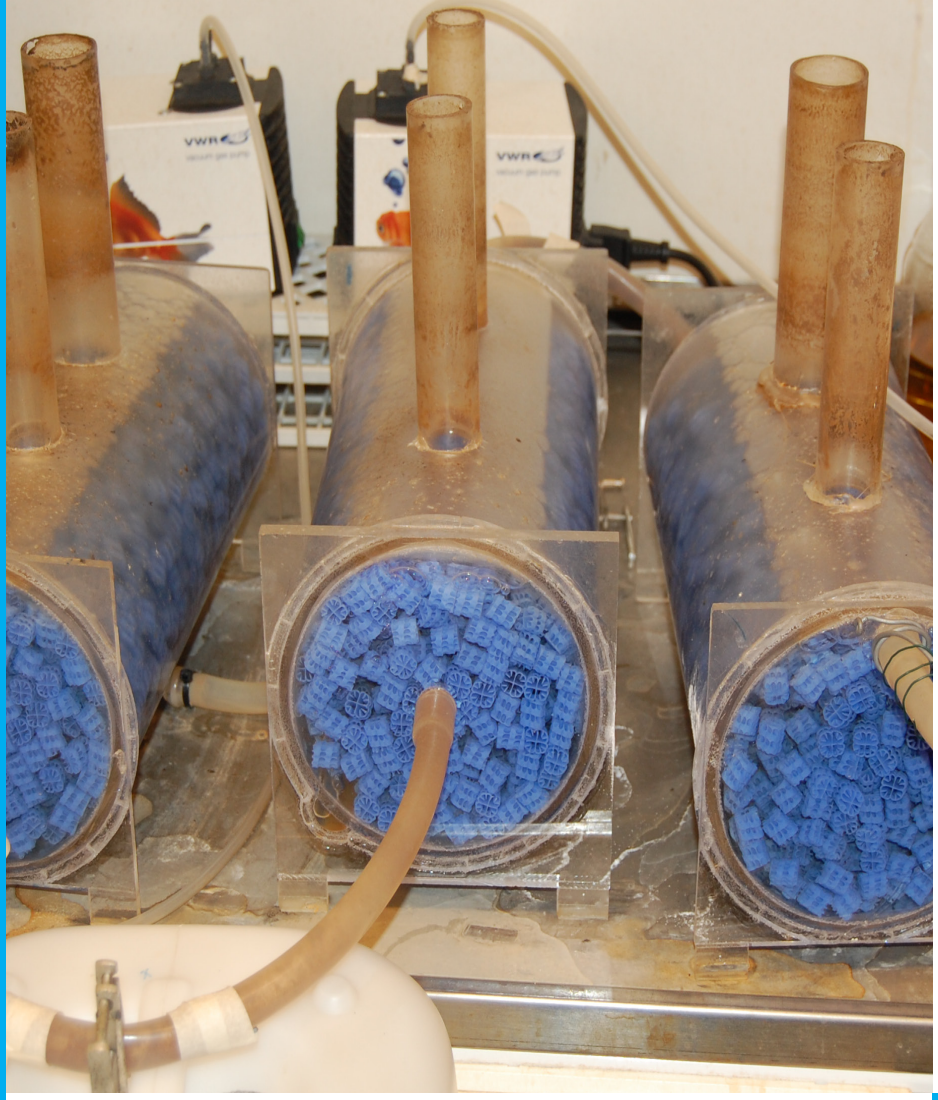

TECHNOLOGY • RESEARCH HIGHLIGHTS • VISIONS • SCIENCE •

98

Waste water treatment by multi-stage biofilm processes

Report of the VESITURVA project

VTT TECHNOLOGY 98

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Results of the VESITURVA project

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Jäteveden puhdistus monivaiheisella biofilmiprosessilla. Tuloksia Vesiturva-projektista. **Tiina Mononen (Ed.), Sebastian Coloma, Martin Romantschuk, Minna Vikman (Ed.), Anu Kapanen, Anna Lehtonen, Elina Saario, Merja Itävaara, Essi Malinen, Silja Kostia &Tuula Tuhkanen.** Espoo 2013. VTT Technology 98. 74 p.

Abstract

Municipal and industrial waste waters in Finland are treated before their release into the environment. New legislation also requires that waste waters from all households with running water are treated before release, whereas the methods for treatment may vary. In the Tekes-Symbio project VESITURVA research groups at the University of Helsinki, VTT, Tampere University of Technology and Lahti University of Applied Science, in collaboration with companies in the field and municipal stakeholders, pooled their resources in an effort to study and improve waste water treatment.

In the case of household waste waters, minimum removal requirements exist only for the bulk components, organic matter (BOD, COD), nitrogen, and phosphorus. While we also monitored the removal of these components in VESITURVA, the main focus was on micropollutants (pollutants that exist in waste water in ng per litre to µg per litre concentrations, for example hormone disruptors, farmaceuticals, musks, components of personal care products etc.) – how they behave and how their removal can be improved.

In VESITURVA we tested waste water treatment methods that are based on biofilms colonising the surfaces of matrix materials using multi-phase water treatment systems, where the water passes through two or three reactors. The model substances for the micropollutant removal process were Bisphenol A (BPA), a component used in the plastics industry, and the commonly used polycyclic musk compound HHCB. Two different types of reactors were used for studying the effect of biofilm activity: Rotating Bed Bioreactors (RBBR), where the biofilm develops on plastic beads with a large surface area, and Fixed Bed Bioreactors (FBBR), where wood chips were used as the support. In both cases a continuous or semicontinuous flow of waste water passed through the reactors. In the RBBR set-up, municipal waste water was led through a three-phase treatment process, while in the FBBR artificial grey water was treated in a two-phase process. In both processes, the removal of the bulk components was good, and substantial reductions were also achieved in the case of the model micropollutants. Multi-stage biofilm reactors seemed to be efficient at removing BPA and musk HHCB from waste water. Major parts of the micropollutants were already removed in the first reactors but the model of sequential reactors enhanced the removal of both BPA and HHCB. Diversity of bacterial communities decreased as a function of time suggesting that the bacterial communities in the

reactors became specialized over time. The same bacterial groups were dominant in all sequential reactors, but differences were observed at genus taxonomic level. Also, the microbial diversity was similar to that seen earlier studies of waste water treatment microbiology. Carrier material (polyethylene, wood chips) affected the biofilm community profile in FBBR.

The performance of FBBR in grey water purification was evaluated in field conditions when the reactor was installed in a detached house. The nitrogen removal efficacy of the reactor was very good and the maximum nitrogen removal efficiency in the system was 84%. Nitrogen removal in grey water treatment system was verified by an evaluation of the abundance of denitrifying microbes. The performance of the grey water treatment system was returned to the original level in 1–2 weeks after the replacement of wood chips, which were used as carrier material. Preliminary results in the laboratory also indicate that nitrogen removal in grey water treatment can be enhanced by using inoculants.

Biological waste water treatment based on RBBRs purified car wash waste waters efficiently, while the reduction of surfactants was at least 95% and the reduction of chemical oxygen demand (COD) between 87 and 95% during the sampling period. Efficient waste water treatment allows automatic car washes to recycle water used for washing. The main challenges for the quality of purified water seems to be optimal nutrient input and an on-line monitoring system for water quality.

We conclude that waste water treatment technology based on microbial biofilms is an efficient alternative, at least in smaller units suitable for single family homes. Although not tested in VESITURVA, we believe that the units and the technology can be upscaled and adapted to at least cover the needs of several families or a small village. Furthermore, the results regarding removal of the micropollutants tested were promising, and it is likely that many other organic micropollutants would behave similarly in multi-phase biofilm treatment systems.

Keywords waste water treatment, biofilm, carrier, pollutant, microbial diversity

Jäteveden puhdistus monivaiheisella biofilmiprosessilla
Tuloksia Vesiturva-projektista

Waste water treatment by multi-stage biofilm processes. Results of the VESITURVA project.
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Tiivistelmä

Yhdyskuntien ja teollisuuden jätevedet puhdistetaan Suomessa ennen kuin ne päästetään ympäristöön. Uusi lainsäädäntö edellyttää myös, että kaikkien juoksevaa vettä käyttävien talouksien jätevesi puhdistetaan, joskin puhdistusmenetelmät voivat vaihdella. Tekesin Symbio-ohjelman projektissa VESITURVA tutkimusryhmät Helsingin yliopistosta, VTT:ltä, Tampereen teknillisestä yliopistosta ja Lahden ammattikorkeakoulusta yhdessä alan yritysten sekä kunnallisten vaikuttajien kanssa yhdistivät voimansa pyrkimyksenään tutkia ja parantaa jäteveden puhdistusta.

Kotitalousjätevesien kohdalla on voimassa vähimmäispoistovaatimuksia vain orgaaniselle kuormalle (BOD/COD), typelle ja fosforille. Vaikka VESITURVA-projektissa seurattiin näiden komponenttien poistumista, päätutkimuskohteena olivat niin sanotut mikropollutantit (haitta-aineet, jotka esiintyvät jätevedessä erittäin pieninä pitoisuuksina, esimerkiksi hormonihäiritsijät, lääkeaineet, hajusteet, hygieniatuotteiden ainesosat) – minne ne kulkeutuvat ja miten niiden poistoa voitaisiin parantaa. Projektissa tutkittiin erityisesti monivaiheisia, biofilmiin perustuvia puhdistusmenetelmiä, joissa mikrobiyhteisöt kolonisoivat kiinteitä alustoja. Puhdistettava vesi kulkeutuu kahden tai useamman peräkkäisen reaktorin kautta ja puhdistuu vähitellen yhä paremmin. Mikropollutanttien malliaineina käytettiin Bisfenoli A:ta, joka on muoviteollisuuden raaka-aine, sekä hajusteena yleisesti käytettyä myskiyhdistettä nimeltä HHCB. Projektissa tutkittiin kahta reaktorityyppiä: pyöriväpatjaista biofilmireaktoria (Rotating Bed Bioreactor – RBBR), jossa biofilmi muodostuu muovisten perforoitujen pallojen pinnalle, sekä kiinteäpatjaista reaktoria (Fixed Bed Bioreactor – FBBR), jossa biofilmi muodostuu puulastujen pinnalle. RBBR-systeemissä kunnallista jätevettä puhdistettiin kolmessa vaiheessa johtamalla jätevettä laboratoriomittakaavan reaktoreiden läpi, kun taas FBBR oli kahden vaiheen systeemi, johon johdettiin keinotekoista harmaavettä. RBBR-systeemiin jätevettä pumpattiin jatkuvatoimisesti, kun taas FBBR-systeemiin synteettinen harmaavesi syötettiin säännöllisissä pulsseissa, koska haluttiin imitoida omakotitalon jäteveden muodostumista. Molemmissa systeemeissä jäteveden pääkomponenttien poisto toimi hyvin, ja merkittävää puhdistumista saavutettiin myös malliaineina käytettyjen mikropollutanttien kohdalla. Suuri osa haitta-aineista poistui jo ensimmäisessä reaktorissa, mutta reaktorien monivaiheisuus paransi poistotehokkuutta. Bakteeridiversiteetti reaktoreissa väheni ajan kuluessa, mikä voi tarkoittaa bakteeriyhteisöjen erikoistumista. Peräkkäisissä reaktoreissa baktee-

riryhmät olivat hyvin samanlaisia, mutta bakteerilajitasolla todettiin eroja. Mikrobisto muistutti yleisesti ottaen aikaisemmin julkaistuja jätevesipuhdistamojen mikrobiyhteisöjä. Kantajamateriaali (polyeteeni, puulastut) vaikutti biofilmin mikrobiston koostumukseen.

FBBR-reaktorien toimivuutta harmaaveden puhdistuksessa arvioitiin myös omakotitaloon sijoitetussa reaktorissa. Typen poisto reaktorissa oli erittäin hyvä, parhaimmillaan 84 %. Typen poiston toimivuus voitiin osoittaa myös sillä, että systeemissä todettiin runsaasti denitrifioivia mikrobeja. Puulastujen vaihtamisen jälkeen reaktorin toiminta palautui alkuperäiselle tasolle 1–2 viikossa. Alustavat laboratoriotulokset myös osoittavat, että typen poistoa voidaan tehostaa käyttämällä siirrosteita.

Pyörivän alustan bioreaktori osoittautui myös tehokkaaksi puhdistamaan automaattisten autopesuloiden jätevesiä. Autonpesuun käytetyn veden tensidipitoisuus väheni 95 % ja kemiallinen hapenkulutus 87–95 % näytteenottoaikana. Pesuveden puhdistaminen mahdollistaa veden kierrätyksen, mikä puolestaan pienentää puhtaan veden tarvetta. Haasteiksi tunnistettiin ravinteidensyötön optimointi sekä veden laadun ajantasaisen tarkkailumenetelmän kehittäminen vikatilanteiden tunnistamiseksi.

Avainsanat waste water treatment, biofilm, carrier, pollutant, microbial diversity

Preface

The Finnish branch organisation Finnish Water Utilities Association FIWA (Vesi ja viemärilaitosyhdistys, recently Vesilaitosyhdistys – VVY) produced a report (VVY, 2008) based on the register (Pollutant Release and Transfer Register/PRTR) published by the European Union. This report examines the environmental release of certain pollutants with industrial and municipal waste waters, and the spread of these pollutants in rivers and lakes. Drainage water from landfills was also included. It is concluded in the report that the concentration of pollutants in both influent and the effluent is so low that the detection level is often not reached. Of the compounds included in the report, only tributyl tin (TBT) frequently exceeded the norms while a number of compounds, such as bentzo(a)pyrene, di-2-ethylheksylphthalate (DEHP), mercury and cadmium exceeded the limits occasionally. It is concluded in the report that due to dilution factors, it is unlikely that the concentrations in the water of the catchment basin would exceed environmental norms.

Harmful substances, also called micropollutants may, however, cause problems in the ecosystem, and are often a challenge for the waste water treatment process, even at low concentrations. Furthermore, many of the micropollutants are novel contaminants for which monitoring data is lacking. Among these are pharmaceuticals, musks, and components in the plastics industry that may function as hormones or hormone disruptors even at low concentrations. It is mentioned in the report that a large part of the micropollutants are precipitated and thereby enriched into the sludge in the waste water treatment process, which may result in high local concentrations, and may render the sludge unfit for further use, e.g. as fertilizer. In many cases the micropollutants are principally biodegradable, but factors such as low bioavailability due to low water solubility, or other reasons for recalcitrance result in poor or no biodegradation in the existing waste water treatment processes. The micropollutants often pass the cleaning process, and are released to either the waterways or the soil.

Even if any one of these compounds alone does not trigger a reaction in organisms of the receiving water body, little or nothing is known about the effect of the mixture of micropollutants that the organisms are exposed to. Another unknown is the final fate of the chemicals. Are they degraded all the way, or are degradation intermediates enriched in the process?

The main idea behind the VESITURVA project was, on one hand, to enrich and optimize biofilms that are able to degrade micropollutants, and on the other hand,

to test the use of a multi-phase water cleaning process, in which the bulk contaminants and substrates for the microbes in the biological cleaning process are degraded in the first phase(s), while the biofilms colonizing the surfaces in the later phases are left with only recalcitrant substances to degrade and use as growth substrates. The hypotheses were that a specialized microbial community would be an efficient micropollutant degrader, and that the multi-phase process would generate water and sludge where the micropollutants are also efficiently degraded all the way to harmless end products. The possibility of combining chemical and biological treatment was also explored in part. The principally very viable concept of treating grey waters (kitchen-bath-laundry) separately from black (toilet) waters was studied. The relative proportion of some micropollutants such as musks is higher in grey water, which is likely to influence their removal.

In the process of testing the method and hypotheses, several laboratory setups were constructed in the laboratories of the research partners in VESITURVA. In addition to scientific and technological efforts, products and practical applications, such as inoculum production, were also considered. At all stages a close contact was maintained to the companies involved in VESITURVA, and international partners were also directly involved in the research and supervision of the project.

The Onsite Waste water System Decree (209/2011) came into force on 15.3.2011, setting minimum standards for waste water treatment systems in rural areas of Finland with no centralized sewerage system. These laws and the set limits are, however, mostly concerned with nitrogen, phosphorus and bulk organic matter, for which there are minimum removal percentages. In VESITURVA the main focus was on determining the fate and degradation of micropollutants, but the degradation of bulk agents was also measured.

Contents

List of symbols

amoA	Ammonium-monohydrogenase
ATP	Adenosine triphosphate
BOD	Biological oxygen demand
BPA	Bisphenol A dimethyl ether
COD	Chemical oxygen demand
CW	Car wash
DEHP	Di(2-ethylhexyl)phthalate
DGGE	Denaturing Gradient Gel Electrophoresis
DN	Denitrification-nitrification
DOC	Dissolved organic carbon
E-PRTR	The European Pollutant Release and Transfer Register
EPS	Extracellular polymeric substances
FBBR	Fixed Bed Bioreactors
FIWA	Finnish Water Utilities Association
HHCB	3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyran
MLSS	Mixed liquor suspended solids
narG	Nitrate reductase
nirS	Nitrite reductase
PAH	Polycyclic aromatic hydrocarbon
PBS	Phosphate-buffered saline
PE	Polyethylene
qPCR	Real-time Polymerase Chain Reaction
RBBR	Rotating Bed Bioreactors
SPE	Solid phase extraction
WD	Wood chips
WWTP	Waste water treatment plant

Research partners and steering group

The project was funded by Tekes – the Finnish Funding Agency for Technology and Innovation and the participating companies and research institutes. The research partners were:

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Kirsi Lipponen, technician, 454 sequencing

Eeva-Marja Turkki, technician, 454 sequencing

Tampere University of Technology

Tuula Tuhkanen, Professor

Piia Nieminen, Master's student

The members of the steering group for the project were: Petri Auvinen, Institute of Biology, University of Helsinki; Merja Itävaara, VTT; Eerik Järvinen, Ramboll Oy; Silja Kostia, Lahti University of Applied Sciences; Mikko Kotro, Nordic Envicon Oy; Eija Lehtinen, Helsinki Region Environmental Services Authority HSY; Antti Leinonen, Bioste Oy; Timo Lukkarinen, MetropoliLab Oy; Leif Lundell, Konva-Center Oy; Yrjö Lundström, Helsinki Region Environmental Services Authority HSY; Esa Mäkinen, Clewer Oy and Tekno-Forest Oy; Jari Männynsalo, Vantaanjoen ja Helsingin seudun vesiensuojeluyhdistys ry; Marjo Mäntylä, Lindström Oy; Kirsti Määttä, Insinööritoimisto Gradientti Oy; Tiina Oksanen, Riihimäen vesi; Niko Rissanen, Ramboll Oy; Martin Romantschuk, Department of Environmental Sciences University of Helsinki; Teppo Tuomikoski, Tekes; Outi Tuovila, Tekes; Gatja Tiusanen, Tekes; Tuula Tuhkanen, Tampere University of Technology.

1. Introduction

Waste water treatment plants, both municipal large scale units and smaller units used for single family homes and cottages in rural areas, do a relatively good job if the reduction in organic contents of the water and nutrients (BOD, COD, N, P) is considered. However, a large part of the chemicalization of the environment that we experience, even in a relatively sparsely populated country like Finland, is a result of the failure of the waste water treatment plants to remove harmful recalcitrant substances that enter the plants as dilute suspensions or solutions. Among the harmful substances that may harm the environment are pharmaceuticals and hormones that pass through patients, compounds used in cosmetics, plastics additives, flame retardants and so on. Higher concentrations of harmful substances may be released from hospitals, landfills, factories, etc. The main outcome of flushing the substances with the rest of the waste water to the treatment plants is so that they are diluted. Much of the substances are removed from the water but end up in sludge, from where they may be spread in the environment. Their concentration in water released from treatment plants is below permissible levels and therefore overlooked (Standley et al., 2008; Lacey et al., 2011). Recalcitrant substances may, however, cause toxic effects even in dilute concentrations or be enriched in the food chains in the streams and lakes, and end up in fish and bottom-dwelling fauna.

A large part of the harmful substances are biodegradable yet poor carbon and energy sources. Furthermore, the low concentration may not be sufficient to induce microbial degradation activities, particularly in the presence of easily degradable components of the waste. In most waste water treatment units the degradation takes place in aerobic and anaerobic pools, where recalcitrant compounds are poorly degraded because of sample supply of good carbon and energy sources.

1.1 Micropollutants

Efficiency of municipal waste water treatment is evaluated by removal of nutrients and organic load measured as biological oxygen demand (BOD). Municipal waste water treatment plants remove phosphorous and BOD at a rate of 95% and nitrogen at 90%. However, numerous organic compounds have been detected in mu-

nicipal waste water in concentrations varying from 0.5 to 50 µg/l. Some of the compounds such as long-chain fatty acids, sterols, caffeine and indol originate from food and faeces; odorants (for example synthetic musks), plasticizers (for example adipates and phthalates), flame retardants (for example chlorinated phosphate esters), preservatives (esters of hydroxybenzoic acid, known as parabens) and antioxidants (substituted phenols) originate from both industrial and domestic products and applications; solvents (for example non-chlorinated alcohols, ethers and ketones) are mainly of industrial origin (Paxéus, 1996). Some 72–99% of removals of micropollutants have been reported at waste water treatment plants (Horii et al., 2007; Pothitou & Voutsa, 2008; Stasinakis et al., 2008). However, micropollutants have also been measured in surface water (Bendz et al., 2005; Andersen et al., 2007).

EU legislation has already been created to protect water ecosystems in Europe, to prevent groundwater pollution and control emissions of pollutants to air, water and soil from various industrial sources (e.g. the Water Framework Directive, the IPPC Directive 2008/1/EC, and REACH EC 1907/2006). The European Pollutant Release and Transfer Register (E-PRTR) was established for monitoring and preventing chemical discharges into the environment from various industrial sources, including municipal waste water treatment plants. Regulation 166/2006 lists 91 priority pollutants that must be reported if their emissions into air, water or soil exceed the limits that are respectively represented in the regulation. The priority pollutant list include compounds like nonylphenols, polyaromatic hydrocarbons, tributyl tin and di(2-ethylhexyl) phthalate. As more knowledge is gained, new pollutants are considered for addition to the list of priority pollutants.

1.2 Biofilm processes

Bacteria in aquatic environments are predominantly not in a free-floating planktonic stage, but have a tendency to attach to surfaces and form multi-species communities called biofilms (Metcalf & Eddy, 1991; Syron & Casy, 2007). The microbes produce extracellular polymeric substances (EPS), which form complexes with the surface materials. A mature biofilm is a complex mixture of active and dormant or drying cells, inorganic and organic materials and tunnels filled with water, and the biofilm contains both aerobic and anaerobic niches (Korber et al., 1995; O'Toole et al., 2000) (Figure 1).

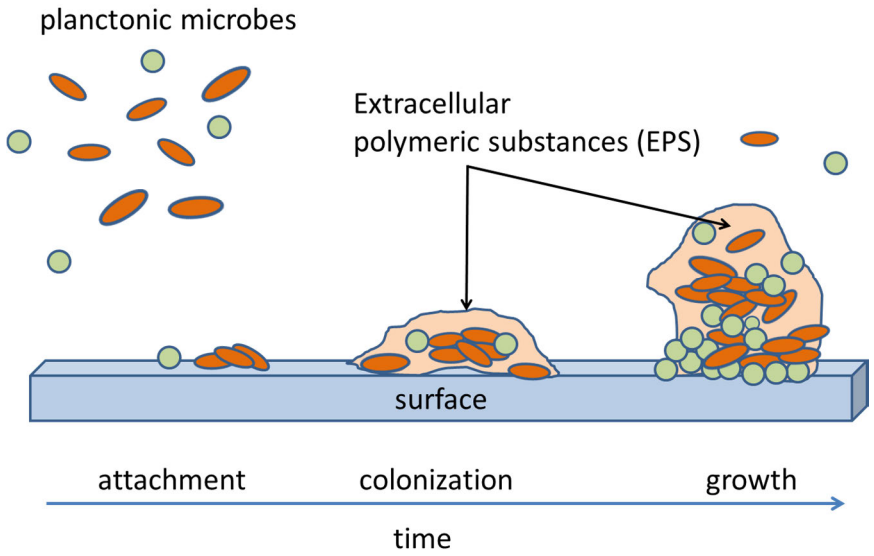


Figure 1. Biofilm formation.

The most commonly used process for the treatment of municipal and industrial waste water is activated sludge process, but increasingly biofilm technologies are utilized. Biofilm waste water treatment processes can be roughly divided into 1) the fixed-medium systems, and 2) moving-medium systems (Rodgers et al., 2003). In the fixed-medium systems (fixed bed bioreactor; FBBR) the biological reactions take place in the biofilm growing on a static medium. In the moving-medium systems the biofilm media are kept in continuous movement by mechanical, hydraulic or air forces. Rotating bed bioreactors (RBBR) are an application of moving bed biofilm reactors with a higher filling rate of carrier elements (www.clewer.com). An essential element of both fixed- and moving-medium biofilm systems are the properties of the carriers, because they directly influence the ability for biofilm growth, the quantity of biomass and the effectiveness of the treatment. Carriers can be found in a variety of shapes, sizes and materials in order to optimize these carriers for the best possible performance of biofilm. Commercial carrier elements are usually made from polyethylene, polypropylene or polyurethane. Porous ceramic materials have been used as carrier material in waste water treatment (Kariminiaae-Hamedaani et al., 2003), especially in packed bed reactors. Inorganic materials have also been incorporated into polyethylene in order to control properties like surface roughness, adsorption ability and density (Jeong et al., 2006).

The process efficiency of biofilm waste water treatment process is affected by the diversity and function of microbes. Biological waste water treatment processes are microbial degradation processes which have been treated as ‘black boxes’ for decades. Traditionally, the microbial communities of waste waters have been analyzed by light microscopy or by cultivation-dependent techniques. Since only

1–5% of all the microorganisms have been found to be cultivatable, the introduction of molecular methods has revolutionized research on microbial communities. In VESITURVA, a variety of methods have been used to analyse microbial composition in biofilms including PCR-DGGE (Denaturing Gradient Gel Electrophoresis), qPCR (Real-time Polymerase Chain Reaction) and 454-pyrosequencing. Pyrosequencing is a method which generates a broad and quite detailed view of the resident microbial community, while the data can be utilized in producing useful inocula, for example.

In addition of novel molecular methods various other techniques can be utilized to study microbial biofilms. These methods can be spectroscopic, surface and interface characterizing techniques or techniques which utilize microsensors (Table 1).

Table 1. Techniques to monitor microbial biofilms (modified from Denkhaus et al., 2008).

Analytical methods			
Molecular techniques	Spectroscopy	Surface and interface characterizing techniques	Microsensors
DGGE FISH *in situ* hybridization immunoassays TGGE = temperature gradient gel electrophoresis DHPLC = denaturing high-performance liquid chromatography qPCR = real-time polymerase chain reaction metagenomic sequencing	AAS = atomic adsorption spectrometry fluorescence spectrometry spectrophotometry	AFM = atomic adsorption microscopy SEM = scanning electron microscopy X-ray microscopy CLSM = confocal laser scanning microscopy NMR = nuclear magnetic resonance photoacoustic spectroscopy	electrochemical microsensors fibre-optic microsensors

Detailed knowledge of the composition of biofilms, when they function properly, as well as when the degradation process is disrupted, is useful when evaluating treatment processes, when producing inocula, and perhaps especially when diagnosing aberrations and suboptimally functioning processes.

1.3 Aims of the VESITURVA project

The aim of the VESITURVA project was to improve waste water treatment processes applicable in various environments and situations, for example households, industrial

plants and other sources of waste water such as landfills, laundries, car washes, hospitals, etc. The aim was also to exploit the tendency of microbes to live on surfaces and feed on organic compounds and nutrients that exist in waste water. A model of sequential bioreactors was studied to enhance the efficiency of waste water treatment. We also aimed to characterize microbial communities in the biofilms from different stages of multi-stage waste water treatment processes. In addition, the efficacies of multi-stage biofilm processes were studied using different sources of waste water: grey water, municipal waste water and car wash waste water.

2. Methods

2.1 Analyses for the control of waste water quality

Table 2 summarizes the chemical methods applied over the course of the project.

Table 2. Chemical methods.

Analysis	Method	Description	See Chapter
Ammonium (NH_4^+)	Hach Lange photometric test or measured by MetropoliLab	Ammonium ions react at pH 12.6 with hypochlorite ions and salicylate ions. Sodium nitroprusside act as a catalyst to form indophenol blue which is measured with a spectrophotometer.	4.2, 4.3, 6.5
Anionic surfactants	Hach Lange photometric test	Anionic surfactants react with methylene blue to form complexes, which are extracted in chloroform and evaluated photometrically.	5.1, 5.3
Biochemical oxygen demand (BOD)	OxiTop (WTW)	The method is based on manometric measurement of carbon dioxide that is produced as organic material is biodegraded in a closed flask.	3.1, 3.2, 4.1, 4.2, 5.1, 5.4, 6.3, 6.5
BPA and HHCB		Solid phase extraction (SPE) was performed to prepare the waste water samples for analyses of BPA and HHCB. Subsamples were derivatized with MSTFA to enhance the analysis of BPA. The equipment for analyses was a gas chromatograph coupled with a mass detector (Shimadzu).	3.1, 4.1
Chemical oxygen demand (COD)	Hach Lange photometric test	Oxidizable substances react with a solution of sulphuric acid and potassium dichromate. Silver sulfate serves as a catalyst. Colour caused by Cr^{6+} is evaluated with a spectrophotometer.	3.1, 3.2, 4.1, 4.2, 5.3, 6.3

Electrical conductivity, oxygen, pH	WTV Multi 3410		5.1
Nitrate (NO_3^-)	Hach Lange photometric test or measured by MetropoliLab	In the presence of sulphuric and phosphoric acid in solutions, Nitrate ions react with 2.6-dimethylphenol to form 4-nitro-2.6-dimethylphenol. The result is gained by a spectrophotometric evaluation.	4.2, 4.3
Nitrite (NO_2^-)	Hach Lange photometric test or measured by MetropoliLab	Nitrites react with primary aromatic amines in acidic solutions to form diazonium salts. These combine with aromatic compounds that contain an amino group or a hydroxyl group to form intensively coloured azo dyes which can be detected with a spectrophotometer.	4.2, 4.3
Nonionic surfactants	Hach Lange photometric test	Nonionic surfactants react with the indicator TBPE to form complexes, which are extracted in dichloromethane and photometrically evaluated.	5.3
Suspended solids	SFS-EN 872	A sample is filtrated through a glass fiber filter and dried at 105 °C for 1 to 2 hours. The mass of the dry material on the filter is divided by the volume of the sample.	3.1, 4.1, 5.1, 5.4
Total phosphorous	Hach Lange photometric test	A sample is hydrolyzed and resulting phosphate ions react with molybdate and antimony ions. The forming antimonyl phosphomolubdate complex is then reduced to phosphomolybdenum blue that is evaluated with a spectrophotometer.	3.1, 4.1, 5.4
Total nitrogen	Hach Lange photometric test	Inorganically and organically bound nitrogen is oxidized to nitrate. As nitrate and 2.6-dimethylphenol react, nitrophenol is formed. The result is gained by a spectrophotometric evaluation.	3.1, 3.2, 4.1, 5.1, 5.4, 6.5
Turbidity			5.3

2.2 Microbial activity and diversity

2.2.1 Microbial activity

Microbial activity in grey water treatment units (wood chips) in the field experiment was monitored by measuring ATP (adenosine triphosphate) concentration using ATP Biomass kit (BioThema) as described by Saario (2012).

2.2.2 Denaturing Gradient Gel Electrophoresis (DGGE)

Microbial diversity in carrier elements (wood chips and polyethylene) and water samples were analyzed with PCR-DGGE (Saario, 2012; Lehtonen, 2011). Water samples targeted for microbial diversity analysis were filtrated with SterivexTM (Millipore) filters, cooled down on dry ice and stored at –80 °C. Biofilm samples from carrier materials were cooled down on dry ice and stored at –80 °C. The similarities of DGGE profiles were analyzed by Bionumerix (Applied Maths, Belgium). Major bands in DGGE gel were cut off and sequenced to define the microbial species.

2.2.3 Real-time Polymerase Chain Reaction (qPCR)

Samples for qPCR were collected in a similar way to sampling for DGGE. The occurrence of the functional genes encoding the enzymes involved in nitrification and denitrification were measured with a quantitative PCR method using Roche LightCycler® 480 (Saario, 2012). The number of active functional genes was quantified with reverse transcription qPCR (RT-qPCR).

2.2.4 Fluoresence *In situ* hybridization (FISH)

Microbial diversity in a rotating bed bioreactor was evaluated by fluorescence *in situ* hybridization as described by Eriksson (2010). Samples were fixed with 96% ethanol or ethanol/PBS mixture on dry ice and stored at –20 °C. Biofilm grown on the surface of PE carrier elements was extracted by sonication with PBS-solution (phosphate-buffered saline). Photographs were taken using an epifluorescence microscope (Olympus BX60).

2.2.5 454-pyrosequencing

454-pyrosequencing has shown to be an effective method for analyzing bacterial diversity in waste water treatment systems. By using this method, more detailed information is gained compared to DGGE. The sequencing was carried out at the Institute of Biotechnology (Helsinki, Finland) using a 454-GS FLX Titanium protocol (454 Life Sciences, Roche Diagnostics, USA). The device used was a Genome Sequencer FLX. Microbial diversities in RBBR were studied by 454-pyrosequencing as described by Coloma (2012).

3. Treatment of municipal waste water

Experiments on treatment of municipal waste water were conducted at the Department of Environmental Sciences at University of Helsinki and VTT. The aim of the experiment conducted at the Department of Environmental Sciences was to have a waste water treatment system that efficiently removes BOD and nitrogen from waste water but also to adapt biofilms in the different stages of the three-phase rotating bed bioreactor system to remove micropollutants from waste water. Bisphenol A (BPA, 2,2-(4,4-dihydroxydiphenyl) propane) and polycyclic musk HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran, known also as Galaxolide) were chosen as model pollutants for the experiments. Polycyclic musks are widely used as fragrance ingredients in detergents and cleaning agents, products for personal care and in other consumer products. HHCB is lipophilic and persistent compound which has proved to be very toxic in the environment (Balk & Ford, 1999). Bisphenol A is mainly used in the chemical industry in the manufacturing of polycarbonates, epoxy resins and flame retardants (Staples et al., 1998). Both HHCB and Bisphenol A have endocrine disrupting activity disturbing the hormonal balance of several aquatic organisms. The aim of the experiments at VTT was to study microbial diversity in biofilms on two different types of carrier materials, wood chips (WD) and polyethylene carrier elements (PE), in laboratory-scale packed bed bioreactors.

Municipal waste water treatment plants and small-scale waste water treatment units were sampled for comparison with laboratory scale experiments. Three municipal waste water treatment plants were sampled to analyze BPA and HHCB in waste water and to study their removal in different stages of a waste water treatment process. Samplings, one per waste water treatment plant, were carried out in April and May 2011. In addition, small-scale waste water treatment units were sampled at a test field of a cooperation partner. Eight small-scale waste water units were installed at the field and municipal waste water was conveyed to them. Each small-scale waste water treatment unit as well as inflowing waste water was sampled in April 2011. Results of these samplings will be published later.

3.1 Rotating bed biofilm reactor in laboratory scale

3.1.1 Experimental set-up

The experimental set-up consisted of a series of three cylindrical vessels (R1, R2 and R3 in Figure 2). Pre-settled municipal waste water was fed with continuous flow to the first reactor in the series, which led to a hydraulic retention time of two days in each reactor; six days altogether. Air was pumped to each reactor to mix and aerate them. Air flow brought the waste water and carrier material in a rotating motion inside the reactors. Plastic carrier material with a large surface area (effective surface area 650 m^2m^{-3}) functioned as a carrier material for biofilms (Figure 2).

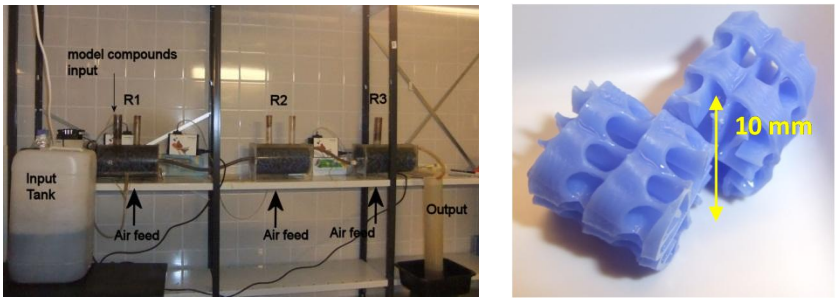


Figure 2. Experimental set-up of RBBR consisted of an input tank and three conjoined reactors, and plastic pieces with maximal surface area served a carrier material for biofilm.

Waste water was run through the series of bioreactors for 4.5 months. No extra inoculation was added since it was expected that the microbes in the waste water to attach to the surfaces of the carrier material and form biofilms. Continuous flow of model pollutants, BPA and HHCB, to the first reactor of the series was started in July 2010.

Samples were taken from the waste water feed, from the water inside the reactors, and from the carrier materials. Water samples were analyzed for BOD, suspended solids, COD, total nitrogen and total phosphorous, and for the model pollutants BPA and HHCB.

3.1.2 Efficiency of laboratory scale RBBR in waste water treatment

The quality of waste water largely varies on a daily, weekly and seasonal basis, and is dependent for example on household activities, temperature and rainfall. Thus it is natural that every grab batch of the waste water feed was somewhat different. BOD of waste water feed varied between 22 and 439 mg/l, suspended solids between 56 and 247 mg/l and pH was neutral. The amount of total phosphorous in the waste water feed was 0.5–2.5 mg/l and total nitrogen 31–61 mg/l.

Since waste water was fetched from a waste water treatment plant every 1–2 weeks, waste water may have also changed during storage.

As was expected, the majority of BOD and a large portion of COD were removed from waste water in the first stage of the RBBR (Figure 3 and Table 3). pH in the reactors usually followed the pH of the influent. The data based on the low number of analyses does not show the removal of total nitrogen in the RBBR. Different forms of nitrogen, that is ammonium and nitrate, were not measured here but they would illustrate the efficiency of nitrification. The aerobic conditions in the RBBR enabled only nitrification. For total removal of nitrogen, an anoxic stage would have been necessary for denitrification that converts nitrate to nitrogen gas. There was a fluctuation in the removal efficiency of BOD, COD and nutrients on a daily basis and no change in the trend of the removals was noticeable after the feed of the model pollutants to the reactors was started.

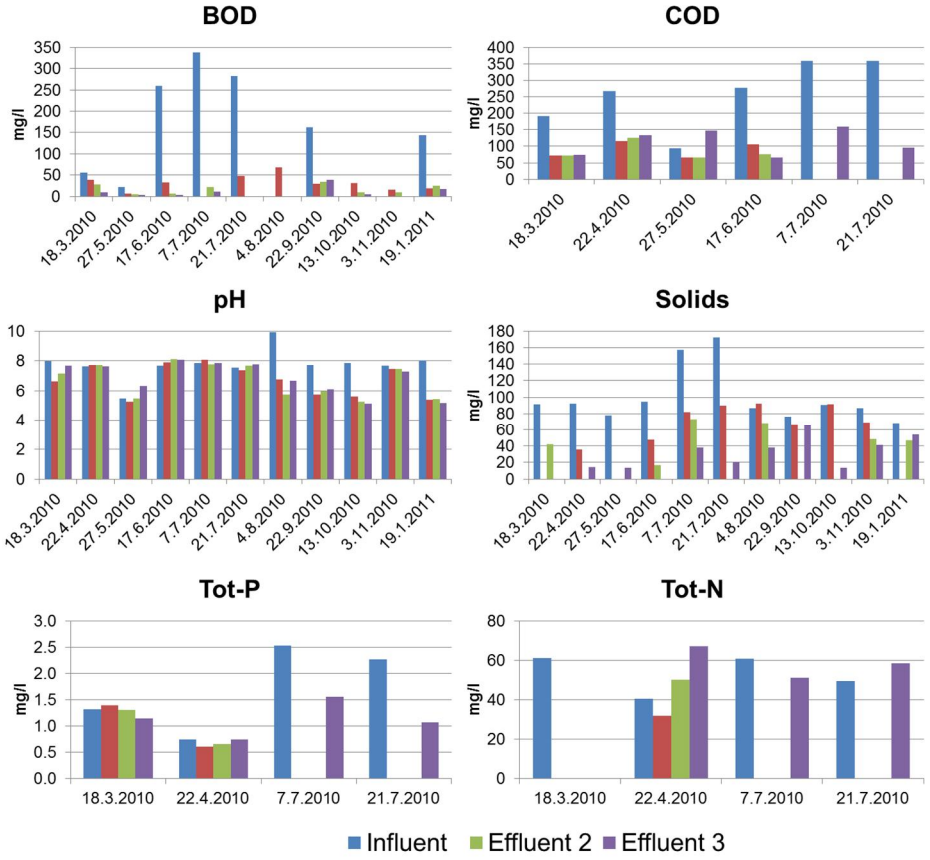


Figure 3. BOD, COD, pH, suspended solids, and total phosphorous and nitrogen were measured to control the performance of the RBBR. The addition of model pollutants (BPA and HHCB) was started on 14.7.2010.

As the majority of biodegradable material was removed from the first reactor of the series, it was expected that more optimal conditions for removal of micropollutants would develop in Reactor 3. Although there was a constant flow of the solution that contained the model pollutants to the first reactor of the series, there was variation in the concentration of BPA (Figure 4). There are many factors that may affect the concentration of BPA in the liquid phase of waste water, for example the existence of other components such as suspended solids. However, this limited data does not support the assumption that BPA bind to suspended solids from the liquid phase of waste water. Momentary changes in the performance of the experiment apparatus or incomplete mixing may also have affected the result.

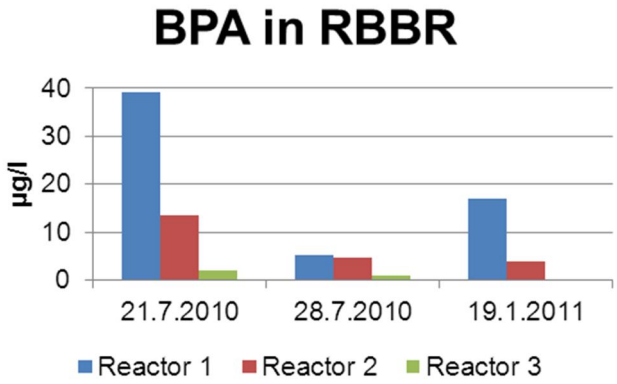


Figure 4. Concentration of BPA in the three sequential reactors of the RBBR. Reactor 3 on 19.1.2011 = not analyzed.

Table 3. Performance of the laboratory scale RBBR in terms of chemical analyses. Change in the value of a parameter (%) is calculated from that of the influent. N.A. = not analyzed.

Date	18.3.2010	22.4.2010	27.5.2010	17.6.2010	7.7.2010	21.7.2010	4.8.2010	22.9.2010	13.10.2010	3.11.2010	19.1.2011
Reactor 1											
pH	-17	+1	-4	+3	+3	-2	-32	-26	-29	-3	-33
Solids (mg/l)	N.A.	-61	N.A.	-49	-48	-48	+6	-13	±0	-20	N.A.
BOD7 (mg/l)	-33	N.A.	-72	-88	N.A.	-83	N.A.	-82	N.A.	N.A.	-87
COD (mg/l)	-62	-57	-29	-62	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
P (mg/l)	+5	-18	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
N (mg/l)	N.A.	-21	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Reactor 2											
pH	-11	+1	±0	+6	-2	+2	-42	-23	-33	-3	-32
Solids (mg/l)	-54	N.A.	N.A.	-82	-54	N.A.	-21	N.A.	N.A.	-45	-31
BOD7 (mg/l)	-50	N.A.	-81	-98	-94	N.A.	N.A.	-79	N.A.	N.A.	-83
COD (mg/l)	-62	-53	-30	-73	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
P (mg/l)	-2	-12	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
N (mg/l)	N.A.	+25	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Reactor 3											
pH	-4	±0	+15	+5	±0	+3	-33	-21	-35	-5	-36
Solids (mg/l)	N.A.	-84	-82	N.A.	-76	-88	-56	-13	-85	-52	-21
BOD7 (mg/l)	-83	N.A.	-86	-99	-97	N.A.	N.A.	-76	N.A.	N.A.	-88
COD (mg/l)	-62	-50	58	-76	-56	-73	N.A.	N.A.	N.A.	N.A.	N.A.
P (mg/l)	-14	±0	N.A.	N.A.	-38	-53	N.A.	N.A.	N.A.	N.A.	N.A.
N (mg/l)	N.A.	+66	N.A.	N.A.	-16	+18	N.A.	N.A.	N.A.	N.A.	N.A.

3.1.3 Microbial diversity in biofilm

Microbial diversity in RBBR was evaluated by PCR-DGGE and microscopically by fluorescent *in situ* hybridization (Figure 5). Both methods showed differences in microbial diversity between the influent waste water and between sequential reactors. Time-dependent changes were also detected (Eriksson, 2010).

The relative amounts of different eubacterial groups (α-proteobacteria, β-proteobacteria, γ-proteobacteria and δ -proteobacteria) measured by FISH *in situ* hybridization are shown in Figure 6. The relative amount of β-proteobacteria was higher in the influent compared to the first and second reactor. In addition, the relative amount of unknown eubacteria was higher in the second reactor.

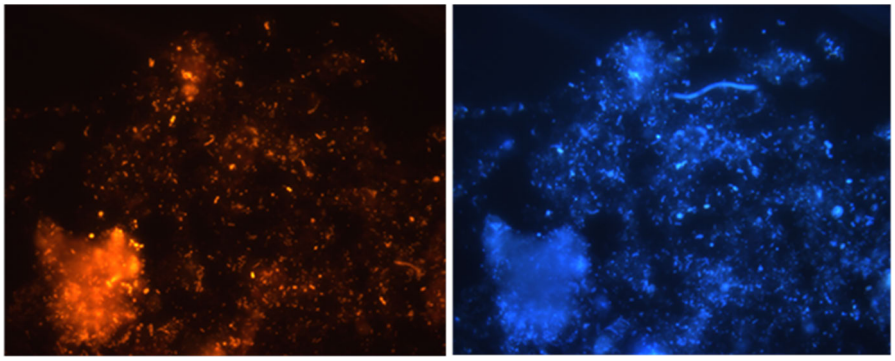


Figure 5. FISH *in situ* hybridization of biofilm sample from the first reactor in RBBR. Biofilm was extracted from the surface of carrier element by PBS-buffer. Epifluorescence micrographs are shown for identical microscopic fields. Probe EUB3381 (Cy3 labelled red and DAPI labelled blue). Samples taken before feeding of pollutants was started (25.5.2010).

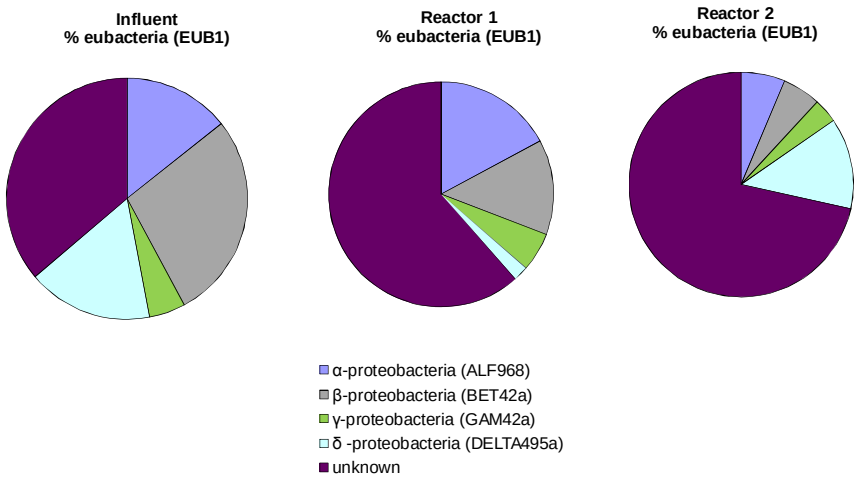


Figure 6. Amount of bacteria as a percentage of eubacteria in influent and first and second reactor in RBBR analyzed by fluorescent *in situ* hybridization. (probe EUB338 I; Cy3, DAPI) (Eriksson, 2010). Values were calculated on the basis of epifluorescence micrographs (DAPI/specific probes). Samples taken before feeding of pollutants was started (25.5.2010).

According to the PCR-DGGE profiles and bionumerics analysis, the microbial diversity of municipal waste water used as influent varied significantly during the testing period of 4.5 months. The bacterial species dominating the biofilm reactors differed from the diversity in influent waste water. Bacterial diversity also varied considerable between three sequential reactors. To get more detailed information, microbial diversity was analyzed by 454-pyrosequencing.

454-pyrosequencing showed that before the addition of the BPA and HHCB, the same bacterial groups were dominant in all reactors, i.e. Proteobacteria (α-, β-, γ-proteobacteria), Bacteroidetes (Sphingobacteria and Flavobacteria), Acidobacteria and Actinobacteria, but different bacterial dominance was observed at genus taxonomic level. Also Deng et al. (2011) have reported that proteobacteria and Bacteroidetes are dominant groups in aerated bed biofilm reactors with municipal wastewater. After the addition of the model compounds into the waste water, Gammaproteobacteria and Nitrospira rose notably in frequency. Bacteria belonging to the genus Pseudomonas declined, and genera Rhizobacter and Nitrospira rose in frequency. With respect to archaeal and fungal communities in the biofilm, their DNA were detected in low proportions (< 5%) compared to the total microbial DNA.

Finally, based on the diversity analysis that indicated a decreasing level of diversity during the experiment in Reactor 2 and 3 together with the significant differences found in the phylogenetic analysis, we could suggest that the bacterial communities in the reactors became specialized over time. The development, the dispersal behaviour of bacterial structures and the variation in the quality of the used municipal waste water were suggested to be responsible for hiding the presumable effect produced by the model compounds in the specialization of the bacterial community in the reactor biofilms. (Coloma, 2012.)

3.1.4 Recovery of the RBBR performance following an operation interruption

Recovery of the RBBR system was tested by stopping the operation for three months. BOD, suspended solids and pH was analyzed prior to the stop, after 12 weeks of interruption before a restart, and then 1 week, 4 weeks and 7 weeks after the restart, which is 13, 16 and 19 weeks from the beginning of the test that is presented in Figure 7. Removal of BOD did not suffer from the interruption – 1 week after the restart removal of BOD was > 97% in all reactors. After 4 weeks from the restart there was a remarkable increase in the amount of suspended solids in all three reactors, but it almost reached the original level seven weeks after the restart. A possible reason for the increase of suspended solids in waste water was that there may have been some dead biofilm that detached from the carrier material. It seems that although removal of BOD and suspended solids normalized quite quickly, more time was needed to stabilize the system since there was still fluctuation in pH 7 weeks after the restart.

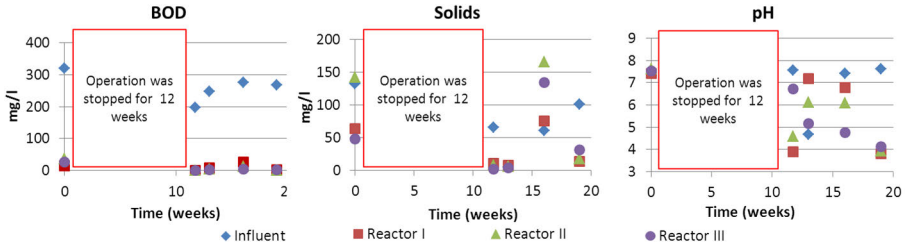


Figure 7. Performance of the RBBR after operation interruption in terms of BOD, suspended solids and pH.

3.2 Effect of carrier material on waste water treatment performance in a FBBR

3.2.1 Experimental set-up

Two sets of fixed bed bioreactors (FBBR) were constructed including two reactors set in series (Figure 8). In the first reactor wood chips were used as carrier material and polyethylene carrier elements (PE) were used in the second reactor. The volume of a single reactor was 250 ml, with a filling rate of 70%, while the used flow rate was 0.5 ml min^{-1}. Experiments were run at room temperature over a period of three months.

The waste water studied was weekly collected from municipal WWTP. Water samples were collected from influent and from effluents from second bioreactors. In the 13th week of sampling, samples from the first bioreactors were also collected. The parameters measured from water samples were dissolved oxygen, COD, BOD, NH_4 –N, NO_2 + NO_3-N, total N and total P. Carrier elements to study microbial diversity were collected from a depth of 5 cm from the top of the bioreactors. Bacterial diversity in carrier biofilms was studied using PCR-DGGE.

With both carrier materials the highest removal rate of BOD, COD and nutrients occurred in the first bioreactor (Figure 9). According to Andersson et al. (2008), the carrier material type can influence denitrification activity. In our experiment the removal rates of NH_4-N, BOD and COD were at the same level for both carrier materials for the first 9 weeks. After 9 weeks the removal efficiencies started to decrease, especially in the case where wood chips were used as carrier material. The study was reported more in detail in Anna Lehtonen's Master's thesis (2011).

Figure 8. Experimental set-up of fixed bed bioreactors.

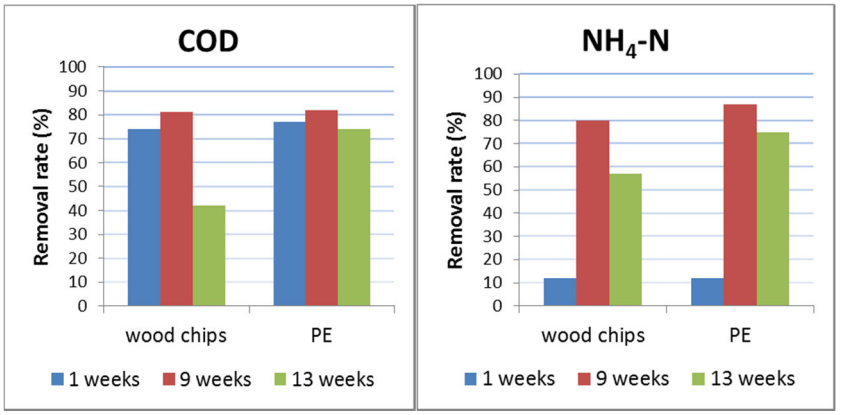


Figure 9. Process performance in a fixed bed bioreactor with two different types of carrier materials used after 1, 9 and 13 weeks. Analyses were made from the influent and the effluent of the second reactors set in series.

3.2.2 Microbial diversity in biofilm

PCR-DGGE analysis indicated that the bacterial species dominating the biofilm reactors differed from the diversity in influent waste water. The difference in the first and second reactor was particularly obvious with the biofilm on polyethylene carrier material. Contrary to the bacterial diversity in the first bioreactor, there was only one major species detected in the second bioreactor. The bacteria detected belonged to the *Rhizobium* genus. Acidobacteria were present in biofilms on both PE and WD, but were not detected from influent. Biofilm on WD, but not on PE, contained Verrucomicrobia and δ-proteobacteria. Furthermore, only one β-proteobacteria sequence was derived from PE biofilms.

4. Treatment of grey water in FBBR

Grey water is defined as waste water from kitchens and bathrooms (in households, offices, public buildings, etc.) excluding toilet waste water. According to a literature survey by Eriksson et al. (2002), grey water is more diluted than in household waste water. For suspended solids, values of 17–330 mg/l were reported, BOD 5–1460 mg/l, COD 13–ca. 8000 mg/l, total nitrogen 0.6–74 mg/l, ammonium < 0.05–25 mg/l and phosphorous 0.1–57 mg/l. The high variation in the composition of grey water is due to household activities, the quality of the water supply and the properties of piping. For example, grey water originating from kitchen contains the highest values of suspended solids and nitrogen, whereas the highest values of phosphorous are measured from grey water from laundry because of phosphorous-containing detergents. Concentrations of heavy metals and inorganic trace elements are low in grey water. However, many of them (aluminium, cadmium, chromium, iron, potassium, sodium, sulphur, tin, and especially nickel and mercury) are caused to some extent by household activities (Eriksson et al., 2010).

The presence of micropollutants in grey water is not as widely studied as the conventional parameters of waste water quality. Eriksson et al. (2010) analyzed 23 organic substances including parabens and sunscreens that are used in personal care products, benzene, chloroform, and octylphenol and iso-nonylphenyl. Of the 23 substances, 18 were found in both raw bathroom grey water and after a grey water treatment process on the µg/l scale. Donner et al. (2010) reviewed articles on grey water and found that polycyclic aromatic hydrocarbons (PAHs), chlorinated aliphatic hydrocarbons such as chloroform and carbon tetrachloride, phenols such as octyl- and nonylphenols, as well as tributyl tin and di(2-ethylhexyl)phthalate (DEHP), for example, have also been detected in grey water on the µg/l scale.

Treatment of grey water with small-scale treatment units was studied both in the laboratory (HU) and through field experiments (VTT). The next chapters will summarize the experiments and their results.

4.1 Pilot scale experiments

4.1.1 Experimental set-up

The pilot scale experiment on treatment of grey water was carried out in a series of two cylindrical tanks made of steel (Figure 10a). Inside the tanks wood chips served as a fixed bed for biofilm growth (Figure 10b). Artificial grey water was pumped twice a day to the first reactor in the series, ten minutes at a time so that the inflow was 100 litres per day. Artificial grey water was prepared by mixing purée soup, sour milk, washing powder, dishwashing gel and shampoo in tap water. BPA and HHCB were mixed with artificial grey water before pumping the grey water to the first reactor in the series. Grey water that flowed down through the reactor was pumped to the top of the second reactor. Samples were taken from the artificial grey water feed and the effluents of reactors 1 and 2, as well as the carrier material. The carrier material was sampled from two depths. Water samples were analyzed for BOD, suspended solids, COD, total nitrogen and total phosphorous, and for the model pollutants BPA and HHCB. PCR-DGGE was used for the study of microbial diversities in the biofilms living on the carrier material.

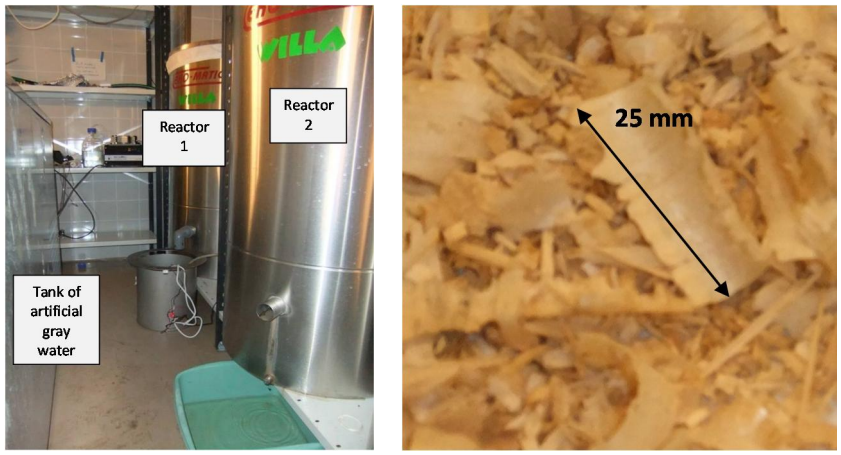


Figure 10. a) Experimental set-up of FBBR system consisted of a tank of artificial grey water, two cylindrical reactors, and pumps. b) Wood chips served as carrier material in FBBR system.

4.1.2 Efficiency of the FBBR in grey water treatment

In spite of the artificial nature of our grey water, there was some variation in the quality in terms of suspended solids, BOD, COD and nutrients. For example, BOD varied between 97 and 155 mg/l and suspended solids 8–70 mg/l. Shortly after the preparation, the pH of the grey water was rather high, about nine, because of the

alkaline components, mainly laundry powder and dishwashing gel. The amount of total phosphorous was about 1.4 mg/l and nitrogen 4.0 mg/l. The grey water feed was stored at ambient temperature and it is possible that some alteration in the quality may have occurred during storage. However, it was considered to mimic the inherent variation in the quality of grey water. Compared to the values introduced by Eriksson et al. (2010), the artificial grey water used here presented typical, although fairly diluted, grey water in terms of the measured parameters.

Efficient removal of BOD, COD and suspended solids was seen after the first reactor, as expected (Figure 11). The level of removal percentages did not decrease during the 10-month follow-up (Table 4) although the wood chip filters were not changed during this period. Nevertheless, our load of 100 litres per day was less than the average household load. The system removed total nitrogen, which indicates that both nitrification and denitrification existed in the system. Surprisingly, minor portions of total phosphorous were removed from the grey water, probably by adsorption. No significant changes were detected in the process performance after feeding of model compounds was started.

Table 4. Performance of the pilot FBBR in terms of chemical analyses, removals (%) calculated from influent. N.A. = not analyzed.

Date	18.3.2010	26.4.2010	25.5.2010	7.6.2010	7.7.2010	21.7.2010	4.8.2010	22.9.2010	13.10.2010	3.11.2010	19.1.2011
Effluent 1											
pH	21	25	5	25	27	30	26	29	28	28	26
Solids (mg/l)	85	N.A.	33	73	71	80	77	77	76	85	80
BOD7 (mg/l)	N.A.	N.A.	68	75	86	N.A.	45	50	78	67	69
COD (mg/l)	71	N.A.	51	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
P (mg/l)	5	3	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
N (mg/l)	N.A.	61	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Effluent 2											
pH	22	24	2	23	26	27	24	25	27	24	23
Solids (mg/l)	N.A.	N.A.	> 97	> 85	> 90	> 93	90	> 92	> 91	> 95	> 93
BOD7 (mg/l)	N.A.	N.A.	92	95	94	90	90	91	N.A.	93	96
COD (mg/l)	83	N.A.	50	67	58	74	N.A.	N.A.	N.A.	N.A.	N.A.
P (mg/l)	16	6	N.A.	N.A.	20	14	N.A.	N.A.	N.A.	N.A.	N.A.
N (mg/l)	N.A.	86	N.A.	N.A.	93	94	N.A.	N.A.	N.A.	N.A.	N.A.

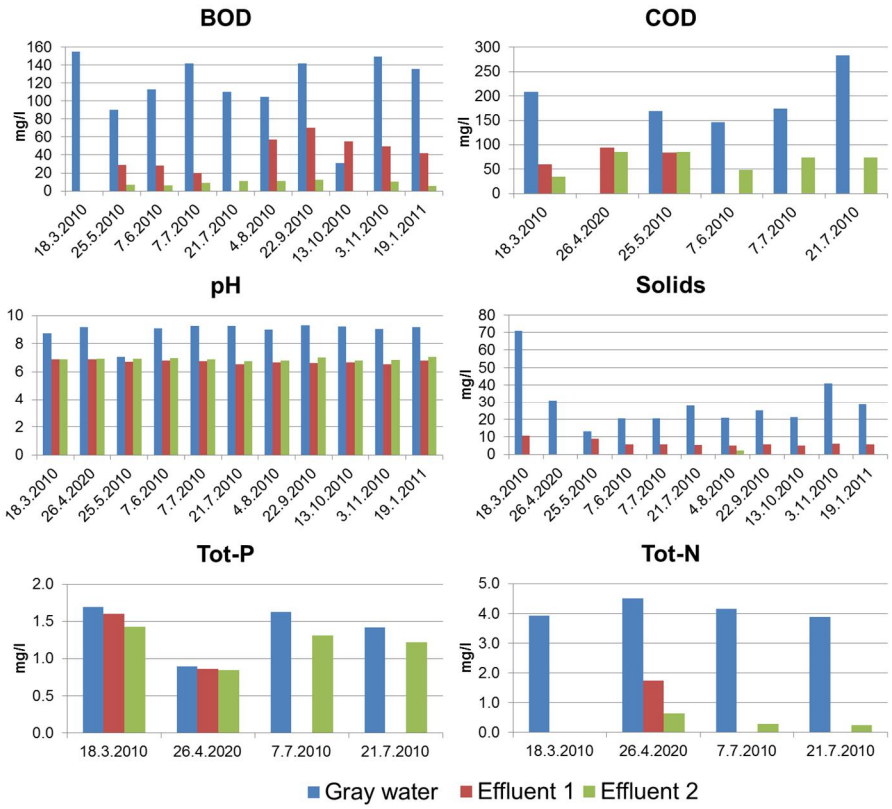


Figure 11. BOD, COD, pH, suspended solids, and total phosphorous and nitrogen were measured to study the performance of the FBBR. The addition of the model pollutants (BPA and HHCB) was started on 14.7.2010.

BPA and HHCB were chosen as model compounds for studying the removal of compounds that exist in waste water in minor concentrations. The model compounds were added to the artificial grey water to be able to work in detectable concentrations from which removal percentages could be calculated. Note that on 7th of July, pollutants were not added to the artificial grey water but there was 4 µg/l BPA and 13 µg/l HHCB in the influent that originated from the constituents of the grey water.

Removal of BPA from artificial grey water was largely the result of the first reactor in the FBBR series (Figure 12; Table 5). Since there was a high removal percentage of the model pollutants in Reactor 1 from the beginning when there was not yet biofilm formation, it may be possible that the removal was caused to some extent by adsorption to the wood chips that served as carrier material. Since the removal percentage of BPA slightly decreased in Reactor 1 along with decreasing concentration of BPA in the influent, it may indicate that its capacity as adsorbent was exceeded. However, adsorption of BPA to carrier material does not exclude

the possibility of biodegradation as a mechanism of BPA removal. The removal of HHCB was over 91% in the first reactor of the series and over 98% from influent in the second reactor of the series in all samplings (Figure 13; Table 6). There was no major difference in the performance of the FBBR system in respect of HHCB removal at different sampling times. Both BPA and HHCB were efficiently removed in the first reactor in the series, but the second phase enhanced the removal. As efficient a removal of pollutants as possible from waste water is important, especially when large volumes of waste water is considered.

Table 5. Concentration of BPA (µg/l) in grey water and influents from reactors 1 and 2.

	7.7.2010	21.7.2010	4.8.2010	21.9.2010
Influent	4	1346	986	698
Reactor 1	3	45	59	68
Reactor 2	1	6	5	1

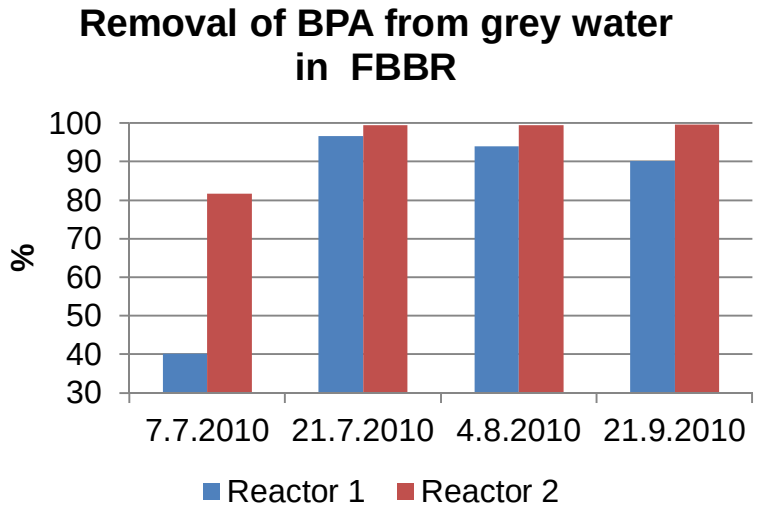


Figure 12. Removal (%) of BPA from grey water in reactors 1 and 2.

Table 6. Concentration of HHCB (µg/l) in grey water and influents from reactors 1 and 2.

	7.7.2010	21.7.2010	4.8.2010	11.8.2010
Influent	13	45	32	28
Reactor 1	1	3	3	2
Reactor 2	0.4	0.2	0.5	0.4

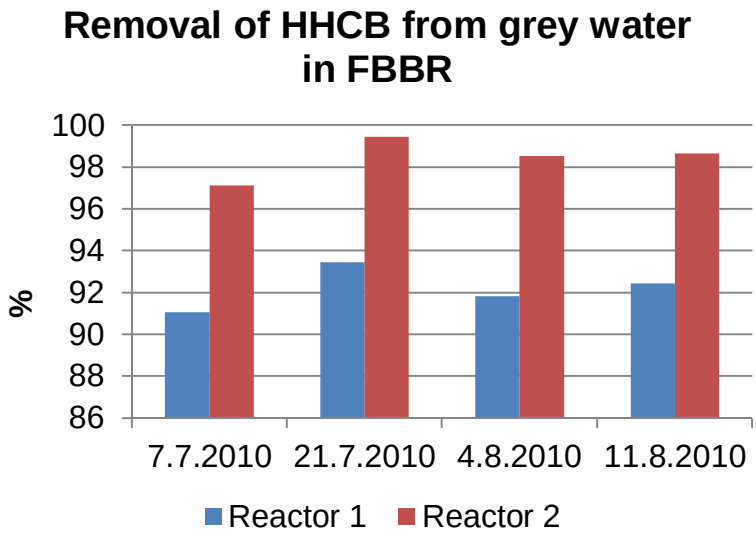


Figure 13. Removal (%) of HHCB from grey water in reactors 1 and 2.

4.1.3 Microbial diversity in biofilm

Bacterial and fungal diversity in wood chips in two sequential reactors and two different depths in the same reactor were analyzed using PCR-DGGE. According to DGGE results and the analysis with bionumerics, bacterial diversity and fungal diversity was quite different between sequential reactors. On the other hand, diversities were quite similar in two different depths (10 and 30 cm) in the same reactor and similarities in the final sampling were 75–98%.

4.2 Field experiments

The aim of this research was to evaluate the performance of FBBR in field conditions. The composition of microbial populations and nitrogen removal efficacy were studied in particular. The study was reported more in detail in Elina Saario's Master's thesis (2012).

4.2.1 Experimental set-up

The process performance was followed for 5 months in the Willa grey water treatment unit (Konva-Center) installed in a detached house. In the Willa, water purification is based on a packed bed process in which wood chips are used as carrier material. Wood chips are replaced at regular intervals to maintain the function of a

treatment system. Phosphorus is removed separately by precipitation as a final stage of the grey water treatment. Samples were taken both from water (influent and effluent) and from wood chips used as carrier material for chemical and microbiological analyses, as described in Figure 14.

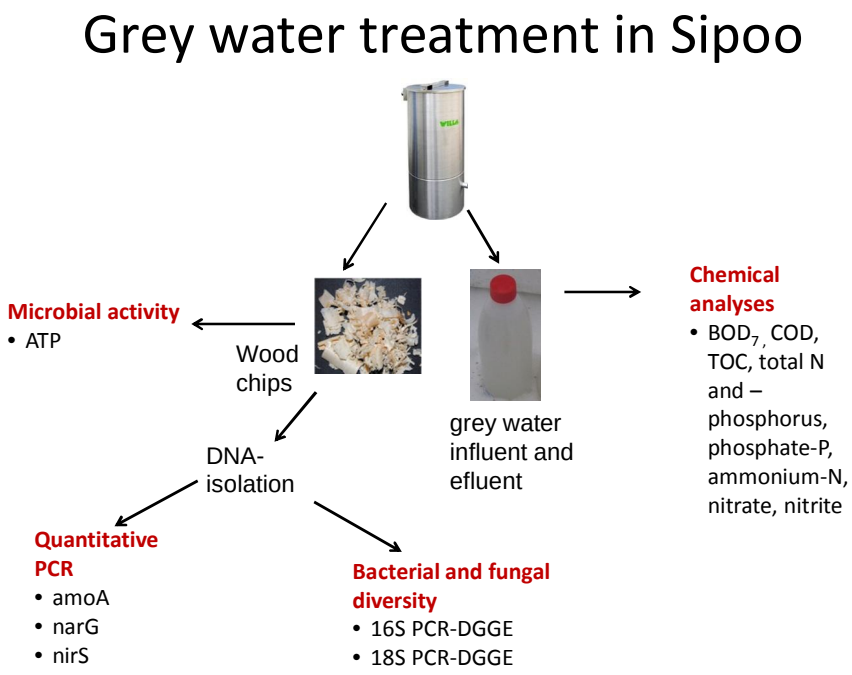


Figure 14. Experimental set-up and analyses in the grey water treatment unit in Sipoo.

4.2.2 Process behaviour

During the measuring period the removal of BOD (biological oxygen demand) and COD (chemical oxygen demand) were 61–71% and 14–57% respectively (Figure 15). The maximum ammonium-N removal during the measuring period was 84% (first sampling). The lowest nitrogen removal was obtained in the second sampling but the removal rates were returned to a higher level 1–2 weeks after the replacement of wood chips.

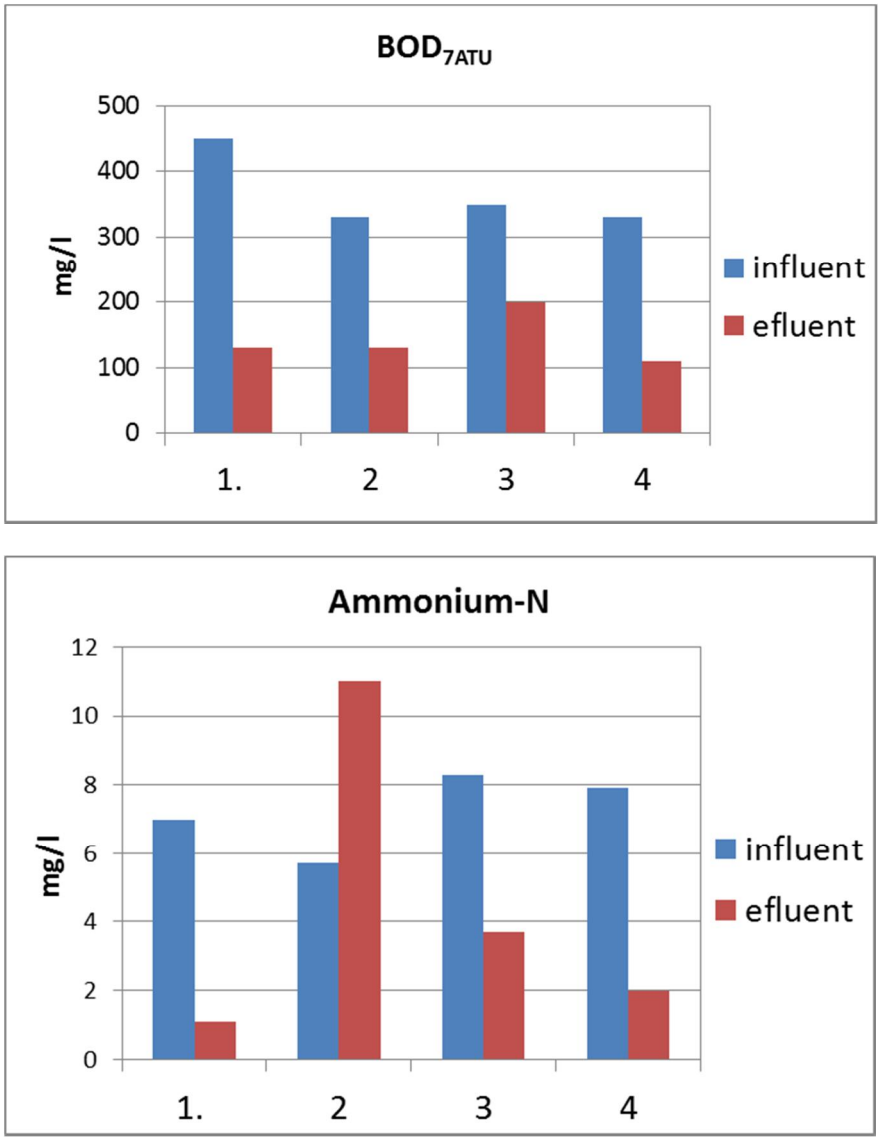


Figure 15. BOD and ammonium-N measured from influent and effluent in a grey water treatment system. Wood chips were replaced after the second sampling. Samplings: 1st (8 weeks after replacement of wood chips), 2nd (28 weeks), 3rd (1 week), 4th (2 weeks).

4.2.3 Microbial activity and diversity in biofilm

Microbial activity decreased gradually during the first and second sampling (Figure 16). After the replacement of wood chips, the activity returned to the original level (third sampling) and even higher (fourth sampling).

Figure 16. Microbial activity in wood chips carrier material measured as ATP activity. Microbial activity was measured 10 cm from the surface.

An abundance of microbes related to nitrogen removal was verified by quantitative PCR measuring functional genes involved in nitrogen removal in the grey water treatment system. A relatively high amount of nitrate reductase (narG) and nitrite reductase (nirS) coding gene copies were detected indicating denitrification potential in grey water treatment system. No ammonium monohydrogenase coding amoA gene copies related to nitrification were detected (Figure 17) and the reason for this should be evaluated in further studies.

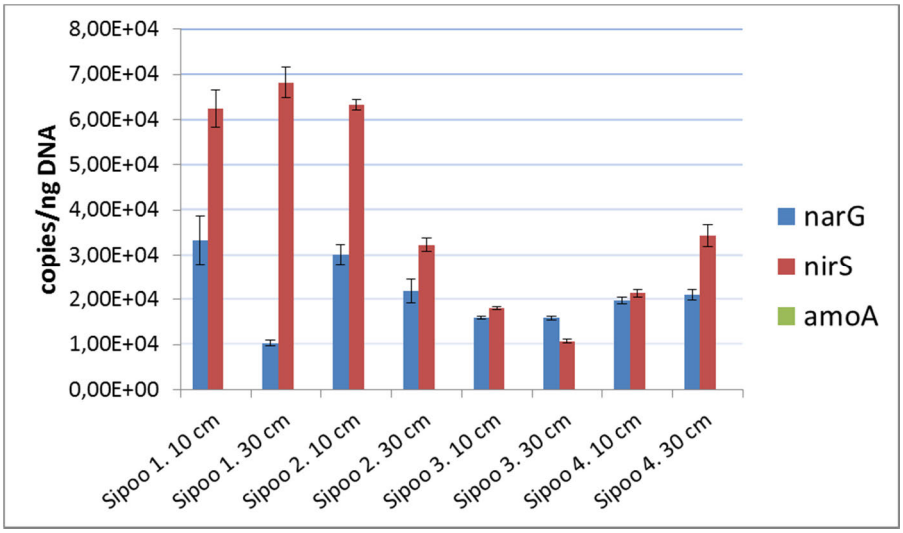


Figure 17. The amount of gene copies related to nitrogen cycle measured from grey water treatment system with qPCR. Wood chips were replaced after second sampling. Samples (wood chips) were taken from two different depths (10 cm and 30 cm from the surface).

4.3 Improving grey water treatment by bacterial inoculation

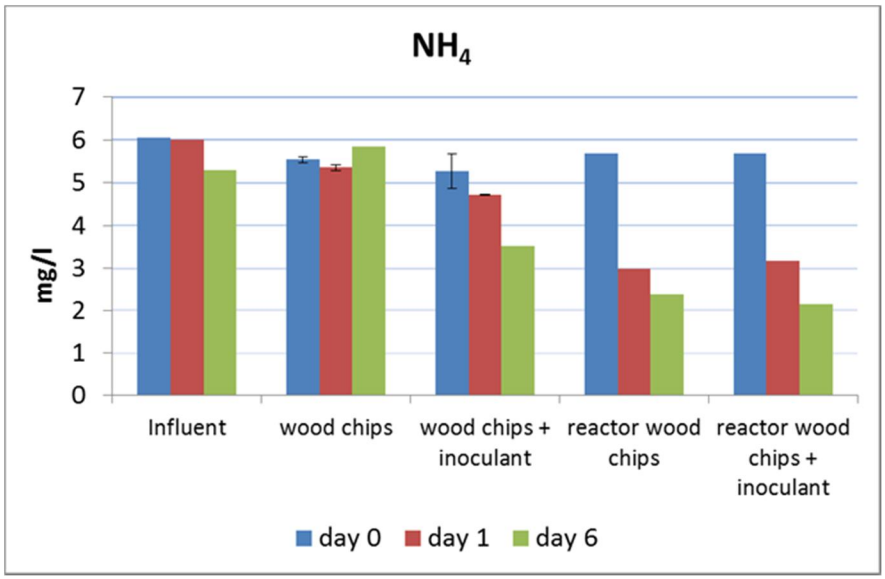
The aims of this study were to enrich nitrifying and denitrifying bacteria and use the enrichment cultures as an inoculant to improve nitrifying and denitrifying processes in grey water treatment process.

4.3.1 Experimental set-up

Wood chips from a small-scale grey water treatment unit and waste water from a municipal WWTP were used in the preparation of enrichment culture. Enrichment of nitrifying and denitrifying bacteria was performed at 25 °C as described by Du et al. (2003). During the enrichment, ammonium, nitrate and nitrite were measured to follow the progress of nitrification and denitrification. The effectiveness of the selected inoculants in removing nitrogen from synthetic grey water was tested by packed bed bioreactors with a volume of 250 ml.

4.3.2 Results

According to the preliminary results, the most effective enrichment culture was selected for further experiments in bioreactors. The addition of inoculant increased the removal of NH_4-nitrogen and the indication of formation of NO_3-N and NO_2-N was detected (Figure 18). The best results were obtained when reactors were filled with wood chips from the operating grey water treatment unit. This shows that wood chips acted as suitable carrier elements for nitrifying and denitrifying microbes. In this case, the addition of inoculant to the bioreactor did not enhance the removal of nitrogen. The results are promising but more research is needed to verify these results.



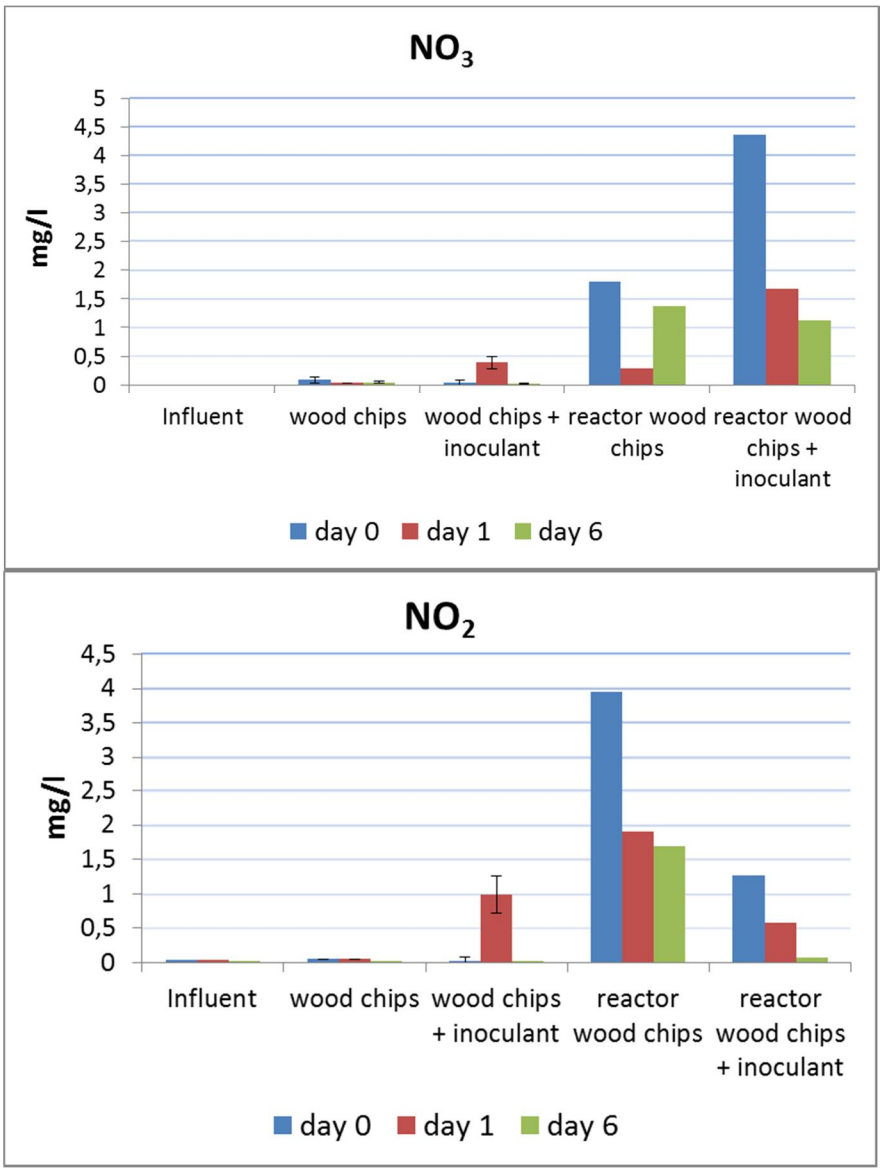


Figure 18. The influence of inoculant on the removal of nitrogen from synthetic grey water in bioreactor experiments. The amount of NH_4-N, NO_3-N, NO_2-N was measured from influent and effluents. Reactor wood chips were collected from the operating grey water treatment system.

5. Biological waste water treatment of car washes

In Finland, there are around 900 automatic car washes, which are often located at petrol stations, and about 10 million cars are washed in them every year. Biological waste water treatment is not usual in car washes and for example in Northern countries the most common waste water treatment technologies are sand and oil separators. Less than 5% of Finnish car washing facilities have an additional waste water treatment method such as peat, activated carbon filter or biological treatment. Waste water from car washes consist of dirt from cars, such as sand and dust, chemicals used for the washing, materials from washing equipment, and lubricants and other chemicals for maintaining the equipment (Norden, 2007).

Biological treatment systems are sensitive to chemicals, so non-solvent type detergents are used for washing. Surface active agents (also called surfactants or tensides) are generally the active agents in car shampoos, rinses and waxes. They lower the surface tension of a liquid, the interfacial tension between two liquids, or that between a liquid and a solid. Usually, anionic and nonionic surfactants are used in washing and cleaning products. Positively charged cationic surfactants are used to neutralize the negatively charged surfaces. Zwitterionic (amphoteric) surfactants have two oppositely charged groups and are anionic in alkaline solution, cationic in acidic solution and neutral in uncharged solution (The Finnish Cosmetic, Toiletry and Detergent Association, 2006). A high concentration of surfactants in waste water can inhibit autochthonous microorganisms. Surfactants increase the permeability of the bacterial membranes and the adsorption of surfactants causes the depolarization of the cellular membranes (Aloui et al., 2009). The surfactant concentration in the medium cannot exceed 1000 mg/l in biological treatment (ADEME, 2001; Aloui et al., 2009). However, more than 90–95% of surfactants can be eliminated by waste water treatment plants under optimized conditions. All surfactant classes undergo primary biodegradation under aerobic conditions, but persistent biodegradation products can form since all compounds do not biodegrade completely (González et al., 2007).

The aim of the car wash study was to examine how efficiently rotating bed biofilm reactors purify car wash waste waters. Altogether, three Bachelor of Engineering reports were prepared during the VESITURVA project. Sampling, measurement and analysis plans were first prepared based on literature, Internet sources

and interviews (Hakala, 2011). In next project, the plan was implemented by taking samples, performing measurements, and analysis (Valtonen, 2011). Due to several technical problems in waste water treatment facilities, the calculation of input load reduction was impossible. When the third project started, waste water treatment facilities were working well, which made the reduction survey possible (Id, 2012). Most of the results presented in this article originate from the third project.

5.1 Sampling, measurements and analysis of car wash waste water

Five automatic car washes (CW) located in Southern Finland were involved in this study. An overview of automatic car washes is presented in Figure 19, and a schematic diagram of the waste water treatment process and reuse of water is presented in Figure 20.

Figure 19. Overview of the automatic car washing process.

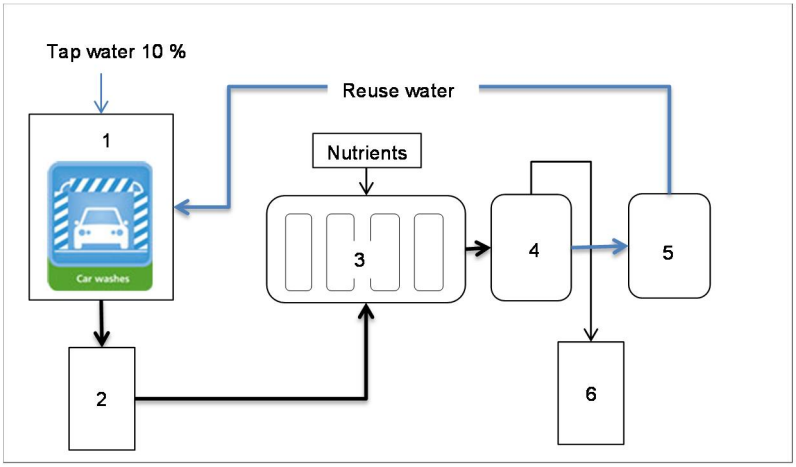


Figure 20. Schematic diagram of the waste water treatment process and reuse of water. 1 car washer; 2 sand separator; 3 rotating bed biofilm reactor (in series or in parallel); 4 flotation unit; 5 clean water tank; 6 sludge tank.

Three of the car washes (CW1, 4 and 5) purify their waste waters using commercially available aerobic rotating bed biofilm reactors and reuse the water for car washing (Table 7). CW1 has been awarded the Swan ecolabel. In CW1 and CW4,

bioreactors are connected in parallel and CW5 in series. In CW2 and 3, waste waters go through a sand filter and oil separator to the sewage and municipal waste water treatment plant. These five car washes were selected for this study because they all use the same Pineline detergents (Swan Label by Ecolabelling Finland) manufactured by Tekno-Forest Ltd (Table 7). In addition to those presented in Table 8, some additional detergents are used in CW2 and 3.

Table 7. Car washes involved in the study.

Name	Car wash type	Sampling period 1	Sampling period 2
CW1	Two bioreactors (15 m^3 each, consisting of two compartments of similar size) connected in parallel and placed underground	Sand filter, bioreactor and purified water	Purified water
CW2	Single door automatic	Sand filter and sewer	-
CW3	Two-door automatic	Sand filter and sewer	-
CW4	Bioreactor, in parallel	Sand filter and purified water	-
CW5	Four bioreactors (5 m^3 each, consisting of two compartment of similar size) connected in series and placed in a container	Sand filter and purified water	Purified water

The first sampling period was from the middle of April to the end of August 2011. Sampling was performed 12 times in total, and samples were taken from 2 to 6 times per individual car wash from the sand filter, bioreactor, purified water or sewer, depending on the car wash. COD, BOD, suspended solids, nitrogen, phosphorus, electrical conductivity, pH and temperature were analyzed every time. Since June 2011, cationic, anionic and nonionic surfactants were also analyzed. Metals and DEHP were analyzed in a commercial laboratory once at the end of April (CW1–CW3). COD, BOD and suspended solids were also analyzed from control samples in a commercial laboratory.

The second sampling period was from February to the middle of May 2012 and included two car washes, CW1 and CW5 both having bioreactors. Sampling was performed every two weeks, for a total of seven times, and samples were taken only from the purified water which is used for washing cars. COD, BOD, suspended solids, nitrogen, phosphorus, surfactants, turbidity, electrical conductivity, oxygen, pH and temperature were analyzed every time. The concentration of chlorides was also analyzed six times in a commercial laboratory.

Table 8. Contents and load of detergents used in CW1–CW5.

Car wash detergent consumption/wash (ml) in CW1/CW5	**Contents**	**Load (CW1 and 5)**
Smart Prewash C Smart Prewash D 72.5 / 58.5	Silicates < 5% Nonionic surfactants > 30% Sodium hydroxide < 2%	Anionic surfactants 300.25 mg/l Nonionic surfactants 50.6 g/l Ntot 7.11 g/l Ptot 39.85 mg/l COD 618.4 g/l
Smart Brush Shampoo 100 / 80	Anionic surfactants 15–30% 2-Propanol < 3%	Anionic surfactants 374.0 g/l Nonionic surfactants 89.3 g/l Ntot 9.48 g/l Ptot 445.0 mg/l COD 2276 g/l
Smart Pressure wax 32 / 15 or when used as drying wax 30 / 60	Cationic surfactants < 5–2% Butoxyethanol 5–15%	Anionic surfactants 12.0 mg/l Nonionic surfactants 132.5 g/l Ntot 7.18 g/l COD 1763 g/l
Smart Polishing wax 48 / 40	Amphoteric surfactants 5–15%	Nonionic surfactants 4.58 g/l Ntot 2.17 g/l Ptot 8.4 mg/l COD 402.4 g/l

Altogether, 14 different washing operations are provided in car washes CW1 and 5 consisting of between one and five units (prewash, washing with or without brushes, etc.). Consumption of detergents varies between different operations. The consumption was experimentally tested in CW1 by measuring the amount of chemicals by weight in a test wash. The detergent consumption in CW5 was calculated using the mean of measurements from spring 2011 provided by the operator. The load of detergents was examined using Hach Lange photometric tests using dilutions from 1:10 to 1:1 000 000. During the sampling period, the number of washes was on average five times higher in CW5 than in CW1 and the relative number of car washes is presented in Figure 21. The numbers of washes increased towards the spring and begin to drop at the end of April. Approximately 13,000 and 17,000 cars are washed per year in CW1 and CW5, respectively.

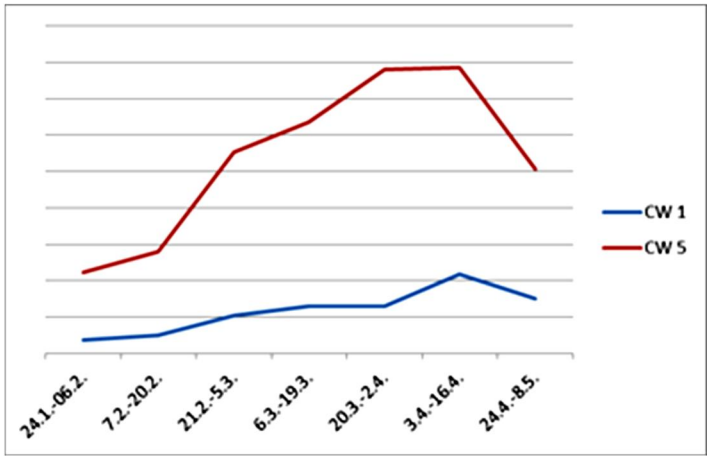


Figure 21. The relative number of car washes during the testing period.

5.2 Calculation of reduction

Reduction of COD and detergent load (anionic and nonionic surfactants) during the purification process was calculated comparing calculated input load (from detergents and reused water) to the analysis result of purified water (outflow). The input load was estimated as follows:

$$((CW \times D \times L) + R) : W$$

(CW) = number of different car washing operations in a two-week period before sampling (information from sales database).

(D) = amount of detergent/wash (Table 8).

(L) = chemical load of five detergents (Table 8).

(R) = chemical load of reused water in a two-week period before sampling calculated using analysis results from purified water and multiplying by the amount of reused water consumed in the two-week period.

(W) = amount of used water in a two-week period before sampling (tap water and reused water). Mean value was calculated using the value from the beginning and the end of the two-week period.

5.3 Reduction of COD and detergents

The reduction of COD and detergent load are presented in Figure 22. The mean of COD and surfactants reductions were calculated using the results from seven sampling times. However, the error situation (3.4.–16.4.2012) of CW5 was exclud-

ed. The average COD reductions were 95% and 87% in CW1 and in CW5, respectively. The reduction of anionic surfactants was almost complete in the aerobic treatment; the average reduction was 99% and 97% in CW1 and CW5, respectively. Also, the reduction of nonionic surfactants was almost complete; with the average reduction being 99% and 95% in CW1 and in CW5, respectively.

During the second sampling period, the reduction of COD and surfactants was better in CW1, which is connected in parallel, than in CW5. In the first sampling period, reactors connected in series seemed to purify surfactants more efficiently than those that were connected in parallel (data not shown) but as mentioned earlier, several error situations took place during the first period. One reason for the worse purification capacity in CW5 than CW1 was most probably the lack of oxygen. The average oxygen concentration in the purified water tank during the sampling period was 7 mg/l in CW5 and 8 mg/l in CW1. The amount of dissolved oxygen was lower in CW5 than in CW1 at all the other sampling times except the first and last. In accordance, turbidity was much higher in CW5 than in CW1 except at the last sampling time (Figure 23). In April the oxygen concentration dropped to almost zero in CW5 and a peak in turbidity was also observed (Figure 23). The reason for that was that the aeration blower of the bioreactor was broken, which was clearly indicated by low dissolved oxygen concentration and turbidity measured in the purified water tank. The number of washing operations was highest when the level of oxygen was lowest in CW5. In the future, turbidity could be used in process control to indicate error situations.

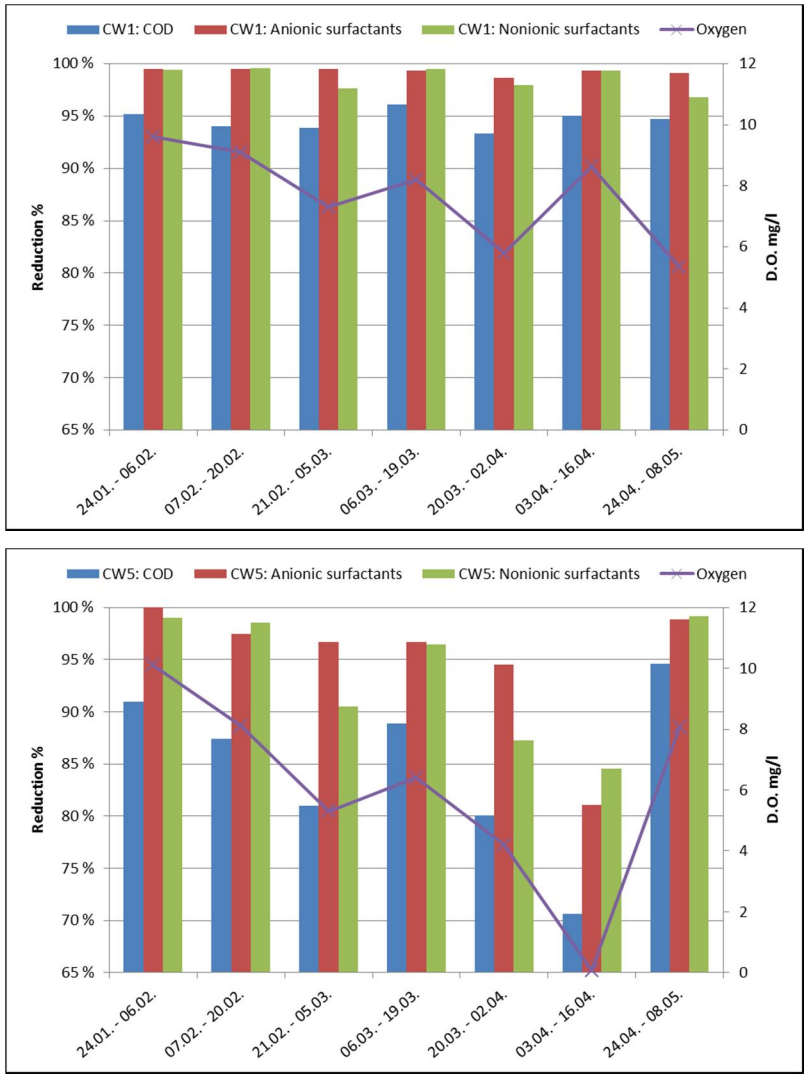


Figure 22. Reduction of COD and surfactants during sampling period 2 in CW1 and 5.

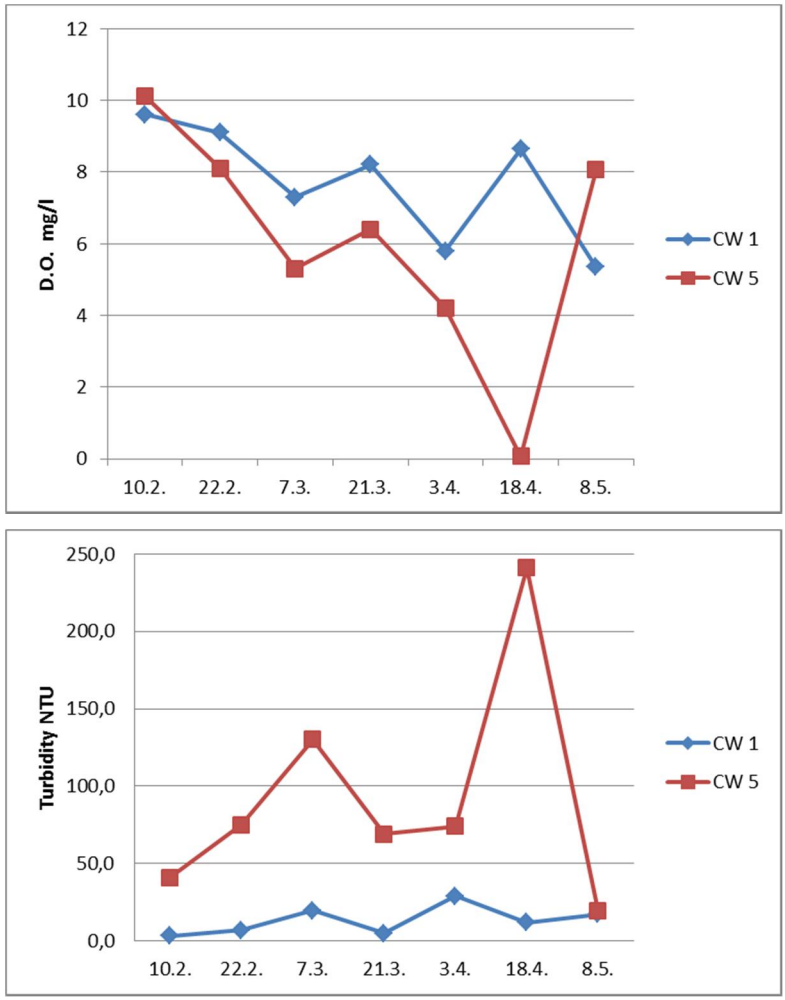


Figure 23. Oxygen and turbidity in CW1 and 5.

pH was slightly alkaline in both car washes. In CW5 the average pH was 7.54 (deviation 0.25) and the temperature varied between 13 and 18 °C. In CW1 the average pH was 7.87 (deviation 0.20) and the temperature was a bit warmer, varying between 17 and 22 °C. The temperature followed the same trend towards spring in both car washes. One object of the study was to find out the effect of temperature on the biological treatment process and the degradation of surfactants. Although there were very low temperatures outside, the temperature inside the bioreactor only varied by ±5 °C. In comparison to oxygen level, the temperature seemed not to have any important effect on purification capacity.

5.4 Quality of reused water

pH, BOD, COD, suspended solids and turbidity analyzed from purified water were compared to standards in Flanders (EU) and China (Table 9). The average values of CW1 are mainly below the standards in Flanders but the average values of CW5 are higher. When the dissolved oxygen levels measured from the purified water tank were at a good level in CW5 (10.2., 22.2., 8.5., see Figure 23) BOD, COD, SS and turbidity values were also much lower and at best below the standards in Flanders. However, mean turbidity exceeded the criteria in China in both car washes.

Table 9. Mean of quality parameters of purified water in CW1 and 5 calculated from values measured during the sampling period (the minimum and maximum is presented in brackets).

Quality parameter	CW1	CW5	Standards in Flanders (Boussu et al., 2007)	Criteria of China (GB/T 18920–2002) (Li et al., 2007)
BOD (mg/l)	17 (4–43) (BOD_7)	146 (20–372)	< 25 (BOD_5^{20})	10 (BOD_5)
COD (mg/l)	126 (87–152)	313 (116–652)	< 125	50 (COD_{Cr})
SS (mg/l)	18 (6–33)	112 (53–218)	< 60	5
Turbidity (NTU)	13 (3–29)	93 (20–241)	–	5
pH	7.87 (7.61–8.11)	7.54 (7.24–8.00)	6.5–9	6.5–9

In first sampling period, high conductivity values were measured from purified water (data not shown). In this study, the chloride concentration was analyzed to find out if there is a correlation between road salting and high conductivity (Figure 24). Based on the results, road salting is not the reason for an increase in conductivity. Chloride concentrations seem to decrease and conductivity increase when the amount of washes increases during the spring (see Figure 24), especially in CW5. The reason for high concentration of chlorides in CW5 must be further studied. The chloride concentration of CW1 was below the criteria of China (300 mg/l) during the whole sampling period.

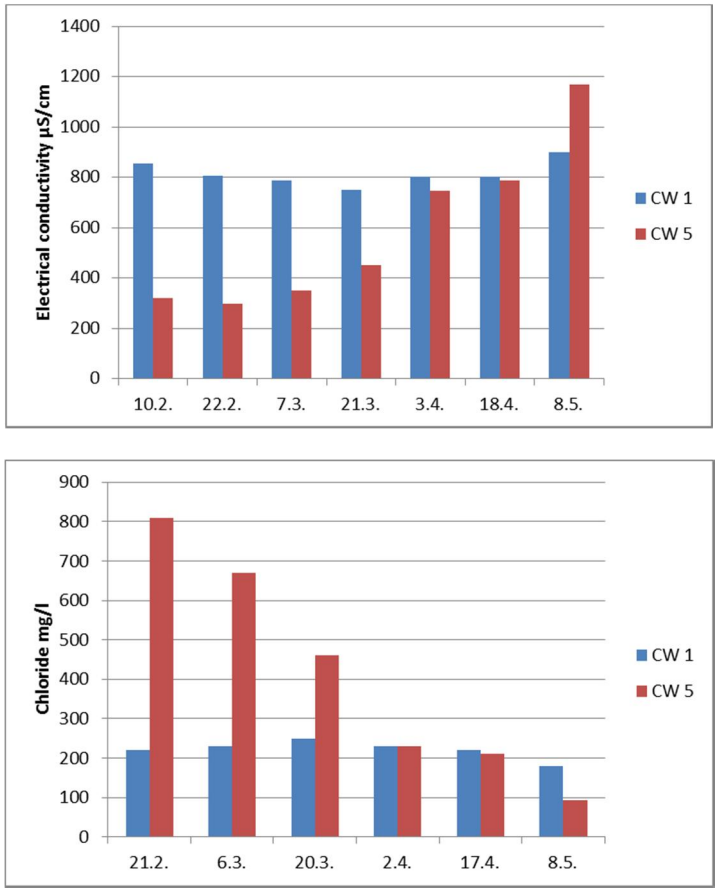


Figure 24. Electrical conductivity and Chloride concentrations (not analyzed on 10 February) in CW1 and 5.

The amount of suspended solids in the purified water was also analyzed and the results followed the same trend as turbidity, which was expected. The concentration of suspended solids was (2–14 times) greater in CW5 than in CW1 during the second sampling period and did not correlate with electrical conductivity but indicated more efficient purification in CW1.

5.5 Nutrient load

The total phosphorus and nitrogen in input and purified water consisted of load from reused water and from surfactants as well as nutrient supply (Figures 25 and 26). Phosphorus and nitrogen are fed into the process manually by an operator and the exact amount of amendment is not known. In an optimum situation, microbes use

all the nutrients during the purification process. In CW1, the nutrient balance is pretty good except for the excess load of phosphorous in April, probably because of the growing number of car washing operations (see Figure 25). In CW5, the optimization of nutrient feed improved during the sampling period. The effect of the broken aeration blower can also be seen in poorer removal of nitrogen by microbes. Biological treatment of car wash waste water needs some extra nutrients but it should be better optimized and controlled.

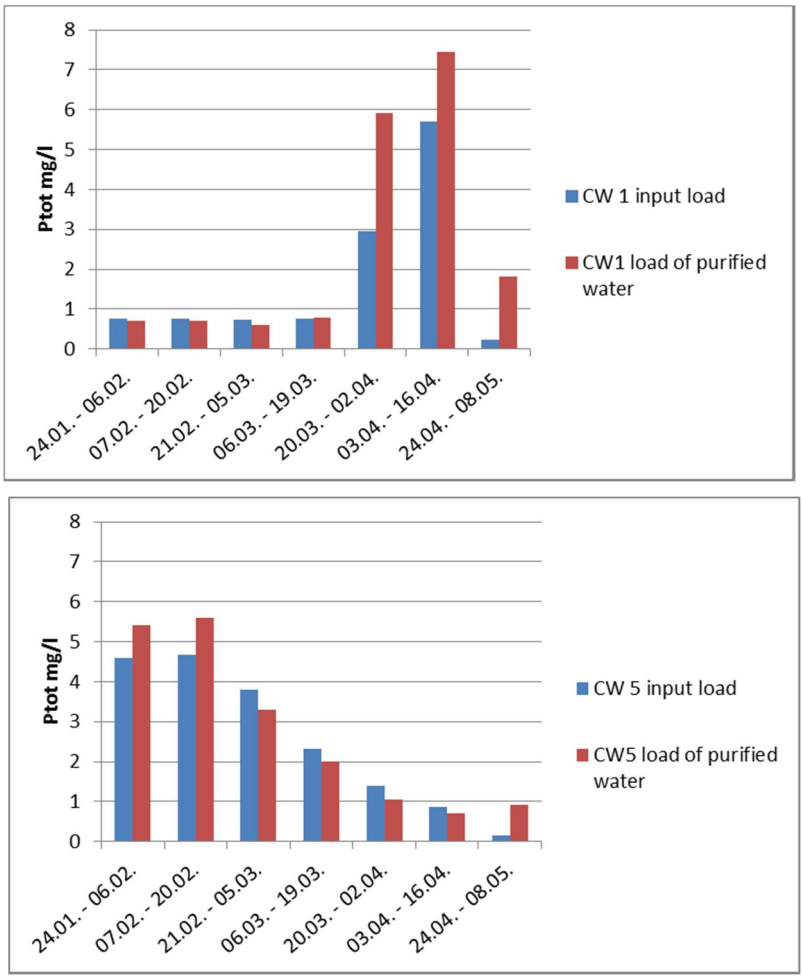


Figure 25. Total phosphorous in CW1 and 5 in input and purified water.

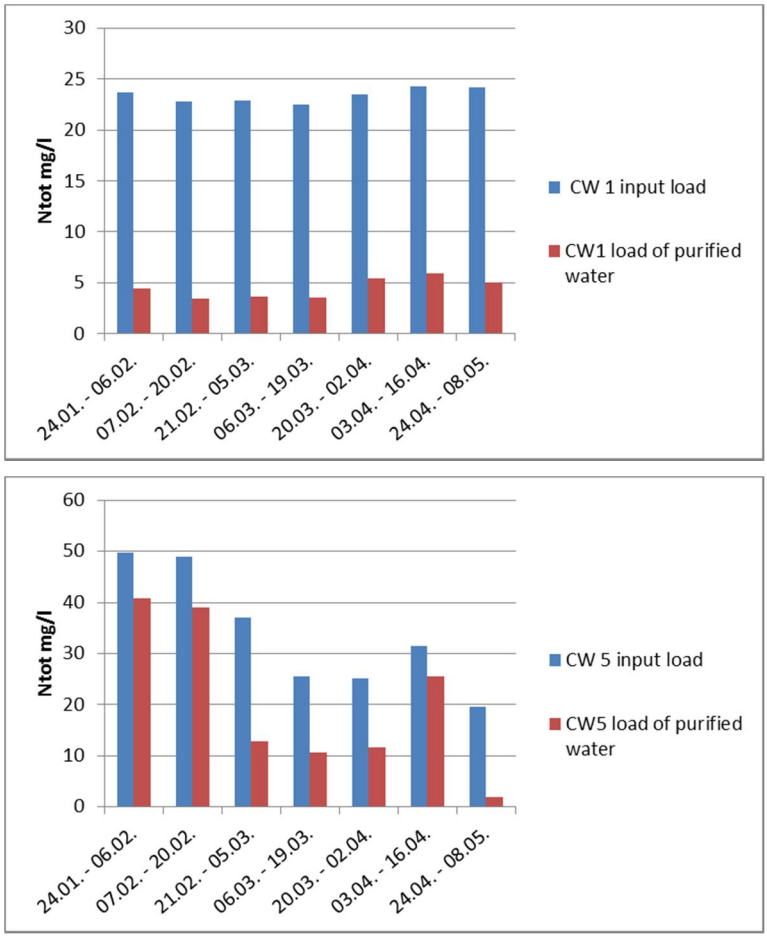


Figure 26. Total nitrogen in CW1 and 5 in input and purified water.

5.6 Analysis of metals

Five metals and Bis(2-ethylhexyl)phthalate (DEHP) were analyzed at the end of April 2011 from samples collected during the first sampling period from three car washes (Tables 10 and 11). Metals did not exceed the maximum concentration limits which are defined by the local municipal waste water treatment plant. There is no defined DEHP limit for incoming waste water in the treatment plant. Concentrations were slightly higher in CW1 where waste waters are treated than in CW2 and 3.

Table 10. Concentrations of metals.

	CW 1 Bioreactor	CW 1 Purified w.	CW 2 Sewer	CW 3 Sewer	Max. concentration
Cadmium (mg/l)	< 0.001	< 0.001	< 0.001	0.001	0.01
Copper (mg/l)	0.31	0.22	0.15	0.14	2.0
Lead (mg/l)	0.014	0.009	0.006	0.007	0.5
Nickel (mg/l)	0.036	0.033	0.010	< 0.01	0.5
Zinc (mg/l)	0.94	0.45	0.41	0.52	2.0

Table 11. Concentration of DEHP.

Car washes	DEHP (µg/l)
CW 1 Bioreactor	5
CW 1 Purified w.	7
CW 2 Sewer	2
CW 3 Sewer	< 1

5.7 Water consumption

Due to diminishing global water supplies and the growing number of cars, purifying and reusing car washing waste water is sensible. The average water consumption per car wash without waste water treatment is 200–500 l. In tunnels, which have a higher washing capacity, water consumption is about 100–250 l/wash. Systems which use recycled water for both body and underbody washing reduce water consumption by 80–90%, and produce usually 20–60 l of waste water per wash (Norden, 2007). Water recycling in car washes is mandatory in some European countries. In Belgium, the water consumption of automatic car washes is on average 400 litres per car and in the future 70% of recycling will be needed to obtain an environmental licence (Huybrechts et al., 2002; Boussu et al., 2007).

Waste water treatment of car washes aims at closed water systems and savings of clean water. In CW1 and 5, only 10% (from 35 to 60 litres) of all the water used for washing is clean tap water, which is used for the final rinsing. They also recover the water that is lost during the process because of evaporation and spillage. Assuming that approximately 30,000 cars are washed in an automatic car wash, and 200 litres are used in car washes without water reuse, 140 litres are saved from every wash in a closed water system, resulting in 4,200 m^3 water saved per year.

5.8 Conclusion and further challenges

We can conclude that biological waste water treatment efficiently purifies COD and surfactants from car wash waste water. The closed water system saves water and does not load the municipal sewage system. On the other hand, nutrient supply is needed and the manual input should be replaced or developed further to avoid an overload of nutrients. Rainwater could be also used as a water source to save valuable tap water even further.

The first sampling period was very challenging because of a number of technical problems. However, many important issues were recognized during the period, which were performed better and studied further in the next sampling period. This study was conducted in uncontrolled situations outside the laboratory, and that is why many questions are still open. On the other hand, it provides an opportunity for the development of an important application of waste water treatment in a real situation.

6. Chemical treatment of landfill leachate

6.1 Aim of the leachate treatment

The aim of the landfill leachate treatment was to enhance the biodegradability of the leachate and the total nitrogen removal. The proposed pretreatment was ozonation. The study was reported in more detail in Piia Nieminen's Master's thesis (2010).

6.2 Sampling

The main aim of the study was to screen the biodegradability of landfill leachate from Kuusamo. Due to the long distances, part of the studied water was collected from the local landfill at Tarastejärvi, Tampere. The sample was taken from a collection well that leads the leachates to the municipal waste water treatment plant in Tampere.

6.3 Characteristics of studied leachates

The landfill leachate from Kuusamo was biodegradable and no pretreatment was needed as was originally planned. Ozonation was not able to increase biodegradability, that is, the BOD value of the leachate. The ozone dose of 0.2 mg O_3/mg DOC decomposed the organic matter measured as BOD at about 20%. The main parameters of the studied water are presented in Table 12.

Table 12. The studied parameters of the waste water from Kuusamo and Tampere.

mg/l	Kuusamo	Tarastejärvi
DOC	153	410
COD	483	476
NH_4-N	84	188
BOD	338	118
Phosphorus	3.5	4.8
Fe	3.4	–
pH	7	8
BOD/COD	0.7	0.25

6.4 RBBR Reactor

In the first phase, the experimental set-up consisted of two aerobic reactors in series (Figure 27). After the performance was stabilized and the nitrification rate was 100%, 3rd anoxic stage was added in front of the two reactors in order to achieve also denitrification (DN-process).

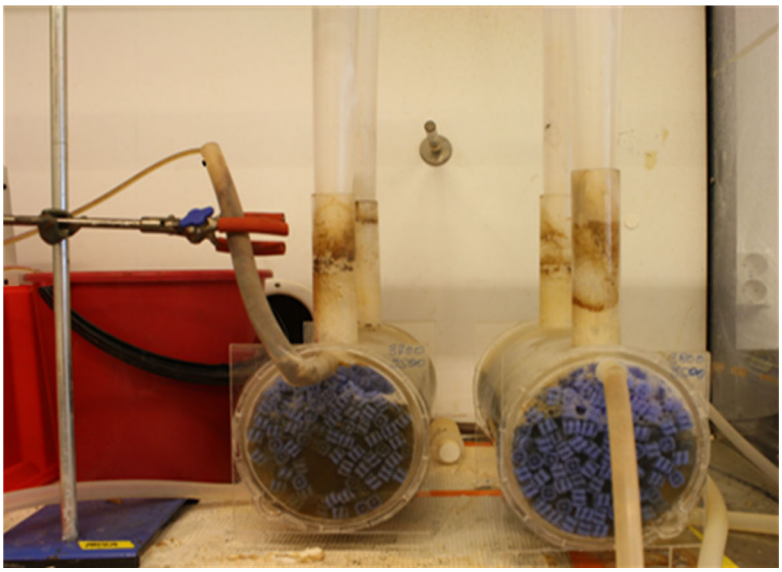


Figure 27. RBBR system: two aerobic reactors in series.

6.5 Results

6.5.1 Aerobic phase

After the addition of activated sludge from the Tampere municipal water works, the performance of the aerobic reactors was established at a level where half of the organic matter was removed (Figure 28). Nitrification was practically complete as can be seen from Figure 29.

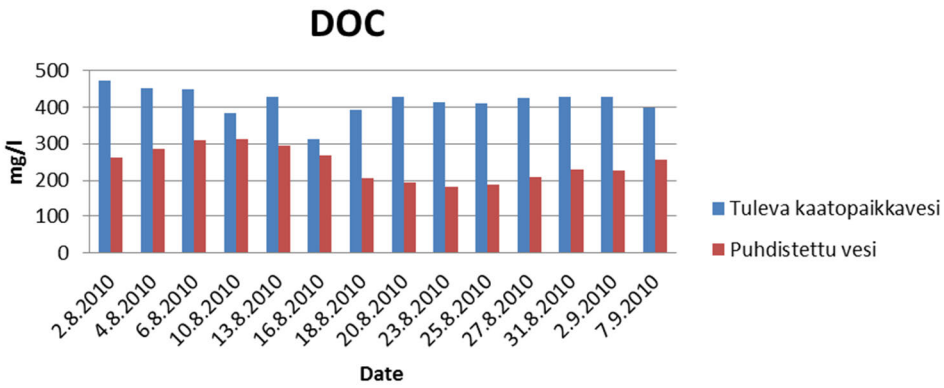


Figure 28. The removal of organic matter measured as Dissolved Organic Carbon. Blue bars: inflowing landfill leachate; red bars: treated landfill leachate.

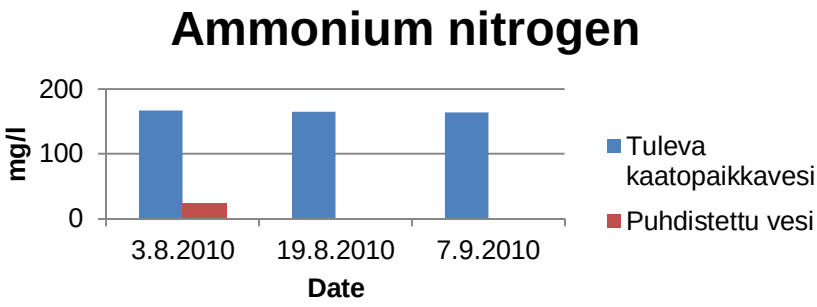


Figure 29. Removal of NH_4-N. Blue bars: inflowing landfill leachate; red bars: treated landfill leachate.

Table 13. The performance of aerobic RBBRs.

	DOC	BOD	COD	Tot- N	NH_4-N	P
Input	410	118	476	188	165	4.8
Output	187	84.4	381	146	0	4.5
Reduction	54%	28%	20%	20%	100%	6%

6.5.2 DN process

After the performance of RBBRs reached the level of 50% DOC removal, an anaerobic reactor was added in front of the aerobic reactors in order to denitrify the nitrate (Figure 30). The removal efficiency of organic carbon deteriorated after

the modification. The removal of ammonia to nitrate was no longer 100% as can be seen from Figure 31. The total nitrogen removal can be seen from Figure 32.

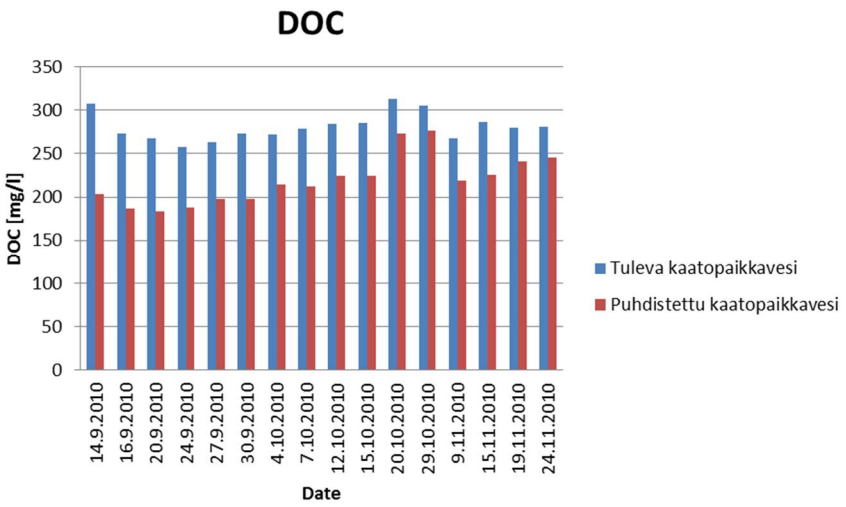


Figure 30. Removal of DOC by DN-RBBR reactor. Blue bars: inflowing landfill leachate; red bars: treated landfill leachate.

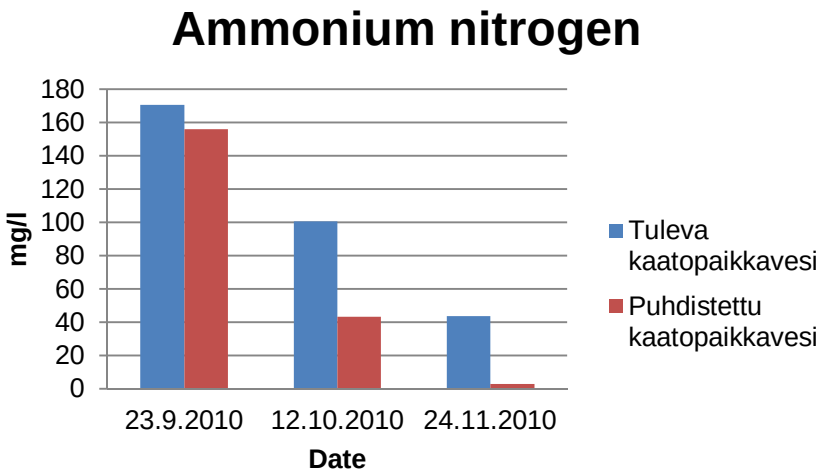


Figure 31. Removal of NH_4-N by DN process. Blue bars: inflowing landfill leachate; red bars: treated landfill leachate.

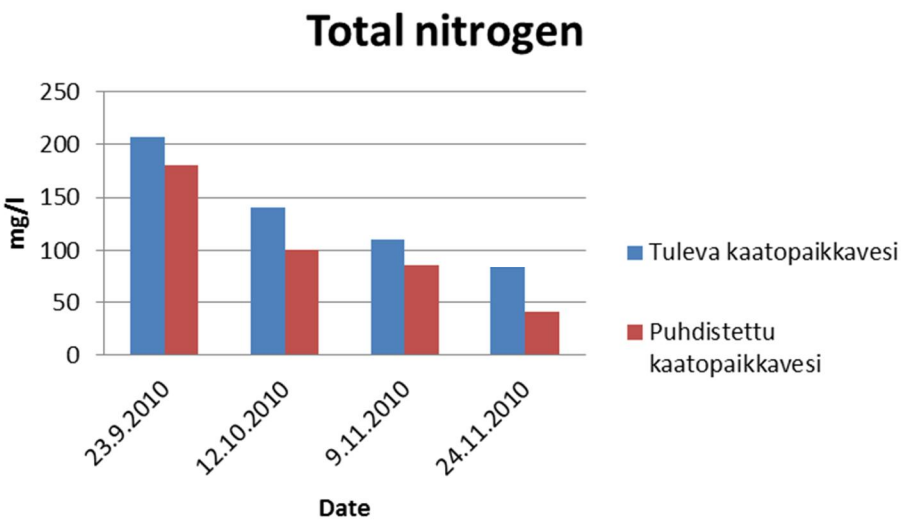


Figure 32. Removal of total nitrogen by RBBR in DN-mode. Blue bars: inflowing landfill leachate; red bars: treated landfill leachate.

6.6 Conclusions

For landfill leachate waters, pre-tests were performed in order to find out how biodegradable the leachate was. Landfill leachate was found to be already biodegradable when the process for removing nitrogen from it with a rotating bed biofilm reactor was started in the laboratory. At first, two aerobic reactors were running in series in a refinery. In this case, 100% effectiveness for the nitrification process was achieved. After this, one anaerobic reactor was added in front of the aerobic reactors, which made the whole treatment system operational. Because only half of the total nitrogen was removed, the treatment system did not work as well as expected.

7. International collaboration

7.1 Microbial activity, diversity and composition in grey water filters

Sahar Dalahmeh M.Sc. from the Swedish University of Agricultural Sciences visited Helsinki University's Department of Environmental Sciences for three weeks in May 2010. During her visit she worked in AlmaLab in the Department of Environmental Sciences to learn the laboratory procedure for extraction of DNA that is performed prior to pyrosequencing. She processed filter materials from different grey water treatment systems that she studied under the supervision of Professor Håkan Jönsson. They achieved a successful removal of pollutants from grey water with bark and activated charcoal, whereas foam and sand were less efficient (Dalahmeh et al., 2012). A publication discussing the results of microbial characterization in the filter materials is forthcoming.

7.2 Fate of Bisphenol A and its metabolite Bisphenol A dimethyl ether in microcosms inoculated with marine and pond sediments

7.2.1 Background

Tiina Mononen MSc (Tech), from Helsinki University Department of Environmental Sciences, visited Rutgers State University of New Jersey, USA from December 2011 to March 2012. During her visit, she concentrated on the study of the environmental fate of one of the model pollutants, BPA, applied in the project. Professor Max Häggblom's research group has previously shown that mycobacteria are capable of metabolizing BPA among other hydroxylated aromatic compounds. Furthermore, they showed that the products of O-methylation of BPA by mycobacteria are more lipophilic and more toxic than the original molecule BPA. (McCormick et al., 2011) However, it is not well known how common O-methylation of BPA is in the environment. The aim was to study whether there is potential in marine and pond sediments for 1) degradation or O-methylation of BPA or 2) degradation of O-methylated BPA, and Bisphenol A dimethyl ether (BPA DME).

7.2.2 Methods

Microcosms were established by spiking BPA or BPA DME in nutrient solution and adding marine or pond sediment as inoculation to introduce environmental bacteria to the mixture. The microcosms were incubated at 21 °C and shaken occasionally to ensure aeration. Microcosms with autoclaved sediments were established to study whether removal of the pollutants was biological. Microcosms were sampled at the beginning of the experiment and 1, 2, 4 and 8 weeks (marine sediment) and 1 and 2 weeks (pond sediment) after the start. The concentrations of BPA and BPA DME in the samples were analyzed by using liquid extraction as a pretreatment and GC-MS for the analysis.

7.2.3 Preliminary results and discussion

The change in the concentration of BPA or BPA DME in marine sediment was less than ± 5% for four weeks from the start of the experiment. After eight weeks there was 24% removal of both BPA and BPA DME (Figure 33). However, removal of BPA and BPA DME was also noticeable in autoclaved controls, 15% for BPA and 7% for BPA DME. Therefore, removal of both BPA and BPA DME may happen due to both biological and physico-chemical processes, such as adsorption. Furthermore, in microcosms spiked with BPA DME, the appearance of BPA was noticeable after eight weeks, which indicates a transformation of BPA DME to BPA.

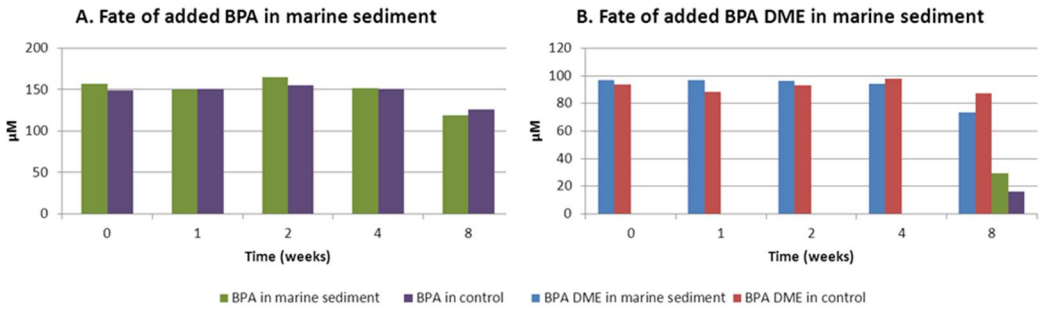


Figure 33. Fate of BPA (A) and BPA DME (B) in marine sediment was similar in respect of the amount of the chemicals. However, it seems that part of BPA DME transformed to BPA.

In the pond sediment, removal of BPA DME was 12% after the first week but it did not increase during the second week. Removal of BPA was 18% after the first week and 27% after the second week. Removal in autoclaved controls was 5% for BPA DME and 4% for BPA after two weeks (Figure 34A and B).

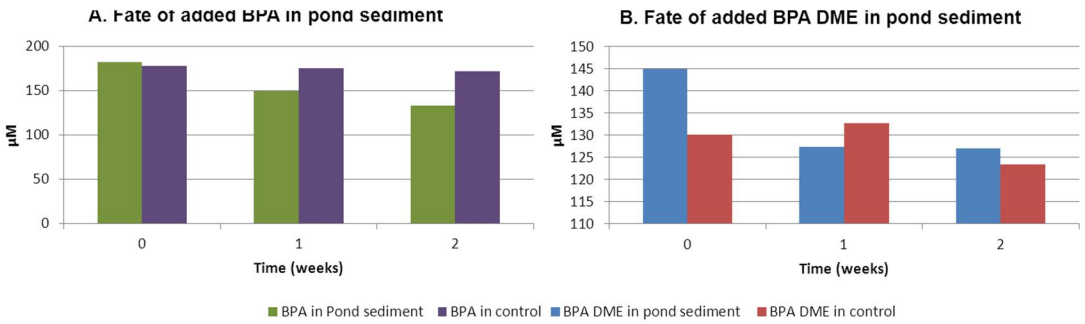


Figure 34. Fate of BPA (A) and BPA DME (B) in pond sediment. In pond sediment the removal of BPA was faster than the removal of BPA DME.

A decrease in BPA and BPA DME was seen in both marine and pond sediments. Further experiments will be needed to clarify the role of biological degradation/alteration and chemical processes in the decrease of BPA and BPA DME. In the marine sediment, alteration of BPA DME to BPA was noticeable but there was no evidence of O-methylation of BPA to BPA DME, which is good in respect of the environmental risks because of the higher toxicity of BPA DME compared to BPA. In the pond sediment the decrease in the concentration of BPA and BPA DME began more rapidly than in the marine sediment. One explanation for this might be in the sediments and their microbial communities. The marine sediment was collected earlier from a polluted site and it had been stored for a longer time than the pond sediment that was collected not long before the start of the experiment. With both sediments a longer incubation time would be important to see the final extent of removal of BPA and BPA DME, as well as to see if a transformation product could be noted and identified. The research into the environmental fate of pollutants is important for risk assessment and for acquiring knowledge for future decisions.

8. Conclusions of the VESITURVA project

The aim of the VESITURVA project was to improve the waste water treatment process performance in different applications. Several kinds of waste waters were included: municipal waste water, grey water, landfill leachate and car wash waste water. Depending on the source of the waste water, it can contain a complex mixture of different kinds of harmful organic pollutants and one aim was to evaluate the removal of these pollutants during treatment processes. Both FBBR and RBBR were studied in laboratory conditions and in the field. In addition, several methods were developed and utilized in order to study the diversity and function of microbes, because these aspects greatly influence the process efficiency of biofilm reactors.

The following conclusions can be drawn from the results obtained in the VESITURVA project.

Multi-stage biofilm reactors:

- The organic matter (COD, BOD) and nutrients were effectively reduced in biofilm reactors. Most of the organic matter and nutrients were already removed in the first bioreactor in the series. This might favour degradation of the more complex substrates in the second/third reactor.
- Multi-stage biofilm reactors seemed to be efficient in removing BPA and polycyclic musk compounds from waste water. Major parts of the micropollutants were removed already in the first reactors but the model of sequential reactors enhanced the removal of both BPA and HHCB.
- It is not known what the role of biodegradation and other mechanisms, such as adsorption, in the removal of micropollutants from waste water is, and this should be evaluated in further studies. In the case of fixed packed bed reactors in particular, other mechanisms such as adsorption could be an essential part in removing micropollutants. On the other hand, plastic materials of rotating bed reactors as well as suspended solids that exist in the waste water may also be capable of adsorbing micropollutants.
- Microbial diversity in incoming waste water was different compared to the microbes grown on the carrier elements in the bioreactors. 454-pyrosequencing

showed that before the addition of the micropollutants, the same bacterial groups were dominant in all reactors, but different bacterial dominance was observed at the genus taxonomic level.

- Time-dependent changes in microbial diversity were detected in multi-stage biofilm reactors. These changes may be due to the feeding of micropollutants to the reactors.
- Diversity of bacterial communities decreased as a function of time, suggesting that the bacterial communities in the reactors became specialized over time.
- Carrier material (polyethylene, wood chips) affected the biofilm community profile in packed bed bioreactors.
- Small scale waste water treatment units did not need a long adaptation period to perform efficiently as it was seen after an interruption in the run on the RBBR and the replacement of the wood chips of the FBBR.

Treatment of grey water treatment in field conditions:

- The maximum nitrogen removal efficiency in the system was 84%. Nitrogen removal in the grey water treatment system was verified by an evaluation of the abundance of denitrifying microbes by quantitative qPCR.
- Preliminary results indicate that nitrogen removal in grey water treatment can be enhanced by using inoculants.

Landfill leachate waters:

- When landfill leachates were treated by multi-stage biofilm reactors, the complete nitrification process was achieved. The addition of an anaerobic phase induced the denitrification but effective nitrogen removal was not achieved.

Car wash waste water:

- Waste water treatment by aerobic bioreactors with a carrier substance efficiently purified COD and surfactants from the car wash waste water. Because a nutrient supply is needed, the manual input should be replaced or developed further to avoid overloading of nutrients. In the future, turbidity could be used in process control to indicate error situations. Rainwater could be also used as a water source to save valuable tap water even further.

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Waste water treatment by multi-stage biofilm processes

Report of the VESITURVA project

Municipal and industrial waste waters in Finland are treated before their release into the environment. New legislation also requires that waste waters from all households with running water are treated before release, whereas the methods for treatment may vary. In the Tekes-Symbio project VESITURVA research groups at the University of Helsinki, VTT, Tampere University of Technology and Lahti University of Applied Science, in collaboration with companies in the field and municipal stakeholders, pooled their resources in an effort to study and improve waste water treatment.

Waste water treatment technology based on microbial biofilms is an efficient alternative, at least in smaller units suitable for single family homes. Although not tested in VESITURVA, we believe that the units and the technology can be upscaled and adapted to at least cover the needs of several families or a small village. Furthermore, the results regarding removal of the micropollutants tested were promising, and it is likely that many other organic micropollutants would behave similarly in multi-phase biofilm treatment systems.

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Title	**Waste water treatment by multi-stage biofilm processes** **Results of the VESITURVA project**
Author(s)	Tiina Mononen (Ed.), Sebastian Coloma, Martin Romantschuk, Minna Vikman (Ed.), Anu Kapanen, Anna Lehtonen, Elina Saario, Merja Itävaara, Essi Malinen, Silja Kostia & Tuula Tuhkanen
Abstract	Municipal and industrial waste waters in Finland are treated before their release into the environment. New legislation also requires that waste waters from all households with running water are treated before release, whereas the methods for treatment may vary. In the Tekes-Symbio project VESITURVA research groups at the University of Helsinki, VTT, Tampere University of Technology and Lahti University of Applied Science, in collaboration with companies in the field and municipal stakeholders, pooled their resources in an effort to study and improve waste water treatment. In the case of household waste waters, minimum removal requirements exist only for the bulk components, organic matter (BOD, COD), nitrogen, and phosphorus. While we also monitored the removal of these components in VESITURVA, the main focus was on micropollutants (pollutants that exist in waste water in ng per litre to µg per litre concentrations, for example hormone disruptors, farmaceuticals, musks, components of personal care products etc.) – how they behave and how their removal can be improved. In VESITURVA we tested waste water treatment methods that are based on biofilms colonising the surfaces of matrix materials using multi-phase water treatment systems, where the water passes through two or three reactors. The model substances for the micropollutant removal process were Bisphenol A (BPA), a component used in the plastics industry, and the commonly used polycyclic musk compound HHCB. Two different types of reactors were used for studying the effect of biofilm activity: Rotating Bed Bioreactors (RBBR), where the biofilm develops on plastic beads with a large surface area, and Fixed Bed Bioreactors (FBBR), where wood chips were used as the support. In both cases a continuous or semicontinuous flow of waste water passed through the reactors. In the RBBR set-up, municipal waste water was led through a three-phase treatment process, while in the FBBR artificial grey water was treated in a two-phase process. In both processes, the removal of the bulk components was good, and substantial reductions were also achieved in the case of the model micropollutants. Multi-stage biofilm reactors seemed to be efficient at removing BPA and musk HHCB from waste water. Major parts of the micropollutants were already removed in the first reactors but the model of sequential reactors enhanced the removal of both BPA and HHCB. Diversity of bacterial communities decreased as a function of time suggesting that the bacterial communities in the reactors became specialized over time. The same bacterial groups were dominant in all sequential reactors, but differences were observed at genus taxonomic level. Also, the microbial diversity was similar to that seen earlier studies of waste water treatment microbiology. Carrier material (polyethylene, wood chips) affected the biofilm community profile in FBBR. The performance of FBBR in grey water purification was evaluated in field conditions when the reactor was installed in a detached house. The nitrogen removal efficacy of the reactor was very good and the maximum nitrogen removal efficiency in the system was 84%. Nitrogen removal in grey water treatment system was verified by an evaluation of the abundance of denitrifying microbes. The performance of the grey water treatment system was returned to the original level in 1–2 weeks after the replacement of wood chips, which were used as carrier material. Preliminary results in the laboratory also indicate that nitrogen removal in grey water treatment can be enhanced by using inoculants. Biological waste water treatment based on RBBRs purified car wash waste waters efficiently, while the reduction of surfactants was at least 95% and the reduction of chemical oxygen demand (COD) between 87 and 95% during the sampling period. Efficient waste water treatment allows automatic car washes to recycle water used for washing. The main challenges for the quality of purified water seems to be optimal nutrient input and an on-line monitoring system for water quality. We conclude that waste water treatment technology based on microbial biofilms is an efficient alternative, at least in smaller units suitable for single family homes. Although not tested in VESITURVA, we believe that the units and the technology can be upscaled and adapted to at least cover the needs of several families or a small village. Furthermore, the results regarding removal of the micropollutants tested were promising, and it is likely that many other organic micropollutants would behave similarly in multi-phase biofilm treatment systems.
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Nimeke	**Jäteveden puhdistus monivaiheisella biofilmiprosessilla** Tuloksia Vesiturva-projektista
Tekijä(t)	Tiina Mononen (toim.), Sebastian Coloma, Martin Romantschuk, Minna Vikman (toim.), Anu Kapanen, Anna Lehtonen, Elina Saario, Merja Itävaara, Essi Malinen, Silja Kostia & Tuula Tuhkanen
Tiivistelmä	Yhdyskuntien ja teollisuuden jätevedet puhdistetaan Suomessa ennen kuin ne päästetään ympäristöön. Uusi lainsäädäntö edellyttää myös, että kaikkien juoksevaa vettä käyttävien talouksien jätevesi puhdistetaan, joskin puhdistusmenetelmät voivat vaihdella. Tekesin Symbio-ohjelman projektissa VESITURVA tutkimusryhmät Helsingin yliopistosta, VTT:ltä, Tampereen teknillisestä yliopistosta ja Lahden ammattikorkeakoulusta yhdessä alan yritysten sekä kunnallisten vaikuttajien kanssa yhdistivät voimansa pyrkimyksenään tutkia ja parantaa jäteveden puhdistusta. Kotitalousjätevesien kohdalla on voimassa vähimmäispoistovaatimuksia vain orgaaniselle kuormalle (BOD/COD), typelle ja fosforille. Vaikka VESITURVA-projektissa seurattiin näiden komponenttien poistumista, päätutkimuskohteena olivat niin sanotut mikropollutantit (haitta-aineet, jotka esiintyvät jätevedessä erittäin pieninä pitoisuuksina, esimerkiksi hormonihäiritsijät, lääkeaineet, hajusteet, hygieniatuotteiden ainesosat) – minne ne kulkeutuvat ja miten niiden poistoa voitaisiin parantaa. Projektissa tutkittiin erityisesti monivaiheisia, biofilmiin perustuvia puhdistusmenetelmiä, joissa mikrobiyhteisöt kolonisoivat kiinteitä alustoja. Puhdistettava vesi kulkeutuu kahden tai useamman peräkkäisen reaktorin kautta ja puhdistuu vähitellen yhä paremmin. Mikropollutanttien malliaineina käytettiin Bisfenoli A:ta, joka on muoviteollisuuden raaka-aine, sekä hajusteena yleisesti käytettyä myskiyhdistettä nimeltä HHCB. Projektissa tutkittiin kahta reaktorityyppiä: pyöriväpatjaista biofilmireaktoria (Rotating Bed Bioreactor – RBBR), jossa biofilmi muodostuu muovisten perforoitujen pallojen pinnalle, sekä kiinteäpatjaista reaktoria (Fixed Bed Bioreactor – FBBR), jossa biofilmi muodostuu puulastujen pinnalle. RBBR-systeemissä kunnallista jätevettä puhdistettiin kolmessa vaiheessa johtamalla jätevettä laboratoriomittakaavan reaktoreiden läpi, kun taas FBBR oli kahden vaiheen systeemi, johon johdettiin keinotekoista harmaavettä. RBBR-systeemiin jätevettä pumpattiin jatkuvatoimisesti, kun taas FBBR-systeemiin synteettinen harmaavesi syötettiin säännöllisissä pulsseissa, koska haluttiin imitoida omakotitalon jäteveden muodostumista. Molemmissa systeemeissä jäteveden pääkomponenttien poisto toimi hyvin, ja merkittävää puhdistumista saavutettiin myös malliaineina käytettyjen mikropollutanttien kohdalla. Suuri osa haitta-aineista poistui jo ensimmäisessä reaktorissa, mutta reaktorien monivaiheisuus paransi poistotehokkuutta. Bakteeridiversiteetti reaktoreissa väheni ajan kuluessa, mikä voi tarkoittaa bakteeriyhteisöjen erikoistumista. Peräkkäisissä reaktoreissa bakteeriryhmät olivat hyvin samanlaisia, mutta bakteerilajitasolla todettiin eroja. Mikrobisto muistutti yleisesti ottaen aikaisemmin julkaistuja jätevesipuhdistamojen mikrobiyhteisöjä. Kantajamateriaali (polyeteeni, puulastut) vaikutti biofilmin mikrobiston koostumukseen. FBBR-reaktorien toimivuutta harmaaveden puhdistuksessa arvioitiin myös omakotitaloon sijoitetussa reaktorissa. Typen poisto reaktorissa oli erittäin hyvä, parhaimmillaan 84 %. Typen poiston toimivuus voitiin osoittaa myös sillä, että systeemissä todettiin runsaasti denitrifioivia mikrobeja. Puulastujen vaihtamisen jälkeen reaktorin toiminta palautui alkuperäiselle tasolle 1–2 viikossa. Alustavat laboratoriotulokset myös osoittavat, että typen poistoa voidaan tehostaa käyttämällä siirrosteita. Pyörivän alustan bioreaktori osoittautui myös tehokkaaksi puhdistamaan automaattisten autopesuloiden jätevesiä. Autonpesuun käytetyn veden tensidipitoisuus väheni 95 % ja kemiallinen hapenkulutus 87–95 % näytteenottoaikana. Pesuveden puhdistaminen mahdollistaa veden kierrätyksen, mikä puolestaan pienentää puhtaan veden tarvetta. Haasteiksi tunnistettiin ravinteidensyötön optimointi sekä veden laadun ajantasaisen tarkkailumenetelmän kehittäminen vikatilanteiden tunnistamiseksi.
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