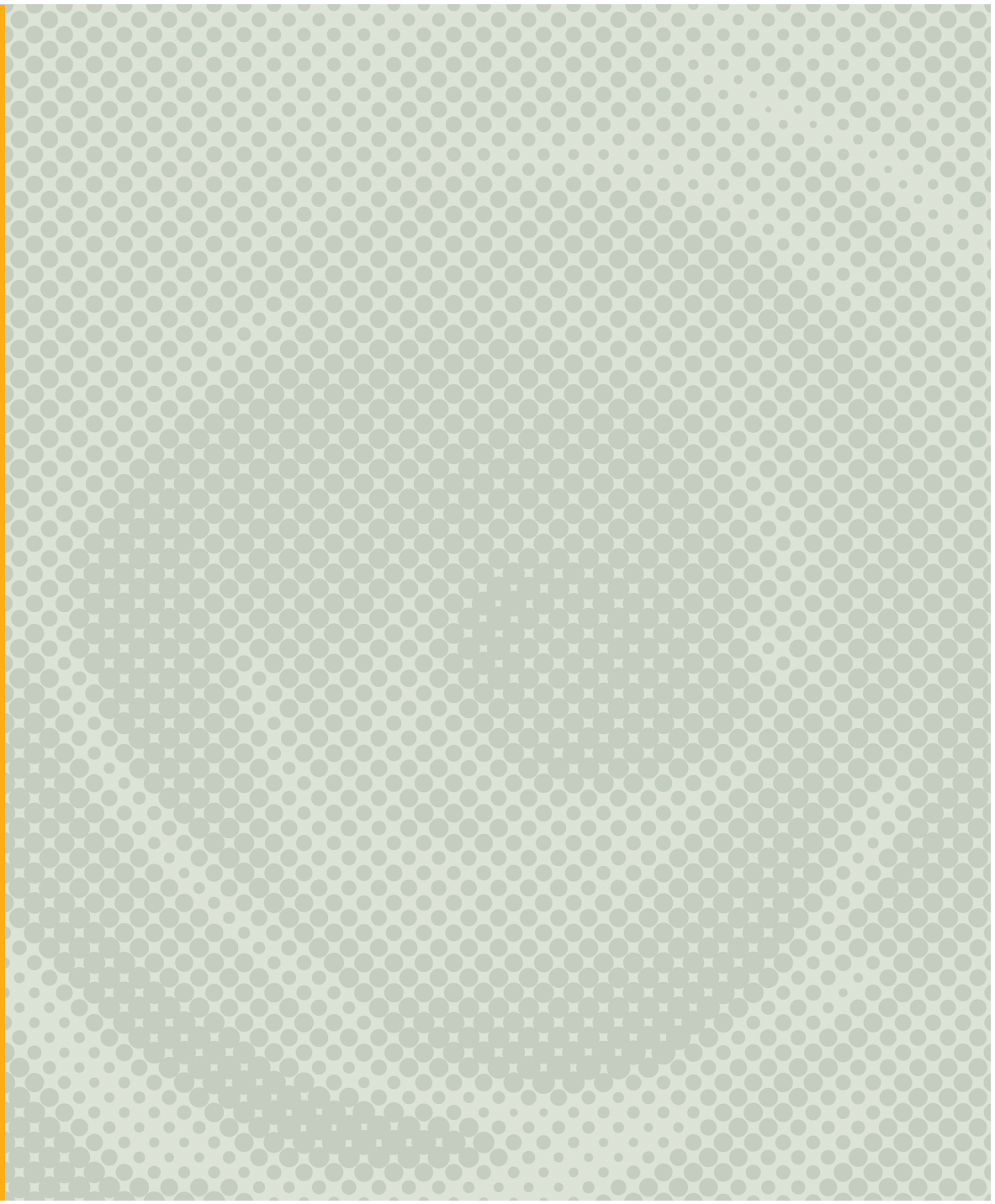
triclosan's interactions in a biological greywater treatment system

anne guri weihe

Norwegian University of Life Sciences
Department of mathematical sciences and technology
Master Thesis 30 credits 2012



PREFACE

This thesis is the final document of the study program Environment and Natural Resources – Sustainable Water and Sanitation, Health and Development by the Department of Plant and Environmental Sciences at the University of Life Sciences (UMB).

The work with this thesis has been long, but interesting. First of all I want to thank my three supervisors, Arve Heistad (IMT), Daniel Todt (IPM) and Jon Fredrik Hansen (IKBM). A special thank to Daniel for always being able to help with everything, and for not letting my stressed, bad mood or his own huge load of work affect him. Thanks to Arve, who are always encouraging and inspiring, and who made a great effort to help me when thousands of technical problems were drowning my results. Last but not least a great thank goes to Jon Fredrik, who always have time for me and who knows exactly what is needed to make progression when everything seems dark.

Secondly I am sending my thanks to my great editors Torbjørn Kornstad and Farshad Tami, who are certainly not responsible for the occasionally poor language I added after midnight.

Lastly I want to thank Anne-Grethe Kolnes who can solve any problem!

Ås, 17.12.2012

Anne Guri Weihe

ABSTRACT

Triclosan have been tested for inhibition effect on greywater bacteria during four correlated experiments. Greywater LECA filters were added triclosan diluted in methanol in one lab-scale and one bench-scale experiments. COD values were used to determine differences between filters with and without triclosan addition. Triclosan was also added in two respiration experiments with microbiological development in closed glass bottles. Logging of pressure fall with WTW OxiTop® equipment made it possible to study respiration curves. This study indicates that triclosan has an inhibition effect on greywater bacteria, but that the effect is dependent on certain conditions. The results from the study also imply that triclosan in those concentrations which have been found in household greywater is not likely to affect the treatment ability of a greywater filter. However, many uncertainties makes more studying of the topic necessary.

Innhold

1. INTRODUCTION

1.1. THEORETICAL FRAMEWORK

Clean drinking water is essential to life, and is defined by the United Nations (UN) as a human right along with the access to proper sanitation (United Nations General Assembly 2010). Both issues are included in the UN Millennium Development Goals (MDGs) which state that the amount of people without access to clean water and proper sanitation should be halved by 2015 (UNDP 2007). A new report shows that the MDG target for clean water was met in 2010, while access to proper sanitation is still far from the goal (UNICEF & World Health Organization 2012). However, regardless of the water goal obtainment, the lack of clean drinking water remains a major challenge for the future, as the report also points out.

With a growing population follows a growing need for food production. This puts a huge strain on our resources; on water in particular, but also on nutrients and agricultural land. Together with the need for more food, an increasingly internationalized population is demanding - also in water and sanitation - more western standards, which often include a larger spending of resources than what is accessible and sustainable in many parts of the world (Jenssen et.al. 2009).

Appropriate treatment and reuse of water and toilet waste can help solve several problems by reducing the consumption of clean water and provide organic matter and nutrients for agricultural use (Jenssen et.al. 2003). These could be key solutions in terms of reaching the MDG sanitation target as well as sustaining and improving the achievements of the water target.

1.1.1. Wastewater contents

The composition and destiny of wastewater varies strongly and is highly dependent on the quality and quantity of the input as well as transportation system and treatment. Wastewater in industrialized countries consists of blackwater, which refers to non-separated wastewater from flushing toilets, and greywater, which is defined as wastewater excluded blackwater. The two fractions are usually collected, transported and treated together, though separation is also possible. In many areas *stormwater*, which is runoff-water from non-permeable surfaces like asphalt or other types of paving, becomes part of the wastewater, either in the sewage system

or at the treatment plant. However, this unnecessarily dilutes nutrients and pollutants present in wastewater, and requires bigger volume capacity of the treatment plants. Hence, more resources are demanded for treatment and reuse of the wastewater (Henze & Comeau 2008).

Separation of the three discussed wastewater fractions might therefore favor better and cheaper treatment. It might also favor better exploitation of nutrients and organic matter present in blackwater, as well as reuse of greywater (and stormwater) for irrigation or other purposes.

Blackwater has high levels of organic matter and consists of most of the nutrients (N and P) found in household wastewater (Jenssen et.al. 2003). Greywater does not contain as much organic matter as blackwater, and the organic content is mainly made up from grease, oil and soap from cooking and cleaning. However, greywater contains to a greater extent than blackwater all chemicals present in household- and personal care products. A study conducted by Eriksson et.al. (2001) investigated the table-of-contents of different Danish household products, and found more than 900 different chemicals which are likely to end up in the greywater.

1.1.2. Wastewater treatment

Many techniques exist within wastewater treatment, which roughly can be divided into mechanical, biological and chemical treatment. Mechanical treatments can be different types of grids and screens, sand- or grease traps and sedimentation. In a wastewater treatment plant (WWTP), mechanical treatment is usually associated with pretreatment. In biological treatment microbiological decomposing like biofilms or activated sludge are used together with sedimentation to remove suspended material. Chemical treatment uses precipitation and flocculants combined with sedimentation to remove dissolved matter from the wastewater. What kind of treatment that is needed depends mainly on the contents of the wastewater. WWTPs are usually combining different techniques (Ødegaard et al. 2012, p. 473-475).

As half of the world's population is living in rural areas (UNDESA 2012), source separation and on-site treatment are highly relevant fields of research. This is especially true for the "developing" parts of the world, but also Norway has a need for decentralized wastewater treatment solutions. In fact, 15.5% of Norwegian wastewater treatment is decentralized

(Statistisk Sentralbyrå, 2011) since 25 % of the population lives in rural areas (Ødegaard et al. 2012, p. 652-653) and many extensively use vacation cabins that are not connected to any wastewater grid.

Different technologies exist that favors reuse; among them are source separating systems and different treatment methods for the separated fractions. Small greywater treatment plants (GWTPs) designed for on-site treatment are often based on sedimentation followed by a fixed-film biofilter where the water undergoes both biological and mechanical treatment on the way (Heistad et.al. 2006).

For this study, a commercially produced greywater filter system was chosen, in which the development of a biofilm in the filter top layer plays an essential role in the filter's ability to remove dissolved organic matter (Heistad et.al. 2006) A *biofilm* is formed when microorganisms attach to carrier media and start feeding on substrate in bypassing water. The biofilm expands as more microorganisms attach, feed and multiply. After a while, parts of the biofilm will detach (Ødegaard et.al. 2012, p. 557-559). In a greywater filter the biofilm is formed on the surface of the filter particles. As the biofilm develops, an increased amount of small particles are retained and decomposed by the microbiological activity in the film. Bacteria present in greywater might also be retained, and predated or inactivated in the biofilm (Ødegaard et.al. 2012, p. 667-670).

The GWTP (*Ecomotive A01*®) in this study uses Lightweight Expanded Clay Aggregate (LECA) as filter media. LECA is used as building material in foundation walls, but it is also well known as filter media for wastewater. Since too much organic matter and particles might clog the filter and thereby lead to shorter lifetime or larger operational costs, LECA is most suitable for greywater.

A01 combines the LECA with a pre chamber for sedimentation, a second filter of coarser LECA and a buffer tank before the water is let out, preferably into a sediment ditch for polishing. (Jets 2011) In the test plant used in this study, only the sedimentation- and the LECA stage of A01 are left. Both stages are dimensioned to correspond with the dimensions of A01.

1.1.3. Triclosan

The idea of this thesis was to gain information about an organic micro pollutant and how it interacts in a greywater filter system. The pollutant had to be a potential source of problems globally, and it had to be present in Norwegian greywater.

Triclosan ($C_{12}H_7Cl_3O_2$) (TCS) disturbs activities in the cell membrane of bacteria. In low concentrations, triclosan is bacteriostatic, which means it is preventing reproduction, while in high concentrations it serves as an antimicrobial agent, i.e. killing the bacterium. As summarized by Fang et.al. (2010), a number of studies have been carried out regarding triclosan as a disinfectant in consumer products. The results from such studies have been contradictory, seeing as disinfection effect has been demonstrated in some of the studies while not in others.

Triclosan is a broadspectered antimicrobial agent not only used in clinical settings, but also to a great extent in household and hygiene products. Makeup, soaps, toothpaste, detergents and hand disinfectant might contain triclosan, as well as deodorants, sports clothing and plastic surfaces (Fang et al. 2010). Because of its wide specter of use much triclosan ends up in our wastewater, and some of it travels through the WWTPs and into water recipients. In aquatic environments, triclosan is reported to be photodegraded into a number of toxic dioxins and chlorophenols, as shown by Buth et.al. (2009). Triclosan is very harmful to aquatic organisms, especially to river biofilm. It is also feared that triclosan might cause resistance to antibiotics, though it is shown that the triclosan resistance of gram-negative bacteria, as *Pseudomonas aeruginosa,* is due to efflux pumps which remove chemicals from the bacterial cell (Fang et al. 2010). However, these concerns make triclosan a high-priority chemical of the Norwegian state Ministry of environment. The goal is to remove triclosan from Norwegian products and wastewater within the year of 2020 but the lack of data concerning use and emissions is slowing the process down (KLIF 2010).

The estimated use of triclosan in Norway is 1.6 tons per year, but the number is assumed to be higher due to unregistered, imported products. Triclosan seems to follow the sludge phase in WWTPs, and it is estimated that 0.2 tons of triclosan is spread in sludge used for agricultural purposes each year (KLIF 2009 and 2010). This pollution might end up in rivers, lakes and

oceans, where it is known to damage aquatic organisms (Fang et al. 2010). KLIF also reports that triclosan has been present in all soil samples analyzed in Norway, which indicates persistence in and extensive use of triclosan. Both air and water is considered to be potential spreading routes (KLIF 2009).

Table 1 shows the amounts of triclosan measured in different wastewaters qualities in Norway, Denmark, Sweden, and Switzerland.

Table 1: Triclosan values for untreated wastewater of different wastewater types. WW = wastewater, TCS = triclosan. All values are given in µg/l.

Reference	Country	WW Type	TCS average	Min	Max
Klif (2010)	Norway	Mixed WW	0.508	1.160	0.141
Eriksson et.al. (2003)	Denmark	Greywater	0.6		
Palmquist (2001)	Sweden	Greywater	3.43	0.56	5.9
Palmquist & Hanæus (2005)	Sweden	Blackwater	2.48	0.5	3.6
Singer et.al. (2002)	Switzerland	Mixed WW		0.07	14000
Almqvist & Hanæus (2006)	Sweden	Greywater	0.19	0.075	0.3

1.2. PROBLEM STATEMENT

As Norwegian cabins become more modernized, cabin wastewater contains more of our daily household- and personal care products than earlier, and a look at how these chemicals act in a treatment plant for greywater will be highly relevant.

1.3. AIMS

The aim of this thesis was to study how triclosan interacts in the biofilm of a greywater filter and find out

1) Does triclosan, as a microbial agent, have an inhibition effect on the microbial activity in the biofilm of a greywater filter system, and by that have a negative effect on the filters ability to treat greywater?
2) How does the greywater filter respond to the addition of triclosan in concentrations which are likely to be found in a normal household greywater? To what extent is it able to retain triclosan?

2. MATERIALS AND METHODOLOGY

2.1. Experiments

As foundational work for this thesis, triclosan has been added to wastewater (mainly greywater) in four correlated experiments; two small-scale respiratory experiments and two filter experiments (table 2)

Table 2: The four correlated wastewater experiments within this thesis:

Bottle experiment 1	Respiratory test to determine a potential inhibition effect of triclosan on the microbiological decomposition activity (February – Mars 2012)
Bottle experiment 2	Respiratory test to determine in what concentration triclosan's inhibition effect starts (October – December 2012)
Filter experiment 1	Filter experiment in lab prototype system (March – July 2012)
Filter experiment 2	Bench-scale filter experiment (October – December 2012)

Powdered triclosan (72779-56-F Irgasan® from Sigma Aldrich®) was used for all experiments, dissolved in ethanol for Bottle experiment 1 and in methanol (LiChrosolv® Methanol for liquid chromatography) for the other experiments. A stock solution of 1 mg triclosan / ml alcohol was made as a basis from which all other concentrations were made.

Both filter experiments were carried out in full at the Department of Mathematical Sciences and Technology (IMT) wastewater lab at the University of Life Sciences (UMB), while the bottle experiment and water analyses were carried out in the Department of Plant and Environmental Sciences (IPM) soillab at UMB.

2.1.1. Greywater source

All wastewater used in all four experiments was originated from Kajaveien 15, Ås, which is a student dormitory area. The wastewater system at Kaja currently separates the wastewater into grey and blackwater. Both fractions are led to one septic tank each before the respective fraction is pumped to holding tanks at the UMB wastewater laboratory at IMT. The greywater septic tank has one pump in each end. From the inlet, the greywater is pumped to a constructed wetland, while the pump leading to the lab is located by the outlet. The estimated retention time for greywater is 1-2 days in septic tank, sewer line and holding tank. The greywater used in the four experiments had usually been *in the laboratory tank* approximately one day when samples were gathered. The wastewater lab was normally held at around 20°C,

but the inside temperature also showed some response to significant temperature changes outside.

2.1.2. Background analyses

Before the start of the test period, greywater was analyzed for different parameters (COD, TSS, BOD, TN, TP, OP and metals) to determine background values. As indicators for what ranges the different parameters could be expected to be found within, results from earlier examinations of the greywater source was used.

The determination of background values was done to better understand sources of error and to compare the greywater quality with other wastewater sources. The screening of metals was also done in case the presence of any of them could affect the behavior of triclosan or the reaction chemicals for the determination of different parameters.

2.1.3. Bottle-experiments

Greywater inoculated with suspended biomass was added to closed glass bottles together with different amounts of triclosan to determine a potential inhibitor effect of triclosan on the microbiological activity.

2.1.3.1. Inoculation culture

Greywater was collected in plastic bottles from the holding tank at IMT. The suspended biomass of Bottle experiment 1 was taken from a pilot treatment plant that was located in the same lab and fed with a mixture of 10% blackwater and 90% greywater. Bottle experiment 2 was inoculated with secondary sludge from Bekkelaget WWTP in Oslo. Both inoculation cultures were adapted to greywater for 7 days in an aerated 1000 ml lab-scale batch reactor (fig. 1).

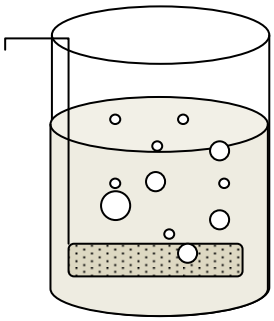


Figure 1: Oxygen needed for aerated conditions was provided by an aquarium pump in the bottom of the flask.

The reactor was fed once per day after allowing it a 30 minutes sedimentation period without aeration. 100 ml reactor liquid was decanted from the supernatant and replaced with fresh greywater. At the same time, potentially evaporated water volume was replaced with fresh greywater. New greywater was fetched from the tank every second day and stored in tightened plastic bottles at 4°C between each use. The growing rate during the adaptation period was monitored indirectly by measuring TSS in the reactor liquid.

2.1.3.2. Respiratory experiment period

Figure 2: The OxiTop equipment inside the 20°C incubation cabinet.

For the respiratory experiment, WTW OxiTop® respiratory test equipment (ref. WTW 2004) (fig. 2) was used. The method was preformed as described in WTW 2010, except that no nitrification inhibitor was added in the respiration experiments. The nitrogen inhibitor is usually added when the OxiTop® is used for the determination of BOD, but in this experiment all bacterial activity and potential inhibition effect on the latter were of interest.

A v/v mixture of suspended biomass and greywater (5/95%) was added to glass bottles. Each bottle was added different concentrations (see table 3) of triclosan dissolved in methanol; two

bottles per concentration*. All bottles received equal amounts of methanol, and two reference bottles received methanol without triclosan. Sodium hydroxide tablets were added to a rubber cap in the top of each bottle for the absorption of CO_2 and the bottles were then closed with an OxiTop® and incubated at 20°C for two days**.

The OxiTop equipment is a system which measures and logs pressure fall inside the bottle. Bacteria feed on the greywater and uses oxygen while producing CO_2. The sodium hydroxide tablets absorb the CO_2 and thereby reduce the pressure. The OxiTop® registers pressure fall inside the bottle several times per hour, depending on the length of the experiment, and makes it possible to study respiration curves. The comparison of curve shapes can indicate if the microbial activity has been affected by the presence of triclosan or not.

* Due to few available bottles only one bottle received the highest concentration of triclosan during Bottle experiment 1.
** The last test of Bottle experiment 2 was incubated for 5 days.

To determine the right program (detection range) for the respiration tests, one bottle (test 1) was run with the amount of methanol (600 µl/l) that was decided for Bottle experiment 2, based on results from Bottle experiment 1. From this test, the 200 mg/l program was decided to be the most sufficient range for a 2 days respiratory test. The rest of the tests were run according to this finding.

2.1.4. Filter experiment 1

2.1.4.1. Equipment

For the bigger-scale filter experiment a lab prototype GWTP (fig.3 and 4) was used. The system was originally designed as an experimental model for the commercial biological GWTP *Ecomotive A01* (see introduction chapter). Only the first two steps of A01; sedimentation and filtration, are included in the prototype plant.

Figure 3: The experimental GWTP with the peristaltic pump in the middle of the sedimentation channels. Each channel is fed through equally long tubes.

To make the right triclosan concentration for this experiment, the triclosan stock solution (1000 mg/l) was further diluted in methanol before being added to a 50% methanol solution. The triclosan dilution was pipetted into the magnetically stirred solution for immediate spreading of triclosan in the solution. This procedure was used to avoid precipitation/decomposition.

2.1.4.2. Technical description of setup

Like described in figure 3, the test GWTP consists of three 1968 x 227 x 227 cm parallel wooden sedimentation channels, covered on the inside with a waterproof plastic fabric, and with a small, separate chamber at the end of each channel. A submerged weir connects the chamber to the rest of the channel. Three 62.7 cm x 1647.5 cm^2 PVC columns, one per channel, are located on top of the channels, at a wooden wrack. The columns are filled up to 49.1 cm with lightweight clay aggregates (Filtralite^{TM} NR 2-4mm) (Fig. 4). The filter inlet is through a hose coupling and a single tangential/fullconical (TF) nozzle in the center of the lid,

and the outlet is through a hose coupling in the center of the base plate. The rack saves space and makes room for the outlet and for easy sampling. (For further details concerning the prototype GWTP, read Ibidapo & Stensen 2007)

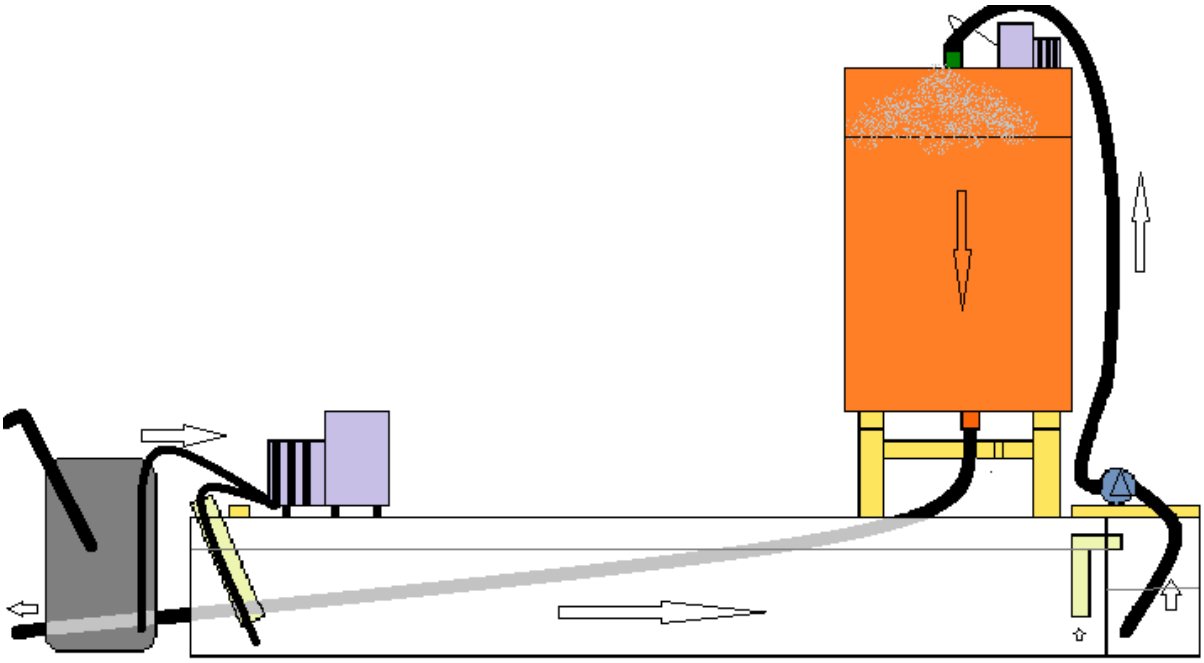


Figure 3: The prototype GWTP used in Filter experiment 1, seen from the side.

Figure 4: Filtralite® inside the filter, with the nozzle in the lid.

2.1.4.3. Running

From the stirred holding tank the greywater was pumped into a plastic barrel in which two floating switches controlled when the pump was starting and stopping. The barrel was not stirred, but the refilling of greywater created turbulence in the barrel. From the barrel a peristaltic pump brought the greywater into the sedimentation stage of the filter system (see fig. 6). The peristaltic pump was operating at a constant speed and provided each filter with a approximate hydraulic load of 0.49 m^3/m^2/day. A separate pumping chamber at the end of each sedimentation channel was reached through a submerged weir. By signals from a floating switch in the pumping chamber the water was pumped up to a nozzle in the lid of each filter. When one of the switches was turned on, all three filter pumps started simultaneously. Switches and pumps were connected through a Programmable Logic Controller (PLC), which controlled the pumping time for each pump. The filter pump was run for 30 seconds. Meanwhile, starting 2 seconds later and running for 4 seconds, a smaller peristaltic pump brought the chemical mixture (see figure 5 and table 3) from a flask and into the filter inlet hose, just above the nozzle. To pierce the inlet hose, a syringe needle, type "butterfly", was mounted at the end of the chemical solution hose (fig. 5).

Fig. 5: (Left): The syringe needle at the end of the chemical solution hose pierces the filter hose just above the nozzle. (Right): The bottles containing the two chemical solutions, on top of the filters.

Figure 6: (Left): Peristaltic pump on top of the sedimentation channels. (Right): Greywater dosing system into the sedimentation channels. Hoses were led into tubes to stay stable.

The plan was to run the filters for four weeks, to be sure that a biofilm had developed. However, technical problems led to a much longer, two-phased experiment. First the filters were run for almost three months with greywater, where pumps were tested, equipment was

adjusted and biofilm was developed. After this the filters were run for one and a half month with chemical addition. When the chemical addition period started, the filters were supplied with 80 liters of greywater per day. Two filters were both added 10 µl of triclosan per liter of greywater, while the third filter did not receive any triclosan.

2.1.4.4. Sampling

Different parameters such as COD-values were measured before and after the filters, and then compared to indicate if triclosan had any impact on the biofilm of the filters. The greywater was also analyzed for triclosan to find background values and indicate if the filters had any impact on the triclosan.

2.1.5. Filter experiment 2

Three PVC columns were packed with moist lightweight clay aggregates (Filtralite™ NC 0,8-1,6 mm), in two cm layers, with manual compaction between each 2 cm layer. The inner diameter of the PVC columns was Ø = 3.5 cm and the filling height was 16 cm. The columns were inoculated with a mixture of untreated greywater and effluent from a biological treatment plant in the ratio 1:1, dosed from a plastic container, by using a Heidolph® PD 5201 peristaltic pump with multichannel pumphead and Marprene^{TM} (Watson-Marlow Ltd) tubing with inner diameter 2.2 mm (see figure 7). During the two days inoculation period the hydraulic loading rate was set to 3.3 m^3/m^2/day. From the fifth day the hydraulic loading rate was adjusted to 0.49 m^3/m^2/day for the rest of the experimental period. During this period the peristaltic pump was operated with approximately 13 seconds runtime and 73 seconds break. On day 12 composite samples were collected from the columns. On day 15 triclosan dissolved in methanol at end concentrations 100 µg/l and 1000 µg/l, was added to the feed solution for two of the columns, while the third column was added the greywater with methanol content equivalent to the two with triclosan. The feed solution was kept in three separate 0.5 l glass flasks. From day 18 the concentration of triclosan and methanol was reduced to 1/10 of the initial concentration. A series of samples were then taken on day 19, 20, 21, 22 and 23, collected in 0.5 l glass flasks.

The greywater and air temperature during the experimental period varied from 12 to 5°C. All samples were stored dark at 4°C until analysis.

Figure 7: The three columns are loaded with feed solution by the use of a peristaltic pump.

2.2. ANALYSIS

WTW OxiTop-equipment and hardware for analyses were borrowed from the IPM lab. ICP-OES-analyses were performed by IPM staff.

2.2.1. Chemical Oxygen Demand

Chemical Oxygen Demand (COD) analyses were done with spectrophotometrical test kits from Hach-Lange™. Both total and soluble COD was determined, but there are gaps when no soluble COD was determined. The range in which the respective samples should be analyzed was chosen based on the expected organic content of the different wastewater qualities within the different steps of the test-GWTP.

Samples were brought to the IPM lab in plastic bottles and analyzed the same day. Samples for $COD_{soluble}$ were filtered through a 1.2 µm Whatman GF/C filter. For this procedure, plastic filter equipment coupled to a vacuum pump was used. Between each use the equipment was thoroughly cleaned with acidic detergent in a laboratory washing machine only used for acid.

2.2.2. Total Suspended Solids

Total Suspended Solids (TSS) was determined with a Whatman GF/C filter.

2.2.3. Biochemical Oxygen Demand

Biochemical oxygen demand (BOD) was measured with the respiratory method (WTW OxiTop (ref. WTW 2004)) (See details regarding this method under Chapter *Respiratory experiment period*).

2.2.4. Triclosan analysis

For the determination of triclosan in greywater, the Abraxis triclosan ELISA (enzyme linked immunosorbent assay) kit with accompanying hardware for analyses was used.

The ELISA method has been developed by Weilin Shelver (the Agricultural Research Service (ARS)), and her team from Abraxis Incorporated (Jan Suszkiw, 2009). In this method, triclosan antiserum, developed in rabbits, are attached to magnetic particles. Triclosan in wastewater reacts with the antiserum and are pushed out of solution when the kuvettes are placed in a magnetic rack. The rack holds the metal particles inside the tubes while excess water is decanted. When all procedures are done, the color of the solution is measured with a spectrophotometer and the triclosan concentration is calculated by the use of a standard curve (see detailed method in Abraxis (2012a and 2012b)).

Two replicates of each sample were analyzed, and the calculations were done from the average value of these replicates.

2.2.5. Other parameters

In addition to COD, TSS and BOD analyses, levels of total nitrogen (TN), total phosphorus (TP) and orthophosphate (PO_4-P) (OP) in source greywater was determined using Hach-Lange^{TM} LCK test kits. Using ICP-OES (inductive coupled plasma - optical emission spectrometry) analysis, a screening of source greywater and tap water for Al, Ca, Cd, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Pb and Zn was performed. The results for tap water were used as reference values.

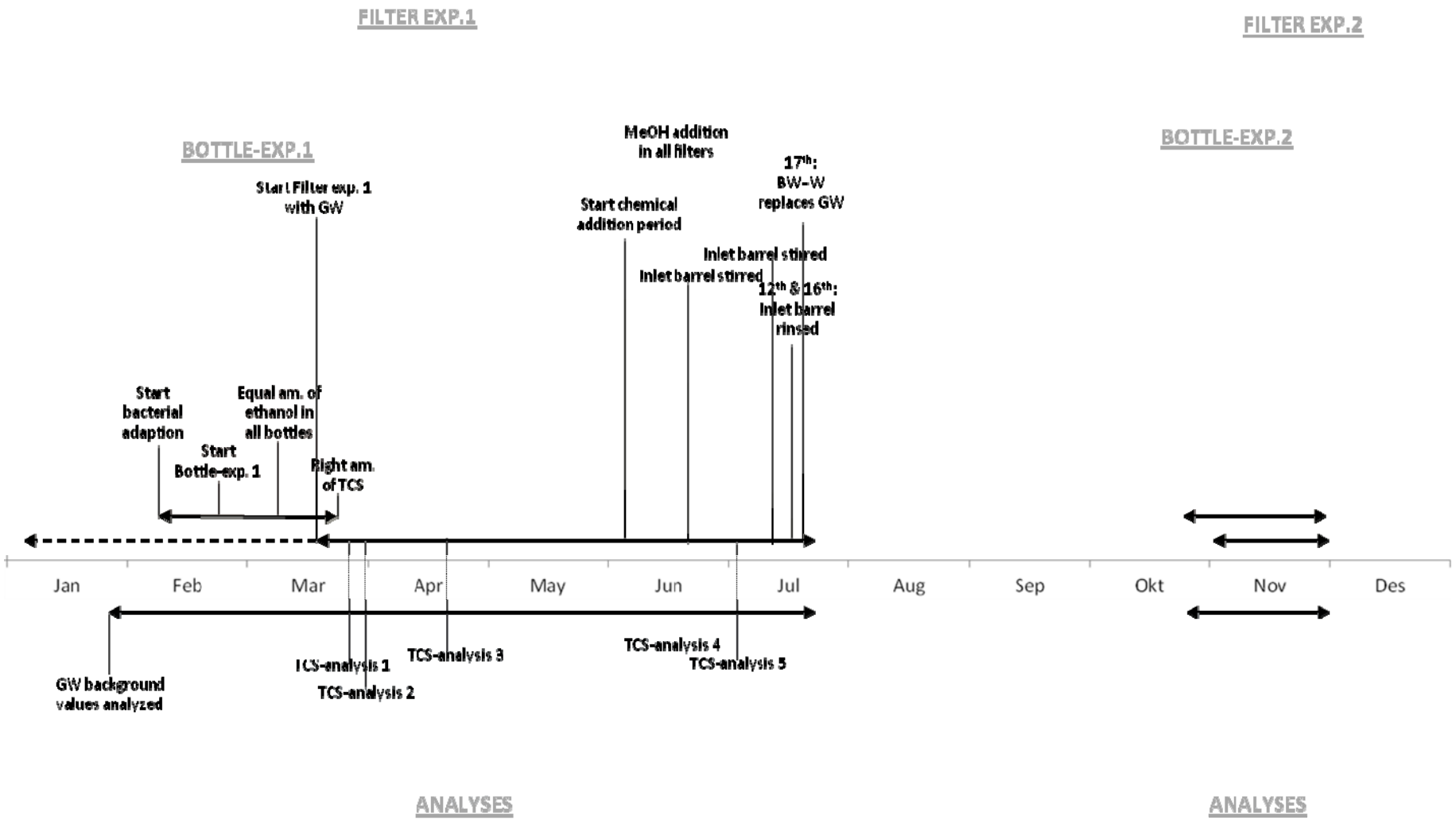


Figure 8: Timeline that gives an overview of important incidents during the four experiments.

3. RESULTS AND DISCUSSION

3.1. GREYWATER, BACKGROUND VALUES

Values of both COD and BOD in the greywater source (table 3) matched average values from earlier studies very good. Figure 9 shows the results from the ICP-OES scan for metals. All background values are shown in the appendix.

Table 3: Background values for greywater, all expressed in mg/l. COD = total COD, COD_S = soluble COD. N = number of analyses from which the average and standard deviations are calculated.

	COD	COD_S	BOD_5	TSS
Average	266.4	120.48	129	55
Std. dev.	8.52	29.70		
N	4	3	1	1

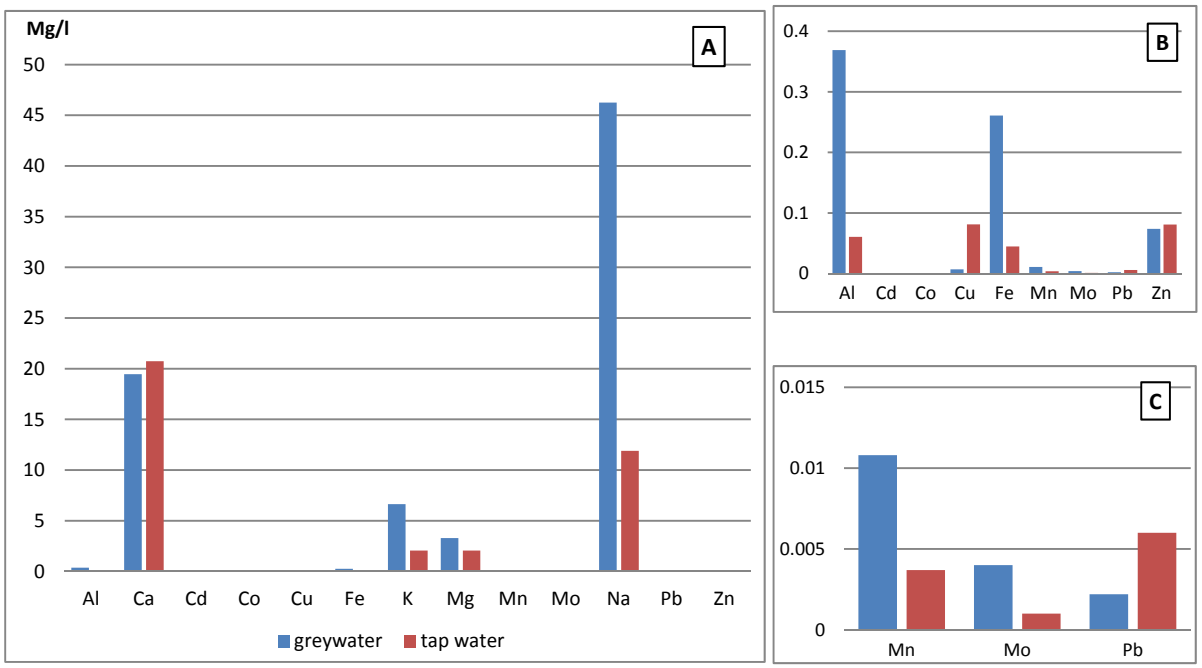


Figure 9: Heavy metals in greywater and tap water, determined by Inductive Coupled Plasma - Optical Emission Spectrometry (ICP-OES). Diagram A shows all elements that were analyzed for, while B and C are magnifications of the elements with the lowest concentrations. (Cd and Co are both given the value "0?" in the results from the ICP-OES, and are therefore not shown in the highest zoom (diagram C)).

Comparison of greywater background values with inlet concentrations of mixed wastewater from three Norwegian WWTPs are shown in table 4. The table shows slightly lower values for almost all parameters in greywater, which could be expected in comparison with municipal wastewater. The data indicate further that heavy metal contamination of the

greywater used in this study is within a normal range for household wastewater. Thus, no exceptional side effects are to be expected on the studied biological treatment system and bench-scale experiments in terms of heavy metals.

Table 4: Greywater background values for Kaja greywater (source greywater), compared with three Norwegian treatment plants for municipal, mixed wastewater.

	COD	BOD_5	TN	TP	Cu	Pb	Zn	Cd
Kaja GW 2012	266.4	129	11.7	1.25	0.007	0.0022	0.0738	0
VEAS 2010*	360	164	29	3.7	0.071	0.00113	0.0771	0.00017
BEVAS 2010*	418	180	32.7	4.0				
FREVAR 2010*	274	112		2.9				

* Source: Ødegaard et.al.(2012), p.349

3.2. RESPIRATORY TEST, BOTTLE EXPERIMENT 1

Test 1 and 2 were run to find the right BOD range and the right greywater/suspended biomass ratio for further tests. Unfortunately, due to an oversight, the tests were run without the CO_2 absorbing tablets, and gave therefore no such indication.

The next two tests (fig.10) received the CO_2 absorbing tablets and different concentrations of triclosan. However, because distilled water was used as diluents instead of ethanol, these tests also received different amounts of ethanol; 1µl ethanol/µg triclosan approximately (table 3). Test 5-7 received the same amount of ethanol for each bottle within the respective test, but, due to miscalculations (liquid volume in each test were not taken into calculation), only test 7 got the *right* concentrations of triclosan; all other tests received *too high* concentrations relative to the plan of 6 and 60 µg triclosan / liter (see table 3 for the respective concentrations).

Table 2: The different concentrations of triclosan (TCS) and ethanol (EtOH) in each test and bottle:

	ZERO		LOW		HIGH	
Test #	TCS (µg/l)	EtOH (µl/l)	TCS (µg/l)	EtOH (µl/l)	TCS (µg/l)	EtOH (µl/l)
3-4	0	0	24	24	240	240
5	0	600	60	600	600	600
6	0	353	35	353	353	353
7	0	60	6	60	60	60

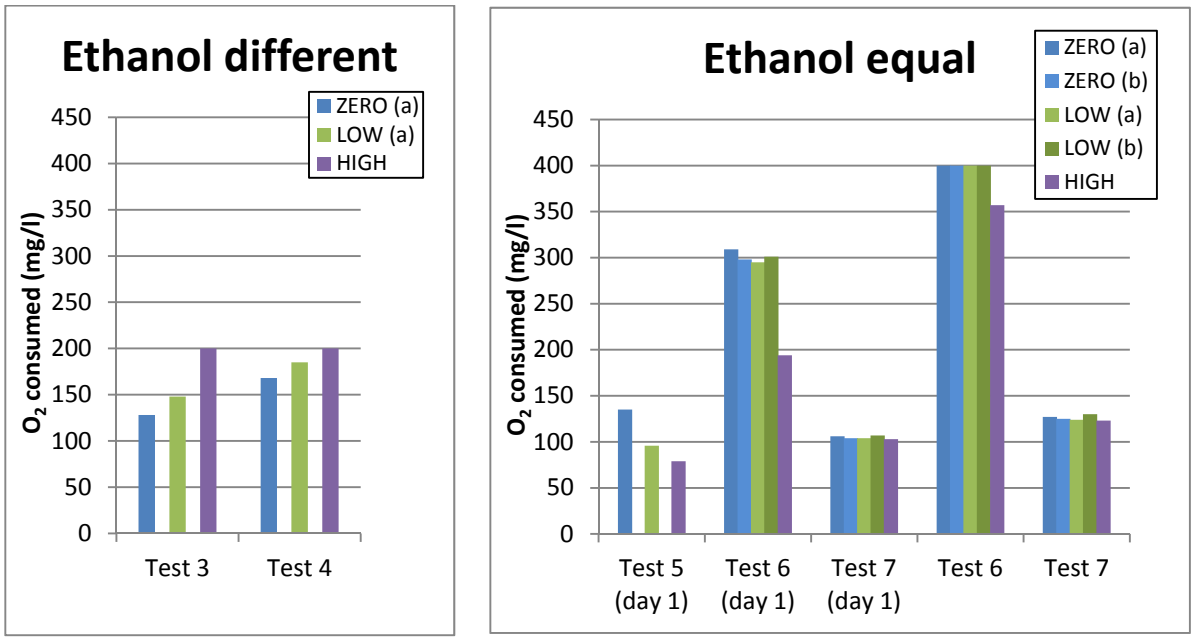


Figure 10: O_2 consumption after two days of incubation for tests 3-7 with triclosan concentrations zero, low and high. Concentrations of ethanol was equal *within* tests 5-7, while increasing with increasing concentration of triclosan in tests 3-4 (see table 3 for all concentrations). Results from day 1 are compared for tests 5-7 because of lost data for test 5 and values above range for test 6. Test 6-7 has two replicates per concentrations "zero" and "low".

As shown in figure 10; in the two tests in which the amount of ethanol was proportional to the concentration of triclosan (ref. table 3), bacterial growth seem to increase with increasing triclosan (and ethanol) concentration. In test 5-7, where the ethanol level was equal in all three bottles, the trend changes to the opposite (fig. 10). The OxiTop® method has a standard error of ca. 20 mg/l (WTW 2010). Some of the differences in test 3, 4 and 5 are within this margin of error. These smaller differences between the samples may therefore be normal sample variations rather than a potential effect by different triclosan concentrations.

The respiration curves of test 6 (figure 13) show a week indication for a *potential* inhibition effect already at 35 µg/l triclosan towards the end of the test. However, the small differences in oxygen consumption within test 6 and 7 (fig. 10) seem to correlate to the COD and TSS concentrations that have been determined in bottles before the start of the test (fig. 11). These findings indicate that the differences in oxygen consumption within test 7 might be related to differences in organic substrate concentration (COD/TSS) rather than a potential effect by triclosan. While the results for test 5 and test 6 indicate a relatively clear inhibitory effect for triclosan concentrations of 350 µg/l and higher, similar effect on lower concentrations (60µg/l

and lower) is unsure and seems to be more or less completely overlaid by other parameters (e.g. substrate concentration) in a treatment system.

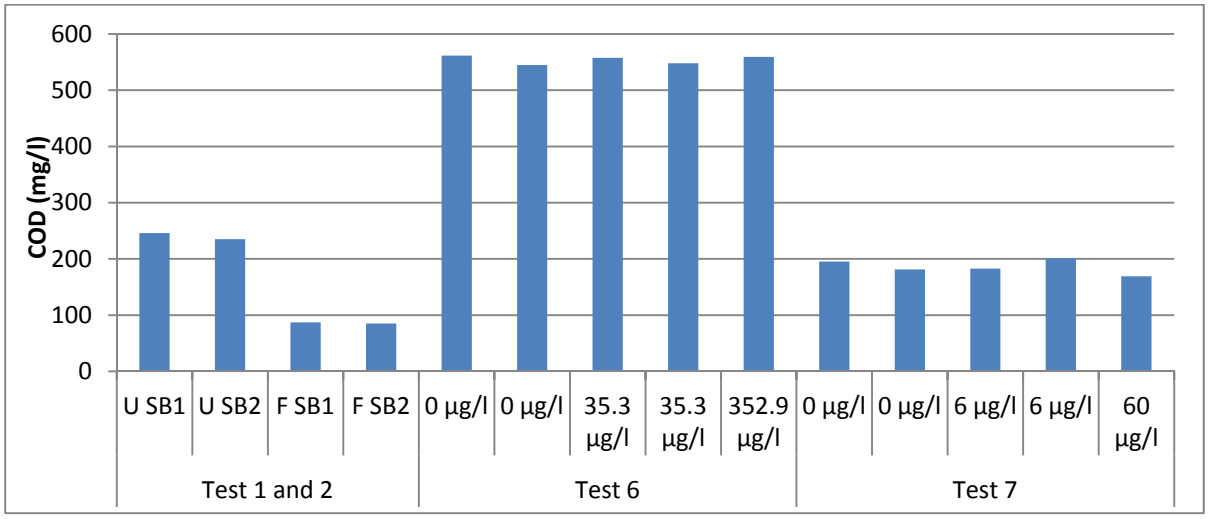


Figure 11: Organic content, expressed as Chemical Oxygen Demand (COD), of test 1, 2, 6 and 7 with respective triclosan concentrations. U = unfiltered, F = filtered, SB*X* = Suspended biomass *and replicate number*. Please note that for test 1-2, COD was measured only in suspended biomass, while in tests 6-7 COD were measured after all components (suspended biomass, greywater, triclosan and ethanol) were mixed.

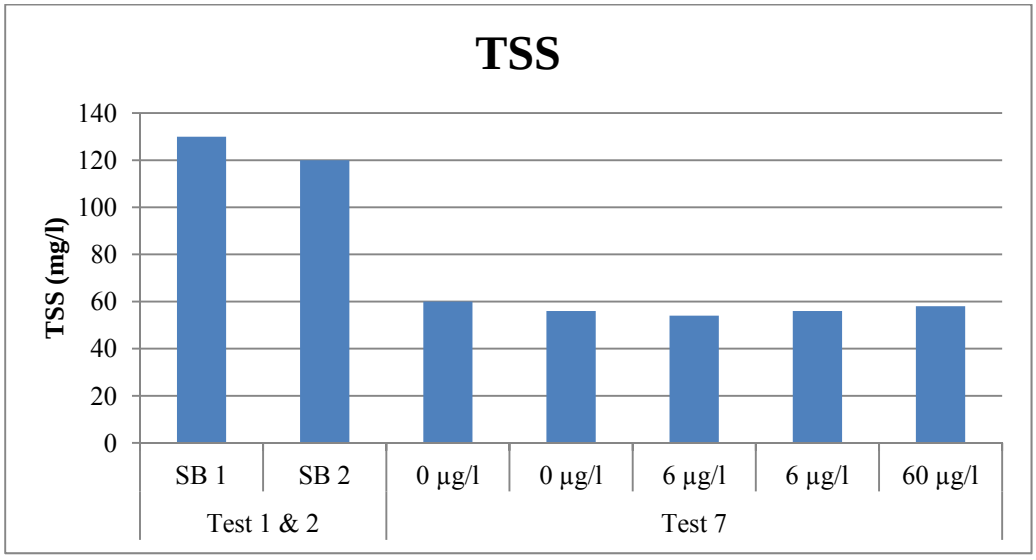


Figure 12: Total Suspended Solids (TSS) in mg/l. Results for Bottle experiment 1. The X-axis shows the different tests and bottles. Please note that test 1-2 is only suspended biomass while test 7 is suspended biomass, greywater, triclosan and ethanol all together. Because only 5 ml of media was filtrated for test 6, compared to respectively 20 and 50 ml for tests 1-2 and 7, the data was considered unreliable and is not presented.

Concerning the graphs:

The differences in time appearances (hours versus minutes) between fig. 13-14 and fig. 15 -19 is due to manual versus electronic logging which gave more detailed data available in fig. 15-

19. In figure 13-14, data were read out of the OxiTop® OC 100 and manually plotted with one value every hour. Data for figure 15-19 were loaded from the OxiTop® OC 100 onto a computer, giving one value every 8 minutes.

Different Y-axis levels are used with the assumption that differences *among* tests are due to the quality of media, and thereby less relevant in this experiment, while differences *within* tests (among bottles) presumably are due to triclosan (or ethanol) concentrations.

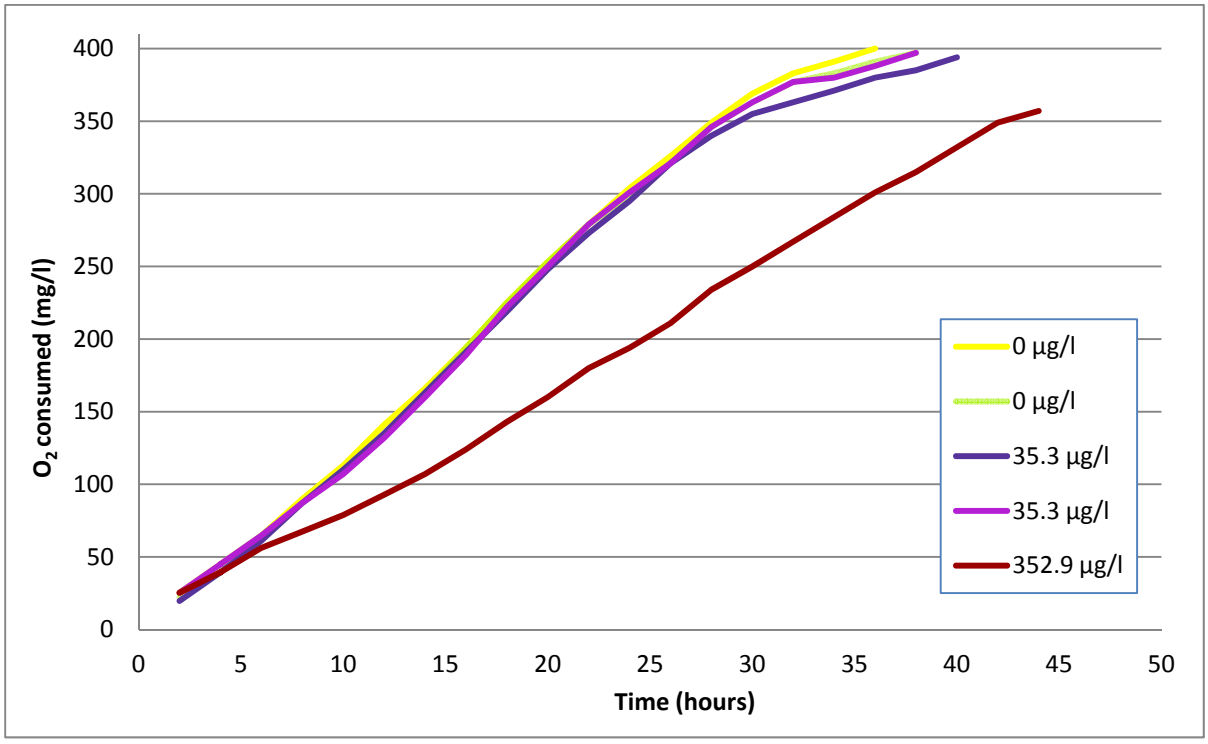


Figure 13: Respiratory test 6 of Bottle experiment 1. The graphs show respiration development in mg O_2 consumed/l for greywater inoculated with different concentrations (µg/l) of triclosan.

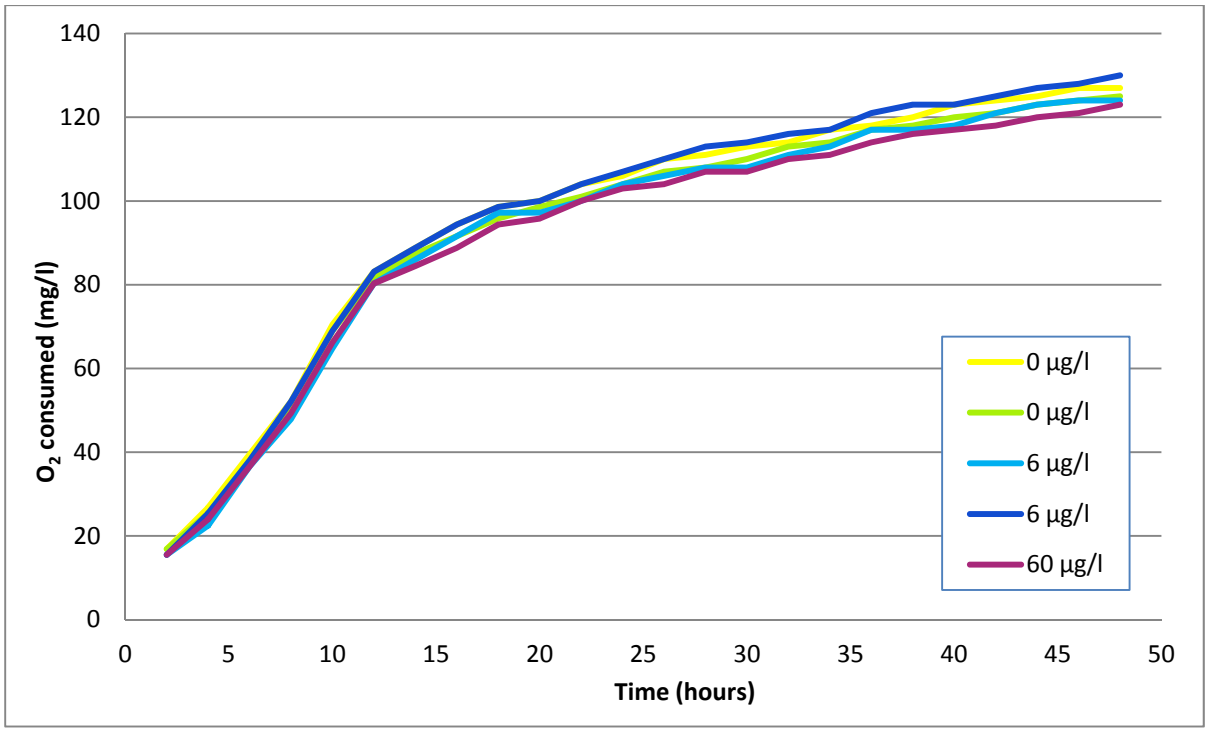


Figure 14: Respiratory test 7 of Bottle experiment 1. The graphs show respiration development in mg O_2 consumed /l for greywater inoculated with different concentrations (µg/l) of triclosan.

3.3. RESPIRATORY TEST, BOTTLE EXPERIMENT 2

Test 2 (fig. 15 and 16) was run with two different greywater qualities: Four bottles with 0, 6, 60 and 600 µg triclosan/l respectively were run with greywater that had been kept in tight plastic bottles at 4˚C for two days (test 2a, fig. 15), while four bottles with corresponding triclosan concentrations were run with fresh greywater from the tank at IMT (test 2b, fig. 16). The respiration curves differed a lot from test 2a - 2b, but showed clear trends among bottles within tests, which support the assumption that differences between tests are mainly due to media. Test 3 (fig. 17) was run with the same triclosan concentrations as test 2, but with two replicates which received equal input, media included. Test 3 received (like test 2b) fresh greywater, and showed different results than the former tests (2a and b).

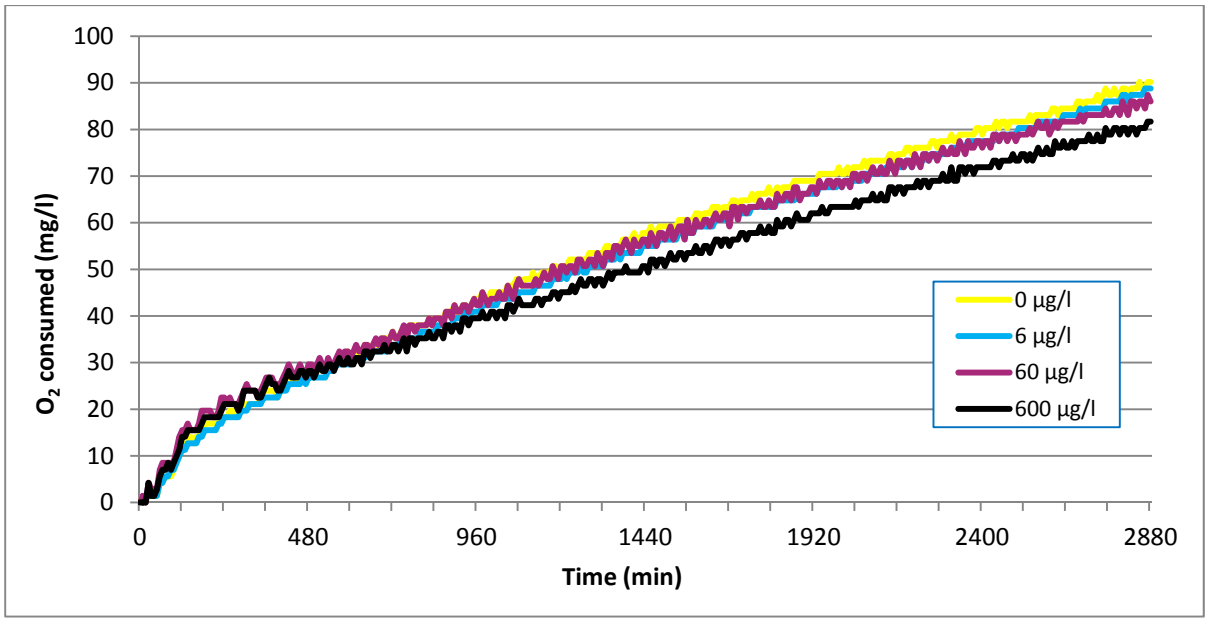


Figure 15: Respiratory test 2a (old greywater) of Bottle experiment 2. The graphs show respiration development in mg O_2 consumed/l for greywater inoculated with different concentrations (µg/l) of triclosan.

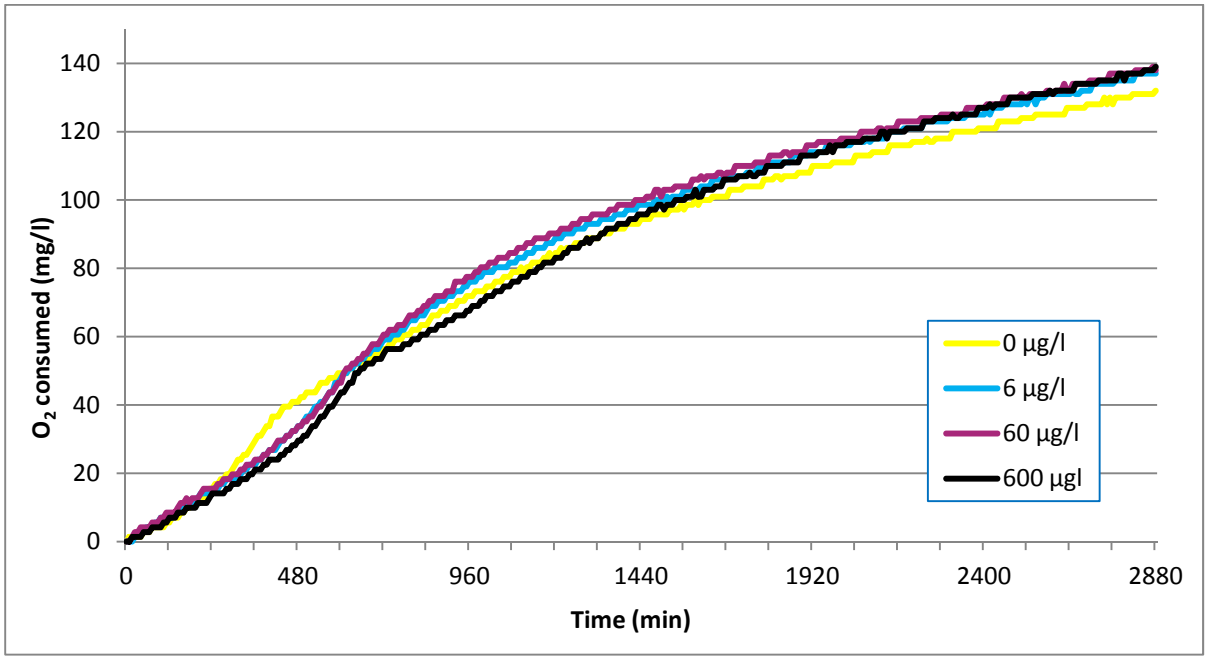


Figure 16: Respiratory test 2b (new greywater) of Bottle experiment 2. The graphs show respiration development in mg O_2 consumed /l for greywater inoculated with different concentrations (µg/l) of triclosan.

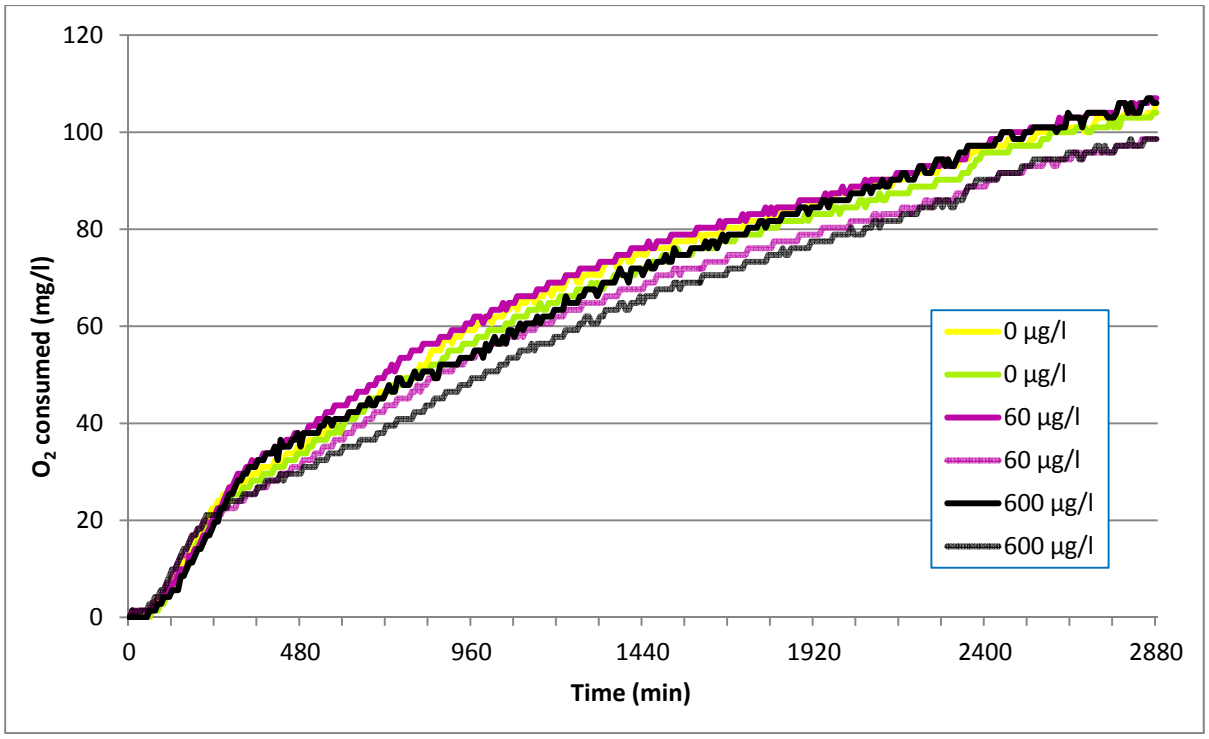


Figure 17: Respiratory test 3 of Bottle experiment 2. The graphs show respiration development in mg O_2 consumed/l for greywater inoculated with different concentrations (µg/l) of triclosan.

Test 2a (fig. 15) implies a small inhibition effect from triclosan; biggest effect of the highest concentration and lower impact from the lower concentrations. Yet the apparent inhibition effect is not as big as suggested in Bottle experiment 1 (fig. 13 and 14), and also, as in Bottle experiment 1, the potential effect is first visible after approximately 12 hours. Test 2b (fig. 16), however, which received newer greywater, did not show the same trend. As can be seen from the graphs, only the area from 8-12 hours shows a potential inhibition effect from triclosan. Like many of the results from Bottle experiment 1 (seen separately), all trends of test 2a and b can be falsified by the 20 mg/l standard error of the OxiTop® method. Seen together though, at least figure 13, 14 and 15 all imply *some* influence from triclosan, although the effects are quite diverse and seem to depend a lot on media or other variables that are not counted with. From the graphs of test 3 (fig. 17), one cannot conclude that triclosan has had an inhibition effect. However, the results from test 3 might support the theory that triclosan's inhibition effect on greywater microbiology is largely dependent on external factors.

During the test temperature was held constant at 20ºC in an incubation cabinet. However samples might have had a different temperature when reaching the lab. If the samples are

cooler than 20ºC, logging of results does not begin until the sample reaches this target temperature. However, if the temperature inside the bottles is higher than the required temperature when the OxiTop system is started, the logging might begin right away (WTW 2004). Since the temperature in the lab where samples are prepared is not constant, this might contribute as a source of error and create different environments for both bacteria and triclosan. Also, the OxiTop method does not require oxygen saturation in samples before start. This might result in quite different respiration conditions for bacteria, and thereby also their ability to restrain stress. Moreover, the ratio between greywater and suspended biomass, as well as the quality and age of both, is highly relevant with respect to type and quantity of bacteria.

To improve the accuracy of the tests and investigate the significance of organic content and access to oxygen on the bacterial response to triclosan, the last two tests were saturated with oxygen and prepared with two different dilutions. Tap water was used as diluents. Also, plain greywater without suspended biomass was tested as media. To minimize potential sources of error, pH and temperature was documented and COD_S was measured before and after tests. Test 5 was run during 5 days instead of 2 to see further development of the respiratory curves. Results from the respiratory tests are shown in figure 18 and 19.

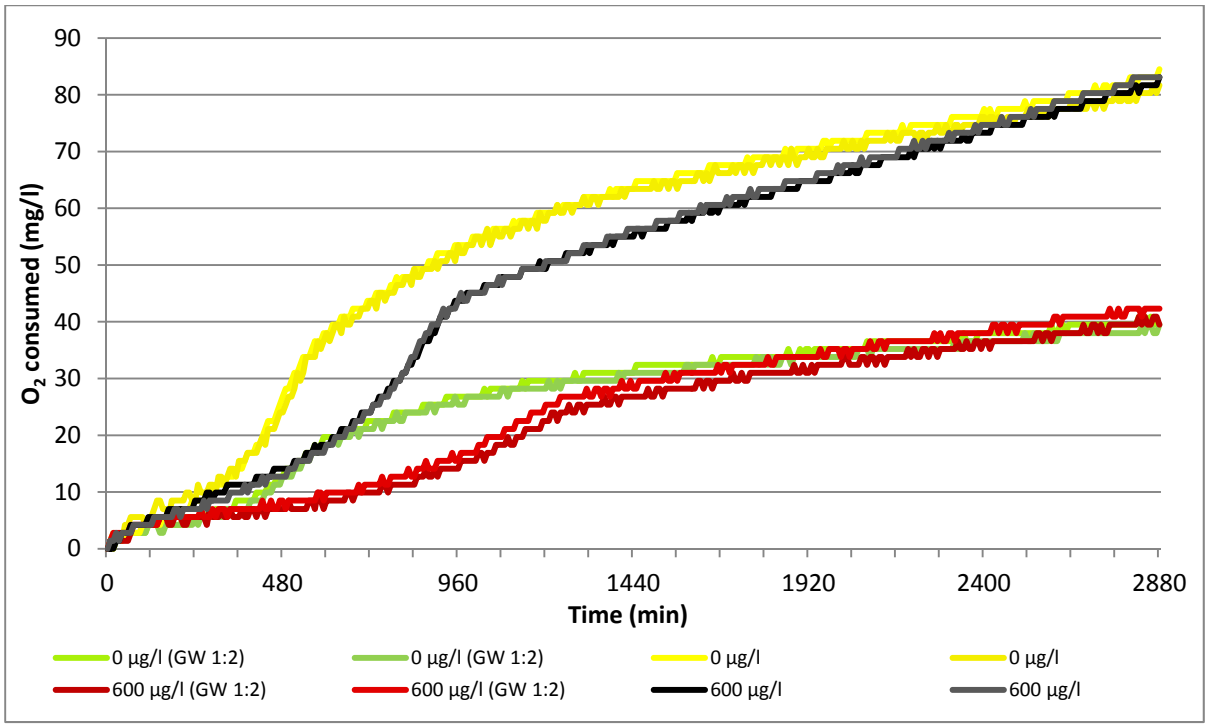
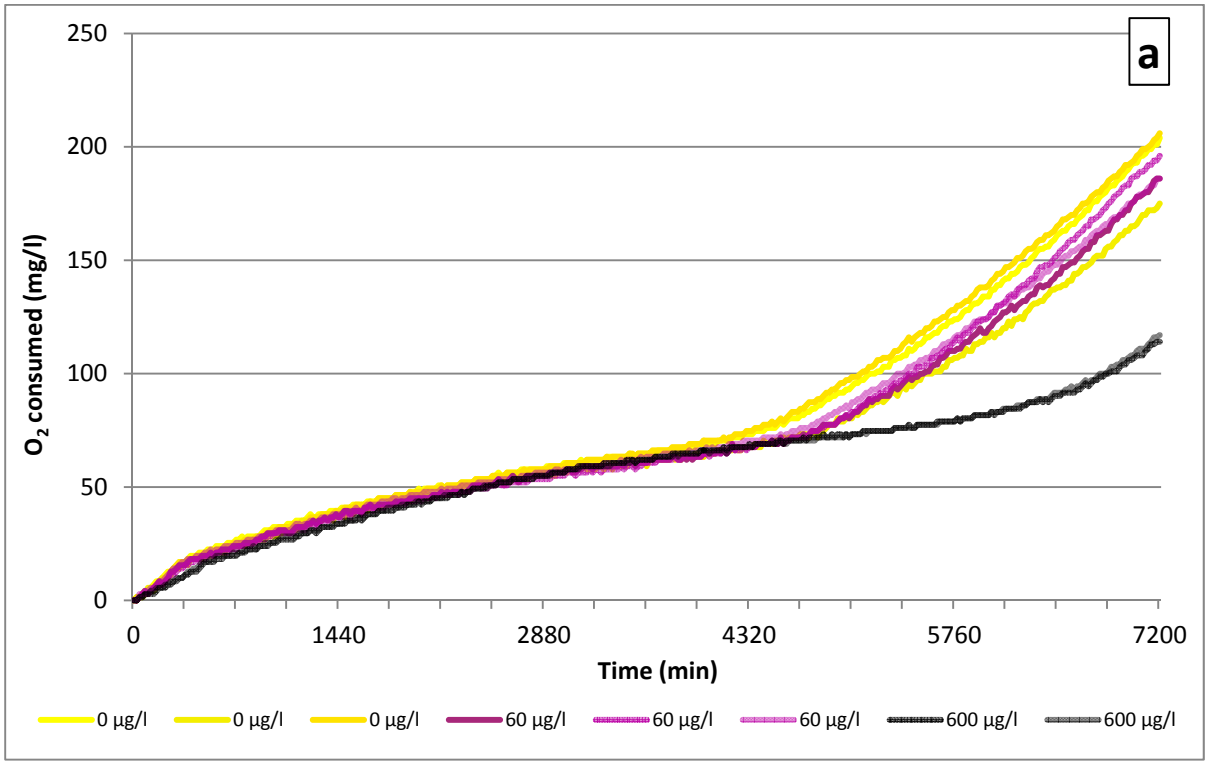


Figure 18: Respiratory test 4 of Bottle experiment 2. The graphs show respiration development in mg O_2 consumed/l for two different dilutions of greywater. Half of the tests are inoculated with triclosan.

Test 4 (fig. 18) has big visible differences between both greywater dilutions and triclosan concentrations. Also, bottles receiving equal dilutions and concentrations show very similar results. Respiration seems to rise with equal speed in all bottles during the first hours. Then comes what appears to be an adaption period ("lag phase") to the new media (methanol/triclosan). This period seems to be overcome after some hours, but the bottles added triclosan exhibits much slower respiration acceleration than those without triclosan. After a period of quick rise, respiration activity decreases, presumably because media and/or oxygen inside the bottle is reduced. By the end of day 2, the bottles inoculated with triclosan have more respiration activity than the bottles without triclosan. This might be due to an earlier consumption of resources in the bottles without triclosan.

The graphs strongly indicate an inhibition effect from the 600 µg/l concentration of triclosan in both diluted and undiluted greywater. It also seems that the oxygen saturation of the sample before test start made the effect more visible. However, it is not clear how the graphs would have developed further after the two days.



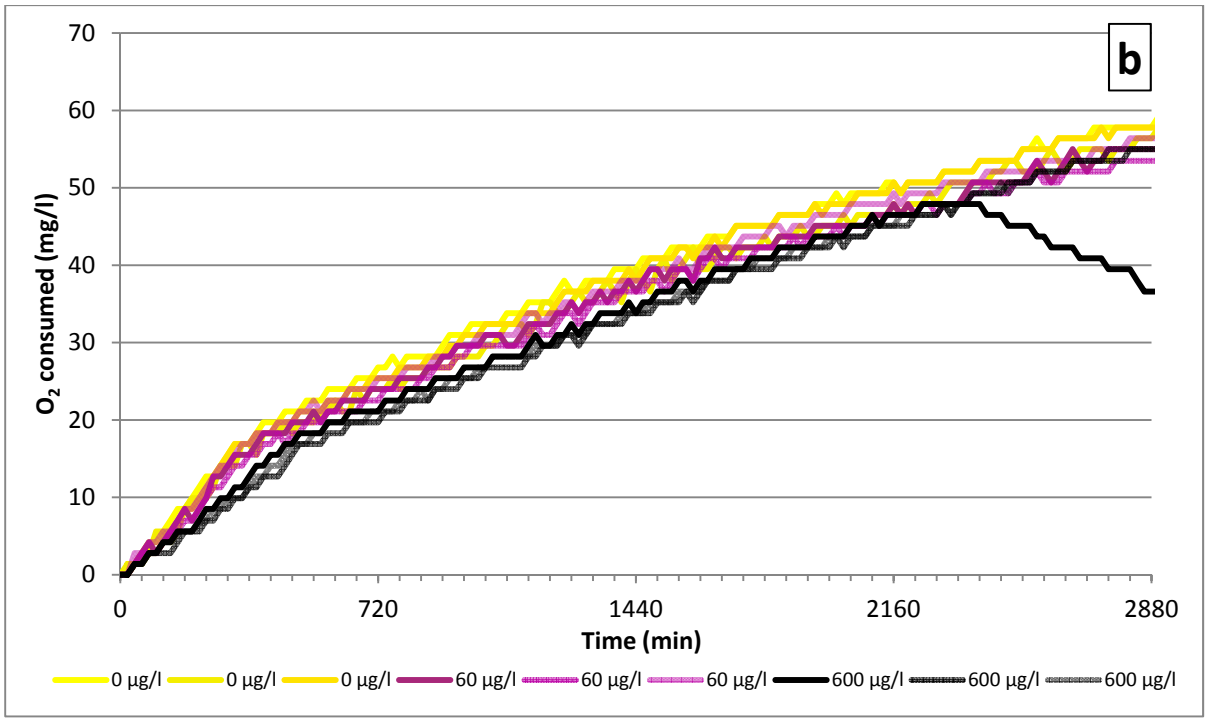


Figure 19 a and b: Respiratory test 5 of Bottle experiment 2. The graphs show respiration development in mg O_2 consumed/l for diluted greywater inoculated with different concentrations (µg/l) of triclosan. Figure a) shows results for 5 days while figure b) gives a magnified view of the first two days of the test period. The sudden drop after 1.5 days for one of the graphs (figure 19b) is presumably due to air breakthrough in the bottle seal. (This graph is removed from the figure 19 a).

Test 4 (fig. 18) illustrates the substantial difference in respiration over time between the two dilutions. Also, the graphs show a much slower start in respiration for the bottles with inoculated triclosan, which can be interpreted as a quite clear indication of inhibitor effect. In test 5, respiration also seems to be affected by the amount of triclosan. Although this is most visible after 3 days and not completely valid during the whole period, figure 12b shows lower respiration curves with higher triclosan concentrations also in the beginning of the test. The curve that falls after 1.5 days is probably due to an air burst into the bottle.

3.4. FILTER EXPERIMENT 1 (LAB-SCALE)

3.4.1. COD analyses

The first 5 days of the chemical addition period of filter experiment 1 filter A did not receive any corresponding methanol to that added with triclosan in filter B and C. This made COD values from these first days difficult to use, since 1 mg of methanol contributes with 1.5 mg COD and 0.7 mg methanol was added per liter. Technical problems led to gaps in the sampling regime, and soluble COD was only determined before and in the end of the

chemical addition period. Challenges and sources of error in sampling were overcome too late and because of this the results are less reliable than the results from the other experiments. See subchapter "Obstacles" for details regarding technical problems and sources of error.

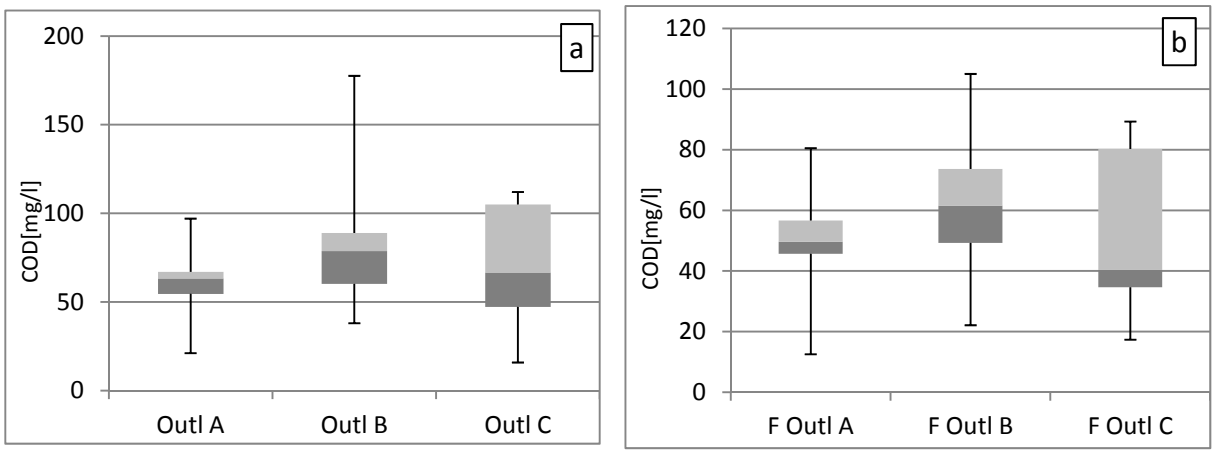


Figure 20: Organic content, expressed in a) COD_{total}, and b) $COD_{soluble}$ (COD_S) of outlet samples from filters A, B and C during the period of triclosan addition. Filter A did not receive triclosan. The center box represents the 1st and 3rd quartile and the median, while the vertical lines show the minimum and maximum values (n = 10 for COD and 7 for COD_S).

Figure 20 shows the differences between outlet values for all three filters. Since all three filters were fed from the same barrel, the inlet COD should be quite equal for all filters. Although several technical problems might have caused unequal treatment for the three filters, these problems are not taken into calculation in the comparison of COD outlet concentrations. This decision was made because all three filters experienced technical problems at some point during the operative period and this makes it likely to believe that the sources of error are evened out by time. This is supported by the even distributions seen in figure 21b.

Total COD (COD) (figure 20a and 21a) is not as reliable as soluble COD (COD_s). Due to sampling techniques, settled or floating material might be present in the samples and thereby create unintended high COD values in some samples. COD is in general more susceptible to random sources of error than COD_s. Also, with respect to the effect of a biofilm, COD_s is more relevant since the main immediate effect of biological processes is related to the degradation of soluble organic compounds. However, the results from COD somehow confirm the differences seen in COD_s between filter A, B and C.

As figure 20 shows, outlet values for filter A is less spread than the others, both in COD and COD_s values. Filter C has the largest spread in both parameters. The median is lowest for filter C in COD_s, however the differences are relatively small and difficult to confirm from a statistical point of view.

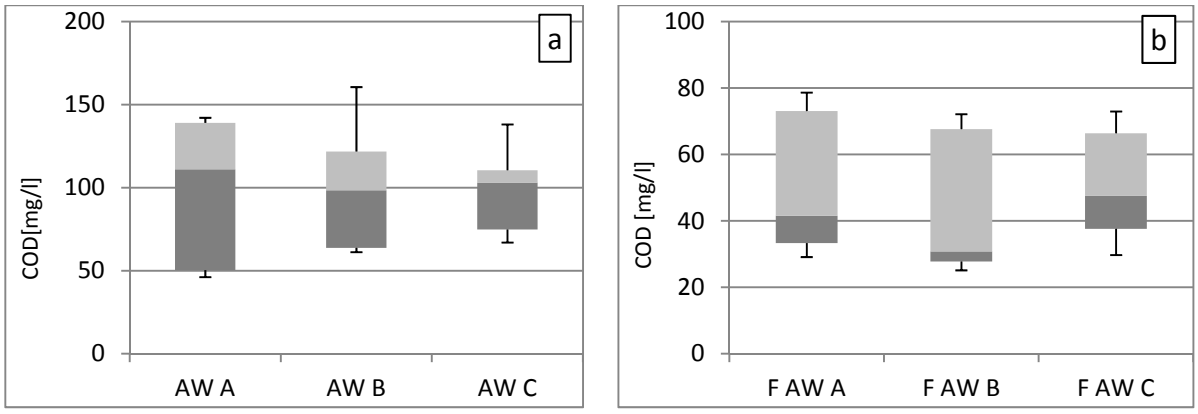


Figure 21: Organic content, expressed as a) COD_{total} and b) $COD_{soluble}$ (COD_S) of samples taken from the pumping chamber (after submerged weir (AW)) of filters A, B and C during the period of triclosan addition. The center box represents the 1st and 3rd quartile and the median, while the vertical lines show the minimum and maximum values (n = 8 for COD and 7 for COD_S).

Values from the pump chambers do not show the same trends as the filter outlet values. Neither the median nor the overall COD values seem to correspond between the inlet (AW) and the outlet of the filters. From this it might be assumed that the differences between filters A, B and C is not due to differences in inlet COD. However, the reason why both organic outlet content and variations in it is different between all three filters cannot (solely) be explained by the inhibition effect of triclosan on the biofilm. This is because the differences in COD_s are minor and because the difference also lies between filter B and C which were inoculated with the same triclosan concentration. Combined with all sources of error experienced during this experiment, it is not possible to conclude that there is an effect from triclosan addition.

3.4.1.1. Obstacles

The test plant had many stops due to power breaks and many different technical issues. During the whole operative period from March – July the feeding pump had to be checked and adjusted every day due to an undiagnosed problem with the pump drive or -head. This led to a less equal and less stable feeding rate than planned (beside being time consuming). Also,

each time the inlet barrel was refilled with greywater, settled material was whirled up and entered the feeding hoses together with a portion of air. This caused clogging and reduced effective suction in hoses, and contributed to inequalities in feeding rates. Different methods were tested to minimize this source of error. The most effective initiative was to make the pump stop during the refill, but several unexpected power breaks made these settings work oppositely. Nonetheless, all inequalities in the inlet organic load were presumed to be less important for the filters because the sedimentation stage was considered to work as a buffer. Also, as mentioned, inequalities in the dosing regime were expected to be evened by time.

During the 2.5 months of biofilm forming, filter pumps was changed from bilge pumps to stronger impeller pumps, and nozzles were cleaned and rinsed for clogging LECA-particles several times. Other obstacles as leakages, temperature changes and lacking equipment also occurred frequently.

3.4.2. Triclosan analyses

Due to limited access to chemicals for triclosan analyses, the greywater was only analyzed for triclosan 5 times. The first two times, wrong type of equipment used and errors concerning the analyzing procedures made the results unreliable. Additionally, all of the samples were above the range of the test. The next two analyses were correctly treated, but most values were under measuring range. However, in test 4, two different dilutions of greywater from the inlet barrel were within measuring range. In test 5, the right dilutions were finally made and all of the samples were within measuring range. However, standard solutions for the determination of a standard curve from which all sample values are determined was completely emptied and this might have affected the results. Due to all these incidents, the error sources were considered too many and too big to be able to trust the results.

Also, when the triclosan ELISA kit was delivered to UMB, it was kept in room temperature for more than three weeks because of confusions with delivery place. This might have damaged the chemicals and contributed to unreliable results.

3.5. FILTER EXPERIMENT 2

When the chemical addition period started, the filters were dosed with 0.57 mg/l of methanol, which proved to be a too high organic load for the filters. When this was noticed, the filters were rinsed with a high greywater load before they were inoculated again, this time with the 10 times diluted chemical solutions. Only results from the last period are presented here. See the methodology chapter for further technical information.

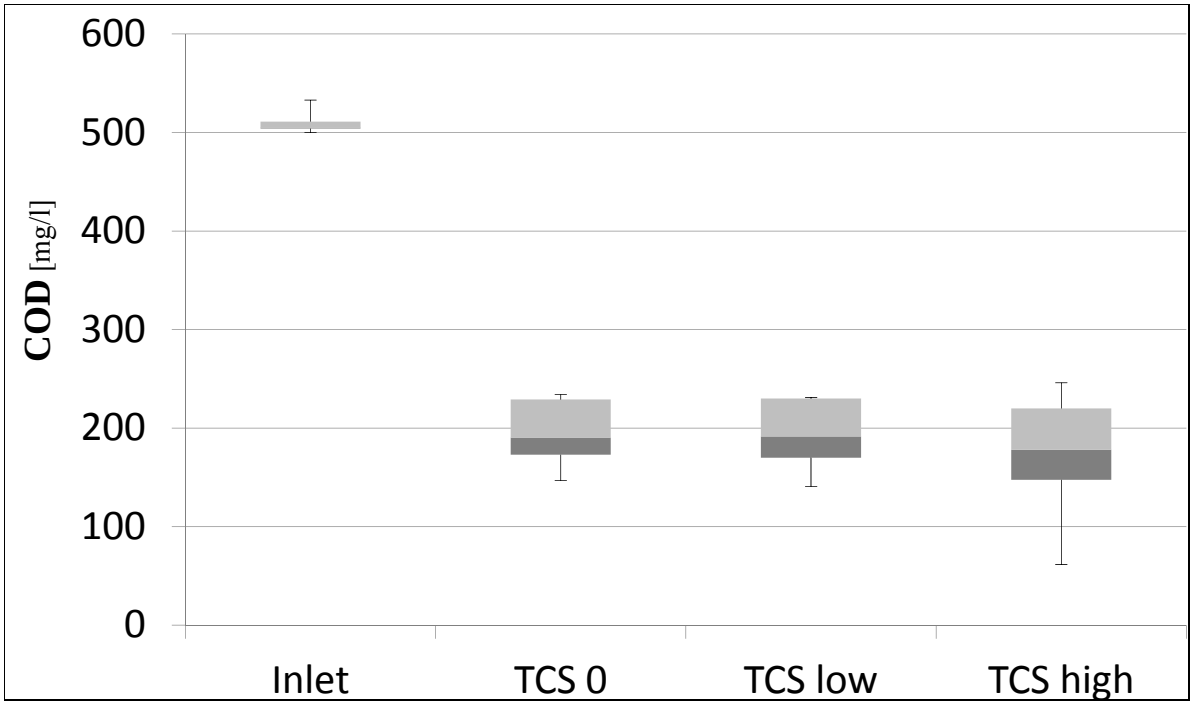


Figure 22: Organic content, expressed as $COD_{soluble}$, from the last five days of the experiment. The center box represents the 1st and 3rd quartile and the median, while the vertical lines shows the minimum and maximum values (n=5).

The first sample, taken before the application of triclosan, showed an initial, average COD removal in the three columns of 66,8 % (data not shown). As expected the percentage COD removal decreased to 61,3 %, mainly as result of the increased COD load added by the methanol in the triclosan solution. If triclosan inhibits the microbial activity, the column receiving high concentration (TCS high) should give lover COD removal. As seen in figure 22 there are only minor differences between the column effluents. The lowest median and minimum value is in fact in the column with the highest triclosan concentration. The lack of (visible) effect from triclosan may be because of the high organic loading of a filter with a poorly developed biofilm. The low temperature during the inoculation may have limited the growth of microorganisms.

4.6. CONCLUSION

This study indicates that triclosan has the ability to inhibit bacterial growth and metabolism in greywater. However, the inhibition effect varies largely and is probably dependent on the strength of the bacterial cultures, substrate availability and other parameters. The bacterial culture seems to adapt to triclosan after some time, spanning from no visible adaption time to a few hours or days and maybe longer, depending on the mentioned conditions. This adaption time might be the reason why no effect could be determined in the lab-scale filters, since none of the two experiments had valid results from the first days of inoculation. This theory is supported by a study of triclosan interactions in simulated drain-field soils (Svenningsen et al. 2011), where the presumed adaption period with "lag phase" and recovery was seen within 7 days after triclosan addition to the soil. The inhibition effect was reported to be observed within the first 2 days, and the study concludes that this short adaption phase might be the reason why a similar study (Ying et.al. 2007 in Svenningsen et al. 2011) concluded with no effect of triclosan.

For a biofilter with retention time of 1-2 days, the inhibition effect from triclosan on the biofilm might be neglectable, at least in the concentrations found in greywater (see table 1). However, as Eriksson et al. (2008) states in their study, greywater loadings, as well as chemical loads, fluctuate a lot during the day. Pulses with high organic and antiseptic loads might lead to different development of bacterial cultures and biofilm adaption than what is observed in this study. In cabins and summer houses, year fluxes presumably causes even bigger differences in biofilm behavior.

4. REFERENCES

Abraxis (2012a) *Triclosan in water*. Users manual triclosan ELISA kit. Abraxis LCC.

Abraxis (2012b) *Triclosan*. Users manual triclosan ELISA kit. Abraxis LCC.

Almqvist, H. & Hanæus, J. (2006). Organic Hazardous Substances in Graywater from Swedish Households. *Journal of Environmental Engineering*. (August 2006).

Buth, J.M., Grandbois, M., Vikesland, P.J., McNeill, K. and Arnold, W.A. (2009). Aquatic photochemistry of chlorinated triclosan derivates. *Environmental Toxicology and Chemistry* 28 (12): 2555-2563

Eriksson, E., Auffarth, K., Henze, M. & Ledin, A. (2001). Characteristics of grey wastewater. *Urban Water* 4: 85–104

Eriksson, E., Auffarth, K., Eilersen, A-M., Henze, M. and Ledin, A. (2003). Household chemicals and personal care products as sources for xenobiotic organic compounds in grey wastewater. *Water SA,* 29 (2): 135-146.

Eriksson, E., Andersen, H.R., Madsen, T.S. & Ledin, A. (2008). Greywater pollution variability and loadings. *Ecological Engineering* 35: 661–669

Fang, J-L., Stingley, R.L., Beland, F.A., Harrouk,W., Lumpkins, D.L. & Howard, P. (2010). Occurrence, efficacy, metabolism, and toxicity of triclosan. *Journal of Environmental Science and Health, Part C,* 28 (3): 147-171

Heistad, A., Paruch, A. M., Vråle, L., Adam, K. & Jenssen, P. D. (2006). A high performance compact filter system treating domestic wastewater. *Ecological Engineering*, 28: 374-379.

Henze, M. & Comeau, Y. (2008). Wastewater Characterization. In Henze, M., van Loosdrecht, M. C. M., Ekama, G. A. & Brdjanovic, D. (eds) *Biological Wastewater Treatment*: 33-52. London: IWA Publishing.

Ibidapo, J. & Stensen, G. (2007). *The performance characteristics of recirculating light weight aggregate filters*. Master thesis. Ås: Norwegian University of Life Sciences, Department of Mathematical Sciences and Technology. 99 pp.

Jenssen, P. D., Heyerdahl, P. H., Warner, W. S. & Greatorex, J. M. (2003). *Local Recycling of wastewater and wet organic waste - a step towards zero emission community*. 8th International conference on Environmental Science and Technology, Lemnos, Greece.

Jenssen, P. D., Bergstrøm, C. & Vatn, A. (2009). From Crap to Food. *The New Economy* (Summer 2009).

Jets (2011). *Grey Water Treatment Plant, Ecomotive, A01*. Produktmanual. Jets.

KLIF (2009). Prioriterte miljøgifter: Nasjonale utslipp – status 2009. Klima- og forurensningsdirektoratet

KLIF (2010). *Undersøkelse av miljøgifter ved fire norske renseanlegg.* Klima- og forurensningsdirektoratet, Rapport nr 10-015. Aquateam - Norsk vannteknologisk senter A/S

Masi, F., El Hamouri, B., Shafi, H. A., Baban, A., Ghrabi, A. & Regelsberger, M. (2010). Treatment of segregated black/grey domestic wastewater using constructed wetlands in the Mediterranean basin: the zer0-m experience. *Water Science and Technology*, 61 (1): 97-105.

Palmquist, H. (2001). *Hazardous substances in wastewater systems a delicate issue for wastewater management*. Licentiate thesis. Luleå Tekniska Universitetet.

Plamquist, H. & Hanæus, J. (2005). Hazardous substances in separately collected grey- and blackwater from ordinary Swedish households. *Science of the Total Environment* 348: 151-163

Singer, H., Müller, S., Tixier, C. & Pillonel, L. (2002).

Statistisk Sentralbyrå (2011). *KOSTRA: Kommunalt avløp, 2011*. http://www.ssb.no/emner/01/04/20/var_kostra/ [12.12.2012]

Suszkiw, J. (2009). New test to determine triclosan in water. *Agricultural Research*, January: 13

UNDP (2007). *MDG Monitor for Goal 7*. United Nations Development Programme. http://www.mdgmonitor.org/goal7.cfm [09.12.2012]

UNDESA (2012). *World Urbanization Prospects: The 2011 Revision, CD-ROM Edition*. United Nations, Department of Economic and Social Affairs, Population Division prospect. http://esa.un.org/unpd/wup/CD-ROM/Urban-Rural-Population.htm [10.12.2012]

UNGA (2010). *The human right to water and sanitation*. United Nations General Assembly Resolution A/64/L.63/Rev.1. 3 pp.

UNICEF and World Health Organization (2012). *Progress on Drinking Water and Sanitation 2012 Update*. WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation Report. 58 pp.

WTW (2004). *Operation of the Single Measurement System OxiTop*. Operation manual WTW. 2004:BA31101e. 4 pp.

WTW (2010). *Respirometric BOD_5 determination of domestic waste water*. Application Report WTW. 2010:lab 02 E (old number 895230*)*. 4 pp.

Ødegård, H., Lindholm, O., Mosevoll, G., Thorolfsson, S., Sægrov, S., Østerhus, S. og Heistad, A. (2012). *Vann- og avløpsteknikk*. 704 pp. Norsk Vann.

APPENDIX

Table 1: Greywater background values, from January and December 2012. All expressed in mg/l. N = number of analyses from which the average and standard deviations are calculated.

	COD	COD_S	BOD_5	TSS	TN	TP	OP (PO_4-P)	NH_4-N	NO_3-N	NO_2-N
Average	266.4	120.48	129	55	11.7	1.25	0.41	7.83	0.17	>0.0015
Std. dev.	8.52	29.70					0.31	0.81	0.05	
N	4	3	1	1	1	1	2	2	2	1

Table 2: Greywater background values compared to tap water values, from January 2012. All expressed in mg/l. Determined by Inductive Coupled Plasma - Optical Emission Spectrometry (ICP-OES).

	Al	Ca	Cd	Co	Cu	Fe	K
Greywater	0.3689	19.46	0?	0?	0.007	0.2608	6.633
Tap water	0.0605	20.74	0?	0?	0.0812	0.0448	2.053
	Mg	Mn	Mo	Na	Pb	Zn	
Greywater	3.295	0.0108	0.004	46.25	0.0022	0.0738	
Tap water	2.058	0.0037	0.001	11.89	0.006	0.081	