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Executive summary

Microbial growth is an important topic in industrial water systems. Problems like the formation of biofouling on pipes and membranes reduce the performances of the systems and microbiological pollutants limit the use of water. Prevention technologies like Denutritor and Nanosilver decrease the use of chemicals for water conditioning and cleaning, and increase the possibilities of water recycling and re-use.

This report describes laboratory tests to evaluate the potential of the use of Denutritor for biofouling reduction and microbial growth in waste water from the chemical industry and rain water for application in food industries, and tests to evaluate the impact of Ag nanoparticles on biological processes in effluent treatment for the paper industry. Denutritor pilot tests in chemical and food industry were started (WP 5.2 and WP 5.3) based on the results of these laboratory tests.

Perstorp Specialty Chemicals, Sweden, intends to re-use effluent from their activated sludge Waste Water Treatment Plant (WWTP) for cooling and other industrial applications. For re-use, biofouling potential and salts concentrations of the WWTP effluent must be reduced. In laboratory tests with synthetic Perstorp WWTP effluent it is demonstrated, that Denutritor can be used to reduce the biofouling potential of this water type. An about 8-fold reduction appears to be within reach.

Unilever Ben&Jerry's Factory in The Netherlands is aiming to re-use collected rainwater for cleaning of packing and transport material. However, rainwater can contain a relatively high concentration of (pathogenic) micro-organisms, which should be removed before the water can be used for these types of application in the food-sector. Laboratory tests with collected and recirculated rainwater showed that Denutritor can reduce coliforms, *E. coli* and pathogenic bacteria present in collected rainwater, but not remove them all. The amount of viable bacteria can additionally be reduced with 90-99% efficiency by exposure to UV doses between 50 and 2500 J/L, to further prevent the downstream process water for application in food industry.

During these laboratory tests, three new types of biofouling monitors were evaluated for use in the on-site Denutritor pilot trials at Perstorp (WP 5.2) and at Unilever, Hellendoorn (WP 5.3). The biofouling monitors were exposed to Denutritor influent and effluent water, to allow the growth of microbial biofilms (biofouling) on their surfaces. The increase of protein concentration on the monitors appeared to be a good indicator of the biofouling potential of the synthetic WWTP effluent water tested. Biofouling monitors made from silicon or polyethylene (PE) tubes worked fine and were finally selected for use during the Denutritor pilots.

The effect of silver nanoparticles has been analysed using model systems for anaerobic and aerobic treatment processes. The impact of nanoscale silver on the respiratory activity of aerobic activated sludge was traced continuously by means of respirometric measurement. Industrial process media (effluents, activated sludge, pellet sludge) from a paper mill were used for the analyses.
Furthermore, trials were conducted in lab-scale at PTS and on mill-site to study the action that surface-bonded Ag nanoparticles had on preventing slime formation.
It was found in model experiments on anaerobic and aerobic biodegradability that concentrations of nanoscale silver <30 mg/l did not cause any impairment of these processes. Contents above this value, however, reduced process efficiency.

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1 Introduction

The development of new sustainable technologies for prevention microbial growth and scaling in industrial water systems is an important topic. Prevention technologies decrease the use of chemicals for water conditioning and cleaning, and increase the possibilities of water recycling and re-use.

This deliverable describes laboratory tests to evaluate the potential use of Denutritor for biofouling reduction and microbial growth in water systems from chemical and food industry under a variety of process conditions, and the impact of nanosilver on paper industry water.

Denutritor
Denutritor is a biofilter which reduces biofouling potential of water by removing substrates required for microbial growth (Gerritse *et al*., 2003; Meesters *et al*., 2003). In Denutritor, microbial communities are grown in biofilms on a filler of polyurethane foams. The micro-organisms in these biofilms degrade organic substrates, which are dissolved in the water. So doing, the source of biofouling is removed, and the potential for re-use of the treated water is enhanced. Denutritor has the potential for water-treatment in various industrial water systems of different sectors, e.g.:

1. Chemical industry;
2. Food industry;
3. Paper industry;
4. Textile industry.

Nanosilver
Silver as an antimicrobial agent has been known for a long time. The concept of “active packaging” deals with the use of several natural and chemical substances, among them silver, to produce packaging materials with antimicrobial or bacteriostatic characteristics for food and non-food packaging (N.N., 2008a). It is expected that nanosilver products will see increasing use in future.

The amounts of nanoscale silver used for this purpose are not yet well known either. Specifications by the manufacturer recommend concentrations of 1 – 25 ppm nanosilver. Depending on the type of use (e.g. use in coating colours) nanoscale silver concentration appears to be low in the paper product.

The entry of these particles in the water circuits and the wastewater is possible because of the intensive use of recycled paper in the case of intensive use of nanosilver products. Even the risk of nanoscale silver affecting human health, when correctly used, is assumed to be negligible (N.N., 2008b). Nanosilver may cause a disturbance of biological effluent treatment processes due to their antimicrobial properties.

Nanosilver products are currently not yet being used on an industrial scale in papermaking (at least not in Germany). At present, they are still being used in the development stage on laboratory and pilot scales.

1.1 State of the art

1.1.1 Denutritor

<u>Chemical industry</u>
Perstorp Specialty Chemicals aims to re-use effluent from their wastewater treatment plant (WWTP) for cooling or other industrial processes. The possibility to cost effectively remove organic compounds, biofouling potential and salts from WWTP effluent, largely determines the re-use potential. To this end, different waste water production and post-treatment systems will be tested on pilot scale during the AquaFit4Use project (WP 5.2, Gerritse and Ter Huurne). The optimal operational conditions for

Denutritor, and the biofouling potential reduction which may be obtained with WWTP effluent are unknown. Therefore, in preparation of the pilot tests, the reduction of biofouling potential was studied with laboratory Denutritor set-ups, fed with "synthetic" effluent from the WWTP plant at Perstorp. Denutritor was particularly tested with synthetic WWTP water at various hydraulic retention times and with different types of biofouling monitors for use during the pilot tests.

Food industry

Unilever Ben&Jerry's Factory in The Netherlands, is aiming to re-use collected rainwater. Rainwater can be used for cleaning of packing and transport material (containers, pallets, etc.) and for sanitation (toilets) at the plant location in Hellendoorn, The Netherlands. The advantage of the use of rainwater is that it is directly available and relatively clean. However, rainwater can contain a relatively high concentration of (pathogenic) micro-organisms, which should be removed before it can be used in the food-sector. The Denutritor can potentially improve the microbial quality of stored rainwater for application in the food sector. The effectiveness of Denutritor to reduce microbial growth and biofilm formation in stored rainwater is unknown, and should therefore be evaluated.

1.1.2 Nanosilver

The production of paper goes hand in hand with the intensive use of water. The effluents of papermaking are contaminated with organic substances which are to a large extent biologically degradable. So the effluents of nearly all paper mills will be treated by mechanical and biological processes to remove the organic load from these effluents. The established processes use different types of anaerobic (e.g. UASB, EGSB) and aerobic biological processes (activated sludge, MBBR, biofilters).

When nanosilver products are used in papermaking, these particles may also be transferred to the process and wastewater streams and eventually reach the effluent treatment plants. Because of the antimicrobial properties of nanoscale silver, disturbances of the biological processes may occur depending on their concentration.

The behaviour of nanoparticles in biological treatment processes and their impact on biomass is currently not well known. On the one hand, adsorption of nanoparticles onto biomass, especial at extracellular substances, on the other hand inhibition of the activity of biomass may be expected (Ganes and Leong, 2006).

Investigations concerning the behaviour of Ceroxide nanoparticles in biological treatment processes showed adsorption onto activated sludge to be the main reason for elimination but also the outwash of nanoparticles to the effluent (6%), obviously dispersed by surface active substances from bacteria (Limbach et al. 2008).

1.2 Objectives

The development of new sustainable technologies for prevention of biofouling and scaling.
Prevention technologies decrease the use of chemicals for water conditioning and cleaning, and increase the possibilities of water recycling and re-use.

1.2.1 Denutritor

The objective was to test and optimize Denutritor biofilters and their operation under different conditions for treatment of different types of industrial water.

1.2.2 Nanosilver

Objective was to elucidate the impact of Ag nanoparticles on biological processes in effluent treatment of paper mills.

1.3 Deviations from original DoW

1.3.1 Description of work related to internal report as given in DoW

1.3.1.1 Denutritor

Laboratory experiments were done to evaluate the effectiveness of Denutritor and re-use possibilities during treatment of water in:

- Chemical industry
- Food industry

More specifically for the Chemical Industry, the aim of the study is:

- Assess the operational stability, optimal process conditions and biofouling potential reduction during treatment of (synthetic) WWTP effluent water.
- Develop and test new biofouling monitors for use during Denutritor pilot tests.

More specifically for the Food industry, the aim of the study is:

- Evaluate the effect of Denutritor on the chemical and microbiological quality of stored rainwater.
- To test the effectiveness of UV-treatment for further improvement of the microbiological quality for use of the stored rainwater for cleaning purposes.

1.3.1.2 Nanosilver

Task 3.2.3 Impact of nanoscale silver:

Nanoscale silver is used in various products/processes, for example in sport clothes, wall colours, water treatment, medicine technology and marine engineering aiming on reducing biological growth. Newer R&D activities deal with implementation of nanosilver in packaging. So, there is a clear demand to ensure stable production, protection of health and environment to provide possible negative developments.

There are four main objectives:

- Measuring methods for nanoscale silver (NSilver) will be used in the processes and water circuits of the paper sector. In a first monitoring the actual concentration level of NSilver will be studied in at least one paper mill.
- Learning from established applications in different sectors: the overall goal is to generate knowledge in terms of using tailor-made nanosilver structures in high loaded microbiological process water and effluent streams. Consequently the need of biocides can be significantly reduced
- Application of effective surfaces in pilot or laboratory trials. This will focus on the differentiation of the effectiveness of dissolved or surface-bounded NSilver and the economic impact.

- Laboratory trials will be carried out to test the impact of NSilver in biological wastewater treatment plant. Objective is the investigation of the impact of different NSilver levels to the degradation level of aerobic and anaerobic water treatment. This will focus on the definition of first critical concentration levels to provide malfunctions of wastewater treatment plants.

1.3.2 Time deviations from original DoW

Because the selection of the two other water types for Denutritor laboratory testing was delayed the research focuses on tests with water from chemical and food industry.

1.3.3 Content deviations from original DoW

Not relevant

1.3.4 Problems occurred

Perstorp WWTP effluent water for laboratory tests with Denutritor was not available.

1.3.5 Actions taken for problem solving

Based on chemical analysis of the composition of Perstorp WWTP effluent water (anions, cations, TOC-content) a similar WWTP water was synthesized for use during the laboratory tests with Denutritor.

1.3.6 Risk register

Not relevant

2 Methods

Experimental set-ups were constructed in the Deltares laboratory for use during tests of Denutritor in combination with various industrial waters (Figure 1 & 2). The lab-scale Denutritor reactors had a volume of 425 ml.

2.1 Denutritor

2.1.1 Tests effluent Chemical industry

For these tests, the Denutritor reactors were filled with three reticulated polyurethane foam elements: course foam (specific surface area about 200 m^2/m^3), intermediate foam (400 m^2/m^3), and fine foam (700 m^2/m^3). The three individual foam elements each had a volume of 130 ml. The course foam was placed in the bottom of the reactor, the intermediate foam in the middle and the fine foam on top. The reactors were operated in an up-flow modus.

An analysis report of the influent and effluent of WWTP water samples, taken at November 15, 2005, was obtained from Perstorp (Appendix A, table 1). Based on this analysis, batches of 50 liter synthetic WWTP effluent water were composed according to Appendix A, table 2. Yeast extract was added as undefined source of carbon, to simulate TOC, and trace nutrients. For use, the synthetic Perstorp WWTP effluent water was stored at 4°C and aerated using a membrane pump.

For startup of a Denutritor laboratory filter, it was first inoculated with micro-organisms by recirculation of 20 l pond water (flow-rate 160 ml/min) through the filter column. After one day, the Denutritor reactor was started by supply of synthetic WWTP effluent water. During the tests the Denutritor filter column was kept at 20°C by using a heating ribbon and temperature controller, to anticipate on the situation expected during the tests with Perstorp WWTP effluent.

Different biofouling monitors were constructed of: polyurethane foam, silicon tubes, or PE tubes, respectively. The biofouling monitors were exposed to Denutritor influent and effluent water to allow the growth of microbial biofilms. In most waste water systems, the major fraction of the macromolecules of microbial biofilms consists of proteins (Jahn and Nielson, 1998). Therefore, biofouling potential was determined by measuring the increase of protein of microbial biofilms growing on the biofouling monitors. After an exposure period of typically one day to a week, the monitors were removed to quantify the microbial biofilms by analysis of the protein concentration on the exposed surface. For this, the proteins were removed from the surface of the monitors by NaOH treatment and quantified colorometrically, according to Lowry *et al*. (1951).

From the incubation time and the exposed surface area, biofouling potential was calculated and expressed as mg protein formed per m^2 monitor per day (mg protein/m^2/day). The biofouling potential reduction was subsequently defined as the difference (%) between the amounts of protein formed on the monitors exposed to the influent or effluent water of Denutritor, respectively.

2.1.2 Rain water tests for Food Industry

Denutritor laboratory experiments were started with rainwater collected from a roof in Apeldoorn, The Netherlands, on October 7, 2009. The rainwater was transported to the laboratory and stored in 50 l reservoir vessels at 20°C. During the tests, the rainwater was recirculated via Denutritor at a flow-rate of 10 l/hr. Denutritor was packed with 420 ml polyurethane foam (specific surface area about 400 m^2/m^3). Two laboratory test systems were constructed: one setup with Denutritor and one setup without Denutritor, which was used as a reference (Fig. 1 and 2). The dimension of the laboratory systems mentioned above were adapted from the situation at the Ben&Jerry's Factory: reservoir tank of 25 m^3, maximum water flow rate = 1500 l/hr. The laboratory experiments were executed in two phases. In the first phase, the effect of Denutritor on the chemical and microbiological quality of the rainwater was tested. In the second phase, the effect of UV-treatment on recirculated rainwater (with and without Denutritor treatment) was tested.

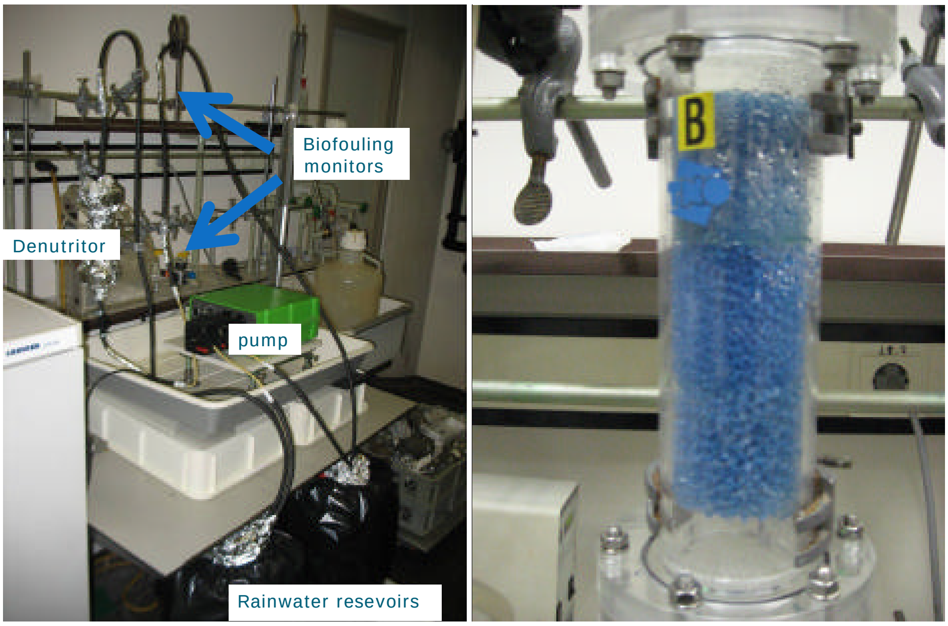
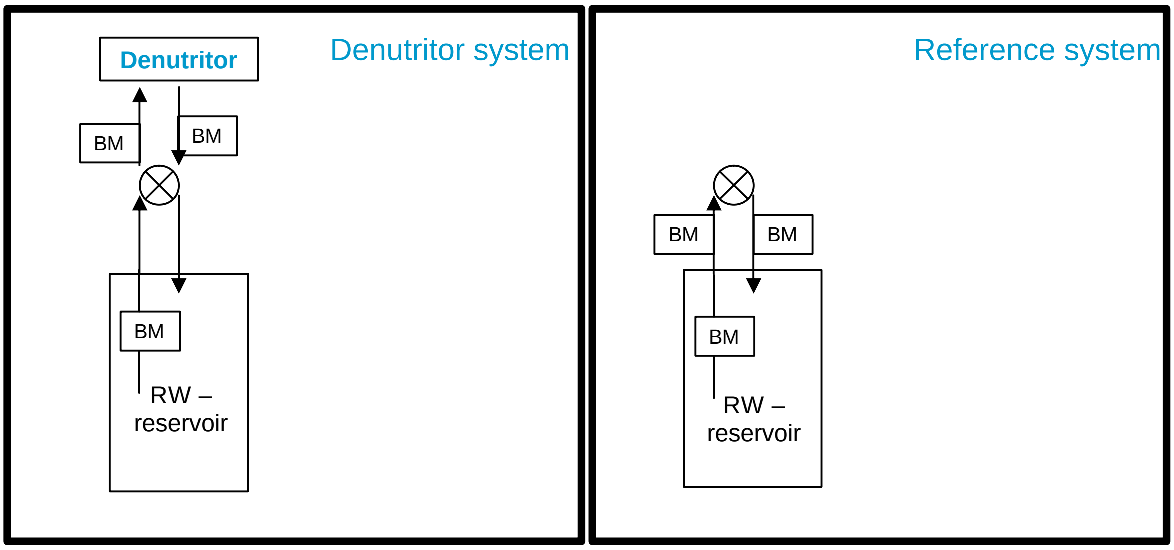


Figure 1: Picture of the Denutritor laboratory set-up (left) and close-up of Denutritor with polyurethane foam (right)

1st phase: Effect Denutritor on water quality

Rainwater was recirculated (10 L/h) at 20°C in a 50 l reservoir, with and without Denutritor. Both test systems run simultaneously, and the chemical and microbiological quality of the rainwater in the reservoirs was monitored by parameters mentioned in Table 1 at t=0, t=14, t=37, t=59 and t=133 days. As exception, AOC was analyzed only at t=0 and t=133 days. Formation of protein was analyzed on duplicate Biofouling Monitors (BMs), which were placed in the recirculation systems (Figure 1 and 2).



BM = Biofouling monitor   RW = Rainwater

Figure 2: Experimental set-up of recirculated rainwater with and without Denutritor treatment

Parameter	Method	Laboratory
Chemical parameters		
pH	Hand-held sensor	Deltares, Utrecht, NL.
TOC (Total Organic Carbon)	TOC-analyser	Deltares, Utrecht, NL.
COD (Chemical Oxygen Demand)	Hach-Lange, LCK414	Deltares, Utrecht, NL.
AOC (Assimilative Organic Carbon)	NEN 6271	KWR, Nieuwegein, NL.
Anions (NO_2^-, NO_3^-, Br^-, PO_4^{3-}, SO_4^{2-})	Dionex	Deltares, Utrecht, NL.
Cations (Na^+, Ca^{2+}, Mg^{2+} NH_4^+).	Dionex	Deltares, Utrecht, NL.
Microbiological parameters		
Biofouling (bacterial protein on monitors)	Protein concentration by Lowry	Deltares, Utrecht, NL.
Aerobe heterotrophic bacteria	Plating on R2A agar (Difco BD)	Deltares, Utrecht, NL.
Coliforms / E.coli	Plating on Chromogenic agar (Oxoid)	Deltares, Utrecht, NL.
Total amount of bacteria	qPCR (16S rRNA genes)	Deltares, Utrecht, NL.
Total number bacteria	FISH	Vermicon, München, D.
Total number of viable bacteria	FISH	Vermicon, München, D.
Pathogens • *Enterobacteriaceae* • *Legionella* • *Ps. Aeruginosa* • *K. pneumoniae* • *Salmonella* • *Listeria* • *E. sakazakii*	FISH	Vermicon, München, D.

Table 1: Overview of parameters analyzed to monitor the chemical and microbiological water quality of the recirculated rainwater

2nd phase: Effect of UV-treatment on rainwater

The effect of UV-treatment on rainwater that was recirculated with or without Denutritor for 133 days was tested in a "once-through" set-up (Figure 3). The rainwater was pumped from the rainwater reservoirs through an UV-filter at 7 different UV-doses in the range of 0 – 2520 J / L. These UV-doses were obtained by pumping the rainwater with different flow rates and various UV-intensities, as indicated in Table 2.

Table 2: UV exposure of the rainwater by 7 different treatments with various flow rate and UV intensity

Treatment	UV intensity	Flow rate	UV exposure
	(Watt)	(L/h)	(J/L)
1	7	10	2520
2	7	50	504
3	7	100	252
4	7	300	84
5	3	100	108
6	1	100	36
7	0	100	0

The effluent water of the UV-filter was collected aseptically, and analyzed for microbiological quality by plate counting on R2A and Chromogenic agar plates (aerobe heterotrophic bacteria and Coliforms / *E.coli*) at 30°C in the Deltares laboratory. Live/dead screening by FISH-analysis was performed in the Vermicon laboratory for the pathogens: *Listeria, Legionella*, *Klebsiella pneumoniae*, *Salmonella*, *Enterobacter sakazakii*, *Enterobacteriaceae*, and *Pseudomonas aeriginosa.* Aerobic heterotrophic bacteria were also analyzed at 30°C and 37°C in the Vermicon laboratory.

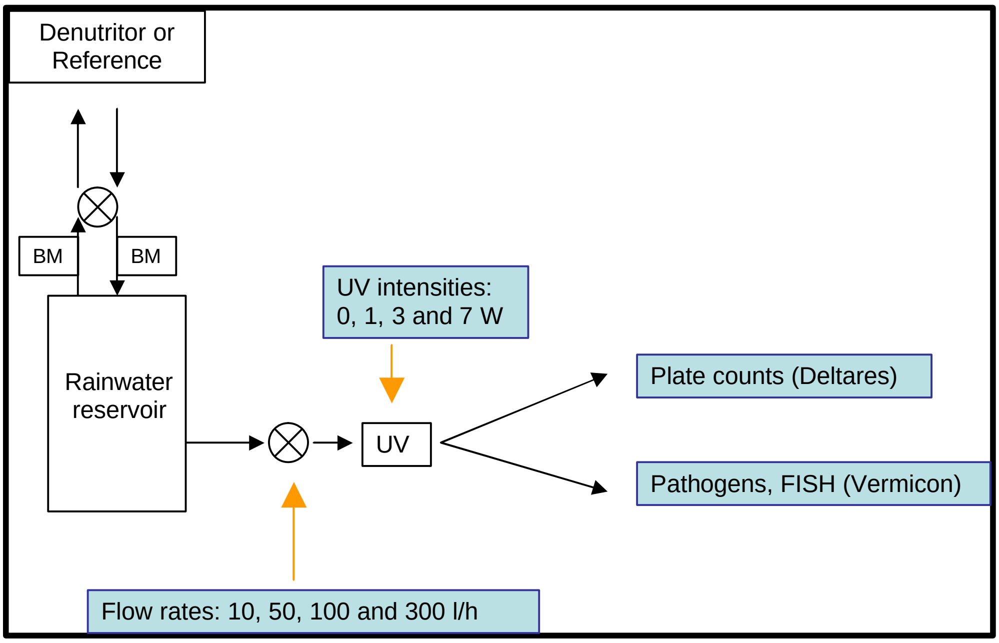


Figure 3: Schematic set-up of the rainwater UV-treatment tests

2.2 Nanosilver

The following methods set forth in Table 3 were used within the scope of the project objectives.

Table 3: Analytical methods

Parameter	Dimension	Method
COD	mg/l	Cuvette test (Hach-Lange)
pH	-	pH electrode, DIN 38404, Part 5
T	°C	DIN 38404, Part 4
DM (dry matter)	g/l	DIN 38409, Part 1
Anaerobic degradability	-	PTS Method PTS-WA 003/9
Aerobic degradability	-	EN ISO 9888: 1999 (adapted)
Respirometric test	-	DIN EN ISO 9408:1999 (adapted)
Particle sizes	nm	Zetasizer Nano ZS (Malvern Instruments) measuring range 0.6nm to 6 µm
Aerobic degradation	-	Zahn-Wellens-Test EN ISO 9888: 1999
Anaerobic degradation	-	Anaerobic batch test (PTS Test Method PTS-WA 003/97
oxygen consumption rates in respirometric testing	-	DIN EN ISO 9408:1999

The nanosilver products used for testing purposes are described in the tables below.

Table 4: Product characteristics nanosilver product used in degradation tests

	Product characteristics
Product name	confidential
Chemical characteristics	aqueous dispersion of nanoscale silver < 10 nm
Ag content	10% (±0,5%)
Application dose	100 – 250 ppm product (10 – 25 ppm Ag)

Table 5: Product characteristics antimicrobial varnish for slime panel tests

	Product characteristics
Product name	confidential
Chemical characteristics	colourless two-component polyurethan-acrylic varnish
Ag content	240 mg/l nanoscale silver (in wet varnish)
Quantity used	80 -100 g/m²

In general, two types of tests were used to describe the impact of nanosilver in papermaking processes and related wastewater treatment processes. First, anaerobic and aerobic degradation tests were used to find out the effect of the nanoscale silver added to the process wastewater in different concentrations.

Another test, using plates which were prepared with and without nanosilver containing coating colour to prevent or to suppress microbial growth, was executed to test the impact of nanosilver on the growth of slime on surfaces of papermaking equipment. The following figure shows the steel panels used for the observation of slime growth. The left picture shows the panel before painting, the right panel is painted with Ag-free coating colour (lower part) and nanoscale silver containing colour (upper part). These panels were positioned at different locations under and near a paper machine to allow the growth of slime to be observed.

Figure 4: Steel panels (left – uncoated panel, right – panel with normal and Ag-doped colour)

The metal sheets that had been prepared in this way were positioned in a paper mill at six different locations in the splash zone of a paper machine and left for 47 days. They were thus exposed to humidity, shower water and spray mist.
Another sheet was suspended directly in the white water of the paper machine. After the exposure time had expired, the periphyton was evaluated visually on the basis of the biofilm and deposits.

The circuit water of the paper mill had the following characteristics:

Table 6: Parameters circuit water

Parameter	**Unit**	**Value**
COD	mg/l	3000
BOD_5	mg/l	1600
Temperature	°C	36 – 40
pH	-	6.6 – 7
NH_4-N	mg/l	0.32
PO_4-P	mg/l	3.1

3 Results and achievements

3.1 Major results and achievements

3.1.1 Denutritor

3.1.1.1 Chemical Industry – Perstorp

Twenty Denutritor laboratory test runs were done with synthetic Perstorp WTTP effluent water. During these runs different operational conditions and three types of biofouling monitors were evaluated.

Initially, three process runs were done in which Denutritor was challenged with 1000 mg/l yeast extract in the influent water. This concentration corresponds to a TOC of 400 mg/l, which is between the Perstorp WWTP influent (830 mg/l) and effluent (110 mg/l) TOC concentrations. During these tests PUR-foam biofouling monitors were used and Denutritor was operated at a hydraulic residence time (HRT) of 130 minutes. Very high biofouling rates, in the order of 300 to 2000 mg protein/m^2 biofouling per day, were observed (Table 7). It appeared that under these conditions, the PUR foam monitors were saturated with microbial biofilms within a few days. Based on protein analyses, a maximum reduction of biofouling potential of 36% was obtained. From the difference between the wet weights of the influent and effluent biofouling monitors a maximum reduction of 49% was calculated. During these experiments the biofouling monitors and the Denutritor filter foams frequently clogged. Therefore it is concluded that 1000 mg/l yeast extract contained too high concentrations of biodegradable substrates for Denutritor to function properly. In a next series of tests, the yeast extract concentration in the influent reservoir vessel was reduced from 1000 to 200 mg/l. Assuming that yeast extract consists for 40% (w/w) of carbon, this brings the TOC concentration in the influent of the laboratory setup at 80 mg/l, which is close to the TOC concentration found in Perstorp WWTP effluent water. However, the PUR-foam biofouling monitors still clogged rapidly during these conditions. So it was concluded that PUR-foam biofouling monitors could better not be used for tests with Perstorp WWTP water.

In a second series of laboratory tests, the PUR-foam biofouling monitors were replaced by silicon tubes (length 10 cm, diameter 0.65 cm). The HRT was reduced to 13 minutes and the influent yeast extract concentration was kept at 200 mg/l. The silicon tube monitors appeared suitable to measure biofouling potential under these laboratory conditions. No clogging problems occurred. Under stable operating conditions, the biofouling potential of the influent water varied from 200 to 500 mg protein/m^2/day. Denutritor reduced this potential by 68-87%. Lower biofouling potential reductions, even to below 50%, were obtained when Denutritor was periodically stopped and not continuously fed with synthetic WWTP effluent water. Also, an increase of the water temperature from 20°C to 40°C reduced the performance of Denutritor. These results indicate that it is important to operate Denutritor at relatively constant conditions.

The silicon tubes worked well for measuring biofouling potential. However, we noticed that material from the silicon could influence the protein analysis. Also, we doubted if the flexible tubes were sufficiently robust for use during the pilot tests. Therefore, a third series of tests was done with stronger polyethylene (PE) tubes as biofouling monitors (length 10 cm, diameter 0.65 cm). With PE-tubes, an average biofouling potential of 364±270 mg/m^2/day (n = 7) was measured with influent water containing 200 mg/l yeast extract at a HRT of 10 minutes. The maximum biofouling potential reduction was 69%. The PE tubes did not influence the protein analyses. Therefore, the PE tubes were chosen for the construction of biofouling monitors for use during the Denutritor pilot trials.

Table 7. Biofouling potential and reduction of simulated Perstorp WWTP water, measured with different types of biofouling monitors

Type of biofouling monitor	Yeast extract concentration (mg/l)	Range of biofouling potential (mg protein/m^2/day)	Maximum biofouling potential reduction (%)
PUR* foam	1000	300 - 2000	36
PUR* foam	200	400 - 1500	23
Silicon tube	200	200 - 500	87
PE** tube	200	150 - 850	69

*PUR = polyurethane
**PE = polyethylene

3.1.1.2 Food Industry

1st phase: Effect Denutritor on water quality

The quality of recirculated rainwater was analysed by monitoring chemical and microbiological parameters over time. Tables 8 and 9 summarize the data of chemical parameters and biofouling formation in the Reference and Denutritor systems.
The pH in both systems remained stable around 6. The concentration of anions and cations fluctuated somewhat over time, but after 133 days the concentrations for Reference and Denutritor system were comparable. The nitrite and bromide concentrations were below detection level in all analysed samples. Remarkably, the concentrations of the anions nitrate, phosphate and sulphate, and the cations manganese and calcium, decreased during the first 59 days, but were increased in both systems in the period between 59 and 133 days after start of the experiments, although absolute values were within the acceptable drinking water range. The increase of ion concentrations might have been caused by resorption of nutrients from precipitates present in the systems.

Table 8: Overview of chemical parameters and presence of bacteria in the recirculated rainwater during the incubation period

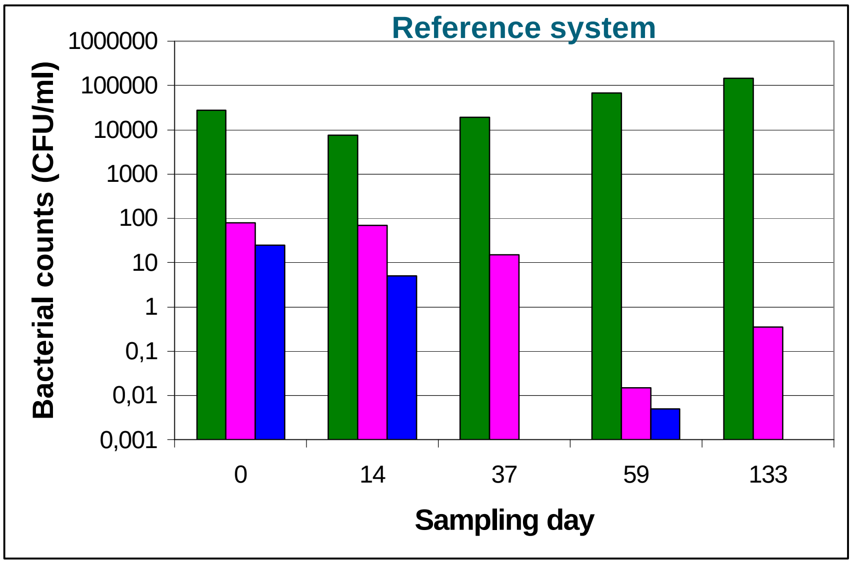
	T=0	T=14 days		T=37 days		T=59 days		T=133 days	
	Rainwater	Reference	Denutritor	Reference	Denutritor	Reference	Denutritor	Reference	Denutritor
pH	5,9	6,0	5,6			6,0	6,0	5,8	5,9
COD (mg/l)	20,0	23,0	25,5	21,4	21,8	20,9	18,5	25,4	23,5
TOC (mg/l)	11,4	11,1	11,6	10,5	10,6	10,9	11,1	11,2	11,5
AOC (ug Ac-C / l)	110							48	27
16S rRNA (gene copies / ml)	3,4E+05	7,4E+05	2,8E+05	1,0E+06	8,6E+05	3,8E+05	1,8E+06		
Anions (mg/l)									
Cl^-	5,0	5,0	5,0	5,1	5,0	5,6	5,8	6,0	6,3
NO_2^-	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
Br^-	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
NO_3^-	14,1	14,2	14,2	14,2	13,4	14,8	14,7	16,0	15,1
PO_4^-	0,7	0,6	0,8	0,3	0,3	<0,1	<0,1	0,5	0,6
SO_4^-	3,9	4,0	4,0	3,7	3,3	4,0	4,0	4,3	4,2
Cations (mg/l)									
Na^+	3,9	4,0	4,0	3,8	3,5	3,7	3,3	3,6	3,7
NH_4^+	0,2	0,2	0,2	<0,1	<0,5	<0,1	<0,5	<0,1	<0,5
K^+	4,3	4,3	4,4	4,5	4,5	4,2	4,5	4,5	4,6
Mg^{2+}	1,1	0,9	0,9	1,3	1,1	1,7	1,1	1,3	1,2
Ca^{2+}	14,2	16,9	18,5	13,3	15,6	12,2	15,2	21,7	20,3

Table 9: Biofouling potential, measured on the Biofouling Monitors (BMs) during the indicated period of incubation. D.o.I. = Days of Incubation

	D.o.I. = 14		*D.o.I.*= 23		*D.o.I.* = 22		*D.o.I.*= 74	
	Reference	Denutritor	Reference	Denutritor	Reference	Denutritor	Reference	Denutritor
Protein BM (ug/m2/day)			10,3	6,8	10,6	7,1	4,0	3,8
16S rRNA (gene copies / m2 / day)	7,1E+07		3,0E+06	3,4E+05	5,2E+06	9,7E+06		

The amount of proteins formed on the biofouling monitors (BMs) in the Denutritor system was >5% lower than in the Reference system after 74 days of incubation. After an incubation period of 23 days, the biofouling formation was 33% less in the Denutritor system compared to the Reference system.

Microbial plate incubations on R2A-agar showed after 14, 37 and 59 days a 2–4 times higher concentration of aerobic heterotrophic bacteria in the Denutritor system compared to the Reference system. However, after 133 days, 50% less aerobic heterotrophic bacteria were detected in the Denutritor system compared to the reference system. Coliform bacteria could be cultivated on chromogenic agar from the Reference water after 14, 37, 59 and 133 days of incubation and *E. coli* cells after 14 and 59 days. In contrast, *E. coli* could not be cultivated from the Denutritor system and coliform bacteria were only found after 59 and 133 days of incubation, at lower concentration than in the Reference system (Figure 5).



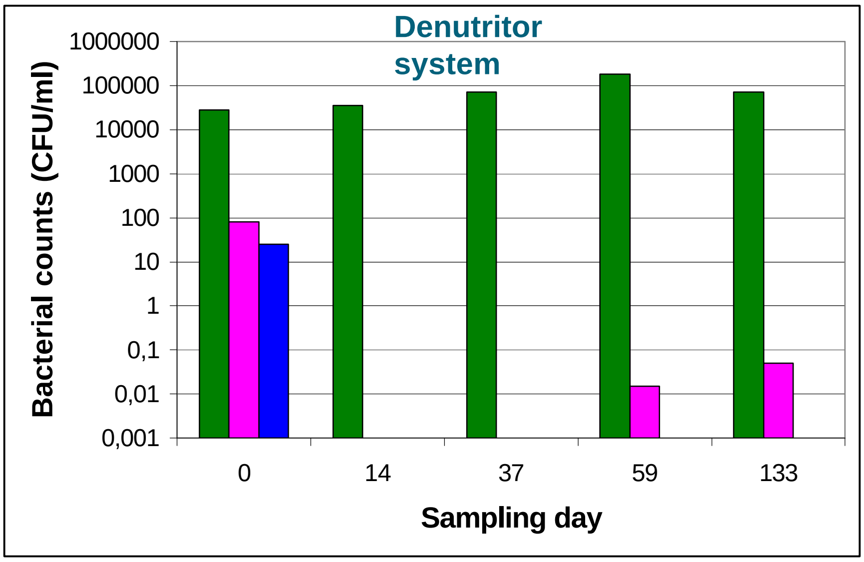


Figure 5: Cultivable aerobic heterotrophic (green bars), coliform (pink bars) and E. coli (blue bars) bacteria in the Reference and the Denutritor rainwater reservoirs during the recirculation period.

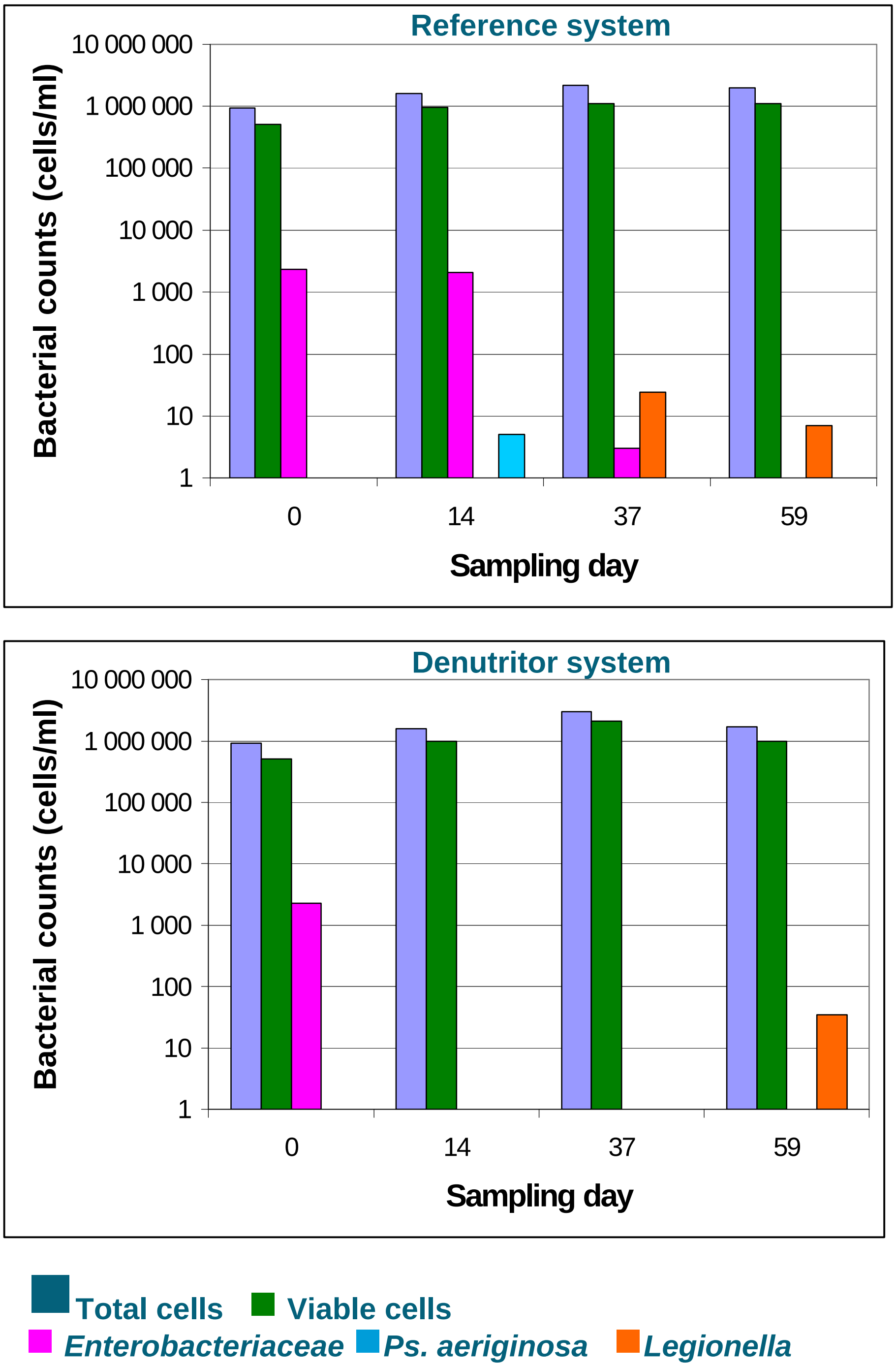
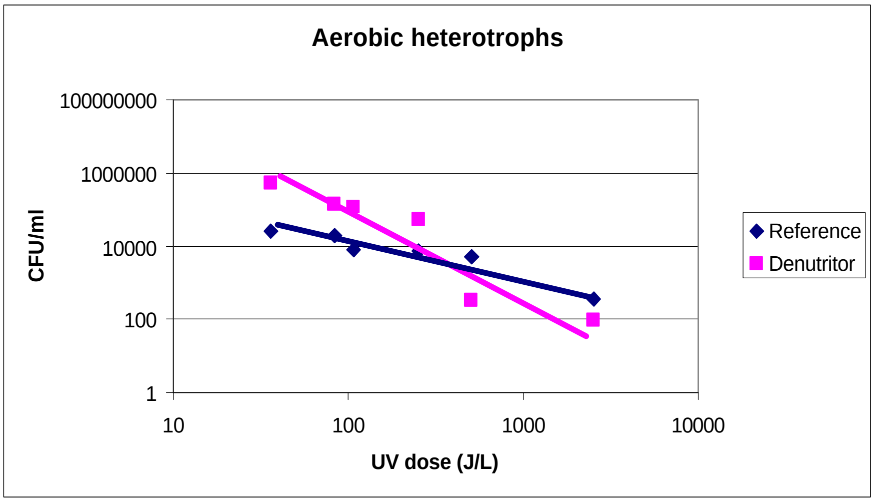


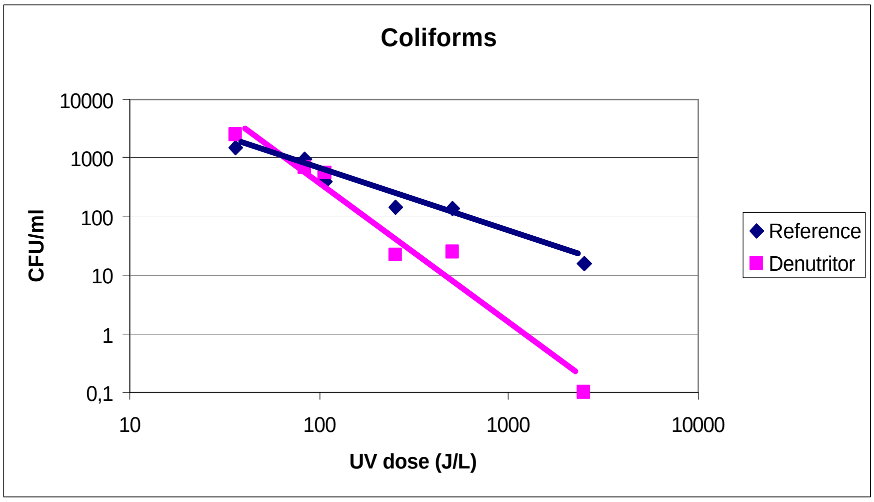
Figure 6: Detected (pathogenic) bacteria in the Denutritor and Reference rainwater reservoirs by FISH-analysis

The total amount of (viable) cells in the Denutritor rainwater reservoir was comparable to these cell numbers in the Reference rainwater reservoir during the incubation period of 133 days. However, in the Reference system higher concentrations and more different pathogenic bacteria were present (Figure 6). *Enterobacteriaceae* were present in the rainwater at t=0, but could not be detected afterwards in the Denutritor systems. In contrast, these organisms were detected in the Reference rainwater reservoir after14 and 37 days of incubation. *Pseudomonas aeroginosa* cells were detected in Reference system after 14 days, but not in the Denutritor system. *Legionella* cells were detected after some incubation time in both systems. However, in the Reference system, *Legionella* was detected after 37 days in the Reference system, while at that moment no *Legionella* was detected in the Denutritor system. Pathogenic bacteria which are relevant for microbial water quality in the food industry, but could not be detected in the tested rainwaters are: *Klebsiella pneumonae, Salmonella, Listeria,* and *Enterobacter sakazakii*.

2nd phase: Effect of UV-treatment on rainwater

In the second phase of the Denutritor laboratory experiments, the effluent of the Denutritor and Reference rainwater reservoirs were treated in a one-through flow with variable UV-exposures (in Joules per Litre rainwater).
In the Denutritor system, higher concentrations of aerobic heterotrophs and Coliform cells were identified at low UV-doses (Figure 7). However, with increasing UV-dose, the cultivability of these organisms, including *E.coli*, decreased more rapidly in samples from the Denutritor system than in samples from the Reference system. This indicates that at higher UV-doses, the treatment was more effective with water from the Denutritor system compared to water from the Reference system. The FISH-analysis did not show major changes in viable cell counts after the UV-treatment (Figure 8). This indicates that the current FISH method can not be used to discriminate between viable cells, and cells that are inactivated by UV treatment.





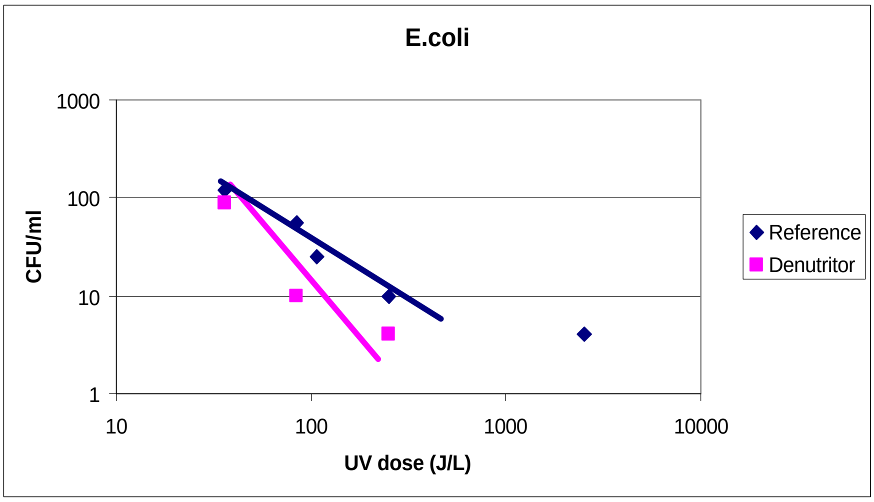
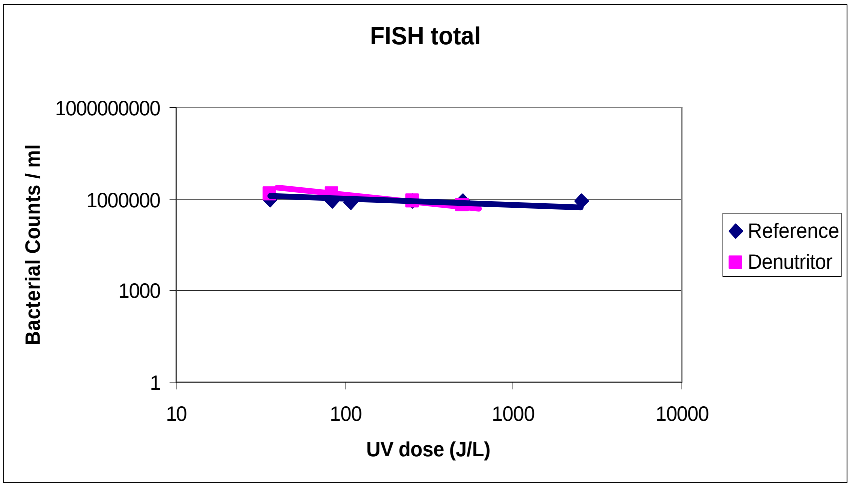


Figure 7: Concentration of aerobic heterotrophic, coliform, and E.coli cells analyzed by plate counting in the effluent of the Reference and Denutritor systems as a function of UV-dose.



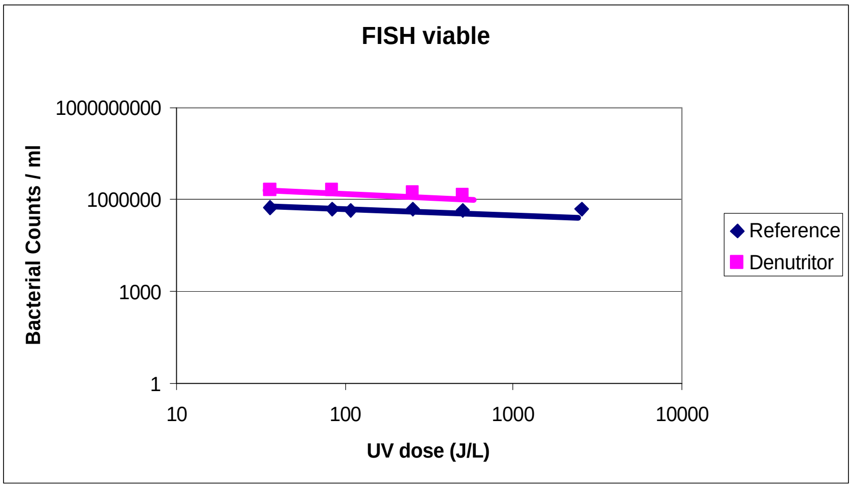


Figure 8: Concentration of bacterial cells (total and viable) in the effluent of the Reference and Denutritor systems at various UV-doses, analysed by FISH.

3.1.2 Nanosilver

The work was divided in two study topics. The first focused on the effect of nanoscale silver on the processes of aerobic and anaerobic wastewater treatment. The second dealt with the prevention of biofilm growth on plates which were prepared with an antimicrobial coating colour, based on nanoscale silver.

The nanoscale silver product was expected to show inhibitory effects on biological degradation processes. Nevertheless, the results show this effect to be limited. Properly used product (concentrations in the range up to 300 mg/l) showed no significant impact on the efficiency of COD elimination in the anaerobic and aerobic tests. Only concentrations of greater than 500 mg/l showed a slightly drop in the degradation rate and COD elimination.
The more sensitive respiration test was the only test in which total inhibition of the activated sludge activity at 1000 mg/l nanoproduct (100 mg/l nanoscale silver) was observed. Because soluble silver in this concentration also shows inhibition effects, this impact is not specific for nanoscale

silver. At lower product concentrations, only a concentration-dependent extension of the lag period was established.

Summarizing these results, nanoscale silver products in concentrations below 300 mg/l of product (30 mg/l Ag) show no evident influence on the processes of biological effluent treatment. Assuming only a fraction of the product used enters the wastewater, there is no danger to be expected by the use of nanoscale silver products.

The use of surface-bonded silver to prevent slime growth under the rough conditions of a paper machine was tested with painted steel panels. The results showed only a small effect. The antimicrobial effect of surface-bounded nanoscale silver seems to be too weak to keep the surface free of slime coating build-up.

Detection of nanoscale silver in process water

The detection of Ag nanoparticles generally encounters a problem with the methods for determining nanoparticles that are conducted with light scattering methods only describe the particle size, but not the chemical character of the particles. Another difficulty in determining nanoparticles in industrial process or effluent matrices arises from the fact that other particles with dimensions less than 1 micron are present in the matrix in addition to the particles to be measured, and the measured size distribution is multimodal. Since there is an exponential relationship between particle size and the measured intensity of the backscattered light to the power of 6, one must be careful to calculate the number weighted particle diameters when evaluating the measurements in order to suppress this effect to a large extent.

Model experiments with nanoscale silver in effluents have demonstrated that Ag nanoparticles in the effluent treatment process are adsorbed on the activated sludge in the biological stage. Although the DLS method is capable of detecting particles in the size of the particles used, the corresponding Ag concentrations, however, are below the detection limit of the chemical method.

No silver could be detected using ICP-MS in measurements of the silver content in process water from two paper mills. This confirms the fact that nanoscale silver is not being used on a large industrial scale at present.

General remarks concerning degradation tests

All degradation tests were performed using different dosages of nanosilver product ranging from 100 mg/l to 1000 mg/l product. The recommended dosage of nanoproduct was 100 – 250 mg/l product. The trials at higher dosages (>500 mg/l) were intended to show if any overdosage of the product (e.g. in case of an accidental release) would lead to major disturbances of the degradation processes. One test series was carried out without application of nanoscale silver and was used as reference series.

In anaerobic and aerobic degradation tests, effluents from a paper mill is used as the substrate and anaerobic pellet sludge from an UASB reactor or activated sludge from the aerobic stage are used as inoculums. The respiration test uses activated sludge as the inoculum and a BOD standard (D-glucose, L-glutamic acid) as the substrate.

Anaerobic test

The results of the anaerobic tests (see Figure) show no drastic reduction in COD elimination rates depending on the concentration of nanoscale silver. A slight lag in degradation rate and a lower degree of COD elimination (approx. 5%) were observed for the highest product concentration of 1000 mg/l. At concentrations below 500 mg/l nanosilver product, COD elimination is marginally smaller and seems to be in the range of experimental error (Graph and Tables in Annex B).

Zahn-Wellens test

The results from aerobic Zahn-Wellens tests (Figure 9) are similar to the results from the anaerobic test. Only at the higher dosages was there a slight decrease in elimination rates but at the end of the test after 21 days they both showed nearly the same COD elimination of 90%.

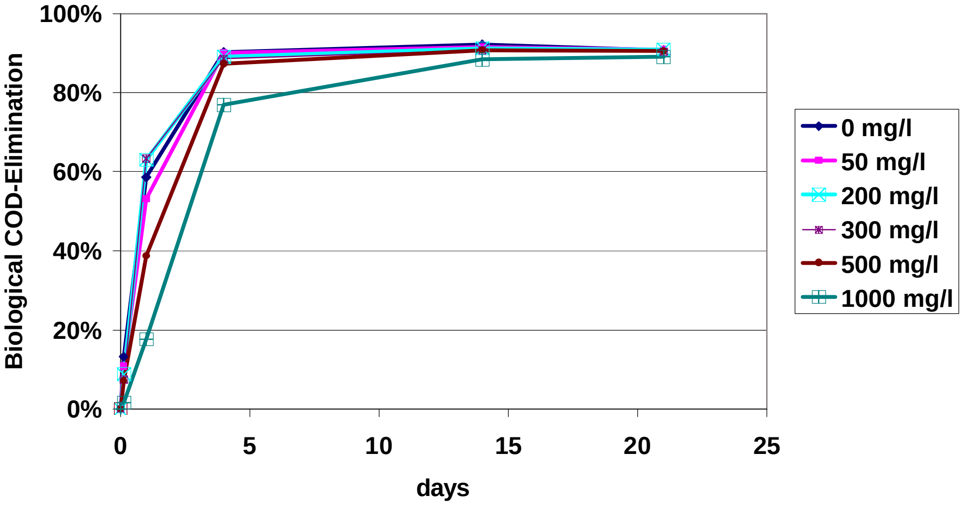


Figure 9: Aerobic degradation test (Zahn-Wellens test)

Photographs below shows selected batch tests at the beginning and at the end of the degradation test. From left to right, the vessels shown are the "reference" (0 mg/l silver), 200 mg/l and 1000 mg/l.

Figure 10: Batches at the beginning (left) and at the end (right) of the Zahn-Wellens test

The obvious effect of Ag dosage can be seen clearly as the coating colour changes to black.

Respirometric test

Respirometric tests were performed in addition to the aerobic degradation test. The test conditions of the respirometric test (Table10) were nearly the same as the Zahn-Wellens test. Especially the relation between nanoscale silver and the dry substance of the biomass was adjusted to the same value. The main difference was the initial sludge load which was 4½ times higher then in the Zahn-Wellens test.

Table 10: Test conditions

parameter	dimension

COD wastewater (BOD-Standard)	~360	mg/l
concentration inoculum	0.08	g/l
initial sludge load	4.5	g COD/g MLSS
N-dosage	1.3	mg/l NH_4-N
P-dosage	97	mg/l PO_4-P
Nano-Ag-product dosage	0 - 1000	mg product / g MLSS

The test shows the oxygen uptake during the elimination of organic substance (BOD standard). The target value was about 200 mg/l and was almost reached in most test runs (Figure 11). The results indicated only a very low influence of nanoscale silver addition at low doses (< 200 mg/g MLSS). Referring to the reference test, only a small extension of the lag phase was observed before the start of the degradation phase. The higher the dose, the longer this lag phase was. At the concentration of 1000 mg product per gram of biomass, total inhibition of respiration was observed during the test time of 100 hours.
These results are in accordance with the results of the aerobic degradation test, which pointed out that nanoscale silver in concentrations resulting from conventional use does not cause a disturbance of the biological treatment processes. However, this short-term trials cannot predict a possible bioaccumulation or long-term inhibition.

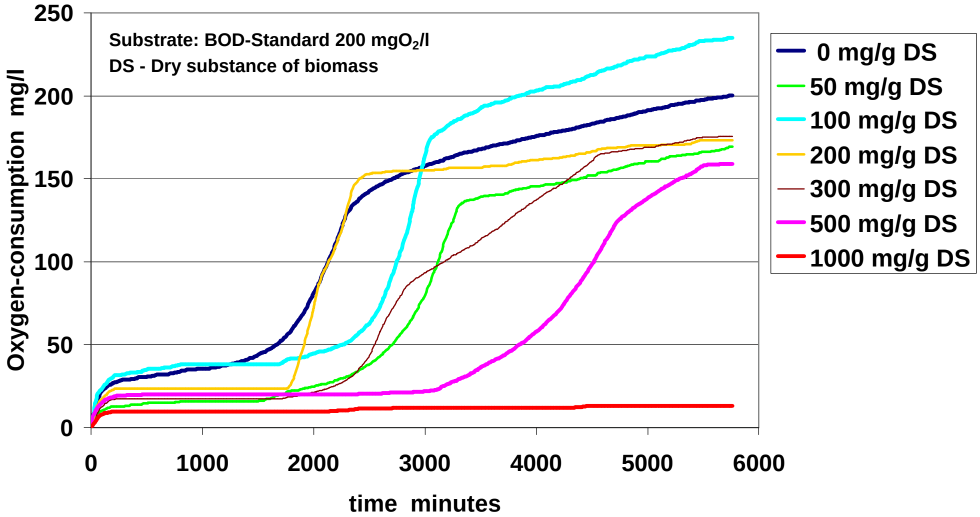


Figure 11: Respiration test run

Testing the effectiveness of surface-bounded nanoscale silver on surfaces

One of the most frequent problems in paper mills is the growth of biofilms in technical equipment. Therefore surfaces with antimicrobial properties to prevent biofilm growth could avoid many resulting problems. The use of surface-bonded nanoscale silver was tested with steel panels which were painted half with normal coating colour and half with nanoscale silver containing coating colour (compare chapter materials and methods). During the exposure time, the panel in the circuit water showed no growth of slime on either coating colours, with and without nanoscale silver.

The following images demonstrate the growth of biofilm and a mixture of fibre and slime on the panels, which were located at different places near the paper machine (wet, warm and misty) for 47 days.

Figure. 12: Growth of biofilm and a mixture of fibre and slime on the panels

Obviously the intensity of the growth of biofilm or slimes is very high and the effectiveness of surface-bonded nanosilver is too weak to prevent a build-up of slime. Furthermore, other principles beside biological growth may contribute to the development of slime and shield the surface-bonded silver.

3.2 Deviations from the DoW

Not relevant

3.2.1 Description of deviations from DoW

Not relevant

3.2.2 Reasons for changing work

Not relevant

4 Exploitation and dissemination of major results

4.1 Knowledge gained and products developed

4.1.1 Description of the knowledge gained and products developed

4.1.1.1 Denutritor

Knowledge gained:

- Denutritor treatment of WWPT effluent water with Denutritor can significantly reduce biofouling potential.
- Polyethylene tubes can be used to measure biofouling potential in a variety of water systems.
- By combining Denutritor with UV, the amounts of viable bacteria can be reduced.

Products developed:

- Laboratory Denutritor setups to test biofouling reduction with various water types
- Novel types of biofouling monitors for application in the laboratory and in different industrial water systems.

4.1.1.2 Nanosilver

The use of nanoscale silver to produce packaging materials with antimicrobial or bacteriostatic characteristics for food and non-food packaging is expected to gain increasing importance in the future. The amounts of nanoscale silver used for this purposes are not yet well known. Specifications by the manufacturer recommend concentrations of 1 – 25 ppm of nanosilver. Depending on the type of use (e.g. use in coating colours) nanoscale silver concentration in the paper product is usually low. The entry of nanoscale silver into the water circuits and wastewater is possible because of the intensive use of recycled paper containing nanosilver. So the real concentrations in the effluent should be low.

The investigations of the impact of nanoscale silver products on biological effluent treatment with anaerobic and aerobic biological test methods have shown that, when used in the dosages recommended by the manufacturer, they have no harmful effects on the biological processes used in effluent treatment. If, however, nanoscale silver products enter the effluent in concentrations > 30 mg/l active ingredient, this may disrupt the processes and reduce the efficacy of treatment. This, however, is only possible in the event of an accident or other instances of improper use.

4.1.2 Links to other Work packages

WP 1.2 Water Quality demands in different industrial processes
WP 2.1.2 Development of specific sampling tools to analyze biofilm and corrosion
WP 5.2.2 Optimization of water quality for different re-use options of treated effluent
WP 5.3.2 Technologies for sustainable water use

4.2 Dissemination

- The results of the laboratory tests are presented on national and international conferences and workshops.
- The results are reported in national and international journals and newsletters.
- An exploitation, dissemination and training plan for Denutritor has been written (Gerritse and Van Tongeren, 2010; Annex C)

- A presentation on the nanosilver experiments was given by R. Spörl to participants of the German seminar “biological wastewater treatment” on 19th October 2010 at PTS Munich
- The internal dissemination of the results of the nanosilver experiments was given by B. Simstich at the AquaFit4Use Project meeting on 20th October 2010 in Maribor

4.3 Exploitation

Denutritor
See exploitation plan Appendix C.

Nanosilver
In its capacity as an independent research and consultancy company, PTS is involved in virtually all areas of papermaking, converting and the associated processes of effectively utilising and conserving resources. The knowledge gathered in this project on the use of nanoscale silver and the possible risks and problems associated with its use will be exploited in the consultancy projects for the paper industry on questions relating to the use and environmentally sound handling of these products.

4.3.1 Patents

Not relevant

5 Partner contribution / progress of work

5.1 First period (month 1 – month 18)

5.1.1 TNO / Deltares

TNO/Deltares have conceived, coordinated and performed the Denutritor laboratory tests.

5.1.2 Perstorp

Perstorp has delivered information on the configuration and operational conditions of their WWTP and the composition of its influent and effluent water.

5.1.3 Unilever

Unilever has delivered information on the configuration and operational conditions of their cleaning facilities, and provided information on chemical and microbiological quality of utility water in food industry.

5.1.4 Vermicon

Vermicon has performed microbiological analysis (FISH and viability tests) on the water samples, and contributed to the interpretation of the laboratory results.

5.1.5 PTS

PTS conducted trials in lab scale relating to the impact of nanoscale silver on the processes used in biological effluent treatment. This involved the preparation, organization of sample transfer, performance of trials, evaluation of results, dissemination and preparation of reports (I3.2.3.1 and D3.2.1).
Both the impact on anaerobic processes (anaerobic degradation test) as well as on aerobic processes (Zahn-Wellens test, respirometric test) were conducted on a laboratory scale and evaluated.

5.1.6 HRT

The paper mill provided support in the studies by taking samples and providing test media. It provided support to PTS during the evaluation of the test results.

5.2 Second period (month 19 – month 36)

5.2.1 TNO / Deltares

TNO/Deltares have conceived, coordinated and performed the Denutritor laboratory tests, and coordinated and prepared Internal report I3.2.1.1 and, in collaboration with partner 5 (PTS), Deliverable report D3.2.1.

5.2.2 Perstorp

Not relevant

5.2.3 Unilever

Unilever has delivered information on the configuration and operational conditions of their cleaning facilities, and provided information on chemical and microbiological quality of utility water in food industry.

5.2.4 Vermicon

Vermicon has performed microbiological analysis (FISH and viability tests) on the water samples, and contributed to the interpretation of the laboratory results.

5.2.5 PTS

PTS conducted trials in lab scale and on mile-site at HRT relating to the impact of nanoscale silver on the processes used in biological effluent treatment. This involved the preparation, organization of sample transfer, performance of trials, evaluation of results, dissemination and preparation of reports (I3.2.3.1 and D3.2.1).

The materials (nano-Ag coating colour, steel plates and suspension devices) necessary to conduct the studies on the action of surface-bonded nanoscale silver were obtained and prepared for the tests in the paper mill.

5.2.6 HRT

The prepared steel plates were installed at the agreed upon positions in the splash zone of the paper machine and in the white water and were checked at regular intervals.

6 Conclusions

6.1 Major achievements

6.1.1 Denutritor

6.1.1.1 Chemical industry – Perstorp

- In laboratory tests with synthetic Perstorp WWTP effluent, it was demonstrated that Denutritor can reduce the biofouling potential of this water type by about 8-fold.
- Biofouling monitors made from polyethylene (PE) tubes were shown to be suited for use during the Denutritor pilot trials.

6.1.1.2 Food industry

Rainwater can contain a relatively high concentration of pathogenic micro-organisms, which should be removed before it can be used in the food-sector. Laboratory experiments with collected rainwater treated by Denutritor indicated that:

- Denutritor did not bring on big changes in total bacterial concentrations in the water
- Coliform and *E. coli* bacteria were reduced in Denutritor treated rainwater
- Pathogens are reduced by Denutritor, but some may remain present

When Denutritor treated water is exposed to specific UV doses before use:

- The amounts of viable bacteria can be reduced
- Lower UV-doses need to be applied for comparable disinfection results, than for untreated rainwater

The FISH-analysis currently used to identify the effectiveness of UV-disinfection did not discriminate viable and unviable cells.

6.1.2 Nanosilver

- Nanoscale silver in the production of special paper with antimicrobial properties is currently still being used only on the laboratory and pilot scale. For this reason, there is no experience regarding the amounts required on an industrial scale.
- Proceeding from the principal intended use of nanoscale silver in coating colours as an antimicrobial finish for paper surfaces, the only feasible way of incorporating nanoscale silver into the process water and effluents of the papermaking process is by slushing the broke or by using an appropriately finished recovered paper. Hence, the load of nanoscale silver in the effluent should be low assuming that the recommended amounts of 1 – 25 ppm are used.
- It was found in model experiments on anaerobic and aerobic biodegradability that concentrations of nanoscale silver <30 mg/l did not cause any impairment of these processes. Contents above this value, however, reduced process efficiency. Hence, when nanoscale silver is used properly, no disruptions of the effluent treatment processes are to be expected.
- Initial studies on the reduction of biofilm growth on surfaces with fixed Ag nanoparticles (nanosilver coating colour) had only a small effect. The test plates that were tested in the splash zone of a paper machine showed considerable microbial growth with a fibre/slime layer irrespective of the foundation. The microbicidal effect of the Ag-doped colour was too weak or it was shielded by the large amounts of fibre/slime mixture that were applied to the surface. Future studies should aim at clarifying the causes of the observed phenomena.

6.2 Future work

6.2.1 Within AquaFit4Use

The Denutritor and nano-silver lab-tests are finished and are concluded in this report.
Rainwater is treated in Denutritor pilot experiments that were started in January 2011, considering the results of the Denutritor labtests.
The gained knowledge will be shared with Aquafit4Use partners and disseminated (SP6).

6.2.2 General recommendations

6.2.2.1 Denutritor

Demonstrate Denutritor on pilot scale in different industrial water systems, in collaboration with a constructor of water treatment equipment and end-users.

6.2.2.2 Nanosilver

The studies have shown that, when the products were properly used, no environmental impacts were discovered under the conditions described here (use of Ag nanoparticles in coating colours and discharge into the water systems of a paper mill via the broke and recovered paper).
The studies on the use of surface-bonded nanoscale silver have demonstrated that, in view of the high exposure of the surfaces to shower water containing fibres and solids, no effect that might prevent the deposits from forming was detected. The tests were intended to be repeated under other conditions, also using other nanosilver-doped coating.

7 Literature

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8 Annex

A

Table 1: Composition of Perstorp WWTP influent and effluent water

Parameter	Influent	Effluent	Unit
AOX	0.44	0.3	mg/l
BOD(7)	940	<8	mg/l
COD	2900	180	mg/l
TOC	830	110	mg/l
Nitrogen-total	83	37	mg/l
Phosphorous-total	1.4	0.11	mg/l
Chloride (Cl)	840	950	mg/l
Sulphate (SO4)	620	580	mg/l
Calcium (Ca)	10.9	11.5	mg/l
Iron (Fe)	1.91	0.0402	mg/l
Potassium (K)	7.88	3.73	mg/l
Magnesium (Mg)	3.24	3.04	mg/l
Sodium (Na)	971	1080	mg/l
Sulphur (S)	207	196	mg/l
Aluminium (Al)	1010	79.5	µg/l
Arsenic (As)	16.8	<20	µg/l
Barium (Ba)	138	56	µg/l
Cadmium (Cd)	1.42	<0.05	µg/l
Cobalt (Co)	6.62	0.754	µg/l
Chromium (Cr)	67.2	4.73	µg/l
Copper (Cu)	18.4	1.44	µg/l
Mercury (Hg)	0.756	<0.02	µg/l
Manganese (Mn)	635	29.1	µg/l
Molybdenum (Mo)	61.9	75.7	µg/l
Nickel (Ni)	63.3	27.4	µg/l
Lead (Pb)	92.8	1.21	µg/l
Antimony (Sb)	1.33	0.852	µg/l
Zinc (Zn)	165	79.2	µg/l

Table 2: Composition of synthetic Perstorp WWTP effluent water

Parameter	Amount	Unit
Yeast extract	200 or 1000	mg/l
Sodium nitrate	100	mg/l
Sodium phosphate	2.2	mg/l
Sodium chloride	306	mg/l
Sodium sulphate	728	mg/l
Calcium chloride	8.3	mg/l
Potassium chloride	1.4	mg/l
Magnesium chloride	5.5	mg/l
Aluminium chloride	14	mg/l
Barium chloride	1.7	mg/l
Copper chloride	60	µg/l
Manganese sulphate	2.1	mg/l
Zinc chloride	33	µg/l

Annex B

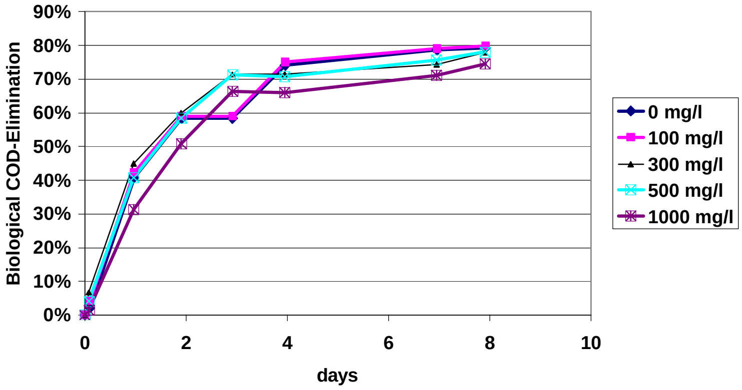

Figure 1: Anaerobic degradation test

Table 1: COD values of anaerobic test samples depending on time and Nano-Ag-conc.

	Concentration of Nano-silver-product				
	0 mg/l	100 mg/l	300 mg/l	500 mg/l	1000 mg/l
Time [days]	COD mg/l				
0	1820	1893	1897	1963	1926
0.10	1780	1813	1769	1883	1897
0.98	1078	1092	1045	1162	1323
1.92	758	775	758	815	947
2.92	758	775	543	564	647
3.96	472	472	541	574	653
6.96	388	397	486	476	555
7.92	377	381	421	430	489

Table 2: COD values of aerobic test samples depending on time and Nano-Ag-conc.

	Concentration of Nano-silver-product						
	0 mg/l	50 mg/l	100 mg/l	200 mg/l	300 mg/l	500 mg/l	1000 mg/l
Time [days]	COD mg/l						
0	1930	1930	1930	1930	1930	1930	1930
0.125	1674	1722	1729	1761	1786	1792	1898
1	798	904	793	713	706	1183	1589
4	187	190	192	209	221	244	445
14	152	163	161	168	177	177	223
21	177	177	179	174	186	183	211

Annex C

Exploitation, Dissemination and Training plan Denutritor

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Date: December 2010

1. General description of the product

Denutritor is a biofilter, which reduces the biofouling potential of water. The water to be treated is lead through columns with a synthetic filler material with high porosity and surface area, on which microbial populations grow in biofilms (Figure 1). The microorganisms in these biofilms degrade organic substrates, which are dissolved in the water and use nutrients and other substances for their growth. So the source of biofouling is removed, and the potential for growth of biofilms is reduced.

Figure 1: Denutritor pilot installation

Denutritor can be applied for different types of biofouling problems. As the process results in the growth of biofilms in the filter, the COD level of the water to be treated should not be too high. In general a maximum COD of 10-25 mg/l is applied. Particles can plug the filter, so if the level of particles is too high a pre-filtration can be needed. On the other hand can the Denutritor also act as a filter for low concentrations of small particles.

Biofouling prevention has a number of advantages. Besides high capacities and operational stability of membrane systems, better heat transfer in cooling water systems, less biofouling also results in less (bio)-corrosion and damage on pipe materials.

The focus in the development of Denutritor within the AquaFit4Use project was on biofouling prevention in membrane systems and cooling water systems and on water re-use in general. In these applications currently a lot of chemicals (organic biocides, chlorine, ozone, hydrogen peroxide) are used to prevent biological growth. Besides, cleaning of the equipment is needed frequently to remove the fouling, which leads to reduced production times. Biofouling also forces to an increased bleed in cooling water systems.

2. Target market

General

As described above Denutritor has a very broad application area. In 2006, the costs of fouling in industrial nations was estimated at 0.17% to 0.25% of their gross domestic product (GDP) (http://en.wikipedia.org/wiki/Fouling). Biofouling in industrial water systems significantly contributes to these costs. Denutritor can be used for treatment of all types of waters with low COD and particle content and a moderate to high biofouling potential. To bring the product to the market the focus will be on two types of applications:

1. Biofouling prevention in membrane systems
2. Biofouling prevention in cooling water systems.

For these two applications successful pilot tests have been carried out in different sectors.

Both target markets are very big. Worldwide membrane filtration installations for water production with a total capacity of 55 million m^3/day are built. The capacity of RO desalination plants worldwide is over 2 million m^3/h.
Also the application for cooling water systems is very broad, both industrial cooling water systems and power plants. A very rough first indication, in America 60 million m^3/h cooling water is used in and in Europe about 40% of the fresh water use is in manufacturing industry and for cooling in energy production.

Sectors

Because of the broad application Denutritor can be applied in different sectors:

- Industry (esp. chemical)
- Drinking water production
- Process water production
- Energy production

First conclusion: There is a stable big market for biofouling reduction in Europe and North America and growing markets in Asia (China, Singapore, etc.) and South America.

Drivers

The main drivers for application of Denutritor are

- Costs
- Legislation
- Water scarcity, water re-use

Besides Denutritor has environmental advantages preventing or reducing the use of chemicals. Within the framework of Corporate Responsibility this can also be an important driver.

Potential competitors

- Biofiltration systems for these applications are sand filters and active carbon filters (e.g. Paques, Norit). Comparable systems for Denutritor are currently not on the market. The potential niches for Denutritor-like systems need to be further explored.
- Suppliers of Waster Chemicals: NALCO, GE-Water, Ciba Kurita, etc.
- Suppliers of AOP systems (Ozone, peroxide, UV): most of the equipment suppliers (Veolia, Suez, Paques, etc.)

Sustainability effects
The application of Denutritor has different sustainability effects like
- water re-use
- less chemicals
- less corrosion
- less cleaning stops
- less pathogens

Customers
- End-users (see above)
- Suppliers of Chemicals and services
- Equipment suppliers

From the project
- Developers: TNO/Deltares, Perstorp, Unilever,
- Interested partners from the project: Veolia, Logisticon, ATM, Enviro-Chemie,

Outside the project
- Big industries (DOW, Chorus, Akzo, Henkel, ..)
- Energy producers
- Suppliers (Chemicals, services); NALCO, GE,
- End-users: DOW, BASF, Solvay, DuPont, Enz
- Equipment: Suez, etc.

3. Development of the exploitation plan

For the further exploitation of the Denutritor technology the next steps have to be carried out:

1. Further elaboration of the potential market

See above. However the market is very broad a further specification of the market in different applications should be made. Part of this is also a SWOT analyses

2. Production of a fact-sheet/ leaflet.

Two different leaflets will be made focusing at the two main topics in relation to biofouling: Cooling water and membrane systems

3. Selection of partners

Potential partners: Supplier of Chemicals and services, possibly in combination with an equipment supplier. TNO will play a role during demonstration and testing for new applications

4. Development of a few demonstrations (with the help if CIP Eco-Innovation?)

The pilots in AquaFit4Use could probably lead to a first demonstration on full-scale at one of the partners (Unilever, PSP, …..).

5. Additional promotion activities
   - Dissemination at next conferences: …
   - Poster sessions
   - Article in Process technology and membrane magazines …
   - EU-sites
6. Full-scale demonstration of Denutritor technology

Timing

- Activities during the project (Steps 2, 3, 4 and 5; see above)
- After the project (Steps 1 and 6; see above)