BONUS OPTITREAT 1 February 2014 - 31 January 2017
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1 Goals and results envisaged at the beginning of the project cycle

Onsite wastewater treatment is of a great concern due to related phosphorous and nitrogen loads contributing to the eutrophication of the water environment. According to the fifth Pollution Load Compilation of the Baltic Sea, scattered dwellings (houses not connected to wastewater treatment plants) contributed ca. 15% of the man-made nitrogen and phosphorous load ending into the Baltic Proper. The onsite wastewater load of pharmaceuticals, household related chemicals and possible transfer of antibiotic resistance into the soil and aquatic environment is a further concern. Although there are numerous technical alternatives for onsite wastewater treatment on the market, removal of emerging substances had been sparsely investigated by the beginning of BONUS OPTITREAT. However, onsite wastewater treatment facilities are relatively cheap constructions and if optimised to mitigate wastewater pollution, they could offer cost-efficient alternatives to wastewater treatment plants. In remote areas, onsite wastewater treatment is the only alternative.

BONUS OPTITREAT aimed at increasing knowledge and understanding of removal efficiencies in onsite wastewater treatment available on the market in the Baltic Sea region. The project set out to achieve this through a holistic assessment of: microbiological characteristics, physical and chemical parameters , removal of nutrients, pathogens, pharmaceuticals, personal care products, and development of antibiotic resistant bacteria. The project envisaged that the increased knowledge on the parameters controlling the removal efficiencies of a large number of pollutants with different physical and chemical properties and antibiotic resistance, would promote development and optimization of the efficiency of small onsite wastewater treatment systems on the market.

The project aimed further to explore the policy tools to regulate the maintenance of the facilities, which is a crucial factor to ensure well-functioning onsite wastewater treatment. based on it's findings, the project suggested recommendations on relevant regulations and support actions. The results were summarized in a benchmark best practice and a desktop study of the policy lessons learnt on maintenance. The project disseminated the results to maintenance and manufacturing SMEs, municipal and governmental stakeholders in the participating countries as well as to the research community.

2 Work carried out in the project

Tests were performed with three different techniques available on the European market for small wastewater treatment facilities. The tests were setup in a natural, full-scale controlled environment at the European test center for CE-branding of certified small onsite wastewater treatment facilities in Aachen, Germany (Figure 1).

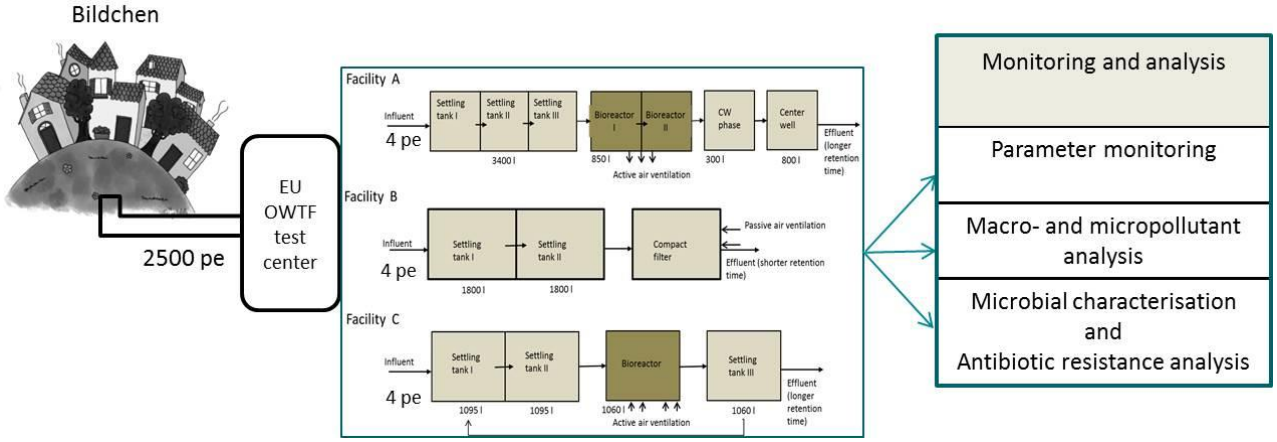

Figure 1. Illustration of the test setup of onsite wastewater treatment failities (OWTF) within BONUS OPTITREAT.

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The three onsite wastewater treatment technologies included in BONUS OPTITREAT use techniques that are abundant on the market: 1) Fixed bed reactor, 2) Trickling filter / Biofilter system, 3) Aerated filter system with fluidized biofilm bed and active sludge process

The test center receives household wastewater collected from about 2500 person equivalents in Bildchen, a suburb of Aachen in Germany, and the wastewater was dosed continuously on the wastewater treatment facilities at their optimal capacity of 4 person equivalents. The tests were first performed according to the EN 12566 testing for CE-branding; 38 weeks' test cycle, and then targeted tests were performed to assess the effect from hydraulic retention time (under- and overloading), power breakdown (oxygen deficit) and different temperature.

Monitoring, analysis and evaluations were performed on influent and effluent wastewater regarding control and functioning parameters as well as macro- and micropollutants (Figure 1). The monitoring and analysis included the following: the control parameters pH, oxygen and temperature; function parameters turbidity, conductivity and settleable solids; the macropollutants total phosphorous, total nitrogen, ammonium and chemical oxygen demand; and the micropollutants included a selection of 32 substances)i.e. pharmaceuticals, hormones and phenolic utility substances, plus analysis in synoptic samples on a set of substances within the household-related chemical group “organophosphates”).

The objectives of the microbiological evaluations were to characterize the microbial abundancy and diversity, antibiotic-resistance bacteria and to find effective biological transformation of antibiotic by microorganisms. Microbial characterisation and antibiotic resistance were analysed in the influent and the effluent wastewater and additionally also on the biofilm within the facilities. Methods for determining microbial functioning by traditional microbiological methods and modern techniques (Biology system and fatty acid analysis) as indicators in wastewater treatment were evaluated. Methods for determining antibiotic resistant bacteria and genes in wastewater were evaluated by the disk diffusion method and DNAs extracted from selected bacteria using the PCR primers specific for the antibiotic-resistance genes. Batch experiments of degradation of the antibiotic norfloxacin by selected bacteria was performed to give new insights to possible future microbial design within wastewater treatment techniques.

In addition to the tests performed in the BONUS OPTITREAT project, over 200 facility test results produced during earlier CE-branding tests at the European test center were included in a benchmarking synthesis to find optimal removal efficiencies for macropollutants. Further, laboratory batch experiments were performed on degradation of antibiotics by bacteria.

BONUS OPTITREAT performed a benchmarking of the project findings towards research literature and earlier test results. The benchmark report was compiled gathering all the results and practical experiences regarding small wastewater treatment facilities performance and control from the BONUS OPTITREAT partners. BONUS OPTITREAT also carried out a survey of regulations and maintenance situation and lessons learnt in Germany, Poland and Sweden. Finally, transfer of knowledge of benchamarking of best practice regarding small wastewater treatment efficiencies was an important part of the project. Stakeholders including water management authorities, municipalities and SMEs were invited to take part in a dialogue with BONUS OPTITREAT.
Project partners in BONUS OPTITREAT were: IVL Swedish Environmental Research Institute in Sweden, PIA-Prüfinstitut für Abwassertechnik GmbH and PIA RWTH Aachen University, Germany and the Institute for Ecology of Industrial Areas (IETU), Katowice, Poland .

PIA Institute for Ecology of Industrial Areas ivl

The project setup a website where all publications arising from the project can be found https://optitreat.ivl.se.

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3 Main results achieved during the project

1 The BONUS OPTITREAT project recommends application of national regulations towards a management control and quality assurance systems, including installation and maintenance of onsite wastewater treatment facilities, similar to the system adopted in Germany.

In Europe, around 20 million onsite wastewater treatment systems are expected to go into long-term operation. This BONUS OPTITREAT project promoted development of the efficiency of small wastewater treatment techniques already available on the market in the Baltic Sea region. If planned, designed, installed, operated and maintained correctly, onsite wastewater treatment systems can be seen as low cost and long-term alternative of wastewater treatment. The BONUS OPTITREAT project however points at many installation failures and what to avoid during installation and operation. Malfunctioning during operation of small onsite wastewater treatment systems can be avoided by a regular and preventive maintenance by trained personnel (from certified companies).

2 The physical and chemical properties of the micropollutants determine much of the fate of the substances during wastewater treatment. The octanol-water partitioning coefficient, biodegradability, water solubility coefficient and acid/base characteristics of the substances show large effects on the removal efficiency.

Wastewater is a major transport pathway of the chemicals used in people's everyday life to the environment, and household wastewater thus contains many of the chemicals on the household market. However, in order to addressprocesses of wastewater removal efficiencies, selection of relevant focus substances to investigate, needed to be done. The selected substances in BONUS OPTITREAT were chosen due to the earlier frequency of detection in wastewater during the Swedish chemicals screening programme, their differences in octanol-water partition coefficient, water solubility and acid-base characteristics as well as aspects of sampling and handling of samples.

Several studies in onsite wastewater treatment facilities show dependencies of higher removal efficiency with higher octanol-water partitioning constant, thus increasing the affinity of substances to sorb to sludge or particles. The substances that are more biodegradable are removed when microbial activity is promoted in the facilities. The acid-base constant is important for the removal efficiency through sorption processes, showing lowest removal efficiency for neutral substances, as the well-known calcitrant pharmaceutical carbamazepine.

3 Onsite wastewater treatment facilities are a pathway of pharmaceuticals, hormones and phenolic utility substances as endocrine disruptors into the soil and aquatic environment. Some substances in the effluent were above critical concentrations and may cause adverse effects on fish.

Thirty micropollutant substances were detected in the effluent wastewater out of 32 substances of pharmaceuticals, hormones and phenolic utility substances that were included. The pharmaceutical simvastatin, and the hormone substances estrone, estradiol and ethinylestradiol were found in the effluent at concentrations above the indicative critical effect concentration of pharmacological effects on fish and diclofenac was found at concentrations above the Swedish freshwater Environmental Quality Standards. Thus there is a risk of adverse effects from these substances in the aquatic environment. However the effluent will most likely be diluted in the environment.

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4 The construction of the treatment facilities is important for the removal of both macro- and micropollutants. The tests of the onsite wastewater treatment facilities verified that the active sludge construction was more efficient in nitrogen and hormones removal than the fixed bed and trickling filter.

The BONUS OPTITREAT results on the test facilities together with the 200 earlier test results verified known effects on the improved removal efficiency of phosphorous in facilities that applied a high pH phosphorous filter, and higher removal efficiency of nitrogen in the facility that applied active sludge technique than the fixed bed and trickling filter techniques. The BONUS OPTITREAT project showed further that the onsite wastewater treatment facility using active sludge processes was more efficient in removing hormones than the other two facilities using biofilm treatment techniques. This results verified findings shown earlier for large wastewater treatment plants.

5 The onsite wastewater treatment facilities on the market can be adjusted for parameters serving for more efficient sorption and /or biodegradation of micropollutants from microbial activity. The onsite facilities would be more efficient in removal of pharmaceuticals and hormones by increasing the hydraulic retention time.

Simulations performed of the theoretical hydraulic retention time and tests performed in practice on the BONUS OPTITREAT three facilities showed that the real retention time was significantly shorter than in theory. This result reveals that the facilities had reactor volumes that were not reached by the wastewater during the treatment process which led to less efficient removal of pollutants. Tests of the effect on the removal efficiency of pharmaceuticals and hormones as a response to the increased hydraulic retention time showed a positive linear relationship. The micropollutants were more efficiently removed with increased hydraulic retention time, which is illustrated in figure 2 as an average of all substances. This implicates the importance of the biodegradation processes in the facilities.

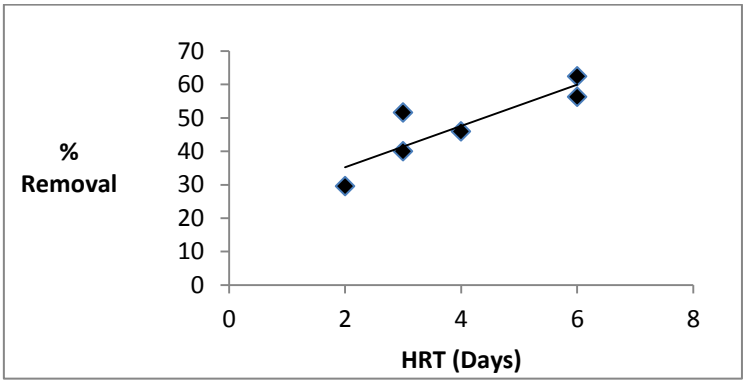

Figure 2. Illustration of the linear relation between the removal efficiency to the hydraulic retention time (HRT) on average for all pharmaceuticals and hormones included in BONUS OPTITREAT.

Oxygen supply is considered one of the most important parameters for efficient removal of both macropollutants and micropollutants due to the effect on microbiological activity. Surprisingly the OPTITREAT results show no significant difference in the removal efficiency of pharmaceuticals during power break tests. Possibly the number of samples was too small to give significant differences or the facilities are robust to shortterm power breakdown.

It is a well-known fact that removal of nitrogen is less effective in cold climate. BONUS OPTITREAT showed further a tendency of increased removal efficiency of many pharmaceuticals at increased temperature (5 degrees Celsius difference), but the increase was not found significant.

The active surface area would be very important for substances prone to sorption removal processes. Very few studies have addressed active surface area in studies of removal efficiency of micropollutants. The BONUS OPTITREAT results showed higher removal efficiency in the facility with smaller theoretical surface area, which may indicate that biodegradation was more important than the sorption removal processes in the test facilities.

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6 Integrating microbial ecology in the design and operation of onsite wastewater treatment is important. The most predominate group of bacteria responsible for organic and nutrient removal was Proteobacteria (21-65%) in which β-proteobacteria was the most abundant class.

The onsite small wastewater treatment facilities represent co-existing microbial populations in wastewaters that change with reactor operational conditions. Integrating microbial ecology in the design and operation of onsite wastewater treatment is important. It allows for better prediction of microbial community assembly and possible variations in the community structure and function in response to environmental changes. Biological interactions are dominant drivers in determining the bacterial community assembly in on-site wastewater treatment facilities whereas environmental conditions explain phylogenetic and quantitative variances and indirectly influence bacterial assembly.

7 The Biolog EcoPlates tests show a strong potential as an effective ecological indicator of changes in ecosystems. The results confirmed that metabolic profiles could be used to monitor treatment processes as valuable biological indicators of onsite wastewater treatment technologies efficiency.

Biolog EcoPlates are useful in differentiating between microbial communities, in the determining factors that most influence the separation of these communities and in identifying which substrates were most utilized by the communities. Community-level physiological profiles (CLPP) successfully discriminated among the microbial communities present in raw and treated wastewaters from different technologies. This information is crucial to understanding the physiology of microbial communities in the various environments. The results obtained confirmed that metabolic profiles could be used to monitor treatment processes as valuable biological indicators of onsite wastewater treatment technologies efficiency. This is the first step toward understanding relations of technology types with microbial community patterns in raw and treated wastewaters.

8 Development of more effective technologies including microbial design, which can degrade antibiotics in the wastewater treatment, is necessary. Three Bacillus subtilis species have the specific properties of biofilm formation and production of biosurfactants and enzymes, and in a cometabolic culture they were shown to be able to degrade norfloxacin.

It is widely known that occurrence and fate of antibiotics in the environment may lead to the development of antibiotic-resistant bacteria. Development of more effective technologies which will prevent the propagation of antibiotic-resistant bacteria and antibiotic-resistance genes is necessary. The knowledge of norfloxacin degrading microbial community can aid in our understanding of the biological process of norfloxacin dissipation. One of the crucial factors for immobilization of bacteria is biofilm formation ability. Characterization and determination of several bacterial features like cell wall properties or autoaggregation are also valuable for effective whole cell immobilization and degradation studies. In conclusion, three Bacillus subtilis species have the specific properties, e.g. production of lipopeptide biosurfactants and various enzymes, biofilm formation, and their consortium was able to degrade norfloxacin in the cometabolic culture. The results obtained from the biodegradation experiment are promising. To our best knowledge, there are no other available reports on the norfloxacin biodegradation by the strains which produced biosurfactants. Moreover, the role of biosurfactants in antibiotic degradation is still unknown.

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9 **The onsite wastewater treatment facilities are a source of a large pool of bacteria resistant to various antibiotics.**

The disc diffusion method and phenotypic microarrays were found to be appropriate tools for the evaluation of multiantibiotic resistance in environmental bacteria.

47% of the bacterial strains in the BONUS OPTITREAT facilities were resistant to more than 5 antibiotics. The highest percentage of multi-antibiotic resistant bacteria were in the biofilm carrier samples.

The widespread emergence of antibiotic resistance, particularly multi-antibiotic resistance, among bacterial strains has become one of the most serious challenges in environmental protection. Environmental bacteria have been shown to be a reservoir of antibiotic resistance genes and a potential source of novel resistance genes in the environmental organisms. The antibiotic susceptibility was evaluated in onsite wastewater treatment facilities within BONUS OPTITREAT. The bacterial strains were analysed for multiple antibiotic resistance. About 20% of tested bacteria showed a 2-5 MAR (i.e resistance from 2 to 5 antibiotics of the 37 antibiotics tested), while 47% were resistant to more than 5 antibiotics. Two species belonging to the Stenotrophomonas (S. maltophila and S. rhizophila) and Variovorax paradoxus were resistant to 40 antibiotics. The highest percentage values of antibiotic resistant bacteria were in the biofilm carrier samples providing a high active surface for growing of microorganisms. The scientific literature on the antibiotic resistance of bacterial communities from small wastewater treatment facilities is limited.

10 **The evaluation of the environmental risk of multi-antibiotic resistance (MAR) needs further attention. The mechanisms that allow resistance genes to migrate from non-pathogenic to pathogenic bacteria are unknown and information regarding resistance of environmental bacteria is still very fragmented.**

The results presented in this project have proven that onsite wastewater treatment plants are sources of antibiotic resistant genes and antibiotic resistant bacteria spread in the environment, e.g. lakes, rivers, soils, sediments, but the mechanisms that allow resistance genes to migrate from non-pathogenic to pathogenic bacteria are unknown and information regarding resistance of environmental bacteria is still very fragmented. Thus the evaluation of the environmental risk of MAR needs further attention.

4 The continuity plan of the project

BONUS OPTITREAT has contributed significantly to the development of new methods of microbiological assessments and antibiotic resistance determinations, as well as facts about development of antibiotic resistance within onsite wastewater treatment facilities. Distinct promising results have been identified of simple ways to improve removal of macropollutants, pharmaceuticals and hormones as well as technological promising results of biosurfactants degradation of antibiotics in onsite wastewater treatment facilities.

There is a lack of investigations on the occurrence and effects of multi-antibiotic resistance caused by onsite wastewater treatment plants in the environment. The evaluation of the environmental and human risk of multi-antibiotic resistance spread from onsite wastewater treatment needs continued research due to the unknown transfer mechanisms between bacteria. Research and development is further needed to increase the use of microbial design in onsite wastewater treatment systems. That could preferably start to increase knowledge of the processes of biosurfactants degradation of antibiotics and other micropollutants.

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