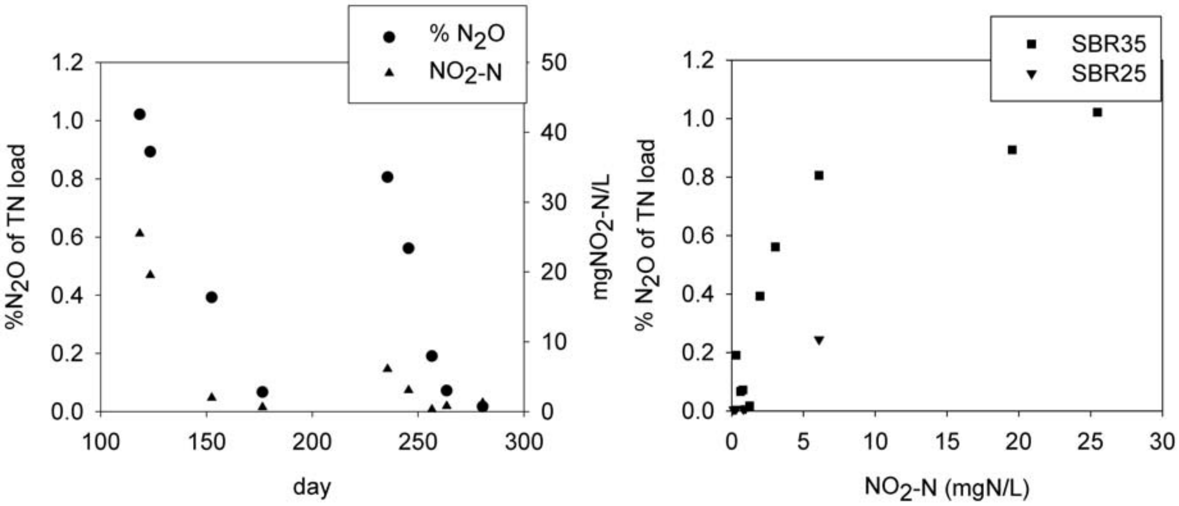

Figure 4.7 left: N_2O production in SBR35 and the NO_2^--N concentration in effluent; right: N_2O production in SBR35 and SBR25 in relation to the NO_2^--N concentration in effluent.

4.4 Discussion

In this study the feasibility and operating conditions of the nitritation-anammox process to remove the nitrogen from anaerobically treated black water were investigated and evaluated. The effect of temperature, calcium levels and the emission of N_2O are discussed below.

4.4.1 Nitrogen removal and the effect of temperature

The two reactor nitritation-anammox process removed 85% to 89% of total nitrogen, which is higher than the nitrogen removal efficiency of 76% reported previously for anaerobic black water treatment in a rotating biological contacter (RBC) (Vlaeminck et al., 2009). This was mainly due to a lower nitrate production than in the “standard” anammox stoichiometry (Strous et al., 1998), probably because of heterotrophic denitrification. In a RBC DO control is difficult and to prevent nitrite oxidation, elevated free ammonia concentrations were induced by increasing the pH and effluent ammonium concentrations were relatively high 100 $mgNH_4$-N/L (Vlaeminck et al., 2009) compared to this research.

In the research of Vlaeminck et al. (2009) the digested black water was stored for a long time before application. This may have resulted in a significant decrease of readily degradable organic compounds. In this research the readily degradable organic compounds were removed for a large fraction in the partial nitritation reactor (chapter 3). In one reactor nitritation-anammox process, like the RBC, these readily degradable organic compounds could interfere negatively with the anammox process by stimulating heterotrophic denitrification (Udert et al., 2008).

Effluent total nitrogen concentrations were 102 – 133 mgN/L of which 25% was organically bound nitrogen. These concentrations are much higher than in effluents of conventional wastewater treatment plants (WWTPs) of 10 mgN/L (CBS-Statline, 2007b). On the other hand, when comparing the loads, the discharge load from conventional WWTPs is still higher (1.2 gN/p/d, based on 124 L/p/d and 10 mgN/L in the effluent, (CBS-Statline, 2007b)) than the load of treated black water, which is 0.7 gN/p/d based on 5 L/p/d.

In this research it was demonstrated that the anammox process can be successfully operated, both at 35 °C and at 25 °C. Similar nitrogen removal efficiencies were obtained and because the specific activity of anammox was lower at lower temperature, efficient biomass retention is crucial for operation at lower temperatures. Operation at elevated temperatures (35 °C) is therefore not necessary (Dosta et al., 2008) and this saves in energy for heating, because the collected black water usually has a lower temperature of about 20 °C (chapter 2).

4.4.2 Calcium as a requirement for biomass retention

It is known that calcium and other polyvalent cations are important for bioflocculation and granulation, because they can form bridges between bacteria and bioflocs that are negatively charged due to the formation of EPS (Exocellular polymeric substances) (Sobeck and Higgins, 2002). By adding extra calcium to the influent, the ratio of monovalent to polyvalent cations ([M]/[P], equivalents) decreased, from 19 [M]/[P] to 12 [M]/[P] equivalents. Other factors to achieve the formation of anammox granules are a suitable selective pressure for settling using a short settling time, a low growth rate and the presence of inorganic precipitates (van der Star et al., 2008). In this research the settling time was 10 minutes in both SBRs, to obtain a selective pressure for settling, however this was not enough for sufficient granulation.

Operation of SBR35 showed that anammox activity could not be sustained without addition of calcium. The increased removal after day 223 and the growth of a thick biofilm on the reactor walls and bottom in SBR35, indicated that the presence of calcium was important for biomass retention. Part of the calcium precipitated as calcium phosphates because the phosphorus concentration in the sludge and the inorganic fraction of the sludge increased as well after day 223. The amount of extra calcium addition should be optimized, because this accumulation of inorganic material is not desired. In SBR25 extra calcium was already added from the start and in this reactor no decrease in removal rate was observed.

Calcium cannot be the only reason for granule formation, as discussed by van der Star (2008). Other research showed that anammox granulation was possible at low calcium concentrations of 5.65 mg/L in membrane SBR (Trigo et al., 2006). In this way a long SRT was achieved and this resulted in a low growth rate favouring granule formation (Trigo et al., 2006; van der Star et al., 2008).

Another aspect that should be taken into account is the availability of free calcium. Calcium can only precipitate and bind EPS if it is available in the free Ca^{2+} form. In black water large amounts of humic acids are present that can form complexes with calcium (van der Stelt et al., 2005) making the calcium less available.

To our knowledge and according to (Abma, 2009) only wastewaters with relatively high calcium concentrations (>80 mg/L) have been treated with the anammox process (e.g. digester effluent from wwtp Dokhoven, Rotterdam, 120 mgCa/L (analyzed twice), or urine with approximately 177 mgCa/L (Wilsenach, 2006)) and there is no experience yet in applying the anammox process for streams with lower calcium concentrations like the black water in this research.

Vlaeminck et al. (2009) operated the one reactor nitritation-anammox process on digested black water for 5 months (155 days) during which no loss of nitrogen conversion was observed. This research (SBR35) also gave stable results for a long period, but showed a decrease in removal rate after 200 days. It is not clear what will happen on the long term in a RBC reactors as used by Vlaeminck et al.(2009). The addition of extra calcium will still be necessary to sustain anammox biomass in the reactor, or the presence of less shear in a biofilm reactor already allows for a satisfactory biomass retention. More research is needed to verify this effect of calcium on granule formation and biomass retention in different reactor configurations.

4.4.3 Nitrous oxide (N_2O) production in the anammox process

The emission of N_2O from both anammox SBRs (0.01% to 1.0% of N load) were comparable with the emission of N_2O in a full scale anammox reactor treating reject water (0.6%) (Kampschreur et al., 2008). Because N_2O does not play a role in the anammox metabolism, Kampschreur et al. (2008) discussed that the main causes for N_2O production in an anammox reactor should be attributed to incomplete regular (heterotrophic) denitrification to N_2O and the denitrification by ammonium-oxidizing bacteria (AOB).

The maximum load of nitrate removed by heterotrophic denitrification on remaining COD from the black water (e.g. maximum 13% of total COD was removed in anammox SBR35 (table 4.3)) was estimated to be 2.2% of influent nitrogen load (assuming that all the COD removed was used and 3 gCOD/ gNO_3^--N was needed). COD from decaying biomass could also have contributed to denitrification (Lackner et al., 2008), but this could not be further quantified. Although some denitrification is likely to have occurred, it is not very likely that all N_2O was produced by incomplete denitrification.

AOBs could have entered the SBRs with the influent, which originated from a partial nitritation reactor and could have produced N_2O using nitrite as electron acceptor under oxygen limiting conditions (Kampschreur et al., 2008). Figure 4.7 shows that N_2O production in the anammox process can be avoided by controlling the process such that nitrite is limiting and the concentration of nitrite in the reactor always is low.

In the partial nitritation reactor the emission of N_2O cannot be avoided because high nitrite concentrations are always present. In this concept for black water treatment this means that 1.9% of the nitrogen will be emitted as N_2O in the partial nitritation process (chapter 3). The emission of N_2O in a full scale one reactor nitritation-anammox process was similar (1.2% of the nitrogen load) as the two reactor nitritation-anammox process (Kampschreur et al., 2009a), therefore the choice between a one reactor or two reactor nitritation-anammox process seems not to be determined by the emission of N_2O. At conventional WWTPs the N_2O emissions vary a lot, depending on the operation and characteristics of the wastewater. More research is needed to determine a quantitative relationship of these factors with the N_2O emission (van Voorthuizen et al., 2009).

4.5 Conclusions

The two reactor nitritation-anammox process removed 85% to 89% of total nitrogen from anaerobically treated black water.

The presence of calcium was crucial for granule formation to obtain high biomass retention and therefore an increasing removal of nitrogen in the reactors. The (free) calcium concentration in black water was too low (42 mg/L) to apply granular processes and addition of extra calcium was necessary to obtain a nitrogen removal of 0.5 gN/L/d, both at 35 °C and 25 °C. The specific activity of anammox biomass was lower at 25 °C, but with efficient biomass retention, operation at elevated temperatures (35°C) is not necessary and energy for heating can be saved.

Nitrous oxide (N_2O) was produced in both anammox SBRs, however only when the nitrite concentration increased because of an inefficient anammox conversion process. By preventing nitrite accumulation in the anammox reactor, N_2O emissions can be prevented.

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5

Phosphorus recovery from concentrated black water

Abstract

Source-separated black water (toilet water) containing 68% of the phosphorus in the total household waste(water) stream including kitchen waste, is a potential source for phosphorus recovery. In this research the fate of phosphorus in concentrated black water (collected with vacuum toilets) during storage and anaerobic treatment was investigated. The phosphorus balance of a UASB reactor treating concentrated black water showed a phosphorus conservation of 61% in the anaerobic effluent. Precipitation of phosphate as struvite from this stream resulted in a recovery of 0.22 kgP/p/y, representing 10% of the artificial phosphorus fertilizer production in the world. The remaining fraction of the phosphorus ended up in the anaerobic sludge, mainly due to precipitation (39%). Simulation of chemical precipitation showed a strong effect of pH and phosphorus concentration in the black water. Low dilution and a high pH favour the accumulation of phosphorus in the anaerobic sludge and this sludge could be used as a phosphorus-enriched organic fertilizer, provided that it is safe regarding heavy metals, pathogens and micro-pollutants.

M.S. de Graaff, G. Zeeman, H. Temmink and C.J.N. Buisman

This chapter has been submitted to Bioresource Technology.

5.1 Introduction

Phosphorus is an important element in food and fertilizers, because it is a vital element of life sustaining compounds such as DNA and ATP. It also is abundantly used to produce chemical compounds such as explosives and pesticides. Phosphorus is produced from mined phosphate rock, mainly for the production of fertilizers (85%, 14.9 million ton P/year) (Cordell et al., 2009). However, phosphorus rock is a limiting, non-renewable resource, and it is estimated that the global phosphate rock reserves will be depleted in 50–100 years (Driver et al., 1999; Cordell et al., 2009). Although there are disagreements on the total global phosphate rock reserves, for many countries these are already becoming a strategic resource (Gilbert, 2009). Mining of phosphate rock also has a large negative environmental impact because it results in the production of gypsum that is contaminated with heavy metals and radioactive elements (Driver et al., 1999; Wilsenach et al., 2003). A large fraction of mined phosphate rock finally ends up in the oceans, causing anoxic events and a negative effect on marine life. Based on this, Rockström et al. (2009) argue that the planetary boundary concerning the phosphorus cycle, defined as the safe operating space for humanity with respect to the Earth's system, almost has been transgressed. Clearly, to prevent a global phosphorus crisis, the focus should be much more on recycling of phosphorus (Gilbert, 2009; Rockström et al., 2009).

Due to over fertilization and the production of large quantities of animal manure a significant fraction of phosphorus finally ends up in soils and inland and coastal waters, together with the phosphorus in treated and untreated sewage originating from human excreta (Cordell et al., 2009). Separation at source of household wastewater would generate a concentrated stream from the toilet, called black water, and a relatively diluted stream from the bathroom, kitchen and laundry, called grey water (Otterpohl et al., 1999). Black water, containing 68% of the phosphorus in the total household wastewater stream including kitchen waste (Kujawa-Roeleveld and Zeeman, 2006), is a potential source for phosphorus recovery. In the Netherlands 7.6 ktP/year are excreted by humans in their faeces and urine (16 million people, average of 1.3 gP/p/d (Kujawa-Roeleveld and Zeeman, 2006)). Worldwide, 3 million tons P/year are excreted by humans, which represents 21% of the total amount of artificial phosphorus fertilizer production (14.9 million tons P/year) (Cordell et al., 2009). Because domestic wastewater contains low amounts of heavy metals compared to industrial wastewater, relatively clean phosphorus rich products can be produced from black water, that could be reused in agriculture as a fertilizer (Winker et al., 2009) or as a raw material for the phosphorus industry (Schipper et al., 2007).

Previous research demonstrated that concentrated black water, collected with vacuum toilets, can be treated in a UASB (Upflow Anaerobic Sludge Blanket) reactor at a relatively short hydraulic retention time (HRT) of 8.7 days (chapter 2), in a UASB-septic tank at a much longer HRT of 29 days (Kujawa-Roeleveld et al., 2006) or in a CSTR at an HRT of 20 days (Wendland et al., 2007). Because of the low biomass yield, nutrients generally are largely conserved in the effluent of these anaerobic treatment systems. Van Voorthuizen et al. (2008) reported a phosphorus removal of only 5% during anaerobic treatment of black water collected with conventional flush toilets, i.e. 95% was conserved in the effluent. However, for more concentrated black water, collected with vacuum toilets, 40% of the phosphorus was removed when applying UASB-septic tank (Kujawa-Roeleveld et al., 2005). Apparently the amount of flushing that is applied seems to be crucial in the level of phosphorus conservation during anaerobic treatment. The lower level of phosphorus conservation in the effluent of anaerobic reactors treating concentrated black water results in a phosphorus richer sludge, which could be reused as a soil conditioner. However, pathogens, heavy metals and micro-pollutants are a potential hazard, and appropriate treatment is required (Winker et al., 2009).

Driver et al. (1999) stated that phosphorus can easily be recovered from a liquid in two inorganic forms: calcium phosphates and struvite ($Mg(NH_4;K)PO_4.6H_2O$). Both products have a high reuse potential. The disadvantage of using calcium is that it will also precipitate with carbonate(s) (Tchobanoglous et al., 2003). This implies that a large amount of calcium is required and the buffer capacity of the liquid will decrease, which in a later process stage is required for biological nitrogen removal (chapters 3 and 4). Furthermore, to obtain high grade and strong calcium phosphate pellets a complex process is needed with subsequent stages of different pH conditions (Driver et al., 1999). Struvite is a slow-releasing fertilizer, and because only magnesium needs to be added to black water, struvite precipitation may be attractive in new sanitation concepts (Zeeman et al., 2008). Struvite can form spontaneously under a wide range of conditions. The optimum pH for struvite precipitation was determined to be between a pH of 8.5 and 9.5, but struvite can also precipitate at lower pH values, depending on the saturation of the liquid and factors such as temperature and the presence of other ions (Le Corre et al., 2009). Full-scale struvite precipitation already has been demonstrated for the treatment of calf manure (Schuiling and Andrade,

1999) and digester effluents (e.g. (Ueno and Fujii, 2001)). It also was shown that phosphate can be efficiently eliminated from source-separated urine by struvite precipitation (Ronteltap et al., 2007b). Struvite was therefore selected as the most appropriate technology to recover phosphorus from concentrated black water.

In this study the possibilities to recover phosphorus from concentrated black water were investigated. The phosphorus balance of a UASB reactor treating concentrated black water was analyzed. Chemical precipitation was simulated to investigate spontaneous phosphate precipitation in black water. Batch tests to recover phosphate as struvite after anaerobic treatment were carried out. Finally, the main options to recover phosphorus-rich products from black water, i.e. struvite and stabilized anaerobic sludge, were discussed.

5.2 Materials and Methods

5.2.1 Anaerobic black water treatment in a UASB reactor

Black water, collected in vacuum toilets, was obtained from the DESAR (Decentralized Sanitation and Reuse) demonstration site in Sneek (Friesland, NL) (Zeeman et al., 2007). Every two weeks jerry cans were filled with black water from the buffer tank at the demonstration site (HRT of 4 h, not cooled), transported to the lab and stored at 4 °C. The black water was used as the influent for anaerobic treatment in a UASB reactor (details are described in chapter 2).

For the experiments in this chapter, black water and sludge and effluent from the UASB reactor were used. During the first period from day 1 to day 518 black water was produced at 5 L/p/d. During the second period from day 519 to 1060 the black water was more diluted, caused by a different operation of the vacuum toilets, and resulting in a production of 7.8 L/p/d (Gorter, 2009). Black water was used to investigate the effect of pH on the phosphorus distribution in black water in batch tests. Effluent from the UASB reactor was used for batch precipitation tests with magnesium addition to remove phosphate from solution as solid struvite.

5.2.2 Phosphorus mass balance of the UASB reactor

The phosphorus mass balance of the UASB reactor was established over the first 951 days of operation (equation 5.1).

$$P_{\mathrm{influent}} = P_{\mathrm{effluent}} + P_{\mathrm{sludge,wasted}} + P_{\mathrm{sludge,reactor}} \tag{5.1}$$

Influent (black water) and effluent samples (125 samples, taken once a week) were analyzed for total phosphorus (TP). $P_{influent}$ and $P_{effluent}$ were defined as the accumulative phosphorus load in the black water and effluent respectively (in gP).
The amount of phosphorus in sludge was calculated based on the TP concentration of the sludge (16 samples of UASB excess sludge). $P_{sludge,wasted}$ was defined as the total amount of phosphorus wasted with excess sludge (in gP). $P_{sludge,reactor}$ was defined as the amount of phosphorus that accumulated in the UASB reactor[11] (in gP), based on a sludge bed volume of 75% of the reactor liquid volume and the TP concentration of the sludge.

5.2.3 Batch tests for pH effect on phosphorus distribution in black water

Stirred beaker (0.6L) batch tests (magnetic stirrer, 100 – 250 rpm) were done (three times in duplo) to investigate the phosphorus distribution in relation to the pH. Black water (0.5 L; samples taken at day 1033, 1043 and 1048 of operation of the UASB reactor) was cooled to 7 °C with ice surrounding the glass beaker to exclude biological conversions. Every 20 minutes the pH was decreased with 0.1 M of HCl to a pH of 7, 6, 5 and 4. Black water samples (10 mL) were taken after adjustment of the pH and prepared for analysis of the dissolved phosphate concentration. Temperature and pH were measured with a WTW pH/cond 340 i meter.

5.2.4 Batch tests for struvite precipitation

Stirred beaker (0.6L) batch tests (magnetic stirrer, 100 – 250 rpm) were carried out to investigate phosphate removal by struvite precipitation in the UASB effluent, by adding a magnesium solution (2M $MgCl_2$). Six duplo tests were carried out, all during the period between day 265 – 410 of UASB reactor operation. Varying initial molar ratios of $Mg:PO_4$ (1.0:1, 1.3:1 and 1.8:1) and two different initial pH's were applied (original pH and pH of 9.0, adjusted with 4 M NaOH before addition of magnesium). Samples were taken before addition of magnesium and 15, 30 and 60 minutes after addition of magnesium. Samples were filtered immediately over an ashless black ribbon paper filter (Schleicher & Schuell) and prepared for analysis of the dissolved phosphate and magnesium concentrations. Temperature, pH, redox and conductivity were measured (WTW pH/cond 340 i meter) before taking the samples.

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[11] The amount of inoculum was negligible (chapter 2).

5.2.5 Microwave destruction for analysis of calcium, magnesium and phosphorus

Black water (5 samples, taken between day 1020 – 1050 of operation of the UASB reactor) was destructed to determine total concentrations of calcium and magnesium. UASB excess sludge (16 samples, taken regularly during the total period of operation of the UASB reactor) was destructed to determine the total concentration of phosphorus.

Destruction was done using the Ethos 1 Advanced Microwave digestion system of Milestone. Two grams of sample were put into a special microwave vessel, 10 mL of HNO_3 (68%) was added and milliQ water was added up to a total volume of 30 mL. The vessels were placed into the microwave. Samples were heated to 180 °C in 15 minutes and this temperature was maintained for another 15 minutes. After cooling down, the contents of the vessels were transferred to a 100 mL flask and diluted to 1% acid for analysis.

5.2.6 Analyses

TP was determined according to standard methods using commercially available DrLange ® test kits (Hach-Lange) (APHA, 1998). Samples were diluted 20 times prior to analysis to exclude possible interference of other ions on the analysis.

The free phosphate (PO_4-P) concentration in membrane filtered samples (0.45 µm Cronus filter PTFE) was determined by ion chromatography (Metrohm 761 Compact IC). Total soluble phosphorus ($TP_{soluble}$) in membrane filtered samples (0.45 µm Cronus filter PTFE) was determined with ICP-OES (Inductively coupled plasma optical emission spectrometry, Perkin Elmer Optima 5300 DV).

Concentrations of Ca, Mg, P in membrane filtered samples (0.45 µm Cronus filter PTFE) were determined by ICP-OES (Perkin Elmer Optima 5300 DV). Before analysis by ICP-OES, samples were acidified to 1% acid (HNO_3).

Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) were determined according to standard methods (APHA, 1998) using the ashless black ribbon paper filter (Schleicher & Schuell).

5.2.7 Analysis of heavy metals in sludge

Three samples of UASB sludge (two from the bottom (day 1060 and one wasted sludge sample on day 729)) and two samples of sludge from the UASB-septic tank (HRT of 20 days) in the demonstration site in Sneek (Friesland, NL) were analyzed for heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb and Zn) by Eurofins Analytico. The sludge samples were destructed and analyzed by ICP-AES (Inductively coupled plasma atomic emission spectroscopy) according to NEN 6966 and EN 1483 for Hg (Eurofins Analytico Food B.V.).

5.2.8 Simulation of chemical precipitation in black water

To gain more insight in chemical precipitation that might occur in the black water and in the UASB reactor, these processes were simulated in the OLI Stream Analyzer (version 2.0, OLI Systems). A theoretical composition of black water was calculated using literature data on the composition of faeces and urine and using the composition of drinking water in Friesland[12] (table 5.1). Two different dilution factors were used to simulate the use of vacuum toilets and conventional flush toilets.

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[12] The composition of drinking water in Friesland (NL) can be found on the website of the drinking water company Vitens: www.vitens.nl, visited on 11th November 2006.

Table 5.1 Calculated composition of black water, collected with a vacuum toilet (dilution factor 5) and with a conventional toilet (dilution factor 21). The calculations are based on average numbers collected by (Kujawa-Roeleveld, 2005); data on concentrations of Na^+, Cl^- and SO_4^{2-} in urine were taken from (Maurer et al., 2006); the composition of drinking water in Friesland (NL) was taken from the website of the drinking water company Vitens (www.vitens.nl).

	Faeces (Kujawa-Roeleveld, 2005)	Urine (Kujawa-Roeleveld, 2005)		Drinking water (Friesland, NL)	Black water vacuum toilet, calculated	Black water conventional toilet, calculated
Flow	0.12 kg_{faeces}/p/d ≈ L_{faeces}/p/d	1.4 L/p/d		6 L/p/d (vacuum) 30 L/p/d (conventional)	7.5 L/p/d	31.5 L/p/d
Dilution factor	-	-		-	5	21
	g/p/d	g/p/d	mg/L (Maurer et al., 2006)	mg/L	mg/L	mg/L
CODt	50.1	11	-	-	8152	1940
TN	1.75	9	-	-	1434	341
NH_4^+-N	-	-	-	-	1200 (urine)*	286 (urine)*
TP	0.5	0.8	-	-	173	41.2
PO_4^{3-}-P	-	-	-	-	107 (urine)*	25 (urine)*
Ca^{2+}	0.53	0.2	-	33	124	54.6
Mg^{2+}	0.18	0.2	-	9.7	51	12.1
K^+	0.9	2.7	-	2	487	116
Na^+	-	-	3450	74	692	221
Cl^-	-	-	4970	31	936	246
SO_4^{2-}	-	-	505	10	101	31.6
NO_3^-	-	-	-	12	9.6	11.4
HCO_3^-**	-	-	-	-	5490	1525

* It is assumed that all soluble nitrogen and phosphorus originates from urine and that it is in the form of NH_4^+-N (product of hydrolysis of urea) and PO_4^{3-}-P (Vinnerås, 2002).

** The bicarbonate concentration was adjusted to fulfil the charge balance.

The numbers of table 5.1 were entered in the OLI Stream analyzer as free ions, except for COD, TN and TP. The dominant charge balance was calculated and the amount of bicarbonate (HCO_3^-) was adjusted such that the stream was neutral in charge. To predict the precipitation potential, the scaling tendency was calculated, which was defined as the ratio of the activity product for an equilibrium to the solubility product

K_{sp} for the same equilibrium by OLI Systems[13]. A pH survey by adding NaOH and HCl was carried out from pH 5 to 10 to determine the effect of pH on the composition of the black water. Because struvite is not present in the main database of OLI Stream analyzer, a separate database was used, provided by OLI Systems Inc. Details about chemical models, databases and about OLI Stream analyzer can be found on the website of OLI Systems[14].

5.3 Results

5.3.1 Phosphorus mass balance of the UASB reactor

Figure 5.1 shows the phosphorus mass balance over the UASB reactor. Only 61% of the TP left the UASB reactor with the effluent, 18% was wasted with the sludge and 8% accumulated in the reactor. About 13% of the TP was missing, which could be due to errors introduced by sampling and analysis. Furthermore some precipitates were observed on the walls and bottom of the UASB reactor, which were not wasted with excess sludge and could therefore partly explain the missing TP. The mean phosphorus content in the UASB sludge was 51 mgP/gTSS (s.d.[15] = 12), which is much higher than the typical content of bacteria cells of 2.0% (20 mgP/g dry weight, (Tchobanoglous et al., 2003)), indicating precipitation.

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[13] When the scaling tendency is equal or greater than 1.0, the solid is said to have a thermodynamic driving force to form and the solid is considered to be at saturation. When the ratio is less than 1.0, the solid does not have the driving force to form. http://www.olisystems.com/oliscale2.htm, visited on 16-10-2009

[14] http://support.olisystems.com/Database/UpdateListings/Update7.0/DatabaseUpdate.html, visited on 16-10-2009

[15] s.d. = standard deviation

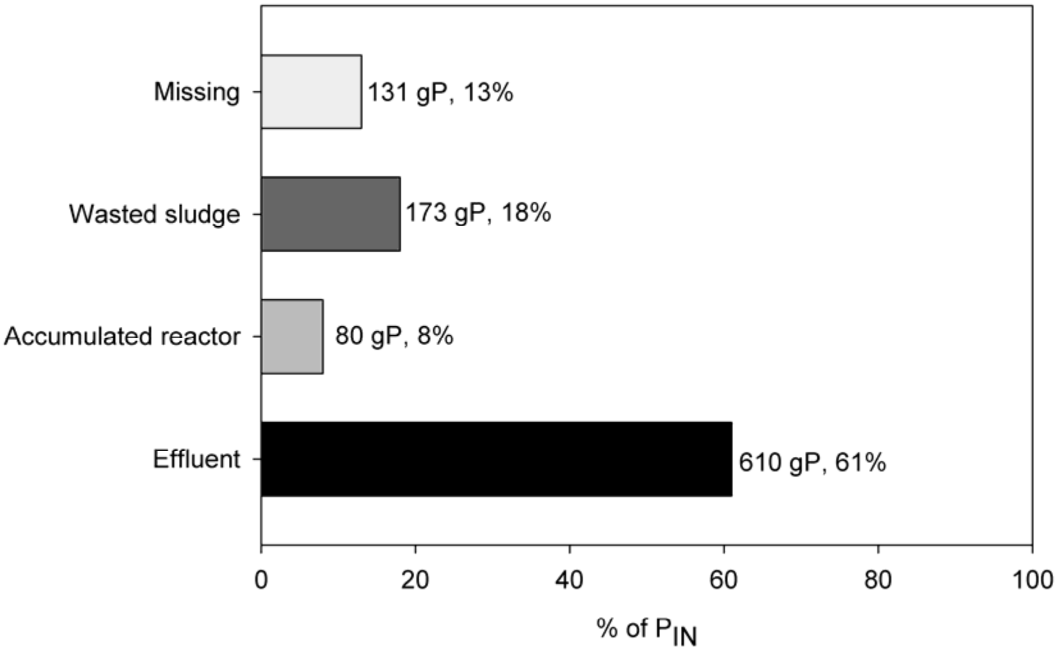

Figure 5.1 Phosphorus balance of the UASB reactor treating concentrated black water over the first 951 days of operation; numbers are the accumulative load of phosphorus (The total amount of TP fed to the reactor during this period was 994 gP).

5.3.2 Characterization of concentrated black water

Table 5.2 gives the measured concentrations of influent black water. The TP of the black water of 220 mg/L in the first period and 148 mg/L in the second period was in a similar range as calculated in table 5.1 (173 mg/L), taking into account that in the first period the black water was produced at 5 L/p/d and in the second period at 7.8 L/p/d.

The results of table 5.2 show that 60% of the phosphorus in black water was present as precipitates or was associated with suspended solids. Because the faeces exist for a large part of bacteria (Vinnerås, 2002), it was estimated that 67 mgP/L in first period and 54 mgP/L in the second period was organically bound in suspended solids (using a typical phosphorus content of bacterial cells of 2.0% (Tchobanoglous et al., 2003)). This corresponds with 52 – 59% of the TP associated with solids. The remaining phosphorus in the suspended solids could be mineral calcium phosphates originating from faeces (Vinnerås, 2002) and other precipitates from urine such as struvite (Udert et al., 2003a), but this was not further quantified.

Most of the soluble phosphorus ($TP_{soluble}$) was present as free phosphate (PO_4-P), which was 36% of TP_{IN} (table 5.2). In table 5.1 a much higher PO_4-P concentration of

107 mg/L was estimated, assuming that all phosphate from urine was excreted in soluble inorganic form (Vinnerås, 2002). During transport and storage of black water free phosphate from urine may have precipitated, resulting in relatively low PO_4-P concentrations in the black water, respectively 79 mg/L and 54 mg/L in the first and second period. Research on black water with a dilution factor of 21, showed a PO_4-P concentration of 27 mg/L (van Voorthuizen et al., 2008), which corresponds well with the calculated value in table 5.1 of 25 mgP/L.

The results in table 5.2 also show that total Ca and Mg concentrations were similar as predicted in table 5.1 and were mostly associated with solids. Batch tests with black water at 7 °C in which the pH was decreased to 6.0, showed an increase of the soluble phosphate concentration by 25% (average result from three tests, results not shown). This indicates that part of the phosphorus was present as inorganic solids and solubilised depending on the pH. Therefore the pH in black water affects the phosphorus distribution between liquid and solids.

Table 5.2 Measured characteristics of influent concentrated black water.

	unit	UASB Influent (concentrated black water) (125 samples)				% of TP_{IN}
		1st period (day 1 – 518)	s.d.	2nd period (day 519 – 951)	s.d.	
pH	-	8.8	0.22	8.6	0.53	
TP	mg/L	220	67	148	64	
TP_{SS}= TP – $TP_{soluble}$	mg/L	130	-	91	-	60%
$TP_{soluble}$	mg/L	90	8.7	57	18	40%
PO_4-P	mg/L	79	8.5	54	27	36%
$Ca_{soluble}$	mg/L	36	18	41	17	
$Mg_{soluble}$	mg/L	3.0	5.4	4.9	4.3	
VSS	g/L	3.3	1.4	2.7	1.4	
Total amount of calcium and magnesium (5 samples)						
Ca total	mg/L	140	14			
Mg total	mg/L	38	5			

s.d. = standard deviation

5.3.3 *Characterization of UASB effluent*

During anaerobic treatment the free phosphate concentration increased from 79 mgPO_4-P/L to 92 mgPO_4-P/L in the first period and from 54 to 69 mgPO_4-P/L in the second period (tables 5.2 and 5.3). This probably was caused by hydrolysis of suspended solids containing organically bound phosphorus and by a decrease in pH during anaerobic treatment (from 8.6 – 8.8 in black water to 7.4 – 7.6 in UASB effluent, tables 5.2 and 5.3) solubilising inorganic phosphates. The effluent contained 61% of the TP in the influent black water, of which the main fraction was present as free phosphate (44% of the influent load of TP). A small fraction of the phosphorus left the UASB reactor associated with solids in the effluent, on average 14% (table 5.3).

Table 5.3 Measured characteristics of the UASB effluent.

	unit	UASB Effluent (125 samples)				% of TP_{IN}
		1st period (day 1 – 518)	s.d.	2nd period (day 519 – 951)	s.d.	
pH	-	7.6	0.13	7.4	0.17	
TP	mg/L	131	15	94	18	61%
TP_{SS}= TP – $TP_{soluble}$	mg/L	28	-	24	-	14%
$TP_{soluble}$	mg/L	103	10	70	11	47%
PO_4-P	mg/L	92	11	69	13	44%
Ca soluble	mg/L	41	21	44	6.5	
Mg soluble	mg/L	7.9	5.7	14	2.7	
VSS	g/L	0.15	0.12	0.053	0.066	

s.d. = standard deviation

5.3.4 Simulation of chemical precipitation

Two aspects seem to play an important role regarding the availability of free phosphate in the black water and during anaerobic treatment for subsequent phosphorus recovery by struvite precipitation. The first aspect was the amount of flushing water as mentioned in the introduction. The second aspect was the pH during transport, storage and anaerobic treatment of black water. Both aspects were further investigated by simulating this chemical precipitation in the black water. The results are presented in table 5.4 and figure 5.2.

Black water with a dilution factor of 21 has lower precipitation potentials and the amount of inorganic precipitate is lower than for concentrated black water with a dilution factor of 5 (table 5.4). The predicted pH in both cases is lower than measured. A reason why the pH was not properly predicted, could be because the composition that was calculated in table 5.1 was not complete. For example, organic acids such as acetate and other ions such as OH^- were not included, which have an effect on the pH as well.

Table 5.4 Simulated precipitation potentials and the amount of inorganic solids in black water at two different dilution factors and two different pH values.

	Concentrated black water, vacuum toilet, dilution factor 5		Black water, Conventional toilet, dilution factor 21	
pH	7.7[a]	8.7[b]	7.7[a]	8.3[b]
Precipitation potential:				
$Ca_5(OH)(PO_4)_3$	1.0	1.0	1.0	1.0
$CaCO_3$	0.72	1.0	0.88	1.0
$Mg(NH_4)PO_4.6H_2O$	1.0	1.0	0.018	0.074
$MgCO_3.3H_2O$	0.0087	0.010	0.0018	0.0067
$MgHPO_4.3H_2O$	0.028	0.0039	0.0019	0.0023
$CaHPO_4$	0.012	0.0021	0.0049	0.0019
Precipitated solids:				
total amount of solids (mol/L)	$1.4*10^{-3}$	$4.5*10^{-3}$	$2.6*10^{-4}$	$3.4*10^{-4}$
$Ca_5(OH)(PO_4)_3$ (mol/L)	$6.1*10^{-4}$	$1.8*10^{-4}$	$2.6*10^{-4}$	$2.5*10^{-4}$
$Mg(NH_4)PO_4.6H_2O$ (mol/L)	$8.1*10^{-4}$	$2.1*10^{-3}$	0	0
$CaCO_3$ (mol/L)	0	$2.2*10^{-3}$	0	$9.1*10^{-5}$

[a] These pH values were calculated by OLI Stream Analyzer

[b] These pH values were measured in this research and in previous research (van Voorthuizen, 2009)

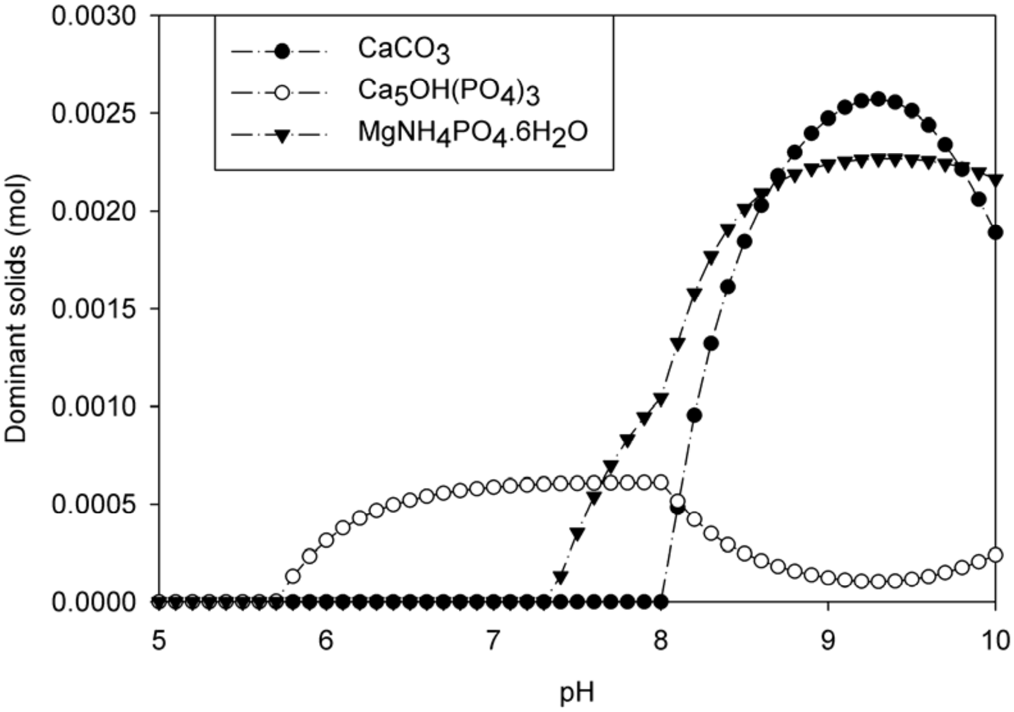

Figure 5.2 The predicted effect of pH in concentrated black water on the precipitation of calcite ($CaCO_3$), hydroxyapatite ($Ca_5OH(PO_4)_3$) and struvite ($MgNH_4PO_4.6H_2O$).

The results in figure 5.2 clearly show that struvite is likely to precipitate when the pH in black water is higher than 7.4. Because the pH of the black water used in this research attained values of 8.6 – 8.8 (chapter 2), struvite and hydroxyapatite will likely precipitate during storage and transport. It should be mentioned that kinetics are not taken into account in the simulation, which will have an effect on the type of precipitates formed (Musvoto et al., 2000). Therefore, the results from the simulation are indicative, showing the possible effect of the amount of flushing water and pH.

5.3.5 Struvite precipitation batch tests with UASB effluent

Batch precipitation tests were carried out at different pH values (8 or 9) and at different initial molar ratios of magnesium to phosphate. The results in figure 5.3 show a fast removal of phosphate from the liquid phase within 15 minutes at a pH of 9.0. Visual observation showed a white and fine precipitate.

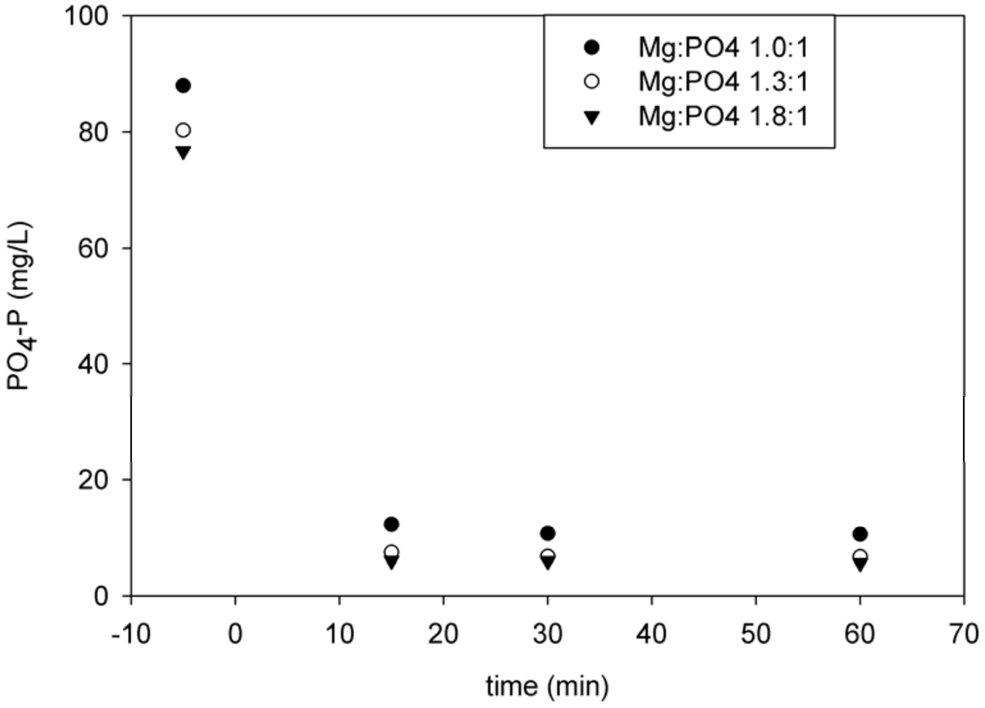

Figure 5.3 Phosphate removal from UASB effluent by the addition of magnesium at pH 9.0 for different starting molar ratios of Mg:PO_4 (average values of duplo tests). At time 0 the $MgCl_2$ solution was added.

The results in table 5.5 show that the removal efficiencies of phosphate depended on the applied pH as well as the surplus of magnesium that was added. Either by adding a surplus of magnesium (e.g. Mg:PO_4 1.5:1 at pH 8.0) or by adjusting the pH to 9.0 at a lower Mg:PO_4 ratio of 1.3:1, a removal efficiency of 90% could be achieved. A higher Mg:PO_4 ratio of 1.8:1 did not significantly contribute to an improved removal efficiency (table 5.5), which was also shown in figure 5.3. Using these results, 0.15 kg PO4-P/p/y can be recovered in the form of struvite (based on a PO4-P concentration of 92 mg/L and a black water production of 5 L/p/d). Because together with the struvite, suspended solids will be removed as well, a maximum of 0.22 kgP/p/y can be recovered from UASB effluent (based on 131 mg TP/L). Obviously, latter will reduce the purity of the produced struvite.

Table 5.5 Phosphate removal efficiencies in UASB effluent by the addition of different amounts of magnesium and at different pH after 60 minutes.

molar ratio Mg : PO_4	pH 8.0 (s.d.)	pH 9.0 (s.d.)
1.0:1	72% (3.5%)	82% (8.2%)
1.3:1	-	91% (0.7%)
1.5:1	90% (0.0%)	96% (0.7%)
1.8:1	-	92% (0.7%)

5.4 Discussion

5.4.1 The fate of phosphorus in black water and during anaerobic treatment

During anaerobic treatment of concentrated black water in a UASB reactor, 61% of the phosphorus was conserved in the effluent, while the remaining phosphorus was mainly discharged with the sludge. Simulation of the phosphorus distribution over the liquid and solid fraction in the raw black water, has shown a strong effect of pH and concentration of the black water (different dilution factors). Similar effects can be expected for the phosphorus distribution in the UASB reactor. This was also shown for the separate collection of urine, where the risk of blockages can be diminished by urine dilution (Udert et al., 2003a).

Although a higher fraction of phosphorus was conserved in the effluent of the UASB reactor at lower black water concentrations (higher dilution factor), a low dilution is to be favoured. A low dilution reduces the amount of water consumption, limits the energy consumption for heating the influent of the anaerobic reactor and can improve the potential to remove pathogens and micro-pollutants in a post treatment step.

The composition of tap water will also have a large effect on the concentrations in black water, because the tap water that was used in this research was relatively soft compared to tap waters in other regions or countries (e.g. tap water in Germany contains up to 199 mg/L of calcium, (Oldenburg et al., 2008)). The use of hard tap water will result in more precipitation of calcium phosphates in concentrated black water, reducing the availability of PO_4-P in the anaerobic effluent.

Because methanogenesis has an optimum between a pH of 6.5 and 7 (Koster and Koomen, 1988), lowering the pH for increased phosphorus conservation might be an option, although the additional chemical costs would be a drawback of such an approach. Acid or the produced CO_2 in the biogas could be used to lower the pH in the UASB reactor. Currently autogenerative high pressure biogas processes to produce biogas with the same quality as natural gas are under investigation[16], which will reduce the pH during anaerobic treatment, because CO_2 remains in liquid phase and possibly helps to release more phosphate into solution.

On the other hand, the precipitation potential of struvite is high during anaerobic treatment of concentrated black water, and struvite precipitation and anaerobic treatment might be combined, producing a P-rich sludge (e.g. (Hwang and Choi, 1998)). This however will require a pH above 8.0 in the UASB reactor. Preliminary results show that this will reduce the specific methanogenic activity and therefore a larger reactor will be required (data not shown). However, in the long term, it is known that methanogenic bacteria can adapt to these higher pH values. More research is needed to investigate the feasibility of this combination, for example with respect to reactor configuration to prevent blockages caused by uncontrolled struvite precipitation (Le Corre et al., 2009).

5.4.2 Reuse of anaerobic sludge

The reuse of UASB sludge as a phosphorus-enriched organic fertilizer would optimize the phosphorus recovery. The quality of the sludge with respect to heavy metals, pathogens and micro-pollutants content will determine the latter possibility.

Present legislation is limited to heavy metals content. For this reason the UASB sludge was analyzed for the presence of heavy metals. Because only a few sludge samples were analyzed, these results are indicative. In table 5.6 the heavy metals content is shown for the UASB sludge in comparison with sewage sludge produced in conventional WWTPs and existing guidelines from the Netherlands and the EU. Except for Cu and Zn all heavy metals were below the Dutch guidelines. As expected,

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[16] http://www.onderzoekinformatie.nl/en/oi/nod/onderzoek/OND1335788/, visited 20th November 2009

sewage sludge contained more heavy metals due to e.g. industrial wastewater, than the UASB sludge. Compared to cow manure the UASB sludge was relatively clean with respect to heavy metals, although commercial phosphorus fertilizer contains even lower Cu, Hg, Pb and Zn concentrations (table 5.7). More research is needed on the amount of micro-pollutants such as hormones and pharmaceutical residues, because their emission into the environment is a growing concern (Daughton and Ternes, 1999; Winker et al., 2009).

Table 5.6 Amounts of heavy metals in UASB sludge and sewage sludge, compared with existing guidelines for application of sewage sludge in agriculture.

Heavy metals	UASB sludge (3 samples) (mg/kg DW)	UASB sludge septic tank (Meulman et al., 2008) (2 samples) (mg/kg DW)	Sewage sludge (mg/kg DW)	Dutch guideline (BOOM[17]) (mg/kg DW)	EU guideline in preparation, long term (mg/kg DW)
reference	This study	This study	(CBS-Statline, 2007a)	IVM/VROM[18]	
As	<10	< 10	10.3	15	-
Cd	0.7	0.87	1.5	1.25	2
Cr	5.7	12.5	42	75	-
Cu	197	255	407	75	600
Hg	0.3	0.4	0.9	0.75	2
Ni	8.8	10.5	32	30	100
Pb	<10	18	137	100	200
Zn	713	900	1032	300	1500

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[17] Dutch abbreviation: BOOM = Besluit kwaliteit en gebruik overige organische meststoffen

[18] http://www.eu-milieubeleid.nl/ch05s10.html, visited on 25-09-2009; Manual of Environmental Policy, in Dutch

Table 5.7 Amounts of heavy metals in UASB sludge in mg/kgP, compared with cow manure and commercial fertilizer.

Heavy metals	UASB sludge (3 samples) (mg/kgP)	UASB sludge septic tank (Meulman et al., 2008) (2 samples) (mg/kgP)	Cow manure (mg/kgP)	P fertilizer (mg/kgP)
	This study	This study	(van Dooren et al., 2005)	(Remy and Ruhland, 2006)
As	< 185	< 185	-	33.2
Cd	13	16	32.7	90.5
Cr	105	232	1145	1245
Cu	3642	4722	14397	207
Hg	6.1	7.4	-	0.7
Ni	162	194	1472	202
Pb	185	333	695	154
Zn	13210	16667	25947	1923

Recently sewage ashes are regarded as a potential source for the phosphorus industry. When application of the UASB sludge as a P-enriched fertilizer is not possible and incineration is unavoidable, this could be an option. One of the requirements is that the iron content is low (Fe/P molar ratio of < 0.2) by applying biological phosphorus removal or precipitation with aluminium (Schipper et al., 2007; Reitsma et al., 2008). The maximum amount that may be recycled from sewage in the Netherlands was estimated to be 12 ktP/year, based on the difference between influent and effluent phosphorus concentrations of WWTPs in the Netherlands (CBS-Statline, 2007b). However, heavy metals like cupper and zinc in the ashes are above the required maximum concentrations (500 mg Cu/mg ash and 1000 mg Zn/mg ash), and are a concern because they tend to build up in the phosphorus production process (Schipper et al., 2007).

5.4.3 Phosphorus removal by struvite precipitation from the UASB effluent

By addition of a surplus of magnesium and/or adjusting the pH to 9.0, 90% of the free phosphate in the UASB effluent can be recovered, which was similar as in other research reviewed by Le Corre (2009) (60 – 98%). In this manner a maximum of 0.22 kgP/p/y can be recovered from the concentrated black water used in this research. This is lower than the total amount of phosphorus present in black water (0.40 kgP/p/y), due to the phosphorus removal that already takes place in the UASB reactor. The recovered struvite would represent 17% of the artificial phosphorus fertilizer production in the Netherlands (21 ktP/year, (CBS-Statline, 2008)) and 10% of the artificial phosphorus fertilizer production worldwide (14.9 million tP/year, (Cordell et al., 2009)).

The quality of the produced precipitate was not further investigated. Because the added magnesium was removed from the liquid phase together with phosphate and ammonium was abundantly present, the precipitate consisted most probably mainly of struvite. However, it is likely that some calcium precipitated as well in the form of calcium carbonate or calcium phosphates. This was also shown by a physical-chemical kinetic model developed to simulate chemical reactions in anaerobic supernatants (Musvoto et al., 2000). For an effective struvite production the Ca:Mg ratio should be lower than 1, and therefore usually an excess of magnesium is required (Hwang and Choi, 1998). In UASB effluent the Ca:Mg molar ratio is 1.9 and by adding a surplus of Mg (in a molar ratio Mg:PO_4 1.3:1) the ratio of Ca:Mg will decrease to below 1.

Precipitation of struvite from urine showed that the struvite can be recovered free from organic micro-pollutants and free from most of the heavy metals present in urine (Ronteltap et al., 2007a). More research with respect to micro-pollutants in struvite recovered from UASB effluent is needed, because the co-precipitated solids and organic material may contain adsorbed micro-pollutants.

Struvite precipitation can also be applied after biological nitrogen removal. Advantages will be that less organic matter is present and that most carbonate has been removed, resulting in a higher grade struvite. Instead of ammonium potassium, which is abundantly present in black water, will be incorporated into the struvite crystal. The production of potassium struvite was demonstrated at full scale for the recovery of phosphate by struvite precipitation from calf manure (Schuiling and Andrade, 1999).

The main challenges to make struvite precipitation for phosphate recovery a successful technology, are the quality of the struvite and the economic value of the struvite (Le Corre et al., 2009). In Japan struvite produced from digester liquor was successfully sold to fertiliser companies (Ueno and Fujii, 2001). Recently the struvite produced from an industrial wastewater treatment plant treating potato wastewater in Steenderen (NL) is being used by a company producing cattle fodder[19], showing an increasing interest for struvite as a valuable product.

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[19] http://www.netserver2.net/waterforum/index.asp?url=/template_a1.asp&que=paginanr=4894, news item in Dutch, 31st March 2009

5.5 Conclusions

Phosphate in highly concentrated black water precipitated for a significant part as inorganic solids during transport and storage. Simulation of chemical precipitation showed a strong effect of pH and concentration in the black water (different dilution factors). In the anaerobic effluent of a UASB reactor treating concentrated black water, 61% of the phosphorus was conserved. Phosphate can easily be removed from anaerobic effluent by struvite precipitation, recovering 0.22 kgP/p/y, which represents 10% of the artificial phosphorus fertilizer production in the world. The remaining phosphorus ended up in the anaerobic sludge due to the low dilution factor. This sludge might be used as a phosphorus-enriched organic fertilizer, provided that it is safe regarding heavy metals, pathogens and micro-pollutants.

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6

Fate of hormones and pharmaceuticals during combined anaerobic treatment and nitrogen removal by nitritation- anammox in vacuum collected black water

Abstract

Vacuum collected black water contains hormones and pharmaceuticals in relatively high concentrations (µg/L to mg/L range) and separate specific treatment has the potential of minimizing their discharge to surface waters. In this study, the fate of estrogens and pharmaceuticals in the anaerobic treatment of vacuum collected black water followed by nitrogen removal by nitritation-anammox was investigated. Even though black water is a very complex matrix, it was possible to detect the presence of a selection of hormones and pharmaceuticals. Anaerobic treatment was only suitable to remove the majority of paracetamol. Metoprolol and ibuprofen were (partly) removed during aerobic treatment. Diclofenac was not removed. The presence of persistent micro-pollutants (carbamazepine and cetirizine), which are not susceptible for biodegradation, makes the application of advanced physical and chemical treatment unavoidable.

M.S. de Graaff, N.M. Vieno, K. Kujawa-Roeleveld, G. Zeeman, H. Temmink and C.J.N. Buisman

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6.1 Introduction

Separation of domestic wastewater at source results in concentrated black water from the toilet (faeces and urine) and less concentrated grey water from showers, laundry, etc. Black water contains half the load of organic material in domestic wastewater and the major fraction of the nutrients nitrogen and phosphorus (Otterpohl et al., 1999; Kujawa-Roeleveld and Zeeman, 2006). Furthermore, black water contains micro-pollutants such as hormones and pharmaceutical residues. In conventional wastewater treatment plants (WWTPs) these micro-pollutants, present in ng/L to µg/L range, are not or only partially removed and present a threat to aquatic life (Schwarzenbach et al., 2006; Ternes and Joss, 2006). The risk micro-pollutants may pose is difficult to assess and not well understood (Schwarzenbach et al., 2006; Schirmer and Schirmer, 2008), but several effects have been reported, such as feminization or masculinisation of fishes already at ng/L levels (Harries et al., 1997). In source separation based treatment concepts the micro-pollutants in black water are present at higher concentrations (µg/L to mg/L range) and specific treatment of this concentrated stream may reduce their discharge to surface waters compared to WWTPs (Larsen et al., 2004; Larsen et al., 2009).

Previous research showed that black water, collected with vacuum toilets, can be efficiently treated in a UASB (Upflow Anaerobic Sludge Blanket) reactor at a relatively short hydraulic retention time (HRT) of 8.7 days (chapter 2). The two reactor nitritation-anammox process successfully removed the ammonium from the UASB effluent (chapters 3 and 4). The biodegradation potential of a number of selected pharmaceuticals under different redox conditions has been investigated in batch tests, but little is known about their removal and biodegradation during continuous biological treatment of concentrated streams (Kujawa-Roeleveld et al., 2008). Under anaerobic conditions and at long retention times (30 days) only a few compounds (acetylsalicilic acid, ibuprofen and fenofibrate) were degraded. Under aerobic conditions the rate of biodegradation of those compounds was much higher (Kujawa-Roeleveld et al., 2008). The fate of estrogens during biological treatment of vacuum collected black water was studied in detail by de Mes (2007) and adsorption and biodegradation were the main removal processes. Furthermore it was shown that a significant amount (> 70%) of the estrogens was present in conjugated form, showing a limited deconjugation during the treatment[20]. Additional treatment is necessary to reduce the emission of estrogens to surface waters, because the effluent concentrations were still in μg/L range (1.4 μg/L E1 and 0.65 μg/L E2, (de Mes, 2007)).

The following compounds were studied, based on their potential harmful effects in the environment, their consumption (amount and frequency), removal in WWTPs and their therapeutic group: antiphlogistics (paracetamol, diclofenac, ibuprofen), antibiotics (tetracycline, doxycycline, ciprofloxacin, trimethoprim), beta blockers (metoprolol and propranolol), antihistamine (cetirizine), antiepileptic (carbamazepine), natural hormones (estrone (E1) and 17β-estradiol (E2), birth control pill (17α-ethynylestradiol (EE2)) and conjugated natural estrogens (E2-17G and E2-3S). Antiphlogistics such as paracetamol are widely used and in the Netherlands can be obtained without prescription. Paracetamol is readily degradable, whereas ibuprofen and diclofenac are only partially removed in conventional WWTPs (Schrap et al., 2003). The antibiotics were selected because of the increasing concern about the formation of resistant pathogen strains (Mückter, 2006). In the Netherlands in 2005, metoprolol was number 1 in the top 10 list of prescribed pharmaceuticals and diclofenac was number 4 in this list (Griens and Tinke, 2006). Beta blockers, such as metoprolol and propranolol, are used for the treatment of vascular diseases and hypertension, and these kind of diseases are the ones for which most money is spent in the Netherlands (Griens and Tinke, 2006). Antihistamines, such as cetirizine are widely used for allergies and asthma and have a high biological activity, posing a potential risk in the environment (Kosonen and Kronberg, 2009). The non-biodegradable and non-adsorptive Carbamazepine (anti-epileptic) was selected, because it was proposed as a marker for the sewage contamination of surface water and groundwater (Clara et al., 2004; Nakada et al., 2008). Human estrogens (E1 and E2) and the synthetic estrogen EE2 are not completely removed in WWTPs and they are mainly responsible for the endocrine disrupting effects seen in the aquatic environment (Desbrow et al., 1998).

In this study, the fate was investigated of the above selected estrogens and pharmaceuticals during anaerobic treatment of vacuum collected black water followed by nitrogen removal by nitritation-anammox. Their removal rates and

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[20] Conjugated compounds are usually the more soluble and inactive forms of the parent compound formed in the human body. In faeces enzymes are present that can hydrolyse conjugates back into their original and therefore active form (Ternes et al., 1999).

possibilities for physical-chemical post treatment in new sanitation systems based on separation at source are discussed.

6.2 Materials and methods

6.2.1 Treatment of black water

Black water, collected in vacuum toilets, was obtained from the DESAR (Decentralized Sanitation and Reuse) demonstration site in Sneek (Friesland, the Netherlands) (Zeeman et al., 2007). Every two weeks jerry cans were filled with black water from the buffer tank at the demonstration site (hydraulic retention time of 4 h, not cooled), transported to the lab and stored at 4 ˚C. The black water was first anaerobically treated in a UASB reactor. The effluent of the UASB reactor was subsequently treated in a two reactor nitritation-anammox process to remove the nitrogen. The treatment concept is shown in figure 6.1. The UASB reactor was operated at 25 ˚C, an HRT of 8.7 day and a SRT of 254 days (chapter 2). The partial nitritation reactor was operated at 25 ˚C, an HRT of 1.7 days and a SRT fluctuating between 1 and 17 days (chapter 3). The anammox SBR35 was operated at 35 ˚C and an HRT of 2.0 to 10 days, depending on the applied hydraulic load (chapter 4).

6.2.2 Samples

Grab samples (15 mL) were taken every 3 to 4 weeks from the black water influent tap (tap 0, figure 6.1) to the UASB reactor (BW), from the UASB effluent, from the effluent of the partial nitritation reactor (PN effluent) and from the effluent of the anammox SBR35 (AMX effluent) and stored at -20 °C. Details about the number of samples and period of operation can be found in table 6.1.

Table 6.1 Details about samples selected for analysis.

Matrix	Nr of samples	Period of operation
BW	15	day 672 – 1070 (UASB reactor, chapter 2)
UASB	18	day 672 – 1070 (UASB reactor, chapter 2)
PN	18	day 425 – 802 (PN reactor, chapter 3)
AMX	18	day 96 – 386 (Anammox SBR35, chapter 4)

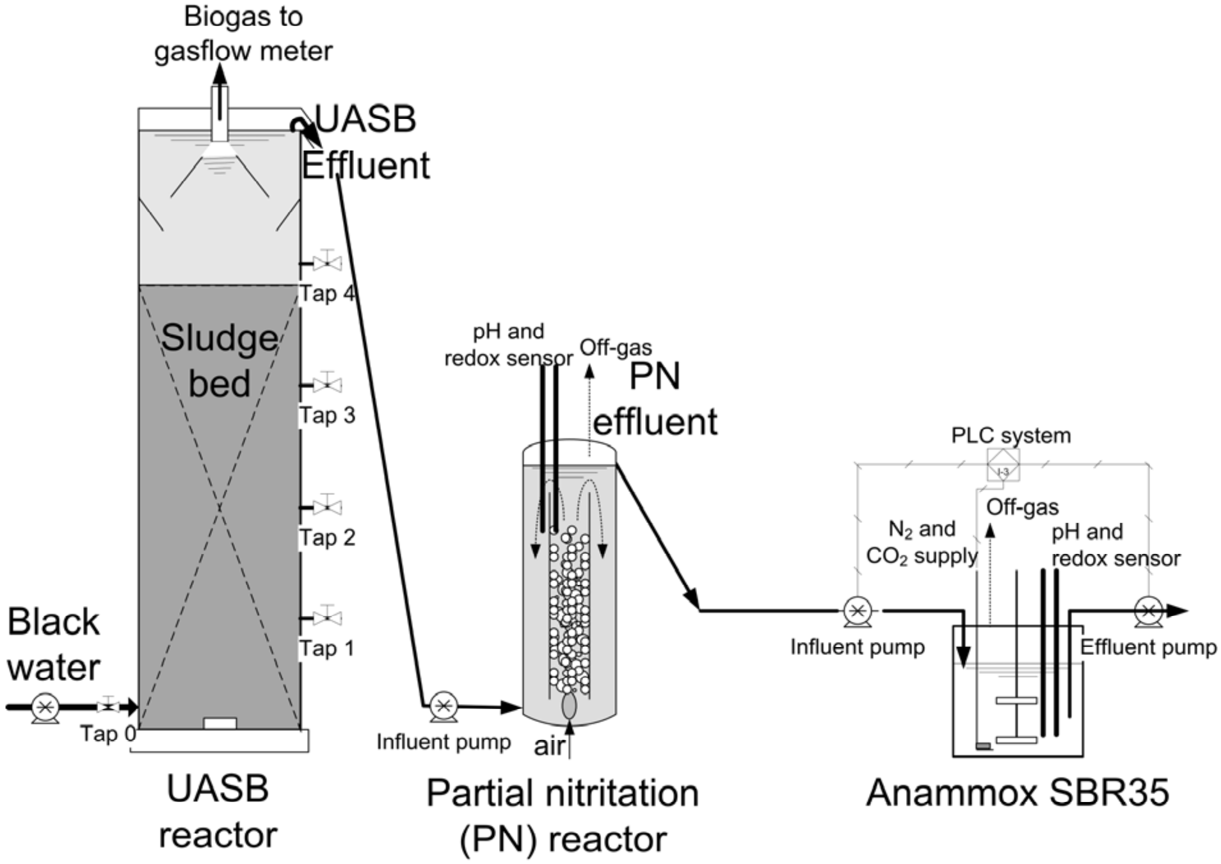

Figure 6.1 Treatment concept for black water.

6.2.3 Chemicals and glassware

All chemicals were purchased from Sigma-Aldrich. Table 6.2 shows the properties of the studied micro-pollutants. Stock solutions of 1 g/L of the analytes were prepared in methanol, except for ciprofloxacin and enrofloxacin which were prepared in a solution of 80% methanol and 20% 0.1M NaOH. From these stock solutions, working solutions of 10 mg/L and 1 mg/L in methanol were prepared.

All the glassware was cleaned and deactivated to prevent interactions of compounds with glass. First the glassware was rinsed with hot tap water several times, then three times with milliQ-water, three times with acetone and once with ethylacetate. The glassware was dried for 10 hours at 350 °C. After cooling down, the glassware was rinsed with saturated methanolic solution of Na_2EDTA and dried over night.

Table 6.2 The studied hormones and pharmaceuticals, their therapeutic use, CAS numbers, molecular weight (MW), $\log K_{ow}$ and pK_a.

Compound	Therapeutic use	CAS number	MW (1)	$\log K_{ow}$ (1)	pK_a
Paracetamol (Acetaminophen)	Analgesic	103-90-2	151.17	0.46	9.38 (1)
Metoprolol	Beta-blocker	56392-17-7	267.37	1.69	9.68 (2)
Propranolol	Beta-blocker	318-98-9	257.34	0.74	-
Cetirizine	Antihistamine	83881-51-0	388.9	-0.61	-
Doxycycline	Antibiotic	24390-14-5	444.45	-	3.02, 7.97, 9.15 (.HCl) (3)
Tetracycline	Antibiotic	60-54-8	444.4	-1.30	3.32, 7.78, 9.58 (.HCl) (3)
Ciprofloxacin	Antibiotic	85721-33-1	331.3	0.28	3.01, 6.14, 8.70, 10.58 (.HCl) (3) 6.27 (-COOH), 8.87 (=NH2+) (4)
Trimethoprim	Antibiotic	738-70-5	290.3	0.91	3.23, 6.76 (3) 6.6 (5)
Carbamazepine	Antiepileptic	298-46-4	236.27	2.47	13.9 (6)
Ibuprofen	Anti-inflammatory	15687-27-1	206.23	3.97	4.91 (1)
Diclofenac	Anti-inflammatory	15307-79-6 (15307-86-5)	296.16	4.51	4.14 (1)
E1	Steroid hormone	53-16-7	270.37	3.13	10.34 (7)
E2	Steroid hormone	50-28-2	272.39	4.01	10.46 (7)
EE2	Steroid hormone	57-63-6	296.41	3.67	10.40 (7)
E2-17G	Conjugated E2	15087-02-2	470.49*	-	-
E2-3S	Conjugated E2	4999-79-5	374.43*	-	-
Internal standards (I.S.):					
Fenoprop	I.S. for ibuprofen and diclofenac	93-72-1	269.51	3.8	2.84 (1)
Meclocycline	I.S. for doxycycline and tetracycline	73816-42-9	476.86	-	-
Diaveridine	I.S. for trimethoprim	5355-16-8	260.3	0.97	-
Enrofloxacin	I.S. for ciprofloxacin	93106-60-6	359.4	0.7	3.85, 6.19, 7.59, 9.86 (.HCl) (3) 6.21 (-COOH), 7.74 (=NH2+) (4)

- Data not found

* Sodium salt

(1)SRC Physprop Database: http://www.syrres.com/what-we-do/databaseforms.aspx?id=386, (2)http://www.drugs.com/mmx/metoprolol-succinate.html, (3)(Qiang and Adams, 2004), (4)(Schmitt-Kopplin et al., 1999), (5)(Beausse, 2004), (6)(Jones et al., 2002), (7)(Hurwitz and Liu, 1977); (8)(Joss et al., 2006b)

6.2.4 Off-line Solid-phase Extraction (SPE)

The detailed procedure for solid-phase extraction is given in appendix 6.I. Black water samples (10 mL) were filtered over a Whatman GF6 filter, which was pre-washed with ethylacetate, acetonitrile, milliQ water (Millipore) and saturated methanolic solution of Na_2-EDTA. The effluents (UASB, PN and AMX) were not filtered. The pH of the sample (filtered black water or effluents, 2.5 mL) was adjusted to a pH of 2 – 3 with 1M of citric acid and with pure formic acid (HCOOH). To separate the analytes from the water phase solid-phase extraction (SPE) was used with Oasis HLB 3cc (60mg) extraction cartridges. The analytes were eluted with 2.5 mL methanol. Part of the extract (0.45 mL) was transferred to a vial for direct analysis. The rest of the extract was evaporated to dryness with a nitrogen stream. The dried extract was dissolved into methanol and internal standard (I.S.) solution to achieve a concentration factor (CF) of 4.1 for the black water samples and a CF of 6.4 for the effluent samples. The extracts were stored at -20 °C until analysis.

6.2.5 Liquid Chromatography (LC)

The LC separations were performed on the LC (Agilent Technologies 1200 series) consisting of a binary pump, a vacuum degasser, a thermostated autosampler, and a thermostated column oven. Analytes were divided into two groups depending on their ionization mode in the electrospray source of the mass spectrometer (MS). Separate LC methods (A and B) were developed for the two groups (Appendix 6.II).

Method A consisted of the analytes that were positively ionized in the MS source. Sample aliquots of 2 µl were injected to Agilent Eclipse XDB-C18 Rapid Resolution HT (4.6 x 50 mm, 1.8 µm) column equipped with Agilent Eclipse XDB-C18 (4.6 x 12.5 mm, 5 µm) guard column. The column was eluted with 0.5 mM NH_4COOH + 0.1 mM oxalic acid + 0.1% HCOOH and acetonitrile at a flow rate of 500 µL min^{-1}. The elution started with 5 % acetonitrile and then with a linear gradient from 5 % to 50 % acetonitrile over the course of 12 min and to 100 % over the next 2 min. The amount of acetonitrile was held at this percentage for 4 min and finally lowered to 5% in 0.5 min. Before the next injection, the system was allowed to equilibrate for 10 min. The column oven was kept at 20 °C. Retention times of the analytes are presented in Appendix 6.II.

Method B consisted of the analytes that were negatively ionized in the MS source. Sample aliquots of 5 µl were injected to Agilent Zorbax Extend-C18 Rapid Resolution HT (2.1 x 50 mm, 1.8 µm) column equipped with Agilent Zorbax Extend-C18 Narrow-bore (2.1 x 12.5 mm, 5 µm) guard column. The column was eluted with 10 mM

NH_4OH and acetonitrile at a flow rate of 300 µL min^{-1}. The elution started with 5 % acetonitrile and then with a linear gradient from 5 % to 65 % acetonitrile over the course of 13 min and to 100 % over the next 1 min. The amount of acetonitrile was held at this percentage for 3 min and finally lowered to 5% in 0.5 min. Before the next injection, the system was allowed to equilibrate for 12.5 min. The column oven was kept at 30 °C. Retention times of the analytes are presented in Appendix 6.II.

6.2.6 Mass spectrometry

The flow from the LC column was transferred to a triple-quadrupole mass spectrometer (Agilent Technologies 6410 TripleQuad LC/MS) equipped with an electrospray ionization source. For both of the methods A and B, nitrogen was used as the desolvation gas at the source at a flowrate of 10 L h^{-1}. Nebulizer pressure was kept at 50 psi and the capillary voltage at 4 000 V. The source temperature was 320 °C. Nitrogen was used as the collision gas. Depending on the method, positive or negative ions were acquired in multiple reaction monitoring (MRM) mode. In order to achieve sensitive and selective detection of the analytes, the MS/MS parameters, i.e. the choice of precursor and product ions, and the fragmentor voltage and collision energy were optimized separately for each analyte (Appendix 6.II). This was done by injecting pure substances at the concentration of 10 µg mL^{-1} to the MS at flow injection analysis mode.

6.2.7 Recoveries

For all four matrices (BW, UASB effluent, PN effluent and AMX effluent) recoveries were determined by spiking them with a stock solution containing all the analytes and enriching with SPE as described above. The spiking level was 10 and 100 µg/L for all analytes and this was done in triplicate.

6.2.8 Adsorption of analytes to glass fibre filters

In order to determine how much of the analytes in black water matrix adsorbed to the glass fibre filter, milliQ water and black water was spiked with 100 µg/L of the analytes in triplicates. After filtration, the filter was washed with 5 mL of methanol (MeOH). Responses were analysed in the filtrates and in the filter methanol. The adsorption on the filter was calculated with the following equations:

$$\text{adsorption milliQ}\,(\%) = \frac{milliQ_{spiked,\,filter,\,MeOH}}{milliQ_{spiked,\,filtrate} + milliQ_{spiked,\,filter,\,MeOH}} \qquad (6.1)$$

$$\text{adsorption}(\%) = \frac{BW_{spiked,\,filter,MeOH} - BW_{blank,\,filter,MeOH}}{BW_{spiked,\,filtrate} - BW_{blank,\,filtrate} + BW_{spiked,\,filter,MeOH} - BW_{blank,\,filter,MeOH}} \quad (6.2)$$

where $\text{milliQ}_{\text{spiked,filter,MeOH}}$ and $\text{BW}_{\text{spiked,filter,MeOH}}$ are the response in the methanol that was used to wash the filter used for the spiked milliQ and spiked BW respectively, and $\text{milliQ}_{\text{spiked,filtrate}}$ and $\text{BW}_{\text{spiked,filtrate}}$ are the response in the filtrate.

6.2.9 Method validation

For each run on the LC-MS system a calibration curve was made from 0.5 to 1000 µg/L (8 points) and the repeatability of the LC-MS system was tested by injecting a standard solution at a concentration of 50 µg/L of all the analytes after each 10 samples. When the relative standard deviation (RSD) of this standard was higher than 10% during the run, the response of the analyte was corrected with the respective internal standard. In table 6.2 it is indicated which internal standard was used to correct the response of the respective analyte. For analytes without an internal standard, no problems were observed with the repeatability of the LC-MS system.

For each set of samples one sample was spiked with 10 µg/L and 100 µg/L to check if the recovery remained the same as for the experiment to determine the recovery in triplicate.

6.2.10 Calibration and limits of quantification

Calibration curves were made for each batch of samples for concentrations between 0.5 µg/L and 1000 µg/L of the analytes in methanol.

Limit of quantification ($\text{LOQ}_{\text{instrumental}}$) for the pharmaceuticals was determined using a method called *the Target RSD* (Vial et al., 2003). The method consisted of the following steps:

1. The target RSD was decided to be 10%.
2. Six samples of each of the following concentrations (in total 36 samples) were made in methanol: 0.5, 1.0, 2.5, 5, 10 and 50 µg/L for the positively ionized compounds and 1, 2.5, 5, 10, 25 and 50 µg/L for the negatively ionized compounds.
3. Each of the samples was analyzed once using the developed LC method.
4. Relative standard deviation (RSD) was calculated for every concentration.
5. Measured data was modeled in SigmaPlot program using Horwitz-like function:

$$RSD = concentration \cdot p_1^{(1-p_2 \cdot \log(concentration))}$$

where p_1 and p_2 are terms determined by the fitting process.

The measured and modelled data were plotted and the $\mathrm{LOQ}_{\mathrm{instrumental}}$ was determined at RSD 10%.

In the matrix samples, the limit of quantification ($\mathrm{LOQ}_{\mathrm{matrix}}$) was determined with the following equation:

$$\mathrm{LOQ}_{\mathrm{matrix}} = \frac{\mathrm{LOQ}_{\mathrm{instrumental}}}{\mathrm{recovery} \cdot \mathrm{CF}} \tag{6.3}$$

where CF is the concentration factor

6.3 Results

6.3.1 Recoveries

The recoveries were determined for black water, effluent from the UASB reactor, effluent from the partial nitritation reactor and effluent from anammox SBR35 (BW, UASB, PN and AMX) (table 6.3). Most recoveries were more than 70%, but due to matrix effects some recoveries were lower than 70%, or in some cases higher than 100%. In all cases the obtained recoveries were consistent with respect to repeatability and no significant differences in recoveries between the four matrices were observed.

For paracetamol in BW the recoveries could not be calculated, because the background concentration in the blank sample was too high. Quantification of paracetamol in BW was therefore not possible.

In the PN effluent the spiked paracetamol the responses were very low, resulting in low recoveries (only 1% for CF=1 and 2% for CF=6.4). This disappearance of paracetamol was also observed with the analysis of PN effluent samples, where one sample was spiked as a control. The same phenomenon was observed for diclofenac in the PN effluent. In milliQ, BW, UASB and AMX this was never observed. Paracetamol is known to be readily degradable (Yu et al., 2006) and could have been degraded during sample pretreatment, but for diclofenac this is not likely as it is only slowly biodegradable (Buser et al., 1998; Kujawa-Roeleveld et al., 2008). It is therefore not clear why the responses for paracetamol and diclofenac in the spiked PN effluent samples were so low.

In BW deviating recoveries were obtained for E1 and E2, 217% and 212% for E1 versus 18% and 5% for E2. This might be explained by the biological oxidation of E2 to E1 which takes place before degradation (Lee and Liu, 2002; Joss et al., 2004), although this did not occur in the other matrices.

For ciprofloxacin (CIP), doxycycline (DOX), ibuprofen (IBP) and diclofenac (DCF) the responses were corrected with an internal standard and this compensated for the decrease in response during a run on the LC-MS system. Because the studied matrices are more complex compared to conventional WWTP influent or surface water, the loss of signal was most probably caused by the pollution of the LC-MS system and matrix signal suppression during the ionization (Cullum et al., 2004). The extracts from the SPE had a strong brown colour, which indicated that compounds such as humic acids were not removed by SPE. Furthermore, the ion source of the MS system needed regular cleaning due to deposition of compounds that did not ionize.

Table 6.3 Recoveries of the studied compounds in black water (BW), effluent from UASB reactor (UASB), effluent from the partial nitritation reactor (PN) and effluent from the anammox SBR35 (AMX) (n=3) (relative recoveries are corrected with the response of the internal standard).

compound		BW		UASB		PN		AMX	
		CF=1	CF=4.1	CF=1	CF=6.4	CF=1	CF=6.4	CF=1	CF=6.4
Paracetamol	abs.	-*	-*	75%	60%	1%	2%	83%	58%
Trimethoprim	abs.	107%	89%	107%	90%	92%	77%	114%	78%
Ciprofloxacin	abs.	135%	93%	649%	152%	673%	84%	751%	55%
	rel.	119%	75%	505%	139%	416%	103%	794%	54%
Tetracycline	abs.	138%	-*	129%	102%	128%	75%	127%	78%
Metoprolol	abs.	152%	82%	114%	125%	84%	80%	114%	94%
Doxycycline	abs.	83%	100%	158%	111%	111%	69%	157%	76%
	rel.	58%	84%	102%	151%	121%	91%	96%	41%
Propranolol	abs.	88%	67%	105%	91%	91%	77%	110%	78%
Carbamazepine	abs.	122%	98%	109%	94%	90%	88%	112%	89%
Cetirizine	abs.	113%	93%	110%	102%	94%	84%	108%	83%
E2-17G	abs.	2%	0%	83%	48%	70%	46%	114%	68%
Ibuprofen	abs.	140%	-*	131%	-*	113%	-*	102%	-*
	rel.	119%	-*	117%	-*	107%	-*	98%	-*
E2-3S	abs.	106%	91%	99%	71%	90%	68%	89%	59%
Diclofenac	abs.	129%	102%	120%	104%	5%	8%	106%	119%
	rel.	112%	99%	106%	85%	5%	7%	103%	103%
E2	abs.	18%	5%	107%	95%	66%	56%	90%	81%
EE2	abs.	103%	51%	110%	93%	58%	70%	94%	117%
E1	abs.	217%	212%	126%	111%	76%	67%	103%	108%

CF	Concentration Factor
abs.	absolute
rel.	relative
-*	could not be determined because of high background concentration
▨	compound seemed to disappear in matrix; standard controls were ok

Because the black water samples were filtered over a glass fibre filter, some analytes adsorbed to the filter and this resulted in a lower recovery. The amount of adsorption of the analytes in the filter is presented in table 6.4. For most analytes adsorption was negligible (maximum 9%), but propranolol adsorbed to the filter by 40% in milliQ samples and 29% in BW samples. Metoprolol adsorbed to the filter as well in milliQ samples (32%), but to a lower extent in the black water sample (7%). The estrogens E2, EE2 and E1 adsorbed to the filter in BW samples to a higher extent than in milliQ samples. The adsorption to filters could have reduced the recoveries of these compounds, but this was not observed in the obtained recoveries, which could not be explained.

Table 6.4 Amount of analyte adsorbed to filter during filtration of spiked milliQ sample and spiked black water samples (n=3).

	milliQ	BW
Paracetamol	5 %	7 %
Trimethoprim	9 %	7 %
Ciprofloxacin	3 %	4 %
Tetracycline	5 %	9 %
Metoprolol	32 %	7 %
Doxycycline	7 %	4 %
Propranolol	40 %	29 %
Carbamazepine	5 %	7 %
Cetirizine	5 %	8 %
E2-17G	8 %	-
Ibuprofen	6 %	7 %
E2-3S	6 %	9 %
Diclofenac	6 %	8 %
E2	6 %	64 %
EE2	9 %	35 %
E1	9 %	26 %

- in this case the adsorption could not be calculated because all the BW samples did not show any response

6.3.2 Calibration and LOQs

The linear range between 0.5 µg/L and 1000 µg/L was determined based on the concentrations found in the matrix and a $R^2 > 0.99$ (Lindqvist et al., 2005). For paracetamol, trimethoprim, metoprolol, propranolol, carbamazepine the upper limit of the linear calibration was 1000 µg/L. For the other analytes the upper limit of the linear calibration was 250 µg/L. The instrumental limit of quantification (LOQinstrumental) and the limit of quantification in the matrices (LOQmatrix) are shown in table 6.5. For ciprofloxacin no linear range was found and therefore quantification of this compound was not possible. Because ciprofloxacin also showed unrealistic recoveries (table 6.3), this compound was excluded from further data analysis.

Table 6.5 Limit of quantification of the direct injection of pure compounds ($LOQ_{instrumental}$) and limit of quantification in the matrices.

compound	LOQ instrumental (µg/L)	LOQ_{BW} (µg/L)	LOQ_{UASB} (µg/L)	LOQ_{PN} (µg/L)	LOQ_{AMX} (µg/L)
paracetamol	1.3	0.3	0.3	not possible	0.3
trimethoprim	0.8	0.2	0.1	0.2	0.2
tetracycline	6.2	1.5	0.9	1.3	1.2
metoprolol	1.1	0.3	0.1	0.2	0.2
doxycycline	3.1	0.9	0.3	0.5	1.2
propranolol	0.5	0.2	0.1	0.1	0.1
carbamazepine	0.5	0.1	0.1	0.1	0.1
cetirizine	0.5	0.1	0.1	0.1	0.1
E2-17G	4.8	not possible	1.5	1.6	1.1
ibuprofen	4.9	1.0	0.7	0.7	0.8
E2-3S	3.4	0.9	0.7	0.8	0.9
diclofenac	3.3	0.8	0.6	not possible	0.5
E2	10.5	not possible	1.7	2.9	2.0
EE2	6.5	3.1	1.1	1.5	0.9
E1	3.1	not possible	0.4	0.7	0.4

not possible: The LOQ could not be estimated because the recoveries were unrealistic (table 6.3).

6.3.3 Fate of the selected pharmaceuticals and estrogens during black water treatment

Black water

Most of the selected pharmaceuticals were detected in all the black water samples that were analyzed (table 6.6). The concentrations of paracetamol in black water were higher than 1000 µg/L in 10 out of 15 samples and because the recovery could not be calculated, these results should be regarded as semi-quantitative. Tetracycline, propranolol and carbamazepine were only detected in a few black water samples. Estrogens were not detected in the black water and responses were under the LOQ, except for one sample where E1 was found in a concentration of 2.4 µg/L. A total estrogen concentration of 43 µg/L in vacuum collected black water was estimated in previous research (de Mes et al., 2006). The difference could be due to differences in the composition of the group of the inhabitants of the housing estate in Sneek (NL) where the black water was collected. For example, pregnant women contribute most to the total amount of estrogens excreted and man and elderly people contribute less (de Mes et al., 2005). Furthermore, in this research conjugated forms of E1 and EE2 were not measured, and de Mes (2007) showed that a substantial amount could be present in a conjugated form of E1.

In table 6.6 the average concentrations measured in this study are compared to calculated predicted concentration (PC) based on the defined daily dose (DDD) and to the concentrations found in WWTP influents in the Netherlands. The concentrations of paracetamol, metoprolol and ibuprofen were in the same range as the PC assuming that one or more persons are using that compound. Compared to the WWTP influents the concentrations found were much higher, which was also expected because the black water is about 25 times more concentrated than conventional domestic WWTP influent (chapter 1). However, the black water was obtained from a community of only 32 houses and therefore the concentrations found in this specific black water may not be representative and the factor of 25 was not found. The concentrations of tetracycline and doxycycline were lower than the PC. This could be due to sorption to the solids because only the liquid fraction of black water was analyzed, and tetracycline and doxycycline are very hydrophobic (Thomas et al., 2007).

Table 6.6 Concentrations of the detected compounds in black water compared with the calculated predicted concentration (PC) based on the defined daily dose (DDD) (WHO, 2006) and the excretion in unmetabolized form (KNMP, 2006) (all in µg/L). The concentrations found in WWTP influents in the Netherlands are given as well (Schrap et al., 2003).

compound	BW This research			Excretion in unmetabolized form (KNMP, 2006)	PC, Calculated assuming that one person in Sneek is using, based on DDD	PC Calculated assuming that all persons in Sneek (32 houses, 2.3 persons per house) are using, Based on DDD	WWTP influents (Schrap et al., 2003)
	average	min	max				
Paracetamol	>1000	747	3432	5%	408	30000	0.33 – 45
Trimethoprim	71	17	124	44%	478	35200	0.083 – 0.51
Tetracycline	107	2.8	358	54%	1467	108000	<0.1
Metoprolol	45	29	68	5%	20	1500	0.25 – 1.8
Doxycycline	3.9	1.8	9.7	40%	109	8000	<0.1
Propranolol	1.0	-	-	96%	417	30720	0.1 – 0.51
Carbamazepine	1.1	0.1	2.5	2%	54	4000	0.5 – 9.5
Cetirizine	1.4	0.2	3.0	100%	27	2000	-
Ibuprofen	147	55	258	1%	33	2400	1.5 – 17
Diclofenac	13	1.9	66	25%	68	5000	1.2 – 13
EE2	<LOQ	-	-	10%	0.0068	0.5	0.0059 [a]

[a] (de Mes et al., 2005)

Removal of the compounds in the treatment concept for black water

In figure 6.2 the average concentrations which were found in all the matrices are shown. In the anammox effluent paracetamol, tetracycline and doxycycline were not detected. The other compounds were still present in the effluent of the anammox reactor in the µg/L range, which shows that biological treatment alone is not enough to eliminate the selected compounds from black water. In appendix 6.III the average concentrations of the selected compounds are presented, including minimum and maximum values.

	BW n > LOQ (15 samples)	UASB n > LOQ (18 samples)	PN n > LOQ (18 samples)	AMX n > LOQ (18 samples)
PAR	15	18	-	0
TRI	2	2	2	2
TC	5	6	1	0
MET	15	18	18	15
DOX	15	18	1	0
PRO	1	11	2	1
CBZ	3	4	11	4
CET	11	17	18	18
IBP	15	18	18	18
DCF	11	17	-	17

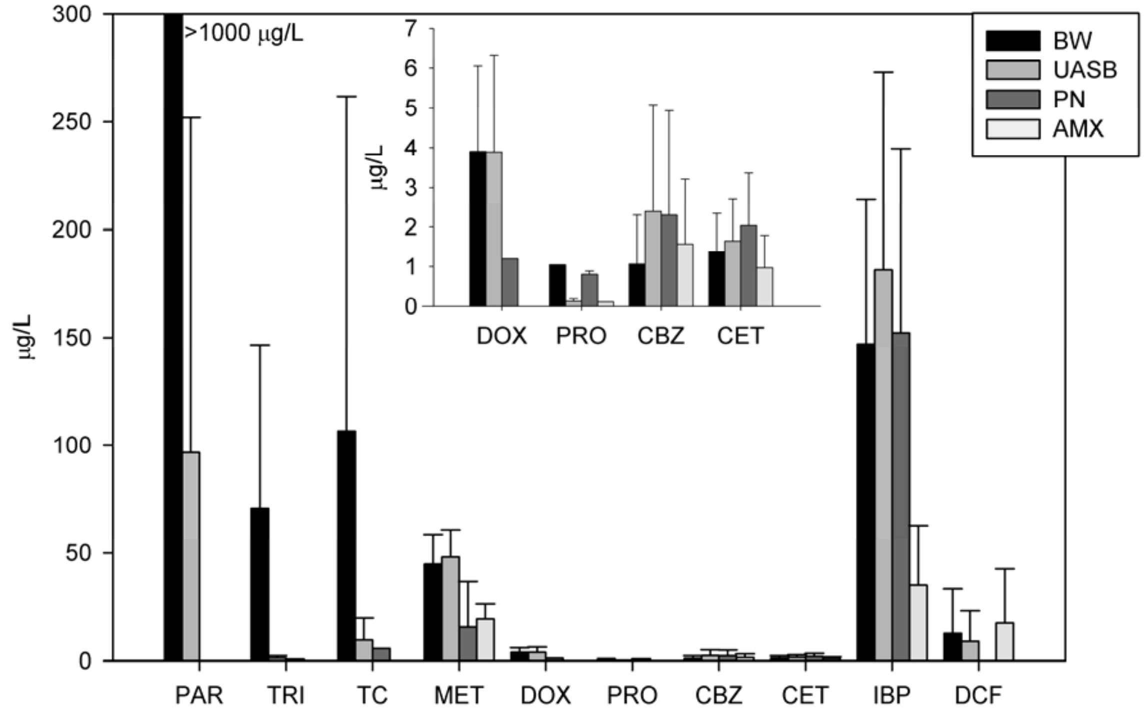

Figure 6.2 Average concentrations of the selected micro-pollutants in black water (BW), effluent from the UASB reactor (UASB), effluent from the partial nitritation reactor (PN) and effluent from the anammox reactor (AMX). A table is included which shows the number of samples that had a response above LOQ.

In table 6.7 the removal for each compound in the treatment concept for black water is shown, together with literature information about the biodegradability under different redox conditions and the tendency to adsorb to sludge. The majority of paracetamol was removed during anaerobic treatment in the UASB reactor, most likely by anaerobic biodegradation (Joss et al., 2006b; Yu et al., 2006). In the effluent from the anammox reactor no paracetamol was detected and all responses were below the detection limit (0.3 μg/L, table 6.5). This corresponds with previous studies where paracetamol was found in only a few WWTP effluents and never in surface waters showing that paracetamol is a readily biodegradable compound (e.g.(Ternes, 1998; Yu et al., 2006)). The remaining amount of paracetamol therefore probably was removed in the PN reactor.

Table 6.7 Removal efficiencies during anaerobic treatment (UASB), aerobic treatment (PN) and anoxic treatment (AMX), literature information about the biodegradability under different redox conditions and the tendency to adsorb to sludge.

Compound	UASB	PN	AMX	Anaerobic bio-degradable?	Anoxic bio-degradable?	Aerobic readily bio-degradable?	Likely to adsorb to sludge?
Paracetamol	95%	x	u.d.	n.f.	n.f.	Yes[1]	No[2]
Trimethoprim	-	-	-	n.f.	n.f.	No[3,4]	No[4]
Tetracycline	-	-	-	n.f.	n.f.	No[3]	Yes[5,6]
Metoprolol	-7%	67%	-23%	No[7]	No[7]	No[8]	No[8]
Doxycycline	0%	69%	u.d.	n.f.	n.f.	No[3]	Yes[6]
Propranolol	-	-	-	n.f.	n.f.	No[8]	No[8]
Carbamazepine	-	-	-	No[7,9]	No[7]	No[11]	No[10]
Cetirizine	-20%	-23%	52%	n.f.	n.f.	No[12]	No[12]
Ibuprofen	-23%	16%	77%	Yes, but limited[7,9]	Yes[7]	Yes[7,13]	No[10]
Diclofenac	22%	x	-75%	Yes, but limited[7,9]	No[7]	No[14]	No[10,14]

x: could not be calculated, because the compounds could not be quantified
u.d.: under detection limit, therefore the removal could not be calculated
- : removal was not calculated because the compounds were detected in only a few samples
n.f.: not found
[1](Yu et al., 2006); [2](Joss et al., 2006b); [3](Alexy et al., 2004); [4](Halling-Sørensen et al., 2000); [5](Kim et al., 2005); [6](Thomas et al., 2007); [7](Kujawa-Roeleveld et al., 2008); [8](Maurer et al., 2007); [9](Carballa et al., 2007); [10](Carballa et al., 2008); [11](Clara et al., 2004); [12](Kosonen and Kronberg, 2009); [13](Buser et al., 1999); [14](Buser et al., 1998)

The antibiotic tetracycline was only found in a few samples of black water and reactor effluents, but always in the samples taken in the same period. Tetracycline is likely to adsorb to the anaerobic sludge, and this was probably the main removal mechanism (Thomas et al., 2007). Doxycycline is also known to adsorb to sludge, but in this research the concentrations found were too low to conclude on this.

Metoprolol was detected in almost all the samples, including samples of the anammox effluent. During anaerobic treatment no removal of metoprolol was observed and this is in agreement with previous research on source separated wastewater streams (Kujawa-Roeleveld et al., 2008). Maurer et al. (2007) found that the removal of beta-blockers depended on the HRT and estimated that 90% of metoprolol could be removed at an HRT of 1 day and a sludge concentration of 4

g_{COD}/L (suspended solids) in WWTPs. In this research 67% of the metoprolol was removed in the partial nitritation reactor at an HRT of 1.7 days. This lower removal of metoprolol could be due to a different sludge concentration and sludge characteristics, i.e. activated sludge versus PN sludge mainly containing ammonium oxidizers (chapter 3). Also the presence of readily degradable compounds can increase the removal of certain compounds, because it is likely that micro-pollutants are degraded during co-metabolism (Alexander, 1981; Alexy et al., 2004; Joss et al., 2006a). Because in the UASB reactor most readily biodegradable organic material already is removed, co-metabolism in the aerated partial nitritation reactor may have been limited.

Carbamazepine and cetirizine were only found at very low concentrations, but were still detected in the anammox effluent, confirming their persistency during biological treatment (Clara et al., 2004; Kosonen and Kronberg, 2009). For the estrogens only E1 was found in a number of UASB effluent samples, in the same range as detected by (de Mes, 2007). In the PN and anammox effluent no estrogens were detected. Because the estrogens were not detected in the BW samples, removal efficiencies could not be calculated and therefore the estrogens are not presented in table 6.7. Ibuprofen was detected in all black water samples, but was not removed during anaerobic treatment in the UASB reactor and during aerobic treatment in the PN reactor. The concentrations in the UASB effluent were even higher than in the black water and this could be due to deconjugation because most ibuprofen is excreted in conjugated or metabolized form (KNMP, 2006). The relatively high removal efficiency in the anammox reactor could have been caused by the higher temperature of 35 °C in this reactor, although no literature information on the effect of temperature on the biodegradability of ibuprofen was found. Diclofenac was still detected in the effluent from the anammox reactor, confirming its reported persistency during biological treatment (Buser et al., 1998).

6.4 Discussion

6.4.1 Analytical method

In this research two methods were developed to analyze a selection of pharmaceutical and hormone residues. For most compounds the analysis was successful showing consistent recoveries between 70% and 120%. For doxycycline, ibuprofen and diclofenac a loss of signal during a run was observed, but this was successfully corrected with the response of the internal standard.

However, not all compounds could be successfully analyzed with these methods. In the black water matrix estrogens were probably converted during sample storage and pre-treatment because the recoveries showed unrealistic values. In spiked milliQ water these problems were not observed. Also with paracetamol and diclofenac in the PN matrix recoveries could not be determined and low responses were obtained. This was probably due to matrix effects either in sample pre-treatment or at the analysis stage, because in spiked milliQ samples these problems were not observed. Especially the black water samples contained lots of matrix components that could have interfered with the analyte in the solid phase extraction, chromatographic separation or ionization stage.

Ciprofloxacin could not be quantified in this method because the calibration curve was not linear and recoveries were not within a reasonable range. Antibiotics are also known to adsorb to glass walls and they can have unwanted interactions with the column (Ye et al., 2007). To properly measure antibiotics a separate method optimized for the analysis of antibiotics might be more suitable, using for example different mobile phases (Lindberg et al., 2004).

Problems during analysis were observed for the column used for the negatively ionized compounds (method B). After about 50 analyses the column got clogged and the pressure increase over the system became too high. This indicated that some matrix components in the samples remained in the column after the analytical runs. Using a new column solved this problem.

To solve these problems observed with the analysis it could be considered to use an additional pre-treatment of the samples by freeze-drying or cleaning up with silica gel (Joss et al., 2006a).

Methods for the analysis of the selected compounds in the sludge were not yet developed. Analysis of the compounds in the sludge matrix is difficult because the complex matrix will cause significant ionization suppression. Recently, methods were developed to analyze beta-blockers in sludge and it was found that appropriate surrogate standards were needed to compensate the matrix-induced ion suppression (Scheurer et al., 2009).

6.4.2 Removal of micro-pollutants in new sanitation concepts

This research showed the fate of selected micro-pollutants during anaerobic treatment combined with the two reactor nitritation-anammox process. In the anammox effluent only paracetamol, tetracycline and doxycycline were not detected. Only paracetamol was removed anaerobically. Aerobic treatment was successful to remove a large fraction of metoprolol. Because several compounds have a relatively slow biodegradation rate, optimization of the biological treatment, for example by increasing the aerobic sludge retention time (SRT) could increase the total removal (Joss et al., 2006b; Jones et al., 2007). In this research the aerobic SRT was relatively short (SRT of 1.0 – 17 days) and may be increased to remove more ibuprofen and metoprolol. However, the increase in the PN reactor is limited to prevent nitrite oxidation (chapter 3) and therefore additional biological post-treatment might be necessary to remove the remaining biodegradable micro-pollutants. Persistent pharmaceuticals such as carbamazepine and cetirizine were still detected in the anammox effluent and were present in the μg/L range. As mentioned in the introduction the risk micro-pollutants may pose is difficult to assess and not well understood (Schwarzenbach et al., 2006; Schirmer and Schirmer, 2008), but several effects have been reported already at ng/L levels. More research is also needed on the combined effect of micro-pollutants present as mixtures in the environment (Schwarzenbach et al., 2006). To which extent the hormones and pharmaceuticals should be removed is therefore not known and requires further research. Another growing concern is the presence of conjugates and metabolites, because a large fraction of the pharmaceuticals is excreted as conjugates and metabolites and not as the parent compound (table 6.6). In most studies these conjugates and metabolites are not analyzed and for example for estrogens it was shown that a large fraction (>70%) of the estrogens was present in the conjugated form after biological treatment of black water (de Mes et al., 2007). Therefore more research is needed on the presence of conjugates and metabolites and their deconjugation and biodegradation rate.

Because some compounds are likely to adsorb to sludge and are not readily biodegradable (e.g. tetracycline and doxycycline), the reuse of the anaerobic sludge in agriculture might be limited (Winker et al., 2009). The conditions during land application may change such that desorption becomes favourable. In this way tetracycline can form a potential risk to the environment (Kim et al., 2005). In this research only liquid samples were analyzed. Further research is needed to develop a method for the anaerobic sludge and to investigate the reuse potential of anaerobic sludge with respect to micro-pollutants.

It was found that pharmaceuticals such as propranolol and diclofenac can inhibit methanogenesis, but only in the mg/L range (Fountoulakis et al., 2004), which is much higher than found in this research. Also inhibition effects of antibiotics were found on nitrite oxidizers at concentrations of 10 mg/L (Dokianakis et al., 2004). Although concentrations in black water are higher than in sewage, they are still in a range where inhibition of biological treatment by pharmaceuticals is not expected. Only in specific circumstances where black water of a small community using a large amount of pharmaceuticals is treated, inhibition on biological processes might occur (Fountoulakis et al., 2008).

To eliminate the persistent micro-pollutants additional physical/chemical treatment such as advanced oxidation, activated carbon adsorption or membrane filtration is needed (Joss et al., 2006a). However, the anammox effluent still contains some organic material and humic acids that will interfere with such post treatment techniques. Furthermore the formation of by-products when e.g. ozonation is applied is of growing concern because they can be more toxic than their parent compounds. On the other hand, ozonation is also known to increase the biodegradability and a combination with biological post treatment might increase the total removal of the micro-pollutants (Li et al., 2006). Promising results were achieved with post-ozonation of WWTP effluent followed by sand filtration requiring an additional energy requirement of 12% (Hollender et al., 2009). Most of the persistent micro-pollutants were removed by ozonation and biodegradable compounds formed during ozonation were additionally removed in the sand filter.

6.5 Conclusions

The methods developed in this research showed the successful detection of a wide selection of micro-pollutants in a complex matrix of vacuum collected black water and effluents of a sequence of biological reactors operated under different redox conditions. To properly measure antibiotics a separate method optimized for the analysis of antibiotics might be more suitable. Further research should focus on the development of methods to analyze micro-pollutants in anaerobic black water sludge. During combined anaerobic treatment and nitrogen removal by nitritation-anammox from vacuum collected black water only a few compounds were (partly) removed. Anaerobic treatment was only suitable to remove the majority of paracetamol. Metoprolol and ibuprofen were (partly) removed during aerobic treatment. Diclofenac was not removed. To eliminate persistent micro-pollutants (carbamazepine and cetirizine) additional physical and chemical treatment will be unavoidable in new sanitation concepts.

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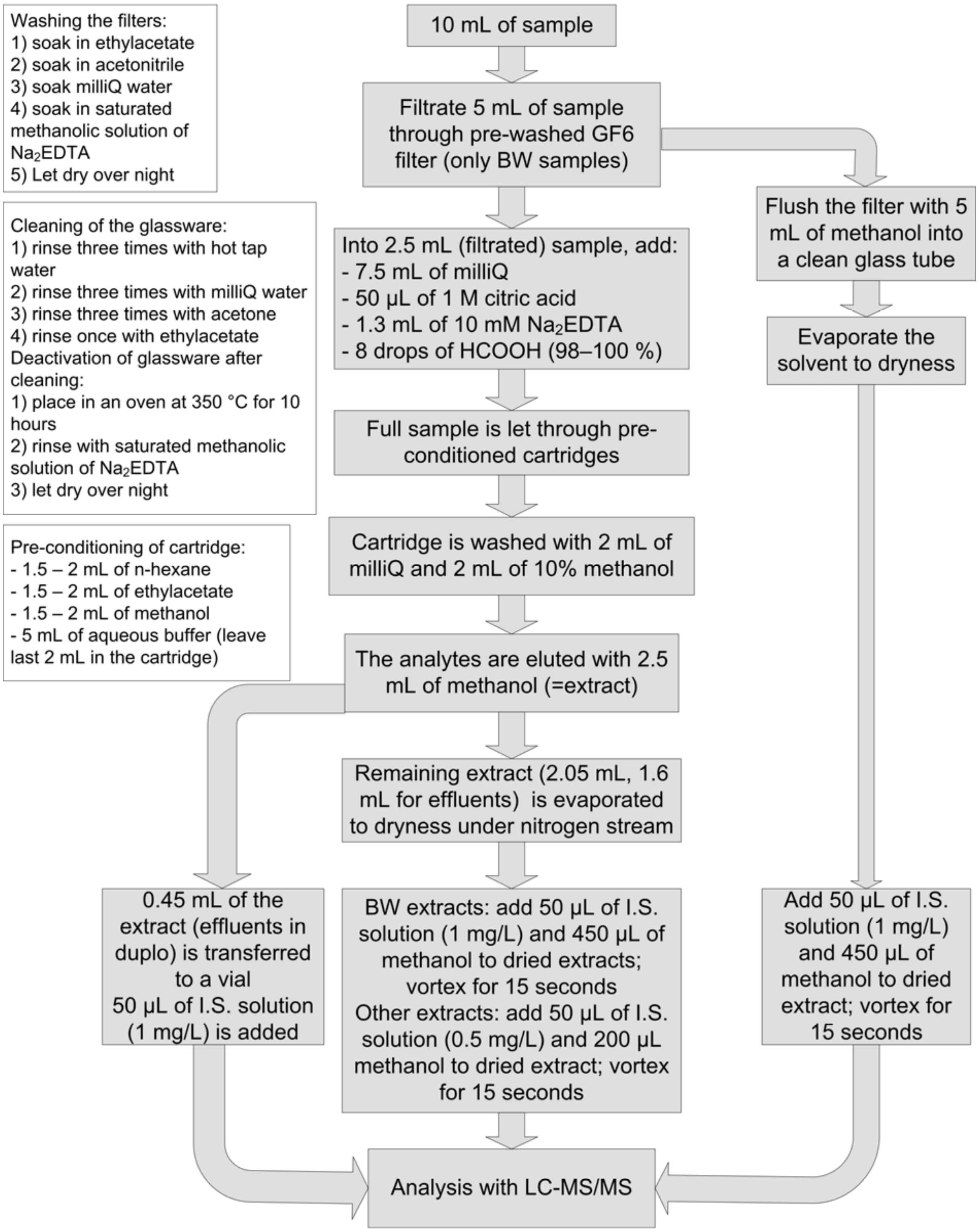
Appendix 6.I Procedure for solid-phase extraction



Washing the filters:
1) soak in ethylacetate
2) soak in acetonitrile
3) soak milliQ water
4) soak in saturated methanolic solution of Na_2EDTA
5) Let dry over night

Cleaning of the glassware:
1) rinse three times with hot tap water
2) rinse three times with milliQ water
3) rinse three times with acetone
4) rinse once with ethylacetate
Deactivation of glassware after cleaning:
1) place in an oven at 350 °C for 10 hours
2) rinse with saturated methanolic solution of Na_2EDTA
3) let dry over night

Pre-conditioning of cartridge:
- 1.5 – 2 mL of n-hexane
- 1.5 – 2 mL of ethylacetate
- 1.5 – 2 mL of methanol
- 5 mL of aqueous buffer (leave last 2 mL in the cartridge)

10 mL of sample

Filtrate 5 mL of sample through pre-washed GF6 filter (only BW samples)

Into 2.5 mL (filtrated) sample, add:
- 7.5 mL of milliQ
- 50 µL of 1 M citric acid
- 1.3 mL of 10 mM Na_2EDTA
- 8 drops of HCOOH (98–100 %)

Full sample is let through pre-conditioned cartridges

Cartridge is washed with 2 mL of milliQ and 2 mL of 10% methanol

The analytes are eluted with 2.5 mL of methanol (=extract)

Remaining extract (2.05 mL, 1.6 mL for effluents) is evaporated to dryness under nitrogen stream

BW extracts: add 50 µL of I.S. solution (1 mg/L) and 450 µL of methanol to dried extracts; vortex for 15 seconds
Other extracts: add 50 µL of I.S. solution (0.5 mg/L) and 200 µL methanol to dried extract; vortex for 15 seconds

0.45 mL of the extract (effluents in duplo) is transferred to a vial
50 µL of I.S. solution (1 mg/L) is added

Flush the filter with 5 mL of methanol into a clean glass tube

Evaporate the solvent to dryness

Add 50 µL of I.S. solution (1 mg/L) and 450 µL of methanol to dried extract; vortex for 15 seconds

Analysis with LC-MS/MS

Appendix 6.II Retention times (RT), monitored ions and MS parameters of the study pharmaceuticals

Compound	RT (min)	Precursor ion	Product ion 1	Fragmentor voltage (V)	Collision energy (eV)
Positively ionized compounds (Method A)					
Paracetamol	5.318	152.1	110.1	120	14
Trimethoprim	7.127	291.2	230.2	160	24
Ciprofloxacin	7.633	332.2	314.2	135	16
Tetracycline	7.852	445.3	410.1	130	15
Metoprolol	8.393	268.2	116.1	120	14
Doxycycline	9.839	445.3	428.3	135	14
Propranolol	10.576	260.3	116.1	115	14
Carbamazepine	12.660	237.2	194.2	155	16
Cetirizine	12.684	389.3	201.2	125	15
Internal standards:					
Diaveridine	6.727	261.2	245.2	155	25
Enrofloxacin	8.078	360.3	342.3	150	18
Meclocycline	10.712	477.2	460.2	145	15
Negatively ionized compounds (Method B)					
E2-17G	6.204	447.1	75	200	20
Ibuprofen	7.253	205.1	161.1	95	0
E2-3S	7.488	351.1	271.1	150	30
Diclofenac	7.942	294.2	250.0	95	5
E2	11.701	271.1	145.1	180	37
EE2	12.421	294.1	144.8	200	40
E1	12.621	269.2	145.2	145	40
Internal standard:					
Fenoprop	6.995	267.0	194.9	85	8

Appendix 6.III Average concentrations of the selected compounds in each matrix

Compound	Black water					UASB effluent					PN effluent					Anammox 35 effluent				
	average	stdev	min	max	above LOQ	average	stdev	min	max	above LOQ	average	stdev	min	max	above LOQ	average	stdev	min	max	above LOQ
paracetamol	1941	1103	747	3432	15 out of 15	96.6	155	4.0	602	18 out of 18										0 out of 15
trimethoprim	70.6	76.0	16.8	124	2 out of 15	1.4	1.0	0.6	2.1	2 out of 18	0.66	0.15	0.56	0.77	2 out of 18	0.32	0.07	0.27	0.37	2 out of 15
ciprofloxacin																				
tetracycline	107	155	2.8	358	5 out of 15	9.6	10.5	1.6	27.5	6 out of 18	5.73		5.73	5.73	1 out of 18					0 out of 15
metoprolol	44.8	13.9	28.5	68.3	15 ouf of 15	48.1	12.8	28.4	73.5	18 out of 18	16.0	21.0	3.22	75.5	18 out of 18	19.6	6.7	8.8	29.3	15 out of 15
doxycycline	3.9	2.1	1.8	9.7	15 ouf of 15	3.9	2.4	1.2	7.8	18 out of 18	1.20				1 out of 18					0 out of 15
propranolol	1.0		1.0	1.0	1 out of 15	0.1	0.1	0.1	0.3	11 out of 18	0.80	0.09	0.74	0.86	2 out of 18	0.11				1 out of 15
carbamazepine	1.1	1.2	0.1	2.5	3 out of 15	2.4	2.7	0.1	6.2	4 out of 18	2.30	2.64	0.10	7.48	11 out of 18	1.57	1.64	0.14	3.25	4 out of 15
cetirizine	1.4	1.0	0.2	3.0	11 out of 15	1.7	1.1	0.1	4.0	17 out of 18	2.04	1.32	0.15	4.59	18 out of 18	0.97	0.82	0.15	2.42	18 out of 18
E2-17G					0 out of 15					0 out of 18					0 out of 15					0 out of 15
ibuprofen	147	67	54.6	258	15 out of 15	181	91.7	87.2	456	18 out of 18	152	85	13.5	337	18 out of 18	35.3	27.5	3.2	80.2	18 out of 18
E2-3S					0 out of 15					0 out of 18					0 out of 15					0 out of 15
diclofenac	13.0	20.3	1.9	65.8	11 out of 15	10.1	14.6	0.7	59.1	17 out of 18						17.8	25.2	2.8	93.4	17 out of 18
E2					0 out of 15					0 out of 18					0 out of 15					0 out of 15
EE2					0 out of 15					0 out of 18					0 out of 15					0 out of 15
E1	2.4				1 out of 15	0.6	0.1	0.4	0.8	11 ouf of 18					0 out of 15					0 out of 15

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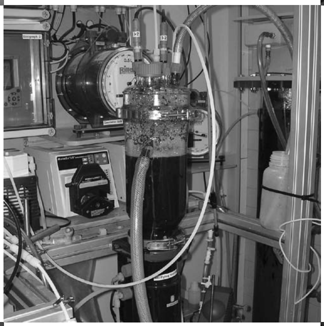

General discussion and outlook

Abstract

By combined anaerobic treatment, autotrophic nitrogen removal and struvite precipitation from concentrated black water, collected with vacuum toilets, high removal efficiencies of 87% COD, 86% nitrogen and 94% phosphorus were achieved. The energy recovered, in the form of electricity and heat, is more than sufficient for the anaerobic treatment, nitrogen removal and phosphorus recovery. Furthermore by struvite precipitation from the anaerobic effluent, 10% of the artificial global phosphorus fertilizer production can be replaced. Nitrogen was successfully removed in a two reactor autotrophic nitritation-anammox process. The use of vacuum toilets saves €12/p.e./year of drinking water. During biological treatment only part of the organic micro-pollutants were eliminated. To remove persistent micro-pollutants, such as carbamazepine, the use of an (advanced) physical or chemical treatment system seems to be unavoidable.

New sanitation systems, based on separation at source, can be attractive from an economic point of view, provided that the recovered resources can be sold. More research is needed to elucidate the mechanisms of nitrous oxide from biological nitrogen removal processes, because this is an uncontrolled source of emission of reactive nitrogen to the atmosphere. The amount of phosphorus recovered from black water can be increased if the excess anaerobic sludge, which contains the remaining part of phosphorus from vacuum collected black water, can be reused as a phosphorus-enriched organic fertilizer, but more research is needed with respect to heavy metals, pathogens and micro-pollutants, such as hormones and pharmaceutical residues. A thorough risk assessment is needed to conclude on the overall effect of micro-pollutants and pathogens in the environment and to determine to which extent they should be removed in a post treatment step.

New sanitation systems require a different infrastructure and management, but would result in a more sustainable concept for sanitation; one in which energy and nutrients are recovered and pathogens and micro-pollutants are removed, producing clean water while also protecting the environment.

M.S. de Graaff, G. Zeeman, H. Temmink and C.J.N. Buisman

7.1 Introduction

New sanitation concepts are based on separation at source of household wastewater streams into grey water and black water (faeces and urine) or into grey water, urine and brown water (faeces), and have a large potential to recover the important resources energy, nutrients and water. They present an alternative approach for the contemporary sewerage combined with centralized, aerobic wastewater treatment plants which are restricted to the removal of organics and nutrients (conventional WWTPs) from wastewater. The design of new sanitation concepts varies with the degree of separation and dilution of black water with flushing and transport water.

In this study treatment of black water was studied. This black water was collected with vacuum toilets using only 1 litre of water for flushing. Black water does not only contain half the load of organic material in domestic wastewater and the major fraction of the nutrients nitrogen and phosphorus, but also most of the pathogens, hormones and pharmaceutical residues. The concept that was investigated consists of a combination of anaerobic treatment for energy recovery, struvite precipitation for phosphorus recovery and autotrophic nitrogen removal in a two reactor nitritation-anammox process. The feasibility of this black water treatment and recovery system will be discussed. In addition, the fate of several selected micro-pollutants in this new sanitation concept was determined and the consequences of this will be discussed. Finally, an outlook with respect to costs, management and acceptance is presented.

7.2 Treatment concept for black water presented in this thesis

7.2.1 Removal efficiencies

High removal efficiencies were achieved for organic pollution (COD), nitrogen (N) and phosphorus (P). The upflow anaerobic sludge blanket reactor (UASB) operated at 25 °C, removed 78% of COD (chapter 2) and an additional 9% of COD was removed in the partial nitritation reactor (chapter 3). Nitrogen was conserved for 91% in the UASB effluent. Subsequently, the two reactor nitritation-anammox process operated at 25 °C, removed 85% of total nitrogen from the UASB effluent (chapter 4), resulting in an overall removal efficiency of 86%. A significant fraction of the phosphorus (40%) was removed in the UASB reactor and a phosphorus rich sludge (51 mgP/gTSS) was produced. The remaining fraction of the phosphorus can be recovered by struvite precipitation from the UASB effluent (90% removal), which was demonstrated in batch tests (chapter 5). Including struvite precipitation in total 94%

of the phosphorus can be removed from black water, of which 55% can be recovered as struvite.

The effluent of the black water treatment system still contained relatively high concentrations of COD, N and P compared to WWTP effluent (table 7.1). However, because of the very high concentrations in black water, similar loads of COD and even higher loads of nitrogen and phosphorus were removed compared to conventional WWTPs. Nevertheless, compared to the maximum tolerable risk (MTR) values required within the context of the European Water Framework Directive (WFD), the effluent concentrations of nitrogen and phosphorus would still be too high and post treatment might be required to remove remaining nutrients and COD. However, this will depend on the type of surface water where the treated black water is discharged and therefore more research is needed to conclude on this.

Table 7.1 Total removal efficiencies in this research compared to conventional WWTPs.

	Effluent concentrations	Effluent load	% Total removal	% removal conventional WWTP (average in 2006, NL) (UVW, 2006)	WFD MTR values
		This research at 25 ˚C			
COD	0.4 gCOD/L	2 gCOD/p/d	87%	90%	-
N	130 mgN/L	0.65 gN/p/d	86%	78%	2 mgN/L
P [a]	13 mgP/L	0.065 gP/p/d	94%	82%	0.15 mgP/L

[a] including 90% removal from the UASB effluent by struvite precipitation (chapter 5)

7.2.2 Anaerobic treatment and energy recovery

Anaerobic treatment of vacuum collected black water in a UASB reactor at 25 ˚C was very successful. Compared to other reactor types reported for anaerobic treatment of vacuum collected black water, such as a UASB-septic tank and a continuously stirred tank reactor (CSTR), a more than two times smaller volume is possible. After water vapor and hydrogen sulfide have been removed from the produced biogas (78% CH_4, 22% CO_2 and traces of H_2S and water (chapter 2)), electricity and heat can be produced in a combined heat and power (CHP) system. These systems allow for an efficiency of 85%, 40% of which is electricity and 60% is heat (van den Berg, 2008). The heat can be directly used in the treatment unit for the biological reactors, because the black water needs to be heated from 15 – 20 °C[21] to obtain the optimal temperature for energy efficient anaerobic treatment (25 °C, chapter 2). The amount of electricity that can be produced is significant in terms of wastewater treatment. Whereas conventional wastewater treatment plants consume 96 MJ/p/y or 26.6 kWh/p/y (Frijns et al., 2008), from the produced methane in the black water treatment concept 56 MJ/p/y can be recovered as electricity and 84 MJ/p/y as heat. When kitchen waste is included, this can even be increased to 116 MJ/p/y or 32 kWh/p/y (Kujawa-Roeleveld et al., 2006). The application of a new type of kitchen grinder, demonstrated in one house at the demonstration project in Sneek, the Netherlands, makes the collection of kitchen waste possible and convenient in places where this is difficult (such as apartment buildings).

An estimation of the energy production and consumption in the black water treatment concept is presented in table 7.2, and is compared with conventional wastewater treatment. Table 7.2 shows that 22.5 MJ/p/y of electricity is gained in the black water treatment concept, whereas in sewerage and conventional WWTPs 107 MJ/p/y is lost. These numbers provide an indication, because energy needed for the production of hardware such as reactors and pumps etc. and heat losses are excluded. Table 7.2 shows that the electricity that is produced from black water can more than compensate for the electricity demand of nitrogen removal and phosphorus recovery. Furthermore, by using less drinking water for toilet flushing, 25% of the total drinking water consumption in a household can be saved, which results in a further saving of about 19 MJ/p/y (table 7.2). More savings could be achieved by reusing the struvite as a P fertilizer, requiring less fossil fuels for synthetic fertilizer production (Maurer et al., 2003). This was however not further quantified.

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[21] Worst case scenario: temperature of tap water is 10 °C, black water with 1.5 L of urine and faeces and 3.5 L of tap water then has a temperature of 18 °C. It was assumed that in total the black water would be heated with 9 degrees, as heat loss can take place during transport and more tap water will be used for cleaning.

Table 7.2 Energy production and consumption in the black water treatment concept and conventional WWTPs.

Black water (5 L/p/d)	MJ/p/y electricity	MJ/p/y heat	Conventional WWTP, domestic sewage (124 L/p/d)	MJ/p/y
Vacuum transport [a]	-14		Sewerage [f]	-43
BW heating [b]		-80	WWTP [f]	-96
Biogas production [c]	+56	+84	Biogas production from sludge digestion [f]	+32
P removal by struvite precipitation [d]	-3.5			
N removal by nitritation-anammox [d]	-16			
Total	+22.5			-107
Savings:				
Drinking water production [e]	+19			

[a]4 kWh/p/y, most energy efficient vacuum toilet (WRS, 2001); [b]increase of 9 degrees (worst case), transfer efficiency of 85%; [c]12.5 L CH_4/p/d, 35.6 MJ/m^3 CH_4, 85% efficiency (40% electricity and 60% heat) (van den Berg, 2008); [d](Maurer et al., 2003); [e]0.47 kWh/m^3 (Vewin, 2006); [f]Including N and P removal, (Frijns et al., 2008)

As will be discussed later on, post-treatment to remove pathogens and micropollutants needs more research and optimization. First estimations show that due to matrix effects ozonation for concentrated streams results in similar energy consumption as WWTP effluents, despite the smaller volume that needs to be treated[22]. When combining ozonation with electrodialysis, and taking into account energy savings of artificial fertilizer production, treatment of source-separated streams could be more efficient than conventional WWTPs, as was already shown for the treatment of urine (Dodd et al., 2008).

A compact grey water treatment system currently is under investigation and this system will require energy for treatment as well. Recent research showed that anaerobic treatment of grey water produced a limited methanization of 25%, mainly due to a large amount of colloidal COD and high concentrations of inhibitory surfactants. Bioflocculation of the grey water to concentrate the colloidal and suspended COD was successful. Combined anaerobic treatment of this concentrate with black water may increase the total biogas production by 50% (Hernandez Leal et al., to be submitted).

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[22] Based on the assumption that the ozone dose mainly depends on the amount of dissolved organic matter (Hollender et al., 2009). This would lead to 5.9 MJ/p/y for black water post treatment (125 mg/L DOC) and 5.7 MJ/p/y for conventional WWTP (5 mg/L DOC).

7.2.3 *Nitrogen removal*

Nitrogen recovery from wastewater has to compete with relatively energy-efficient ammonia production from atmospheric nitrogen. Calculations by Maurer et al. (2003) for nitrogen recovery from urine showed that this is possible. However, this was only valid for applying thermal volume reduction or struvite precipitation (including addition of phosphate to remove all nitrogen), and taking into account the energy savings of artificial nitrogen and phosphorus fertilizers. For more diluted streams, in the range of 0.1 – 5 kgN/m^3, autotrophic nitrogen removal, consisting of partial nitritation and anaerobic ammonium oxidation (anammox), is regarded as a promising technology to remove nitrogen (Jetten et al., 1999; Mulder, 2003). Therefore, in this research autotrophic nitrogen removal was selected to remove the ammonium in black water to nitrogen gas. Nitrogen was successfully removed in a two reactor autotrophic nitritation-anammox process (chapter 3 and 4). Compared to conventional nitrogen removal by nitrification and heterotrophic denitrification, expensive and non-sustainable carbon sources such as methanol can be omitted and due to a much lower oxygen demand energy consumption is limited. However, to produce a nitrogen fertilizer, the nitrogen gas, abundantly present in the atmosphere needs to be fixed into a reactive form such as ammonia, using for example the Haber-Bosch process. This would require an energy consumption of 32 MJ/kgN (Moulijn et al., 2001).

A disadvantage of applying anammox bacteria is that they are slow growing organisms and that they are sensitive to for example high nitrite concentrations. By applying sufficient biomass retention and good process control these problems can be overcome, explaining why more and more full scale anammox reactors are built (Abma et al., 2007). This research showed that the calcium concentration in the black water was too low for optimal granule formation and biomass retention (chapter 4). This means that either calcium needs to be added, or other reactor types have to be developed. A rotating biological contacter (RBC), as researched before for the application of the one reactor nitritation-anammox process for the treatment of black water (Vlaeminck et al., 2009), showed good biomass retention, probably because of less shear. Such a RBC may provide a good alternative for the two reactor approach in this research, preventing the addition of extra calcium. However, in a one reactor nitritation-anammox process dissolved oxygen (DO) control is important to inhibit nitrite oxidation. In a RBC DO control is difficult and to prevent nitrite oxidation, elevated free ammonia concentrations were induced by increasing the pH (Vlaeminck et al., 2009). This limits the nitrogen removal efficiency, because an effluent NH_4

concentration of 100 mgN/L in the effluent was necessary to achieve sufficient high levels of free ammonia (3 $mgNH_3/L$ at pH of 7.7) to prevent nitrite oxidation. Both systems, one and two reactor nitritation-anammox reactors, therefore need optimization, either for sufficient biomass retention without addition of calcium, or for appropriate DO control to limit nitrate production.

This research showed that a higher nitrogen removal efficiency was achieved compared to conventional WWTPs (table 7.1). However, the production of nitrous oxide (N_2O) should be taken into account as well, because N_2O has a 300 times stronger global warming gas potential than carbon dioxide. This research showed that N_2O is produced in the two reactor nitritation-anammox process, mainly during partial nitritation. In the partial nitritation reactor the N_2O emission fluctuated between 0.6% to 2.6% (average 1.9%) of the influent load of nitrogen. Because high nitrite concentrations are always present in the partial nitritation reactor, and these are known to stimulate N_2O production (Kampschreur et al., 2009b), it seems that N_2O emissions cannot be avoided. Although in the anammox reactor N_2O emission possibly can be prevented by avoiding nitrite accumulation (chapter 4), more research is needed to elucidate the mechanisms of N_2O emission, in particular because in a one reactor nitritation-anammox process with low nitrite concentrations also N_2O was emitted (1.2%, (Kampschreur et al., 2009a)). These emissions are higher than the default N_2O emission factor of 1.0% of the nitrogen load applied by the IPCC and the Dutch government for the emissions of greenhouse gases from wastewater (VROM, 2008). A recent study towards N_2O emissions at Dutch WWTPs raised more questions than answers and it showed that the IPCC emission factor probably is not representative. The emission of N_2O from three different domestic sewage WWTPs ranged from 0.04% to 6.1% (van Voorthuizen et al., 2009). The following factors can be related to the N_2O emission: process configuration, oxygen control, nitrogen load, temperature and the BOD/N ratio. More research is needed to determine a quantitative relationship of these factors with the N_2O emission. If the N_2O production cannot be reduced by changing process conditions in autotrophic nitrogen removal from concentrated black water, recovery of nitrogen may become more realistic.

7.2.4 Phosphorus recovery

Energy aspects are not important for phosphorus recovery. Maurer et al. (2003) showed that struvite production costs less energy than artificial P-fertilizer production. Distribution of phosphorus in black water over liquid and solids was shown to be highly dependent on the black water concentration and the pH. In the anaerobic effluent 61% of the phosphorus was conserved and can be recovered as struvite, representing 17% of the artificial phosphorus fertilizer production in the Netherlands (10% of the global artificial phosphorus fertilizer production, chapter 5). When more diluted black water was treated in a UASB reactor, 95% of the phosphorus could be conserved in the effluent (van Voorthuizen et al., 2008).

The amount of phosphorus that can be recovered from black water could be increased if the excess anaerobic sludge, which contains the remaining fraction of phosphorus from vacuum collected black water, can be reused as a P-enriched organic fertilizer, provided that it is safe regarding heavy metals, pathogens and micro-pollutants. Preliminary results (chapter 5) indicated that the UASB sludge would reduce the amount of heavy metals applied to agricultural fields in comparison with the use of cow manure. Prevailing Dutch standards for the application of sewage sludge might however forbid the application of UASB sludge[23]. Also, more research is needed about the presence of organic micro-pollutants in the UASB sludge, such as hormones and pharmaceutical residues, for which the emission into the environment is a growing concern (Daughton and Ternes, 1999; Winker et al., 2009). When reuse of the sludge is not possible, incineration of the sludge may be an option, because recently, ashes of sewage sludge were regarded as a resource for the phosphorus industry. However, the amount of iron and other heavy metals may be the limiting factor for this reuse route (Schipper et al., 2007). No data on the energy requirements for this process was found, but it is expected that this will be substantial, especially for the dewatering of the sludge and removal of heavy metals.

Another potential source of phosphorus recovery is animal manure. Worldwide 12 – 15 million tP/year is produced by domestic animals, and this is about 4-5 times more than what is produced by humans (Cordell et al., 2009; Smit et al., 2009). Most of the animal manure is spread on land, but in countries with a surplus of manure, the soil is saturated with phosphates and the phosphorus finally is lost to inland or coastal waters (Cordell et al., 2009). The treatment concept developed in this research might also be used for phosphorus recovery from digested manure. Human excreta and animal manure are potential sources for phosphorus recovery and controlled reuse of phosphorus products derived from these sources can contribute to a reduction in phosphate lost to inland or coastal waters (Rockström et al., 2009).

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[23] http://www.eu-milieubeleid.nl/ch05s10.html, visited on 25-09-2009; Manual of Environmental Policy, in Dutch

7.2.5 Hormones and pharmaceutical residues

Even though black water is a very complex matrix, it was possible to detect several micro-pollutants. During biological treatment a limited number of the selected organic micro-pollutants was eliminated (chapter 6). Only paracetamol and compounds that adsorb to sludge, such as tetracycline, were removed during anaerobic treatment in the UASB reactor. It is not expected that anaerobic treatment can contribute significantly to the removal of organic micro-pollutants (Kujawa-Roeleveld et al., 2008). Several micro-pollutants are known to biodegrade slowly and are better removed in WWTPs which operate at relatively long aerobic sludge retention times (SRT) (Jones et al., 2005). In this research for example, the aerobic SRT in the partial nitritation reactor was relatively short and fluctuating and by increasing this, a higher removal efficiency of for example metoprolol may be achieved. However, this is limited to prevent nitrite oxidation (chapter 3). To remove persistent polar micro-pollutants, such as carbamazepine, the need for an (advanced) physical or chemical treatment system seems to be unavoidable. This was confirmed by other research as well (de Mes, 2007; Kujawa-Roeleveld et al., 2008).

Advanced treatment processes should be investigated for their feasibility to remove micro-pollutants, in combination with the removal of pathogens, because advanced treatment processes such as ozonation can provide disinfection, color removal and removal of micro-pollutants (e.g. (Hollender et al., 2009)). Ozonation is also known to increase the biodegradability and a combination with biological post-treatment might increase the total removal of the micro-pollutants (Li et al., 2006).

Little is known about the toxicological effects of pharmaceuticals in the environment, but their biological activity and the increasing use of pharmaceuticals, necessitates the research on their fate during wastewater treatment and the need for advanced treatment to prevent the entry of these compounds to the environment (Jones et al., 2005; Schwarzenbach et al., 2006; Schirmer and Schirmer, 2008). Additionally, a thorough risk assessment is needed to analyze the total combined effect of persistent micro-pollutants in the environment. The problem associated with the presence of micro-pollutants in wastewater and surface waters can be much broader than only pharmaceuticals. Grey water contains most personal care products, such as fragrances and UV-filters. These compounds can have an estrogenic or other negative effect on the environment as well (Ternes and Joss, 2006). Several ecological tests exist to determine estrogenicity and toxicity of a mixture of compounds. These tests can be used to screen for the overall toxicity and estrogenicity of several compounds, as was shown for urine and wastewater (Escher et al., 2005; Escher et al., 2006). A combination of chemical analysis and bioassays will be necessary to monitor treatment efficiencies and to determine ecotoxicological effects in the environment.

7.3 Outlook

7.3.1 New sanitation systems

For each situation, rural or urban, a concept can be designed in which wastewater is not treated as ‘waste’, but as a resource. This is important to protect world water resources and to contribute to the recycling of nutrients, energy and water. The choice of the degree of separation, e.g. separation of black (urine and faeces) and grey water, or separation of urine, brown (faeces) and grey water, depends on local circumstances and will result in different treatment techniques to recover different products. For example, in this research the UASB effluent was further treated to remove the nutrients, but depending on the distance to agriculture it may also be possible to use it as a liquid fertilizer after proper treatment to remove pathogens and micro-pollutants (Winker et al., 2009). Struvite production from black water represents 17% of the artificial phosphorus fertilizer production in the Netherlands and when used properly this could reduce the amount of fossil fuels that is used for the artificial phosphorus fertilizer production and the amount of mined phosphate rock.

To achieve successful implementation of new sanitation concepts a new planning and design paradigm is needed, in which attention is given to stakeholder interests. Cooperation within the whole water chain is required, for example between municipalities, drinking water companies and agriculture. Economics and process performance are not the only factors for success and sociological aspects such as public acceptance should also be taken into account (Guest et al., 2009). New sanitation concepts require a different infrastructure than conventional sewer systems and could therefore require a different management with different stakeholders involved. Companies or institutes that treat the wastewater should become companies that produce irrigation water, fertilizers and energy. End-users are crucial actors and their role in wastewater management is very important for the success of new sanitation concepts. For example, new sanitation systems are linked with nature and end-users can be empowered citizen-consumers capable of changing the system of sewerage and centralized wastewater treatment (Hegger, 2007).

Governments can contribute significantly to the development and implementation of new sanitation concepts by giving their financial support. For example in China, the government has supported the implementation of millions of biogas plants for single rural families and this contributes significantly to the emission reduction of CO_2 and SO_2 by replacing fossil fuels with biogas (Zhang et al., 2007). The same should be possible to implement new sanitation systems in urban areas. Furthermore, developed countries such as the Netherlands, should serve as a model to show that wastewater treatment systems should be designed as resource recovery systems.

7.3.2 Costs

The costs for conventional WWTPs depend largely on the scale: e.g. for 10.000 p.e. the costs are € 50/p.e./year and for 100.000 p.e. the costs are € 25/p.e./year (UVW, 2006). However, larger scale usually also means that more costs are involved for transport. On average the costs for wastewater treatment, including transport and sludge treatment, was € 40/p.e./year in 2006 in the Netherlands (UVW, 2006).

First estimations show that a new sanitation system for a community of 500 houses will be close to € 40/p.e./year (Meulman, 2009), without taking into account the benefits from the produced resources such as struvite. In this costs estimation advanced treatment for the removal of micro-pollutants was not included. An important aspect with respect to costs in new sanitation systems, is that there are a lot of opportunities to save energy and resources and therefore money. For example, applying vacuum toilets saves 25% of the total drinking water consumption, which saves €12/p.e./year[24]. As was mentioned earlier, struvite can be used as a fertilizer, replacing 17% of the artificial phosphorus fertilizer production in the Netherlands and 10% worldwide (chapter 5). The produced 0.22 kgP/p/y of struvite could have a value of €0.33/p.e./y (€200/t struvite (12.7% P) (Ueno and Fujii, 2001)). Furthermore, as mentioned before, recovering thermal heat from grey water has a large potential in recovering energy and thereby reducing the consumption of natural gas.

A recent study for new sanitation systems in urban planning in China showed that the economical benefits of sustainable sanitation concepts are attractive (Kerstens et al., 2009). In this study different scenarios were evaluated including separation at source of black and grey water. Membrane bioreactors (MBRs) were used to produce high quality effluent, in combination with anaerobic treatment to produce biogas and struvite precipitation. Provided that the recovered resources can be sold, already after

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[24] Based on €1.30/m^3 (www.vitens.nl , visited on 23rd December 2009) and a saving of 24% on 124 L/p/d (Kanne, 2005).

5 years the break even point can be reached compared to treatment in conventional WWTPs.

Especially in areas where there is no central sewerage and wastewater treatment, such as several areas in China, implementation of new sanitation systems is promising. Furthermore, in densely populated areas upgrading existing WWTPs is often not possible because of lack of space and new sanitation systems with a smaller footprint can be an alternative instead of transporting wastewater over long distances. Investment and capital costs will be higher for new sanitation systems than conventional WWTPs, but running costs will decrease when the recovered resources can be sold.

7.3.3 Recommendations for further research

This research showed that a new sanitation concept for black water can be self sufficient in energy. To maximize the energy recovery, combined anaerobic treatment of black water and bioflocculated grey water is promising and can increase the biogas production with 50% (Hernandez Leal et al., to be submitted). Another aspect of grey water that is gaining more attention is its thermal energy content. In the Netherlands yearly $5.8*10^3$ MJ/p/y (ref Frijns et al. 2009) is used for warming tap water and this is about 60 times more than the energy needed in WWTPs (table 7.2). By installing heat recovery systems from the drain, significant energy recovery can take place at household level[25].

For nitrogen recovery, energy consumption is the decisive factor, and in general biological nitrogen removal is preferred over its recovery. However, N_2O emissions potentially present a disadvantage of applying autotrophic nitrogen removal and this needs more research. To increase the amount of phosphorus recovery from black water, more research is needed for the reuse possibilities of anaerobic sludge. Another nutrient that is abundantly present in faeces and urine, is potassium. This is one of the macronutrients needed for growth and it is a limiting resource, although not to the same extent as phosphorus (Jönsson et al., 2004). To maximize the amount of recovered nutrients, further research should focus on how to recover potassium as well. Potassium could be recovered together with phosphorus in the

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[25] e.g. : http://www.toolbase.org/Technology-Inventory/Plumbing/drainwater-heat-recovery; http://www.groene-energiewinkel.nl/Douche+warmte-terugwinning/Informatie+over+douche+warmteterugwinning/ (visited on 12th December 2009)

form of struvite, where it replaces the ammonium (Wilsenach et al., 2007), although this would require the addition of extra magnesium and phosphate.

Recently a lot of research is dedicated to algae to achieve combined wastewater post-treatment and production of algae as a biofuel or fertilizer (Munoz et al. 2006; Mata et al., 2010). Algae remove nutrients, such as nitrogen and phosphorus, from wastewater and they consume carbon dioxide. Current limitations of this technology are the harvesting of biomass, high land requirement and high construction costs. Currently algae are investigated for its application to upcycle nutrients from urine and anaerobically treated black water[26], which could be a promising alternative for nutrient recovery.

A thorough risk assessment is needed on the fate and effects of micro-pollutants in the environment. Although there are major concerns about the effects of micro-pollutants in the environment, the question "Do we need to remove micro-pollutants from wastewater?" is not answered yet (Larsen et al., 2009). Measures taken at the source to reduce the consumption rate of these compounds and to apply biodegradable compounds should be investigated as well, similar to what was done to control the use of pesticides (Gerecke et al., 2002).

With respect to pathogens, new sanitation concepts should even have a higher level of hygiene than conventional sewerage and WWTPs. In new sanitation concepts removal of pathogens from black water is of importance because distances between discharge and build environment are generally smaller than in conventional centralized WWTPs, potentially increasing the risk of spreading diseases. A thorough risk assessment is needed to conclude on this. More research is also needed with respect to guidelines, because current regulatory standards for recreational areas and swimming waters based on indicator organisms may not be sufficient to mitigate the problem of pathogens (Crocket, 2007; Wen et al., 2009).

The emission of greenhouse gases should be taken into account as well when designing new sanitation concepts. Apart from nitrous oxide, the emission of methane should be prevented. Although most of the methane that is produced, will leave the system in the collected biogas, a small fraction can dissolve and depending on the conditions, can be released in later stage to the atmosphere. Diffuse emissions can already occur in sewer systems and collection tanks (Guisasola et al., 2008). New sanitation systems might prevent this.

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[26] http://www.ete.wur.nl/UK/Research/Reusable+Water/Biological+Water+Treatment/Algae+to+upcycle+nutrients+from+concentrated+urine+and+UASB-digested/ , visited 14-12-2009

Demonstration projects for new sanitation, like the Decentralized Sanitation and Reuse (DESAR) project in Sneek, the Netherlands, are a good start to demonstrate that wastewater is a resource. Because new sanitation concepts require a different infrastructure than conventional sewerage and WWTPs, new housing estates and renovation projects have the opportunity to show that wastewater should be treated as a resource in industrialized countries such as the Netherlands. Furthermore there is a large potential for new sanitation systems in countries with growing urbanization, where no wastewater infrastructure is present yet, such as China (Kerstens et al., 2009).

Summary

New sanitation concepts are based on separation at source of household wastewater streams into grey water and black water (faeces and urine) or into grey water, urine and brown water (faeces), and have a large potential to recover the important resources energy, nutrients and water. They present an alternative approach for contemporary sewer systems combined with centralized, aerobic wastewater treatment plants (conventional WWTPs), which are confined to the removal of organics and nutrients from wastewater. In this thesis several aspects of a treatment concept for black water were investigated.

Chapter 1 gives a literature review regarding the components in source separated black water and treatment and/or recovery techniques for these components. Depending on the degree of separation and the degree of dilution with flushing water, different treatment concepts and reuse options are possible. Black water not only contains half of the load of organic material and the major fraction of the nutrients nitrogen and phosphorus in domestic wastewater, but also most of the hormones, pharmaceutical residues and pathogens. Grey water has a high potential for reuse as e.g. irrigation water, because it is the major volume fraction (70%) of domestic wastewater and is relatively low in pollution.

A promising treatment system for highly concentrated black water, collected with vacuum toilets, would consist of anaerobic treatment followed by struvite precipitation for phosphorus recovery and autotrophic nitrogen removal. This would enable a production of 56 MJ/p/y of electricity and a reduction of the global artificial phosphorus fertilizer by a maximum of 21%. Nitrogen recovery from wastewater has to compete with the relatively energy-efficient ammonia production from atmospheric nitrogen. For streams with nitrogen concentrations in a range of 0.1 – 5 kgN/m^3, which includes black water, biological nitrogen removal therefore is preferred from energy perspective. The issue of hormones, pharmaceuticals and pathogens in black water and their entry to the environment requires more research to determine their fate during treatment and the necessary degree of removal to exclude negative effects.

The black water in this research was collected with vacuum toilets, installed at a small community in Sneek (Friesland, the Netherlands). Black water, collected with vacuum toilets, is 25 times more concentrated, than the total wastewater stream from a typical Dutch household. A UASB (Upflow Anaerobic Sludge Blanket) reactor was

selected for energy recovery from the organic pollutants in black water. The performance of this UASB reactor is described in **chapter 2**. It removed on average 78% of the influent load of organic pollutants expressed in chemical oxygen demand (COD) at an hydraulic retention time (HRT) of 8.7 days, a temperature of 25 °C and a loading rate of 1.0 kgCOD/m^3/d. The methane production was 1.8 m^3 CH_4 per m^3 of black water, which can be converted to 56 MJ/p/y as electricity and 84 MJ/p/y as heat by combined heat and power (CHP). The minimum reactor volume needed for a full scale application was calculated to be 63L per person (for black water containing 16 gCOD/L, produced at 5 L/p/d). This is more than two times smaller than other type of reactors for anaerobic treatment of concentrated black water, like a UASB-septic tank or a continuously stirred tank reactor (CSTR). The effluent of the UASB reactor needs further treatment to remove remaining COD (1.2 g/L) and biochemical oxygen demand (BOD_5) (0.48 g/L). Furthermore, 91% of the nitrogen and 61% of the phosphorus was conserved in the effluent of the UASB reactor.

Because the COD/N ratio of the UASB effluent is very low (~ 1), autotrophic nitrogen removal was selected to remove the nitrogen from black water. This was done in a two reactor nitritation-anammox process. Partial nitritation of the ammonium in the UASB effluent was successfully achieved in a continuous system operated at 34 °C and at 25 °C (**chapter 3**). Without strict process control an effluent with a stable ratio of 1.3 NO_2-N/NH4-N was produced for a period of 400 days at 25 °C. Nitrite oxidizers were successfully outcompeted due to inhibition by free ammonia and nitrous acid, and due to a fluctuating solids retention time (SRT) (1.0 to 17 days) and pH (from 6.3 tot 7.7).

The anammox process was applied to remove the ammonium and nitrite from the effluent of the partial nitritation reactor (**chapter 4**). The main fraction, 85 to 89%, of total nitrogen could be removed. This however could only be accomplished when the (free) calcium concentration of the black water of 42 mg/L was increased by an additional 39 mg/L. This resulted in a better granulation of the biomass, a better biomass retention and, irrespective of the temperatures that were applied of 35 °C and 25 °C, a nitrogen removal rate of 0.5 gN/L/d.

The emission of the strong global warming gas nitrous oxide (N_2O) is a growing concern in biological nitrogen removal processes. In the partial nitritation process 0.6 - 2.6% (average 1.9%) of the total nitrogen load was emitted as N_2O. In the anammox reactors N_2O was produced as well, but only when incomplete anammox conversion was accompanied by elevated levels of nitrite. In the anammox process N_2O can therefore be avoided, by preventing nitrite accumulation by strict process control. However, because high nitrite concentrations are always present in the partial nitritation reactor, and these stimulate N_2O production, it seems that N_2O emissions in the partial nitritation reactor cannot be avoided. More research is needed to elucidate the mechanisms of N_2O emission, in particular because also one reactor nitritation-anammox processes with low nitrite concentrations emit significant amounts of N_2O.

In **chapter 5** the fate of phosphorus in concentrated black water during storage and during anaerobic treatment was investigated. The phosphorus balance of a UASB reactor treating concentrated black water showed a phosphorus conservation of 61% in the anaerobic effluent. Precipitation of phosphate as struvite from this stream can result in a recovery of 0.22 kgP/p/y, representing 10% of the global artificial phosphorus fertilizer production. The remaining part of the phosphorus ended up in the anaerobic sludge, mainly due to precipitation (39%). Simulation of chemical precipitation showed a strong effect of the pH and the phosphorus concentration on the phosphorus distribution in the black water. A low dilution and a high pH favour the accumulation of phosphorus in the anaerobic sludge, and this sludge may be used as a phosphorus-enriched organic fertilizer, provided it is safe with respect to heavy metals, pathogens and micro-pollutants.

The fate of a number of selected hormones and pharmaceuticals during anaerobic treatment of vacuum collected black water followed by nitrogen removal by two reactor nitritation-anammox process was investigated in **chapter 6**. Vacuum collected black water contains hormones and pharmaceuticals at relatively high concentrations (μg/L to mg/L range). Even though black water is a very complex matrix, it was possible to detect the presence of a selection of hormones and pharmaceuticals. Anaerobic treatment was only suitable to remove the majority of paracetamol. Metoprolol and ibuprofen were (partly) removed during aerobic treatment in the partial nitritation reactor. Diclofenac was not removed. The presence of persistent micro-pollutants (e.g. carbamazepine and cetirizine), which are not susceptible for biodegradation, makes the application of advanced physical and chemical treatment unavoidable. The risk that micro-pollutants may pose, and therefore the extent at which they should be removed, is difficult to assess and not understood very well, although several negative effects have been reported already at ng/L levels. More research is also needed to better understand the combined effect of micro-pollutants in the environment.

In **chapter 7** the results of this research are discussed in a general context. By combined anaerobic treatment, autotrophic nitrogen removal and struvite precipitation from concentrated black water high removal efficiencies of 87% COD, 86% nitrogen and 94% phosphorus were achieved. The amount of energy that can be recovered in the form of electricity and heat, is more than sufficient for the anaerobic treatment, nitrogen removal and phosphorus recovery from black water. Furthermore, by struvite precipitation from the anaerobic effluent, 10% of the global artificial phosphorus fertilizer production can be replaced. The use of vacuum toilets saves €12/p.e./year of drinking water.

New sanitation systems can be attractive from an economic point of view, provided that there is a market for the recovered resources. More research is needed on the production of nitrous oxide from biological nitrogen removal processes, because this is an uncontrolled source of emission of reactive nitrogen and greenhouse gases to the atmosphere. The amount of phosphorus recovered from black water can be increased if the excess anaerobic sludge, which contains the remaining part of phosphorus from vacuum collected black water, can be reused as a phosphorus-enriched organic fertilizer, but more research is needed with respect to the presence of heavy metals, pathogens and micro-pollutants in this sludge. A thorough risk assessment is needed to conclude on the overall effect of micro-pollutants and pathogens in the environment, and to determine to which extent they should be removed in a post treatment step.

New sanitation systems require a different infrastructure and management, but would result in a more sustainable concept for sanitation; one in which energy and nutrients are recovered and pathogens and micro-pollutants are removed, producing clean water while also protecting the environment.

Samenvatting

Nieuwe sanitatie concepten zijn gebaseerd op het scheiden aan de bron van huishoudelijk afvalwater in grijs water en zwart water (faeces en urine) of in grijs water, urine en bruin water (faeces), en hebben een groot potentieel om belangrijke grondstoffen zoals energie, nutriënten en water terug te winnen. Deze concepten zijn een alternatief voor de huidige rioleringssystemen en centrale aerobe afvalwaterzuiveringen die beperkt zijn tot het verwijderen van organische componenten en nutriënten. In dit proefschrift worden diverse aspecten van een concept voor de behandeling van zwart water besproken.

Hoofdstuk 1 geeft een literatuuroverzicht betreffende de belangrijkste componenten in brongescheiden zwart water en de technieken om deze componenten terug te winnen of te verwijderen. Afhankelijk van de mate van scheiding en de mate van verdunning met spoelwater, zijn verschillende behandel- en hergebruikconcepten mogelijk. Zwart water bevat niet alleen de helft van de organische belasting in huishoudelijk afvalwater en het grootste deel van de stikstof en fosfor, maar ook het merendeel van de pathogenen, hormonen en medicijnresten. Grijs water heeft een groot potentieel om hergebruikt te worden als bijvoorbeeld irrigatiewater, omdat het de grootste volume stroom is (70%) van huishoudelijk afvalwater en relatief weinig vervuild.
Een veelbelovend behandelingsproces voor geconcentreerd zwart water, verzameld met vacuüm toiletten, bestaat uit anaerobe behandeling gevolgd door struviet precipitatie voor fosfaat terugwinning en autotrofe stikstofverwijdering. Dit levert een energieproductie op van 56 MJ/p/jaar aan elektriciteit en kan de wereldwijde fosfaat meststofproductie verlagen met maximaal 21%. Stikstofverwijdering uit afvalwater moet concurreren met het relatieve energiezuinige ammonia productie uit atmosferisch stikstof. Voor stromen met stikstofconcentraties van 0.1 – 5 kgN/m^3, waaronder zwart water, heeft biologische stikstofverwijdering daarom de voorkeur vanuit het oogpunt van energie. De emissie van hormonen, medicijnresten en pathogenen naar het milieu moet nader onderzocht worden om te bepalen wat het lot is tijdens zuivering en in hoeverre deze componenten verwijderd moeten worden zodat ze geen effect hebben op het milieu.

Het zwart water in dit onderzoek werd verzameld met vacuüm toiletten, die zijn geïnstalleerd in een kleine gemeenschap in Sneek (Friesland, Nederland). Zwart water, wat verzameld is met vacuüm toiletten, is 25 keer geconcentreerder dan de totale huishoudelijke afvalwaterstroom van een Nederlands huishouden. Een UASB (Upflow Anaerobic Sludge Blanket)[27] reactor was geselecteerd om de organische last te verwijderen en energie terug te winnen uit het zwart water. De resultaten van het opereren van deze reactor staan beschreven in **hoofdstuk 2**. Gemiddeld werd 78% van de ingaande hoeveelheid organisch materiaal uitgedrukt in chemisch zuurstofverbruik (CZV) verwijderd bij een hydraulische verblijftijd (HRT) van 8.7 dagen, een temperatuur van 25 °C en een belasting van 1.0 kgCZV/m^3/d. De methaan productie was 1.8 m^3 CH_4 per m^3 zwart water, wat gebruikt kan worden om 56 MJ/p/jaar aan elektriciteit en 84 MJ/p/jaar aan warmte te produceren met warmte kracht koppeling (WKK). Het minimale volume van de reactor nodig voor toepassing in de praktijk is 63 L per persoon (voor zwart water met 16 g CZV/L). Dit is meer dan twee keer kleiner dan andere type reactoren toegepast voor anaerobe behandeling van zwart water, zoals een UASB-septic tank en een CSTR (continu geroerde tank reactor). Het effluent van de UASB reactor moet verder behandeld worden om het overgebleven CZV (1.2 g/L) en biologisch zuurstofverbruik (BOD_5) (0.48 g/L) te verwijderen. Bovendien was 91% van het stikstof en 61% van het fosfor geconserveerd in het effluent van de UASB reactor, wat verwijderd moet worden voordat het geloosd mag worden.

Autotrofe stikstofverwijdering was geselecteerd om het stikstof uit het zwart water te halen, omdat de CZV/N verhouding van het UASB effluent laag is (~ 1). Dit werd gedaan in een twee reactor nitritatie-anammox process. Partiële nitritatie van het ammonium in het UASB effluent was succesvol in een continu systeem opererend bij 34 °C en 25 °C (**hoofdstuk 3**). Zonder strikte controle van het proces werd een effluent met een stabiel gehalte van 1.3 NO_2-N/NH_4-N behaald voor een periode van 400 dagen bij 25 °C. Nitriet oxideerders werden uitgespoeld door inhibitie komende van vrij ammoniak en salpeterig zuur en door de fluctuerende condities in de slib verblijftijd (SRT, 1.0 tot 17 dagen) en pH (van 6.3 tot 7.7).

Het anammox proces was toegepast om het ammonium en nitriet te verwijderen uit het effluent van de partiële nitritatie reactor (**hoofdstuk 4**). Het grootste gedeelte van de totale stikstofvracht, 85 tot 89%, werd verwijderd. Dit werd echter alleen bereikt wanneer de calcium concentratie in zwart water van 42 mg/L werd verhoogd met een extra 39 mg/L. Dit resulteerde in een betere korrelvorming, een betere

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[27] Een UASB reactor is een anaerobe reactor met een slib-bed waardoor de vloeistof omhoog stroomt.

biomassa retentie en, onafhankelijk van de temperatuur die was toegepast (35 °C en 25 °C), een stikstofverwijdering van 0.5 gN/L/d.
De emissie van het sterke broeikasgas distikstofoxide (lachgas, N_2O) staat steeds meer in de belangstelling in biologische stikstof verwijderingprocessen. In het partiële nitritatie proces werd 0.6 – 2.6% (gemiddelde 1.9%) van de totale stikstofbelasting als N_2O geproduceerd. In de anammox reactoren werd N_2O ook geproduceerd, maar alleen wanneer incomplete conversie gepaard ging met een verhoogde concentratie van nitriet in de reactor. De productie van N_2O in het anammox proces kan daarom vermeden worden, door te voorkomen dat nitriet ophoopt door het toepassen van een strakke proces controle. Echter, omdat hoge nitriet concentraties in de partiële nitritatie reactor altijd aanwezig zijn, en dit de N_2O productie stimuleert, lijkt het erop dat N_2O productie in de partiële nitritatie reactor niet voorkomen kan worden. Er is meer onderzoek nodig om uit te zoeken wat de mechanismen zijn achter N_2O productie, ook omdat in een één reactor nitritatie-anammox proces met lage nitriet concentraties ook een significante N_2O productie was gerapporteerd.

In **hoofdstuk 5** wordt de verdeling van fosfor in het geconcentreerde zwart water tijdens transport en anaerobe behandeling behandeld. De fosfor balans van de UASB reactor liet een fosfor conservering van 61% in het anaerobe effluent zien. Precipitatie van fosfaat in de vorm van struviet van deze stroom kan resulteren in een terugwinning van 0.22 kgP/p/jaar, wat zo'n 10% van de wereldwijde fosfor meststofproductie omvat. De rest van het fosfor eindigde in het anaerobe slib, voornamelijk door spontane precipitatie (39%). Simulatie van chemische precipitatie liet zien dat de pH en de fosfor concentratie belangrijk zijn voor de verdeling van fosfor in het zwart water. Een lage verdunning en een hoge pH leidt tot de ophoping van fosfor in het anaerobe slib, en dit slib zou hergebruikt kunnen worden als een fosforrijk organische meststof, mits het veilig is wat betreft zware metalen, pathogenen en microverontreinigingen.

Een selectie van hormonen en medicijnresten was onderzocht in de anaerobe behandeling van het zwart water gevolgd door de stikstofverwijdering met het nitritatie-anammox proces en gerapporteerd in **hoofdstuk 6**. Zwart water, verzameld met vacuüm toiletten, bevat hormonen en medicijnresten in relatief hoge concentratie (μg/L tot mg/L). Ondanks dat zwart water een complexe matrix is, was het mogelijk om de aanwezigheid van een selectie van hormonen en medicijnresten te detecteren. Anaerobe behandeling was alleen voldoende om het grootste deel van paracetamol te verwijderen. Metoprolol en ibuprofen werden alleen gedeeltelijk verwijderd tijdens aerobe behandeling in de partiële nitritatie reactor. Diclofenac werd helemaal niet verwijderd. Omdat er in het effluent van de biologische behandeling nog steeds persistente microverontreinigingen aanwezig waren, die niet biologisch werden afgebroken, is de toepassing van geavanceerde fysische en chemische behandeling onvermijdelijk. Ondanks dat er al verschillende negatieve effecten gerapporteerd zijn op ng/L schaal, is het risico van microverontreinigingen in de natuur moeilijk te bepalen en relatief onbekend, en daarmee ook tot welk niveau ze moeten worden verwijderd. Er is ook meer onderzoek nodig om beter het gecombineerde effect te begrijpen van microverontreinigingen in het milieu.

Hoofdstuk 7 bevat een algemene discussie over de resultaten van dit proefschrift. Relatief hoge verwijderingpercentages werden bereikt in de gecombineerde anaerobe behandeling, autotrofe stikstofverwijdering en struviet precipitatie van 87% CZV, 86% stikstof en 94% fosfor. De hoeveelheid energie die teruggewonnen kan worden in de vorm van elektriciteit en warmte, is meer dan voldoende voor de anaerobe behandeling, stikstof verwijdering en fosfor terugwinning uit zwart water. Bovendien kan 10% van de wereldwijde fosfor meststof vervangen worden. Het gebruik van vacuüm toiletten bespaart € 12 /p/jaar aan drink water.

Nieuwe sanitatie systemen kunnen aantrekkelijk zijn, ook vanuit een economisch perspectief, mits er een markt is voor de teruggewonnen grondstoffen. Er is meer onderzoek nodig naar de productie van N_2O uit biologische stikstof verwijderingprocessen, omdat dit een ongecontroleerde bron is van emissie van reactief stikstof en broeikasgas naar de atmosfeer. De hoeveelheid fosfor die teruggewonnen kan worden, kan verhoogd worden als het anaerobe spuislib hergebruikt kan worden als een fosforrijke organische meststof, maar er is meer onderzoek nodig betreffende de aanwezigheid van zware metalen, pathogenen en microverontreinigingen in het slib. Een grondige risicoanalyse is nodig om te bepalen wat het totale effect is van microverontreinigingen en pathogenen in het milieu, en om te bepalen in hoeverre deze verwijderd moeten worden in een nabehandelingstap.

Nieuwe sanitatie systemen vereisen een andere infrastructuur en management, maar kunnen resulteren in een duurzaam concept voor sanitatie, waarin energie en nutriënten worden teruggewonnen en waarin pathogenen en microverontreinigingen worden verwijderd, zodat schoon water geproduceerd wordt en het milieu beschermd wordt.

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Wow I did it! What a crazy job, writing a PhD thesis, but I'm really proud and happy.

I learned about Wetsus already during my Master thesis. A fellow student was doing his PhD there as well, but I thought: 'No Leeuwarden is in Friesland, I will not go there'. Then I saw an advertisement about my subject and I could not get it out of my head. Well, one interview at Wetsus, one interview in Wageningen and already during that second interview I was told that I got the job. I was surprised, but 'tja' crazy people that want to work with black water are scarce ...!? I visited Wetsus a second time to check if I would really enjoy going all the way to Leeuwarden. The atmosphere was great, Guillo thanks again for the great tour you gave me; this was for me the decisive factor. Yes, I will go to Leeuwarden!
First of all I would like to thank Cees and Johannes for building up Wetsus and for giving me the opportunity to do the research in a completely new lab with new equipment. It was great to work in such a fast growing research institute with good facilities. I would also like to thank Janneke, Jelmer, Wim, Harm, Harrie, Jan T, Pieter, Petra and Bob for all their help in the lab: constructing my set-up, providing assistence in case of difficulties and analyzing the huge number of samples.

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In June 2006 the pilot in Sneek started and finally there was real water for Lucia and me. But how to get the water to Leeuwarden? Well, first using the car of Gert-Jan and a trailer. For a long time we used a rental car. Then suddenly there was a real Wetsus car, not really perfect, but it worked. The maximum was 4x20L jerrycans of black water and 8 grey water tanks (right Lucia?). Once I was almost finished there was a real Wetsus caddy, perfect for the trips to Sneek. Lucia, thanks for the company during the rides to Sneek! Luckily later we both had a number of students who did most of the trips.

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Marthe de Graaff
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About the author

Marthe de Graaff was born on 23rd May 1980. She studied Biochemical Engineering at the Technical University in Delft and obtained her MSc degree (with honors) in August 2005. Her MSc thesis subject at DHV Water (Amersfoort) was on the design of a static tool for the implementation of the BABE technology for upgrading existing wastewater treatment plants. In November 2005 she started her PhD project at Wageningen University, stationed at Wetsus in Leeuwarden. Since January 2010 she is working as a scientific researcher at KWR Watercycle Research Institute in Nieuwegein.

SENSE

Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment

CERTIFICATE

The Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment
(SENSE), declares that

Marthe Sophie de Graaff

Born on: *23 May 1980* at: *Geldermalsen, The Netherlands*

has successfully fulfilled all requirements of the
Educational Programme of SENSE.

Place: *Wageningen* Date: *16 April 2010*

the Chairman of the
SENSE board

Prof. dr. R. Leemans

the SENSE Director
of Education

Dr. A. van Dommelen

The SENSE Research School declares that Ms. Marthe Sophie de Graaff has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 38 ECTS, including the following activities:

SENSE PhD courses:

- **Environmental Research in Context**
- **Research Context Activity: “Organizing a Career Orientation Event for PhD Students within WETSUS (20 November 2008)”**
- **Biological processes in Environmental technology**
- **Basic statistics**
- **Advanced statistics**

Other PhD and MSc courses:

- **Safe and sustainable sanitation (Internet course), Tampere University of Technology**
- **PhD competence assessment**
- **Presentation skills**
- **Teaching and supervising thesis students**
- **Techniques for writing and presenting scientific papers**
- **ETE Department trip**

Oral Presentations:

- **SENSE Symposium Sensible Water Technology, 12 April 2007, Leeuwarden, The Netherlands, "Energy and nutrient recovery and removal of micro-pollutants from black water"**
- **Sanitation Challenge: An International Conference on New Sanitation Concepts and Models of Governance, 20 May 2008, Wageningen, The Netherlands, "The Challenge to treat concentrated black water"**
- **IWA 2nd specialized conference Nutrient management in wastewater treatment processes, 9 September 2009, Krakow, Poland, "Combined anaerobic treatment and autotrophic nitrogen removal from black water"**

Mr. J. Feenstra
SENSE Coordinator PhD Education and Research

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