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TITLE:	Enhanced degradation of organic micro-pollutants in aerobic post-treatment of digested sludge
TOPIC:	Master Thesis
PERIOD OF WORK:	1. September 2008 to 15. June 2009
SUPERVISOR:	Dr. Kai Bester

ABSTRACT:

Organic micro-pollutants (e.g., fragrances, bactericides, pharmaceuticals, and flame retardants) can be detected in surface waters, outlet from the WWTPs and sludges [3-6], which proves the ineffectiveness of existing water purification technologies in removal of these compounds. The compounds are frequently used in personal care products, plastic and textiles applications. Some have been classified as a priority pollutant, suspected to have mutagenic, carcinogenic or toxic effects [1, 2].

Sorption to the sludge is often the major removal mechanism for the organic micro-pollutants from the WWTPs, due to the high octanol-water partition coefficient K_{ow}. The presence of the organic micro-pollutants in the sludge has recently been target of growing interest regarding the safe disposal of the sludge.

The focus of this study was to investigate the enhanced biodegradation of organic micro-pollutants in the digested sludge under aerobic, anoxic and anaerobic conditions. For this reason, lab-scale sludge reactors were employed. Additionally an aerobic treatment of the digested sludge from Ejby Mølle WWTP was investigated in order to remove organic micro-pollutants, decrease the amount of the sludge and hence save money for the disposal. For these purposes the compounds were extracted from the sludge by liquid-liquid extraction (LLE), condensed in toluene to 1 mL and quantified by use of gas chromatography - mass spectrometry (GC–MS).

The most rapid elimination for most of the examined organic micro-pollutants in activated sludge was observed under the presence of oxygen. The highest removal rates were observed for the light PAHs, OTNE, TnBP and DEHP, intermediate removal rates for triclosan, HHCB and some of the heavy PAHs, while low or no removal was observed for AHTN, nonylphenols and transformation products HHCB-lactone and triclosan-methyl in activated and digested sludge. Moreover, a test on a possible performance of an existing tank at Ejby Mølle WWTP was made. The calculations showed that after the available retention time during the aerobic treatment in the existing tank all the compounds' concentrations would be below the limit except the concentration of nonylphenol isomers, which is far above the Danish cut-off value.

Preface

The Master Thesis: *Enhanced degradation of organic micro-pollutants in aerobic post-treatment of digested sludge* was written at Aalborg University for attainment of the academic degree of **Master of Science** by Karolina Furgal.

The references in this project are given in numbers [xx]. The reference is put before a dot. As a supplement to the report a CD has been attached. On the CD all data and calculations can be found along with a PDF file of the project.

My deep appreciation goes to Dr. Kai Bester for his enthusiastic supervising and dedication through the whole project. It goes without saying that without his guidance and support this study would not have been possible.

I would like to thank PhD. David Cecil and Odense Vandselskab for giving me the opportunity to carry out experiments at Ejby Mølle WWTP, which significantly contributed to this project.

Aalborg, 15 June 2009

Karolina Furgal

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1. Introduction

Organic micro-pollutants like fragrances, bactericides, pharmaceuticals and flame retardants are frequently used in personal care products, plastic and textiles applications. Some of them have been classified as priority pollutants, suspected to have mutagenic, carcinogenic or toxic effects [1, 2]. They were detected in surface water, outlet from the wastewater treatment plants (WWTPs) and sludges [3-6] which proves the ineffectiveness of existing water purification technologies in removing organic micro-pollutants. Moreover, the presence of these compounds in the sludge raises concerns about its safe disposal. Current knowledge of the fate of organic micro-pollutants after a release to the environment is scarce. Only very few studies has investigated forms of organic contaminants appearing in soils, sediments and the toxicity determined by these compounds [7]. There are no studies taking the fate of emerging organic micro-pollutants and their forms in sewage sludges into account. Furthermore, the knowledge about the total xenobiotics content does not always provide sufficient information regarding the possible risk. There are some concerns about the transformation products of organic micro-pollutants that could possibly be more persistent or toxic than parent compounds [3, 8]. However, only the parent compounds are regulated.

The focus of this study was on the organic micro-pollutants that tend to sorb to organic matter and can be detected in the sludge as an end point after the water treatment. An attempt was made to develop a method to remove these compounds and hence to enable a safe disposal of the sludge. Therefore, a degradation experiments were performed to gain insight into the removal of the organic micro-pollutants from the sludge. An effort was made to investigate behaviour of the organic micro-pollutants, the environmental factors and crucial parameters influencing their removal.

The term “degradation” in this study was avoided, but “elimination” and “removal” was used if the concentration of the compound was decreasing, but the reactions are unknown. Term “transformation” was used when the conversion of an unknown degree of the parent compound to another has occurred.

1.1 Activated-sludge treatment

The activated-sludge treatment is a biological method for treating wastewater that utilizes a variable and mixed community of microorganisms. In brief, these microbes convert carbon to cell tissue and to carbon dioxide and water. Some of these microorganisms are able to obtain energy from oxidizing ammonia nitrogen to nitrate nitrogen in nitrification process. The basic activated-sludge treatment consists of three main parts: a reactor, where microorganisms are suspended and aerated, liquid-solid separation unit (clarifier) and a recycle system for sludge to return to the process. *Figure 1* illustrates activated-sludge treatment with plug-flow reactor.

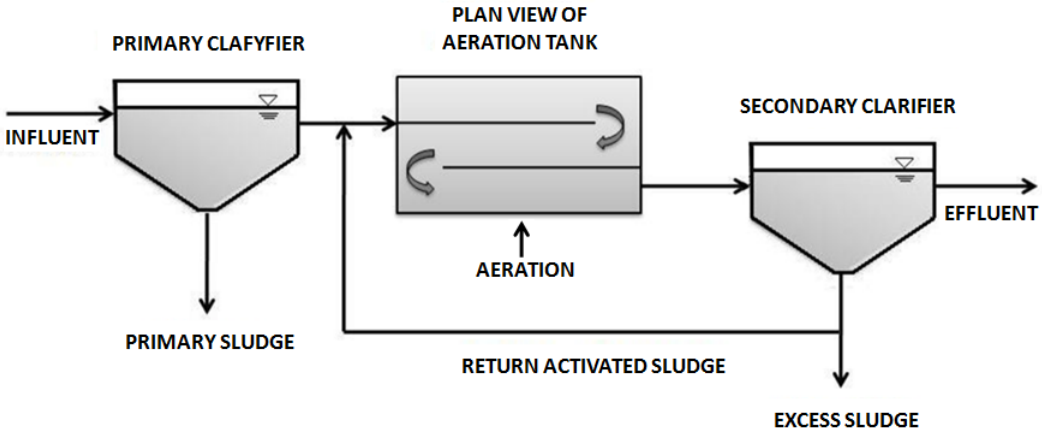


Figure 1 Simplified activated - sludge treatment with plug-flow reactor

General goal of the activated-sludge treatment is to remove organic waste material in the metabolic reactions by the microorganisms, separation and settling of activated bio-solids, in order to create acceptable quality of wastewater effluent. In the primary treatment of water, usually 50-60 % of suspended solids are removed from water and 30-40 % of BOD [9]. The secondary sludge collected after the biological treatment is partially recycled to the biological processes to keep microorganisms at high level within the process. The other fraction is pumped into the digester (excess sludge). Both primary and secondary sludge consist mainly of water 97-99% in the digester [9] and require further treatment before disposal as a waste. The main aim of the sludge treatment is to reduce solids mass, health problems due to the relatively high pathogens and microorganisms content and to convert putrescible organic matter to inert organic matter. There are different routes of sludge treatment, usually starting with sludge thickening, where sludge volume can be reduced up to one third of initial

volume. Thickened sludge requires further stabilization in order to eliminate pathogens and produce odour free sludge, suitable for disposal or recycle, that does not undergo any reactions.

1.2 Sludge stabilization

Sludge stabilization is a treatment method for excess and primary sludge (from the primary settlers) and treated sewage sludge that result in volume reduction and pathogens destruction. Stabilization combines treatment of solids and biogas production (60-70 vol percentage of methane (CH4)) [10]. It involves several groups of microorganisms with different optimum conditions. Stabilization can be realized both aerobically and anaerobically (digestion) and in different temperature ranges. The mesophilic temperature range is approximately from 25° C to about 45° C. An optimum growth for thermophilic conditions is around 50^{o} C or more and with maximum up to 70^{o}C. The temperature has an important influence on the growth rate and metabolism of microorganism in the anaerobic reactor. Generally, mesophilic temperature is applied more often since control is a sensitive issue for thermophilic stabilisation.

1.2.1 Anaerobic digestion

Anaerobic digestion is usually used for larger WWTPs (>20.000 PE). Four steps describe anaerobic digestion. First - hydrolysis, where suspended organic matter and high molecular weight compounds such as lipids, polysaccharides, proteins and nucleic acids are converted to soluble organics. Second - fermentation, called also acidogenesis, where soluble organic matter is transformed into Volatile Fatty Acids (VFA). Third - acetogenesis, where the higher organic acids and alcohols are converted to acetic acid, CO_2 and H_2. Fourth - methanogenesis, where methanogenic microorganisms are producing methane and CO_2 [10]. *Figure 2* illustrates all four steps of anaerobic digestion.

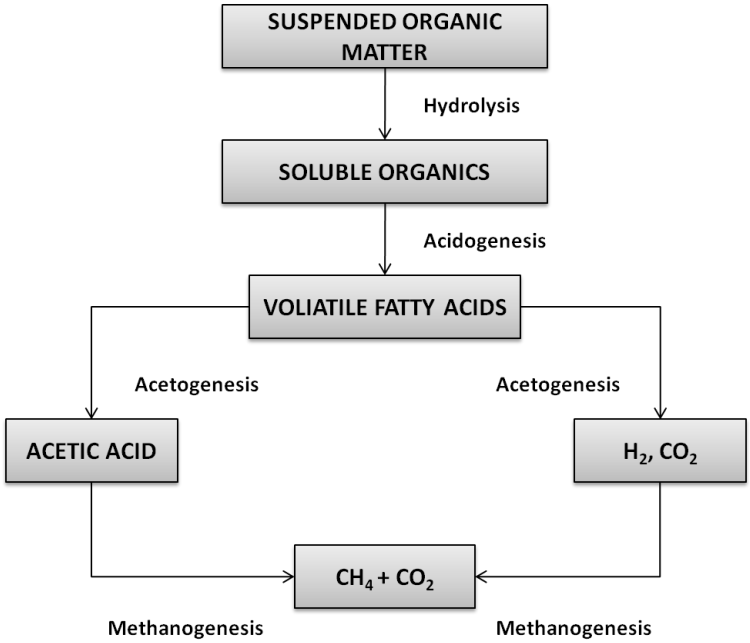


Figure 2 Subsequent steps in the anaerobic digestion process [10]

Temperature [11, 12], pH [13], alkalinity [14], solids and hydraulic retention time [12] are most important parameters affecting the anaerobic digestion. Besides, strict anaerobic conditions are required. The aim of anaerobic digestion is to reduce and stabilise organic solids, production of energy and pathogens inactivation [15]. An advantage of the anaerobic digestions is the improvement of sludge dewaterability. Hydrolysis is however usually a limiting step for digestion. This is due to the release of readily available organic matter during hydrolysis and major constituent of this material are cells that show resistance to biodegradation [11, 16]. There are some others limitations, such as slow reaction rate, only partial organic matter decomposition, presence of inhibitors, poor supernatant quality increasing heavy metal and organic micro-pollutions concentration [10]. The last one often is an obstacle in beneficial recycling, like application of sludge to agricultural land. In Denmark, cut-off values for organic micro-pollutions are almost 50% lower compared to EU standards [17, 18], however 60-90% of sludge can be disposed on agricultural land [18]. EU and Danish standards for organic compounds in the sewage sludge are presented in the ***Table 1*** below [18-19]. Similar strategy is in Sweden, where returning nutrients from urban to agricultural soil is of a high importance. There is a policy to stimulate the use of sewage sludge and treated organic waste in agriculture [24]. Different approach is present in Germany and Switzerland, where the use of sludge in agriculture has been questioned, in particular because of the inorganic and organic pollutants, including traces of drugs and hormones, heavy metals in the sludge [25, 26]. Organic farming has long banned the use of sewage sludge in Switzerland [25]. Less that, 10% of the

municipal sludges could be utilised agriculturally in Germany in 2005 [26]. Therefore, incineration is expected to be the main route for sewage sludge disposal in these countries.

Table 1 EU and Danish Cut - off values for organic compounds in the sewage sludge

	Cut-off values for use in agricultural land	
Compounds	EU [23]	DK [20-22]
DEHP[1]	100	50
LAS[2]	2600	1300
Nonylphenol[3] + NPEO	50	10
PAHs[4]	6	3

All numbers are in mg dry kg^{-1}.

[1] Bis (2-ethylhexyl) phthalate.

[2] Linear alkylsulfonates.

[3] Nonylphenol and NP-1-2 ethoxylates.

[4] Polycyclic aromatic hydrocarbons, i.e. sum of acenapthene, phenanthrene, fluorene, fluoranthene, pyrene, benzofluoranthenes(b + j +k), benzo(a)pyrene, bezo(ghi)perylene, indeno(1,2,3-cd)pyrene.

1.2.2 Aerobic stabilization

Aerobic stabilization is described by two steps performed by heterotrophic aerobic microorganisms: removal and utilization of biodegradable organic matter to cellular material and oxidizing of the cellular material in endogenous respiration [28]. In the first step, a fraction of organic matter is utilized for synthesis of new cells and thus biomass is increasing. The remaining material is oxidized to carbon dioxide, water and soluble inert material. When cellular material is the only available energy source, microorganisms will consume its own cells to maintain reactions in second step. The cellular material is oxidized to water, carbon dioxide and ammonia. Ammonia is further oxidized to nitrate. The processes last until biomass is considerably reduced, and rest will remain at a low energy level that can be considered stable and suitable for disposal or further treatment. In general, aerobic stabilization can be described by following equations:

$$organic\ matter + NH_4^+ + O_2 \longrightarrow cellular\ material + CO_2 + H_20$$

$$cellular\ material + O_2 \xrightarrow{microorganisms} digested\ sludge + CO_2 + H_20 + NO_3^-$$

Aerobic stabilisation usually occurs in ponds, which in fact are large shallow excavations. These excavations act as holding basins for secondary wastewater treatment. However, these ponds require regular maintenance to avoid odours. Temperature and oxygen are most influential parameters for this process. Some of advantages of the aerobic stabilization when comparing to anaerobic digestion are lower BOD concentration in supernatant, odourless biological stable final product and relative simple operation. Disadvantages from the other side are higher power cost due to the oxygen supply, poor dewatering characteristics of final product and process is highly dependent amongst others on the temperature, tank geometry, solids concentration [9].

1.3 Disposal of sewage sludge waste

High demand for improvement of the wastewater quality and efficient strategies of sustainable sewage sludge waste disposal routes are WWTPs' principles. Sludge management, however have not been as advanced as wastewater technologies and therefore became an important problem which usually represents up to 50 % of total cost of WWTP [9]. Effective control from authorities, source identification, improvement treatment and after-care methods, are one of actions for improving sludge quality [18]. There are three directives regulating the disposal methods for sludge waste: Council Directive 91/271/EEC of 21 May 1991, concerning urban waste-water treatment [29], Council Directive 99/31/EC of 26 April 1999 on the landfill waste [30] and Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture [31]. *Figure 3* illustrate in general routes of disposal of sludge after digestion.

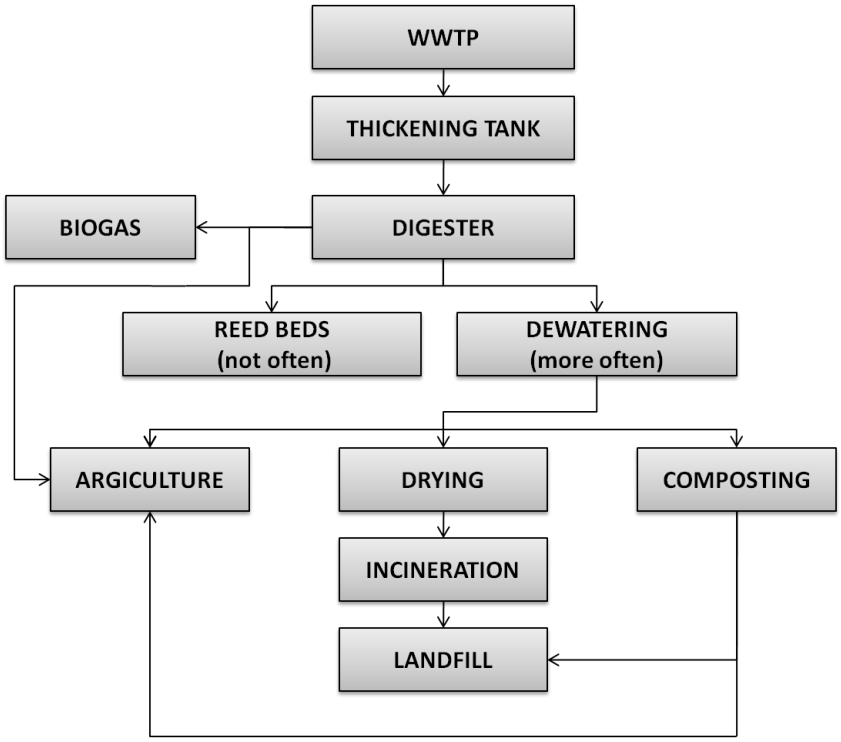


Figure 3 Process flowcharts of the sludge processing steps after digestion

1.3.1 Disposal of sewage sludge waste

Incineration and nowadays rather unpopular - landfills are disposal routes for sludges. The capacities of landfills have been reduced and new landfills that meet strict requirements are difficult to find. As the EU Landfill Directive stipulate, waste with organic content (TOC) more than 5% cannot be disposed on landfill area, only a small amount of sludges still can be disposed in traditional methods. Therefore, landfill will have lower priority in the future and have to give the place to more innovative methods. In some countries (e.g. Germany), incineration of the sludges is expected to increase due to the hazardous organic compounds in the sludge [26]. However, requirements that are more stringent will have to be met for flue gas quality in Germany [26]. Incineration is also highly taxed in Denmark (approximately 30 Euro per t raw waste) [32]. Therefore, a completely combustion of sludges is often solution only for the big municipalities due to the financial issues.

1.3.2 Land application and composting of sewage sludge waste

Alternative to disposal of sludges is recycling like utilization in agriculture (land application) and composting. Land application of sludge is resulting in utilisation of nutrients (phosphorus, nitrogen) and organic substances for soil improvement. Before that however, sludges require pre-treatment to reduce pathogens and organic micro-pollutants. Utilization in agriculture is often the cheapest alternative, however the main disadvantage is insufficient knowledge about organic micro-pollutants, pathogens content and small capacity (sludge can only be spread a few times a year on certain areas). Despite that, the European Sewage Sludge Directive supports utilization in agriculture as a recycle method for sewage sludge waste [30]. Composting is a more costly choice and it involves aerobic degradation of organic matter under variable temperature conditions, pathogens destruction and decrease of water content. The aim of this process is to obtain a stable final product suitable for agricultural or other recycling. However, heavy metals and organic micro-pollutants are difficult to remove and at least partially are present in the composted sludge. The Miljøstyrelsen (The Environmental Protection Agency, EPA) in Denmark together with the Danish Ministry of Environment has specified sharp limit values for heavy metals and organic toxic contaminants in sludge [32]. Considering significant contamination of sludges, (e.g. Ejby Mølle Plant) the price for composting is high what makes it a less attractive option. Preventing this situation, the aim should be to improve or find new methods in wastewater techniques or in sludge treatment (pre-treatment, post-treatment).

1.4 Post-digestion aerobic treatment of the sludge

Post aeration is an introduction of the oxygen to further reduction of organic matter (BOD and COD). Temperature, pH, oxygen level, retention time are some of important parameters for aeration process control. Some lab-scale investigations showed possibility of stabilisation enhancement of anaerobically digested sludge by post aeration treatment [33]. Several Miljøstyrelsen research projects showed possibility to reduce levels of nonylphenols (NPs) by aerobic post treatment of digested sludge [19]. Post aeration (SRT ≈ 6d; 36 °C) of the anaerobically digested sludge was also found to decrease organic content (COD, VSS mass balance) by 16 % at an Australian large WWTP [34]. Moreover, it improved also nitrogen removal by 5.5 % at the WWTP. In one study, the post aeration of the digested sludge was investigated concerning potential of organic micro-pollutants reduction. Both post aeration and composting were found to have potential in reducing DEHP [35]. In

another study, the post aeration of anaerobically digested sludge reported 30 - 40% reduction in DEHP [36]. It would be useful to investigate if other persistent organic micro-pollutions can be reduced by post aeration of digested.

1.4.1 Aerobic biodegradation of recalcitrant organic compounds

Microorganisms are nature's original recyclers that are able to convert toxic compounds to energy and CO_2. The use of organisms for degradation of pollutions has been increasingly applied as the self-sustaining and inexpensive clean-up technology [37]. In recent years compounds previously considered recalcitrant (resistant to biodegradation), are also to be degraded by microorganisms, e.g. before 1980s no biodegradation of high-molecular PAHs was reported [38]. That suggests that microorganisms are able to adapt to degrade resistant organic compounds, by means e.g. of induction of specific enzymes capable of metabolizing these compounds [39].

Different pathways in aerobic degradation of organic compounds are possible. At high concentrations of organic compounds, mineralisation (complete utilization to carbon dioxide, water) may occur, while at trace concentrations transformation (to another organic compound) is more probable [39]. However, mineralisation is also possible at trace levels of organic compounds. In both cases, these organic compounds could potentially serve as primary substrate (compound of concern serves as carbon/energy source) or as secondary substrate through co-metabolism, (growth-supporting substrate present in the process) [39]. In co-metabolism, enzymes involved in the metabolism of growth-supporting substrate are also able to transform the organic compound. An important example of co-metabolism is co-oxidation of chlorinated solvents in presence of methane [40]. During the aerobic degradation in the presence of organic compounds, microorganisms' growth rather than sustaining biomass is expected. For the growth of microorganisms the presence of electron acceptor, donor, carbon source and nutrients are essential [40]. Organic compounds contain different functional groups (-OH, -Cl, $-NH_2$, $-NO_2$,$-SO_3$) that can be used as electron donors, carbon or nutrients source ($-NH_2$, $-NO_2$, $-S0_3$). During aerobic degradation, organic compounds are oxidized or reduced in presence of oxygen that acts as electron acceptor or as direct reactant [40]. Compound concentration, redox conditions, temperature, presence of enzymes, pH, HRT (hydraulic retention time), SRT (solids retention time) are only few factors that determine the rate of biodegradation [39, 40]. However, the potential to degrade organic compounds in aerobic conditions is well-established [41], still too little is known about microbiological background of recalcitrant organic compounds biodegradation.

1.5 Organic micro-pollutants

Anthropogenic organic micro-pollutants (xenobiotics), such as fragrances, bactericides, are used frequently in personal care products. Flame-retardants and plasticizers are used in plastics and textile applications. They are ubiquitous in the environment since their usage has increased to the range of thousands of tonnes annually. Organic micro-pollutants usually are detected in the WWTPs inlet, outlet and sludge. Some of them found also in the surface water near the WWTPs. This indicates that current WWTPs' mechanisms are not yet sufficient in removing of the organic compounds. Moreover, removal efficiency of these compounds is often contributed by a sorption of the organic fraction to the sludge (relatively high K_{ow}, ***Table 2*** and ***Table 3***, hydrophobic nature). The presence of organic micro-pollutants in the sludge is currently a subject of discussion, since it is an obstacle for the safe sludge disposal. Persistence in the environment and frequent usage of the organic compounds, includes in this study, attract more attention of the fate in the environment. Structural formulae and general parameters of the organic compounds analysed in this study are given in *Table **2*** and *Table **3***.

1.5.1 Triclosan

Triclosan (2,4,4-trichloro, 2-hydroxy-phenylether) is a bactericide used frequently in various personal care and customer products, such as toothpaste, soaps, deodorants, detergents, sport clothing. Triclosan is a non-polar, lipophilic compound, found in biota (fish) samples [42] and human milk [43]. Triclosan as a biocide can block lipid biosynthesis and may induce development of resistance microorganisms [44]. Triclosan has been commonly detected in influent from several WWTPs at concentrations ranging from few µg L^{-1} to several hundred µg L^{-1} [3, 46]. Toxicity data on triclosan are still rather scarce. A median effective concentration (EC_{50}) for triclosan equals to 20 mg L^{-1} and 239 mg L^{-1} based on estimations on oxygen consumption and glucose utilization, respectively [45]. Moreover, other studies reported a strong potential for corresponding effects on the structure and function of natural stream ecosystems that receive WWTP effluents containing triclosan, based on the algal bioassays [47]. Nowadays, WWTPs eliminate triclosan with high (87-96 %) removal efficiency [3]. Although triclosan is significantly removed in WWTPs [3, 48], not much is known about removals mechanisms. Some studies reported that 79% of triclosan was biodegraded, while 15% was sorbed to the sludge [48]. Other studies reported 30% [3], 50± 19% [49] accumulation in the sludge of total mass of triclosan entering WWTP. Transformation of triclosan into metabolites, such as

methyl triclosan is also possible [3]. This transformation product may exhibit higher accumulation potential, than triclosan itself. Formation of heavier and more hydrophobic products via dimerization and polymerization can occur during transformation of triclosan [44]. This can lead to decrease in triclosan mobility and thus lower the bioavailability.

1.5.2 AHTN&HHCB

Synthetic polycyclic musk fragrances such as HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[c]-2-benzopyran); common trade names e.g.: Galaxolide®, Abbalide® and AHTN (7-acetyl-1,1,3,4,4,6-hexamethyl- 1,2,3,4-tetrahydronaphthalene); common trade names e.g.: Tonalide®, Fixolide® are used as fragrances in wide range of personal care products, such as shampoos, softeners in washing powders. The usage of these has increased, as the usage of nitro musk decreased due to their reputed toxicity in late 1980s [50]. Over 2000 t of AHTN and HHCB are used annually in the Europe [51], while 6500 t was used in United States in 2000 [50]. Due to their presence in the different cleaning products, they can reach the WWTP shortly after application. Some studies revealed AHTN, HHCB and HHCB-lactone (transformation product of HHCB) in the WWTPs [52]. A primary aeration basin (AB) with intermediate settlement tank (IST) was found as most effective in HHCB and ATHN removing (70% removal efficiency) [3]. Due to their hydrophobic nature, polycyclic fragrances can easily be sorbed to the sludge particles. Both AHTN and HHCB reached concentrations of 0.7±12.1 mg dry kg^{-1}, in all types of examined sludge in one study [5]. As reported, 54-73% and 57-78% of HHCB and AHTN respectively was sorbed to the sludge in a German WWTPs [3]. Mass balance analysis in another study suggested only 30% of HHCB and AHTN entering the WWTP was found in the effluent and the sludge [53]. When attaching to the solids, musk fragrances are not directly available for microbial degradation, resulting in low biodegradability. AHTN and HHCB were found in human tissues and breast milk [54], detected in the marine ecosystem of the German Bight of the North Sea [3] and surface waters, thus polycyclic fragrances are ubiquitous in the environment.

1.5.3 OTNE

OTNE ([1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethylnaphthalen-2yl] ethan-1-one); trade name e.g.: Iso E Super®, a more fruitier fragrance. Removal of OTNE in the WWTPs occurs mainly due to the

sorption to sludge. Transformation to other compounds may be possible, thought that is still not well known [55]. Elimination efficiency of OTNE reported to be equal to 92% and 29% by current WWTPs' technology; activated sludge and settling respectively [56]. Concentration of OTNE in two digested and dewatered sludges from USA found to be equal to 7,3 ± 1,4 μg dry g^{-1} and 30,7 ± 3,7 μg dry g^{-1} [6]. It was lately discovered that only approximately 5% of Iso E Super® mixture causes characteristic (wanted) woody-amber odour, and became available as a pure compound under trade name Iso E Super Plus ® [57]. Therefore, efforts to investigate the fate and behaviour of OTNE in the environment are even of a higher importance.

1.5.4 DEHP

DEHP (bis-(2-ethylhexyl) phthalate), plasticizers phthalate esters, is a common additive in the polyvinyl chloride (PVC) manufacture. DEHP is not chemically bond to the products and may migrate slowly into the environment from polymer products during their entire lifetime [58]. Between one and four million tonnes of tonnes of DEHP are used annually which is approximately 40 -50% of the annual global phthalate production [19]. DEHP has a high octanol–water partition coefficient K_{ow}, ***Table 2***. This indicates a high partitioning to the sludge. Like other plasticizers, DEHP, can persist in the treatment in the WWTPs and accumulate in the sludge. This can be problematic when considering the beneficial recycling of the sewage sludge waste (e.g. agricultural use) [8]. Due to the persistence in the environment and problematic properties, DEHP is in the list of undesirable substances in Denmark (EPA, 2001) [18] and is prioritised under the Water Framework Directive (EU, 2000) [23]. DEHP is currently substituted by other lipophilic phthalate such as bis nonylphthalate (EC, 2008) [59]. As recently demonstrated, biodegradation of DEHP with pure microbial cultures can lead to transformation into metabolites including monoesters 2-ethylhexanol, 2-ethylhexanal and 2-ethylhexanoic, acid [60], which are known to be even more toxic than DEHP and cannot be further depredated [61]. Moreover, DEHP and its metabolites were found in all type of sludges from Quebec, Canada investigated in one study; primary, secondary, digested, dewatered and dried [8], what makes sludge a significant plasticizer source in the environment. The concentration of DEHP in this study was in the range from 15 mg kg^{-1} to 346 mg kg^{-1} in dried and secondary sludge, respectively. DEHP was also detected in the anaerobically digested sludge from Ejby Mølle WWTP with concentration 26 mg dry kg^{-1} [62]. Several studies have reported the aerobic degradation of phthalates in sludge [19, 63]. Oxygen, moderate temperature increases and addition of specialized microorganisms found to simulate DEHP biodegradation in activated sludge [2].

1.5.5 Nonylphenols

Nonylphenols (NPs) are endocrine disruptors that interact with hormone receptors. They are produced in the decomposition of nonylphenol polyethoxylates (NPnEOs), which have been used as 'inert' additive in surfactants and pesticides [64]. NPnEOs are a group of non-ionic surfactants that were widely used in detergents, cosmetic products and textiles [65] before the banning in Europe in 1991 (reinforced in 1999) [66]. Under aerobic conditions decomposition of NPnEOs to nonylphenoxy acetic acids (NPnEC) and NPnEC to nonylphenol mono-ethoxylate (NP1EO) and further to nonylphenol carboxylate (NP1EC) occurs [67]. NPs formation occurs subsequently under aerobic conditions from NP1EO and NP1EC [67]. This is important to understand the fate of the NPs in the WWTP. The term NPs refers to a group of compounds since technical nonylphenol is a mixture of over 100 isomers with different structure and position of the alkyl moiety at the phenol ring [68]. Nonylphenol was found in German WWTPs effluent with concentration approximately 14 ng L^{-1} [4]. NP was also detected in anaerobically digested sludge from Ejby Mølle WWTP with concentration 19 mg dry kg^{-1} [62]. NP was banned in Europe and listed on the OSPAR list of Hazardous Substances [66]. NP is also listed as undesirable substance in Denmark (EPA, 2001) [18].

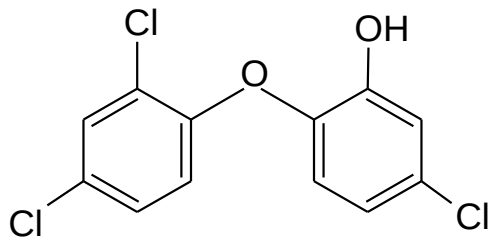
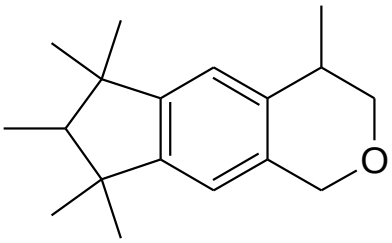
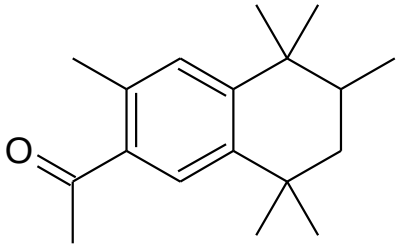
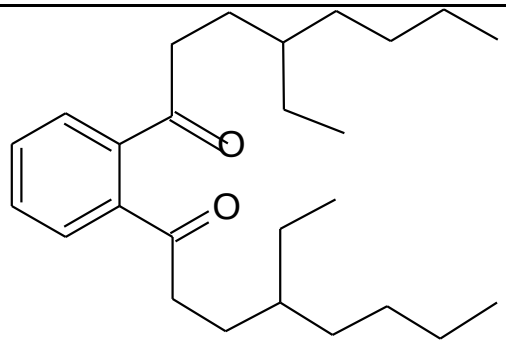
1.5.6 TCPP

Tris-(2- chloro-1-methylethyl) phosphate (TCPP) is one of recently introduced chlorinated organophosphates, marketed by various manufactures under brand names such as Fyrol PCF and Antiblaze TMCP [3]. TCPP is used as a flame-retardant agent in flexible and rigid polyurethane foams, which are used as thermal insulation material in constructions. The concentration of TCPP was identified in the influent and effluent of one WWTP as 520 ng/L and 380 ng/L, respectively, (mean values). Additionally the concentrations in sewage sludge of the same plant were determined (mean value 5100 ng/g dry weight; 1700 ng/g wet weight, respectively) [87]. No elimination of the chlorinated flame retardant TCPP was observed in any of the sampled WWTPs in another study [86]. The chlorinated organophosphate TCPP is a persistent organic pollutant that was found in surface waters and in marine water samples [3]. There are no data on the TCPP's degradation. Its presence in seawater at nanogram per liter levels makes it a priority compound under the OSPAR commission regulations. Moreover, TCPP is suspected to be carcinogenic and its genotoxicity and carcinogenicity have been recently discussed [85].

1.5.7 TiBP & TnBP

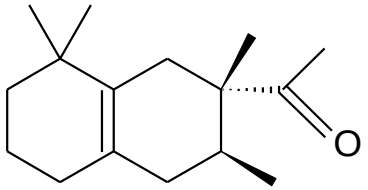
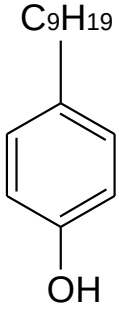
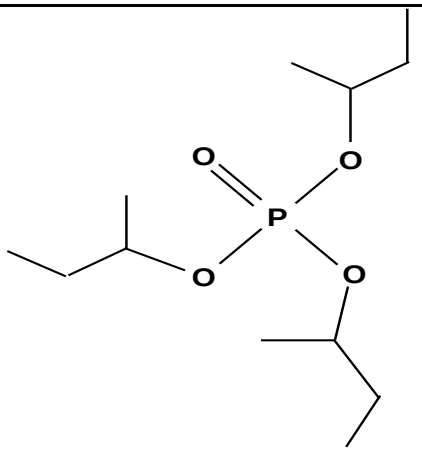
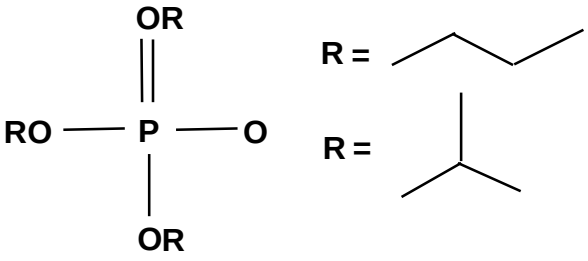
The non-derivatized alkyl phosphates tributyl phosphates (*iso*-and *n*-isomer; T*i*BP and T*n*BP, respectively) are one of the non-chlorinated organophosphates. TiBP and TnBP are mostly used as plasticizers and lubricants and to regulate pore sizes, e.g., in concrete [3]. TiBP and TnBP were detected in the WWTPs effluent in concentrations 120-1,000 and 50-60 ng/L [88]. In other study the elimination efficiencies of the TiBP and TnBP in two different WWTPs were determined. The elimination rates ranged from 57–86% for TiBP, TnBP in both WWTPs [86]. Moreover, TnBP is considered to be neurotoxic [3].

Table 2 Structural formulas and general information of organic compounds relevant for this study

Triclosan (2,4,4-trichloro, 2-hydroxy-phenylether)	
	Empirical formula: $C_{12}H_7Cl_3O_2$ Molar weight: 289,55 g/mol CAS: 3380-34-5 Consumption: 1500 t/a [48] (worldwide) 350 t/a [48] (Europe) Log K_{ow}: 4,3 [48]
HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8--hexamethylcyclopenta[c]-2-benzopyran)	
	Empirical formula: $C_{18}H_{26}O$ Molar weight: 258,41 g/mol CAS: 1222-05-5 Consumption: 1000-5000 t/a [51] (Europe/worldwide) Log K_{ow}: 5,9 [69, 70] K_H: 11,3 [70]
AHTN (7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene)	
	Empirical formula: $C_{18}H_{26}O$ Molar weight: 258,41 g/mol CAS: 1506-02-1 Consumption: 1000-5000 t/a [51] (Europe/worldwide) Log K_{ow}: 5,7 [69, 70] K_H: 12,5 [70]
DEHP (bis-(2-ethylhexyl) phthalate)	
	Empirical formula: $C_{24}H_{38}O_4$ Molar weight: 391 g/mol CAS: 117-81-7 Consumption: 221 000 t/a (Europe) [19] Log K_{ow}: 7,5 [1] K_H: 4,4 [88]

All the K_H values are in [atm · m^3/mol]

Table 2 Continued

OTNE ([1,2,3,4,5,6,7,8-octahydro-2,3,8,8--tetramethylnaphthalen-2yl] ethan-1-one)	
	Empirical formula: $C_{16}H_{26}O$ Molar weight: 234 g/mol CAS: 54464-57-2 Consumption: 2500-3000 t/a (worldwide) [57,71] Log K_{ow}: 5,7 [71, 70] K_H: 31,8 [70, 71]
Nonylphenol - a technical mixture of around 100 isomers	
	Empirical formula: $C_{15}H_{24}O$ Molar weight: 220,18 g/mol CAS: 84852-15-3 Consumption: 340 000 t/a (worldwide) [3] Log K_{ow}: 4,48 [72] K_H: 11 [88]
TCPP -tris-(2- chloro-1-methylethyl) phosphate	
	Empirical formula: $C_9H_{18}Cl_3O_4P$ Molar weight: 327,6 g/mol CAS: 13674-84-5 Consumption: 40.000 t/a (worldwide) 5.000 t/a - (Europe; organophosphates) [85] Log K_{ow}: 2,6 [3]
TiBP, TnBP - non-derivatized alkyl phosphates tributyl phosphates (*iso*- and n-isomer)	
	Empirical formula: $C_{12}H_{27}O_4P$ Molar weight: 266,32 g/mol CAS(TiBP/TnBP): 126-71-8/126-73-8 Log K_{ow}: 4,0 [3]

All the K_H values are in [atm · m^3/mol]

1.5.8 PAHs

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of over 100 compounds that are formed during the incomplete combustion of organic substances such as fossil fuels, woods and mineral oil [73]. The main source of these compounds is the combustion from traffic and commercial processes. When entering WWTPs, PAHs tend to accumulate in the sludge due to their hydrophobic nature [74] (log K_{ow}, *Table 3*). Some studies reported PAHs occurrence in the digested sludge with concentration 5 mg dry kg^{-1} [19], 2,2 mg dry kg^{-1} [62], expressed as a sum of nine PAHs (acenapthene, phenanthrene, fluorene, fluoranthene, pyrene, benzofluoranthenes(b + j +k), benzo(a)pyrene, bezo(ghi)perylene, indeno(1,2,3-cd)pyrene). Although these compounds have hazardous properties, they are not on the list of undesirable substances in Denmark (EPA, 2001) [18]. PAHs are known to be toxic and persistence in all environmental media, though a combination of sorption and biodegradation could have a great potential in the treatment of wastewater polluted with PAHs [75]. Even thought biodegradation rates are inversely correlated with the increasing molecular weight of PAHs, a high number of aerobic microorganisms are expected to degrade both light and heavy PAHs in the sewage sludge [74]. PAHs with low molecular weight (2-3 aromatic rings) usually serve as a primary food source. Biodegradation of PAHs with 5-6 aromatic rings can only occur when a growth-supporting substrate is present in the process (co-metabolism). Both degradation pathways require oxygen to cleave the rings [19]. Moreover, PAHs biodegradation is likely limited by their low bioavailability resulting from their strong adsorption onto organic particles [74]. Addition of methanol and increase of temperature was found to enhance the bioavailability of PAHs in the sludge [74].

1.6 Aerobic degradation pathways of xenobiotics - degradation of aromatic compounds

Aromatic hydrocarbons as derivatives of benzene are very stable and hence very resistant to degradation. Though, many bacterial species have evolved a use of these compounds as source of energy [76]. In general, three parts of the aerobic assimilation of aromatic compounds can be considered. Firstly, as the main strategy is to convert the compounds to a key intermediate, such as catechol, by a usage of different enzymes [77]. There are two different mechanisms of the catechol formation, through monooxygenase or dioxygenase reactions. Numerous compounds are attacked through dioxygenase reactions, amongst others, aromatic hydrocarbons, such as benzene and naphthalene [76]. *Figure 4* shows the initial attack on benzene ring through dioxygenase reaction.

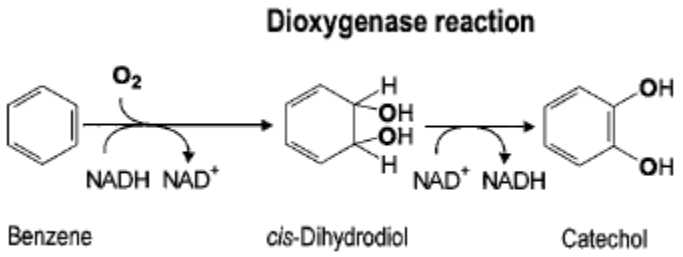


Figure 4 Initial attack on xenobiotics by oxygenases (redrawn from [78]). Dioxygenases incorporate both atoms into the substrate

After the formation of catechol, the second step is to open the aromatic ring by a further dioxygenase reaction by either ortho- (intradiol) of meta- (extradiol), two families of ring-cleavage enzymes [76, 78]. Dioxygenases break one of the carbon-carbon bonds of the ring by the addition of molecular oxygen and subsequently producing an unsaturated aliphatic acid [77]. The intradiol (or *ortho)* dioxygenases are Fe^{3+} enzymes and produce *cis, cis*-muconic acid (or a derivative) and the extradiol (or *meta)* dioxygenases are Fe^{2+} enzymes and which produce 2-hydroxymuconic semi-aldehyde (or a derivative) [77]. *Figure 5* shows the ortho- and meta-cleavage of catechol degradation.

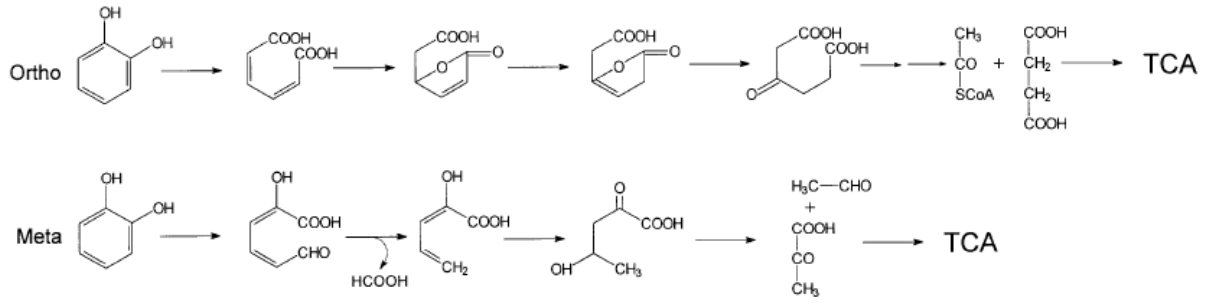
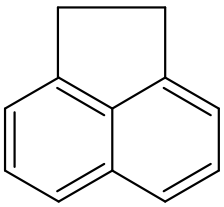
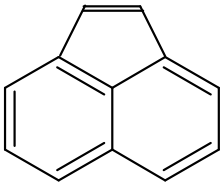
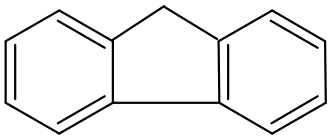
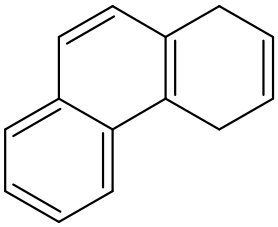
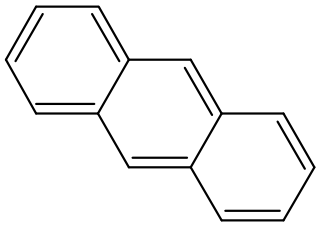


Figure 5 The ortho-(intradiol-) pathway and the meta-(extradiol-) pathway of catechol degradation. TCA (pyruvate and acetaldehyde; succinate and acetyl-CoA, in the ortho- and meta-pathway, respectively) (redrawn from [76])

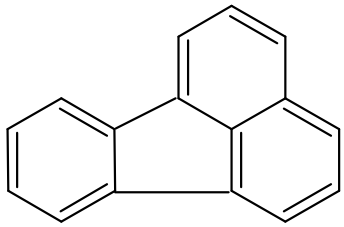
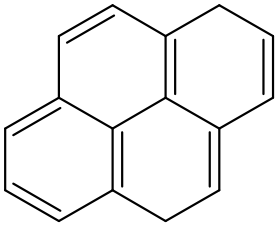
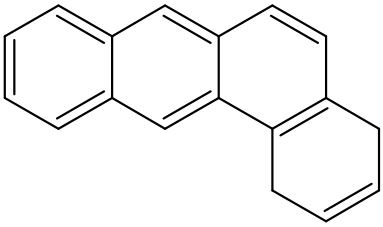
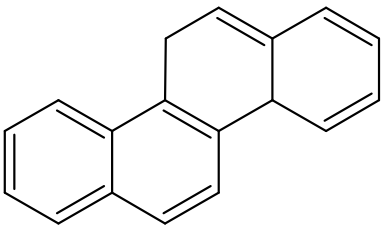
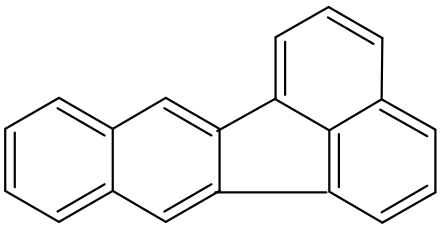
After opening of the catechol ring, the third step is to form small aliphatic compound from by a conversion of the ring cleavage product. In the ortho-pathway catechol is cleaved into pyruvate and acetaldehyde, while in the meta-pathway into succinate and acetyl-CoA. The aliphatic compounds can afterwards enter the central metabolism [76, 77].

Table 3 Structural formulas and general information of PAHs relevant for this study

Acenaphthene	
	Empirical formula: $C_{12}H_{10}$ Molar weight: 154,2 g/mol CAS: 83-32-9 Log K_{ow}: 3,92 [19] K_H: $6{,}0\cdot10^{-3}$ [19]
Acenaphthylene	
	Empirical formula: $C_{12}H_8$ Molar weight: 152,2 g/mol CAS: 208-96-8 Log K_{ow}: 4,07 [79] K_H: $1{,}14\cdot10^{-4}$ [79]
Fluorene	
	Empirical formula: $C_{13}H_{10}$ Molar weight: 166,2 g/mol CAS: 86-73-7 Log K_{ow}: 4,18 [19] K_H: $4{,}1\cdot10^{-3}$ [19]
Phenanthrene	
	Empirical formula: $C_{14}H_{10}$ Molar weight: 178,2 g/mol CAS: 85-01-8 Log K_{ow}: 4.57 [19] K_H: $1{,}6\cdot10^{-3}$ [19]
Anthracene	
	Empirical formula: $C_{14}H_{10}$ Molar weight: 178,2 g/mol CAS: 120-12-7 Log K_{ow}: - K_H: $4{,}1\cdot10^{-3}$ [19]

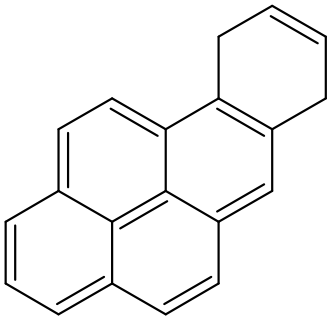
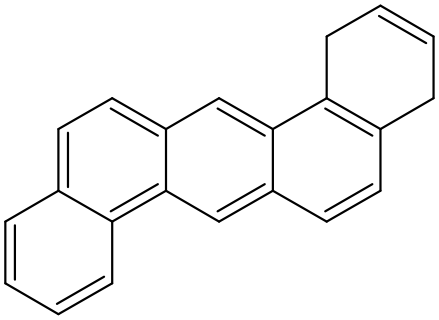
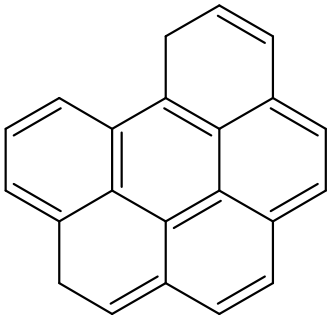
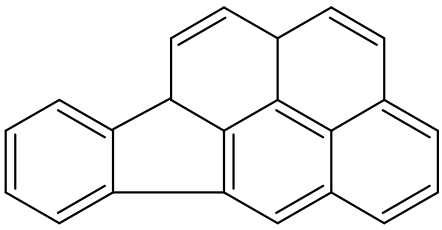
All the K_H values are in [atm · m^3/mol]

Table 3 Continued

Fluoranthene	
	Empirical formula: $C_{16}H_{10}$ Molar weight: 202,3 g/mol CAS: 206-44-0 Log K_{ow}: 5,22 [19] K_H: $5,0 \cdot 10^{-4}$ [19]
Pyrene	
	Empirical formula: $C_{16}H_{10}$ Molar weight: 202,3 g/mol CAS: 129-00-0 Log K_{ow}: 5,18 [19] K_H: $4,4 \cdot 10^{-4}$ [19]
Benzo(a)anthracene	
	Empirical formula: $C_{18}H_{12}$ Molar weight: 228,3 g/mol CAS: 56-55-3 Log K_{ow}: 4,98 [79] K_H: $8,0 \cdot 10^{-6}$ [79]
Chrysene	
	Empirical formula: $C_{18}H_{12}$ Molar weight: 228,3 g/mol CAS: 218-01-9 Log K_{ow}: 5,78 [79] K_H: $5,0 \cdot 10^{-6}$ [79]
Benzo(b+j+k)fluoranthene	
	Empirical formula: $C_{20}H_{12}$ Molar weight: 252,3 g/mol CAS: 207-08-9 (k) 205-82-3(j) Log K_{ow}: 6,6 [19] K_H: $1,6 \cdot 10^{-5}$ [19]

All the K_H values are in [atm · m^3/mol]

Table 3 Continued

Benzo(a)pyrene	
	Empirical formula: $C_{20}H_{12}$ Molar weight: 252,3 g/mol CAS: 50-32-8 Log K_{ow}: 6.5 [19] K_H: $2.0 \cdot 10^{-5}$ [19]
Dibenzo(a,h)anthracene	
	Empirical formula: $C_{22}H_{14}$ Molar weight: 278,3 g/mol CAS: 53-70-3 Log K_{ow}: 6,58 [79] K_H: $1,7 \cdot 10^{-6}$ [79]
Benzo(ghi)perylene	
	Empirical formula: $C_{22}H_{12}$ Molar weight: 276,3 g/mol CAS: 191-24-2 Log K_{ow}: 6.9 [19] K_H: $5,6 \cdot 10^{-6}$ [19]
Indeno(1,2,3-cd)pyrene	
	Empirical formula: $C_{22}H_{12}$ Molar weight: 276,3 g/mol CAS: 193-39-5 Log K_{ow}: 7,66 [49] K_H: $2,86 \cdot 10^{-7}$ [79]

All the K_H values are in [atm · m^3/mol]

The organic micro-pollutants presented above were chosen in this study as markers. They were analysed in the activated and digested sludges from the three Danish WWTPs. The elimination pathways and behaviour of these compounds were further presented and discussed.

1.7 Ejby Mølle Wastewater Treatment Plant

1.7.1 Description of Ejby Mølle WWTP

Ejby Mølle-the biggest water treatment plant in the Odense municipality is treating wastewater form the largest part of the urban area in the Komunne. With capacity of 410.000 person equivalents (PE), the plant is treating 20-25 million m^3 of wastewater annually. There are five main parts of the plant; a mechanical part, including screens, grit and grease chambers; an activated sludge unit; two sets of trickling filters, where most of the organic matter is broken down; a sand filter, where removal of suspended matter and phosphorus, that was not removed in previous steps occurs; sludge stabilization and sludge dewatering unit. Chemical phosphorus removal is performed by addition of $FeCl_2$. The activated sludge unit has biological nitrogen and phosphorus removal and operates with the Bio-Denipho method: the anaerobic tank is followed by an alternating aerobic/anoxic conditions tank, where nitrification and denitrification occurs. The plant has also an additional aeration unit to make sure that water has a high oxygen concentration on the outlet. *Figure 6* below illustrates the overview of the plant.

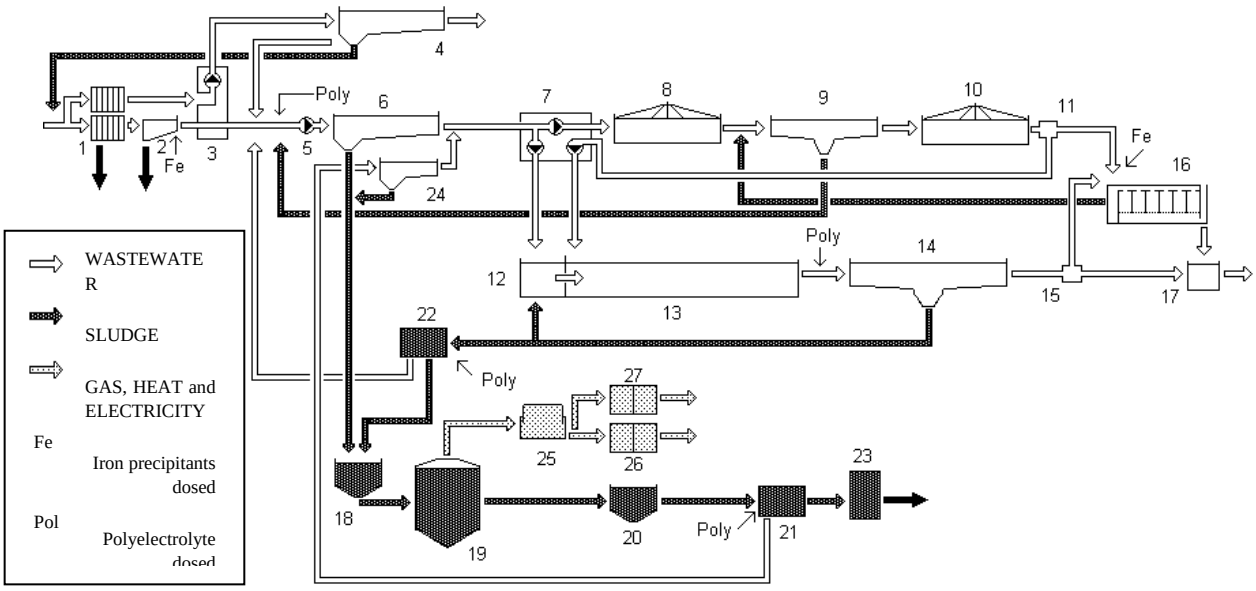


Figure 6 Ejby Mølle WWTP overview

1.7.2 Sludge management at Ejby Mølle WWTP

The sludge production at Ejby Mølle WWTP is approximately 65 tonnes of wet sludge per day. The treatment technologies for solids processing is focused on stabilization during anaerobic digestion and dewatering with centrifuges. Before digestion, sludge is thickened. In the digester, sludge is treated in a mesophilic reactor and biogas production is performed. Currently sludge disposal occurs through composting and land applications. The sludge from the primary treatment is firstly mixed with excess sludge, digested and afterwards composted. When the composting is finished, the product is used as fertilizer in agriculture and forestry. *Figure 7* shows the routes of sludge treatment and recycling at the plant. Stricter regulations concerning sludge volume and quality and organic micro- pollutions content, became an important and expensive problem for Ejby Mølle WWTP. In order to decrease the amount of solids, for safe and more cost-effective disposal, an aerobic treatment of the digested sludge is currently discussed. A decrease of micro-organic pollutants concentrations during an aerobic treatment of the sludge is also of Ejby Mølle WWTP's interest.

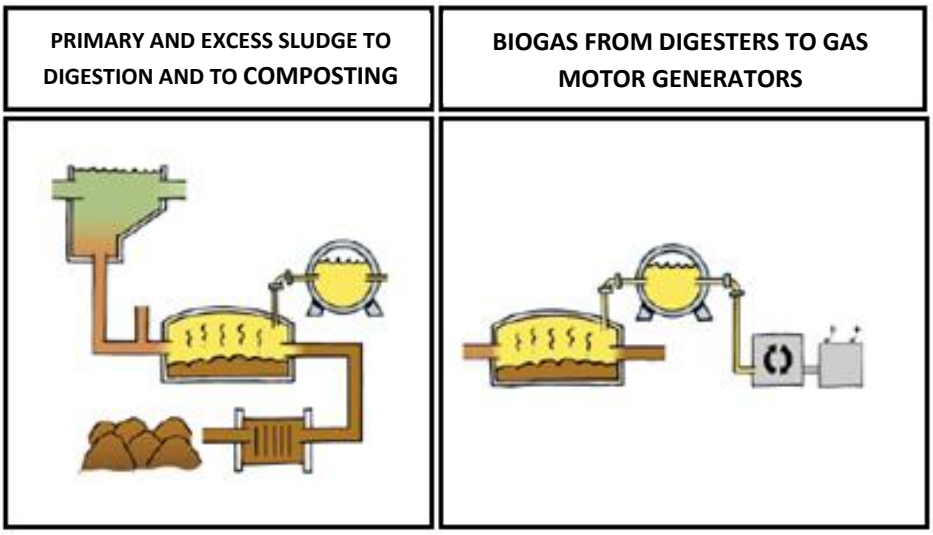


Figure 7 Sludge management at Ejby Mølle WWTP

2. Objectives

Due to the hydrophobic nature of organic compounds, they tend to sorb to the sludge after the treatment. Sludge stabilization and other treatment methods can only remove organic micro-pollutants to some extent through degradation and transformation to volatile compounds [65]. Moreover, their behaviour in the WWTPs is not yet fully understood. Sewage sludge contaminated with the organic micro-pollutants, once applied to the land works as pollutant's reservoir that slowly releases them to the environment. Whether adverse effects on human health and wildlife due to the organic compounds exist when they enter the food chain or the groundwater remains a point of controversial discussion. The sewage sludge represents the unique opportunity for removal of organic contamination to prevent their release to the environment [74]. Even though a basic knowledge of the biodegradation of recalcitrant organic compounds exists, deeper insight into metabolic pathways is necessary.

The purpose of this study was to investigate the enhanced biodegradation of organic micro-pollutants in the digested sludge under aerobic, anoxic and anaerobic conditions. For this reason, lab-scale sludge reactors were employed. Concentrations of the organic micro-pollutants, first order biodegradation rates and half-lives were determined. Additionally an aerobic post-treatment of the digested sludge from Ejby Mølle WWTP was investigated in order to remove organic micro-pollutants, decrease the amount of the sludge and hence save money on the disposal.

1

3. Materials and method

3.1 Wastewater Treatment Plants (WWTPs)

The sludge samples for the degradation experiments were collected from three different Danish WWTPs.

1. Ejby Mølle WWTP (Odense), with capacity of 410.000 PE, 20 - 25 million m^3/a of wastewater has a sludge production of 65 t/d (see 1.7.1). Digested sludge samples were collected from the digester (mesophilic, approximately 36°C, 25-30 days). Digested sludge from this plant was used to investigate the removal of the organic micro-pollutants and decrease the amount of sludge in the aerobic post-treatment at Ejby Mølle plant.
2. Frederikshavn WWTP, with capacity 130.000 PE and 6 millions m^3/a of wastewater is producing approximately 10 t/d (dry matter) of sludge. One third of the total wastewater treated at this plant originates from industry and storm water, the residual is municipal. This WWTP performs biological removal for nitrogen, while phosphorus is removed in both chemical and biological processes. The excess sludge and the primary sludge are thickened together and digested in mesophilic conditions (approximately 36°C). The digested sludge is dewatered and dried afterwards. The digested sludge samples and activated sludge samples were taken from the digester and aeration tank, respectively. Frederikshavn WWTP was chosen as the treatment processes are very similar to those at Ejby Mølle plant.
3. Activated sludge from Aalborg East WWTP, with capacity of 100.000 PE, 8 millions m^3/a of wastewater and a sludge production 10-14 t/day. The wastewater at the plant is in 80% municipal and in 20% from local industries. The activated sludge plant operates with a Bio-Denipho configuration, where an anoxic/aerobic tank follows the anaerobic tank. The plant has a biological removal for nitrogen as well as chemical and biological removal for phosphorus. The excess sludge is thickened to approximately 96% water content and digested in thermophilic conditions (approximately 55°C). After digestion, the sludge is dewatered to approximately 70% water content and dried afterwards. Activated sludge samples were taken from the return sludge pump station at the plant. Sludge samples from this plant were used in the initial experiments, where removal of the organic micro-pollutants in different electron acceptor regimes was investigated.

3.2 Degradation experiments

Five different experiments were performed at the university's laboratory and two experiments were performed at Ejby Mølle WWTP's laboratory. Each of them was started the same day the sludge was taken because of the impracticality of preserving the sample.

- Experiment 1: electron acceptor regimes I. Degradation was performed in three different electron acceptor regimes: reactor I - aerobic, reactor II - anaerobic and reactor III - anoxic. Activated sludge was collected at Aalborg East WWTP. Triclosan was spiked to the sludge. After oxygen was found as optimal electron acceptor for degradation process, experiments were performed under aerobic conditions only.
- Experiment 2: electron acceptor regimes II. Only one reactor with sludge under the aerobic conditions was employed. OTNE was spiked to the sludge. Activated sludge samples were collected at Aalborg East WWTP.
- Experiment 3: triclosan. Only one reactor with sludge under aerobic conditions was employed and triclosan was spiked to the sludge. Digested sludge samples were collected at Ejby Mølle WWTP.
- Experiment 4: molasses. Aerobic sludge and molasses were added as aerobic microorganisms and carbon sources, respectively. DEHP was spiked to the sludge. To avoid water loss during experiments, the airflow was decreased to approximately 1 L/h. Digested sludge samples were collected at the Frederikshavn WWTP.
- Experiment 5: ultrasound. The ultrasonic treatment was introduced to the digested sludge before start of the experiment. No compound was spiked to the sludge in this experiment. Digested sludge samples were collected at the Frederikshavn WWTP.
- Experiment 5 and 6: oxygen concentration I and II. Two reactors were employed: reactor I - lower oxygen concentration (setup 1) and reactor II – higher oxygen concentration (setup 2). No compound was spiked to the sludge. Digested sludge samples were collected from Ejby Mølle' digesters.

*Table **4*** shows an overview on the experiments performed in this study.

Table 4 Description of the degradation experiments performed in this study

<table>
<tr><td></td><td colspan="8">EXPERIMENTS</td></tr>
<tr><td>No.</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td colspan="2">6</td><td>7</td></tr>
<tr><td rowspan="2">Name of the experiment</td><td colspan="2">Electron acceptor regimes</td><td rowspan="2">Triclosan</td><td rowspan="2">Molasses</td><td rowspan="2">Ultrasound</td><td colspan="2">Ejby Mølle experiments - Oxygen concentration</td></tr>
<tr><td>I</td><td>II</td><td colspan="2">I</td><td>II</td></tr>
<tr><td>Type of sludge</td><td colspan="2">Activated sludge (Aalborg East WWTP)</td><td>Digested sludge (Ejby Mølle WWTP)</td><td colspan="2">Digested sludge (Frederikshavn WWTP)</td><td colspan="2">Digested sludge (Ejby Mølle WWTP)</td></tr>
<tr><td>Amount of the sludge</td><td colspan="5">1 L</td><td colspan="2">2,5L</td></tr>
<tr><td rowspan="2">Compound spiked</td><td>TCS*</td><td>OTNE</td><td>TCS*</td><td>DEHP</td><td rowspan="2">-</td><td colspan="2" rowspan="2">-</td></tr>
<tr><td colspan="4">100 µg/L</td></tr>
<tr><td>Reactor I</td><td colspan="3">Aerobic Aquarium pump (60L/d air flow)</td><td colspan="2">Aerobic Pressure pump (1L/d air flow)</td><td colspan="2">Aerobic 2 Aquarium pumps (60L/d air flow)
Oxygen level control 0.2 - 0.4 mg/L | Oxygen level control 2 - 4 mg/L</td></tr>
<tr><td>Reactor II</td><td colspan="2">Anaerobic N_2 gas flushed</td><td>-</td><td colspan="3">Aerobic Pressure pump (1L/d air flow)
Aerobic sludge addition (100ml)</td><td colspan="2">Aerobic 2 Aquarium pumps (60L/d air flow)
Redox potential control 80 - 100 mV | Redox potential control 250 - 270 mV</td></tr>
<tr><td>Reactor III</td><td>Anoxic KNO_3 addition (44g N/d)</td><td>-</td><td>-</td><td>Aerobic Pressure pump (1L/d air flow)
Activated sludge addition (100ml)
Molasses addition (143mg added; 138mg molasses/100mg COD)</td><td>Aerobic Pressure pump (1L/d air flow)
Activated sludge addition (100ml)
Ultrasound (1h)</td><td colspan="2">-</td><td></td></tr>
<tr><td>Duration</td><td colspan="3">4 days</td><td colspan="5">1 week</td></tr>
<tr><td>Sampling</td><td colspan="3">1 day – every 1 hour
2 day – every 2 hour
3 day – every 4 hour
4 day – one sample</td><td colspan="5">1 day – every 2 hour
2,3 day – every 3-4 hour
From day 4 – one sample a day</td></tr>
</table>

*TCS = Triclosan

3.2.1 Oxygen optimisation – experiments conducted at Ejby Mølle WWTP

During the two experiments performed at Ejby Mølle WWTP's laboratory oxygen concentration, pH, redox potential and temperature were constantly monitored. Oxygen concentration was measured with electrodes In Pro 6800 Series O_2 Sensor and Cello Ox.325 O_2 connected to the transmitters 4100 ppb Toledo and WTW Oxi 330i. Redox potential was measured with Bradley James electrode and WTW SenTix ORP electrode connected to Yokogawa pH 202G(S) and WTW pH 171i transmitters. The pH was measured with Hamilton and WTW pH electrodes connected to pH transmitters Yokogawa pH402G and WTW pH 197i. Temperature was measured in the setup 1 only, with the pH electrode. *Figure 8* shows a picture of one of the reactors during the experiment performed at Ejby Mølle WWTP.

Figure 8 Picture of the reactor with digested sludge during the experiment performed at Ejby Mølle WWTP

Signals from the meters were recorded by a Modicon Compact TSX programmable logic controller (PLC). This PLC is from the same manufacturer as the PLC's used in the WWTP control system. The PLC was connected to the plant control system such that the recorded data and control inputs and

outputs could be viewed and downloaded via the plants supervision, control and data acquisition system (SCADA). ***Table 5*** shows the analogue to digital conversion which takes place in the PLC. The analogue input to the Modicon PLC was converted to a digital value by a 12 bit analogue to digital converter.

Table 5 Analogue to digital signal conversion in the Modicon1 Compact TSX PLC

Setup	Measurement	Measurement range	Meter analog output	PLC analog input	Digital span used	Recorded resolution
1	Oxygen	0-10 mgO2/L	0-20 mA	-20 – 20 mA	2047	0,005 mg O_2/L
	Temperature	0-100 ^{o}C	0-20 mA	-20 – 20 mA	2047	0,05 ^{o}C
	pH	0-14	0-20 mA	-20 – 20 mA	2047	0,007 pH
	Redox	-500 – 500mV	4-20 mA	-20 – 20 mA	1637	0,6 mV
2	Oxygen	0-20 mgO2/L	0-2 V	-10 – 10 V	409	0,05 mg O2/L
	pH	0-14	0-1,4 V	-10 – 10 V	286	0,05 pH
	Redox	-500 -500mV	-0,5–0,5V	-10 – 10 V	204	5 mV

1 Schneider Electric SA, France

Oxygen concentration I and II experiments were performed under aerobic conditions. Setup 1 was used for the lower oxygen concentration because of the better resolution of the oxygen signal in the PLC. Setup 2 was used for the for the higher oxygen concentration, *Table* ***4*****.** During oxygen concentration II experiment, the redox potential electrode was found to be more stable than the oxygen electrode (not affected by the air bubbles). Thus in this experiment, redox potential was kept in the low range in the first reactor and in the high range in the second reactor, *Table* ***4*****.** These redox potential ranges were related to the oxygen concentration in the sludge form Oxygen concentration I experiment.

There were two oxygen set points chosen to keep the oxygen concentration in the expected level. The rules of the control strategy were:

- If the oxygen concentration dropped below the lower set point the PCL turned on both air pumps.
- If the oxygen concentration was between the two set points only one pump was on.
- If the oxygen concentration was greater than the high set point both pumps were turned off.

This strategy worked well as long as the sludge was well mixed. When mixing failed the oxygen was not evenly distributed in the sludge and air bubbles collected on the oxygen sensor.

Figure 9 shows data from setup 1 from the oxygen concentration I experiment where the oxygen set points were in the low range. The oxygen concentration varied between values above and below the set points. The control is not perfect primarily because there were delays between aeration and the oxygen concentration. Most of the delay was to due to the response time of the oxygen meter. There was also a delay because oxygen had to be transfer from the air bobbles to the sludge. These delays were the reason that the oxygen concentration continued to increase after both air pumps were turned off. It was also the reason that the oxygen concentration had decreased for a short time after the aeration was turned on.

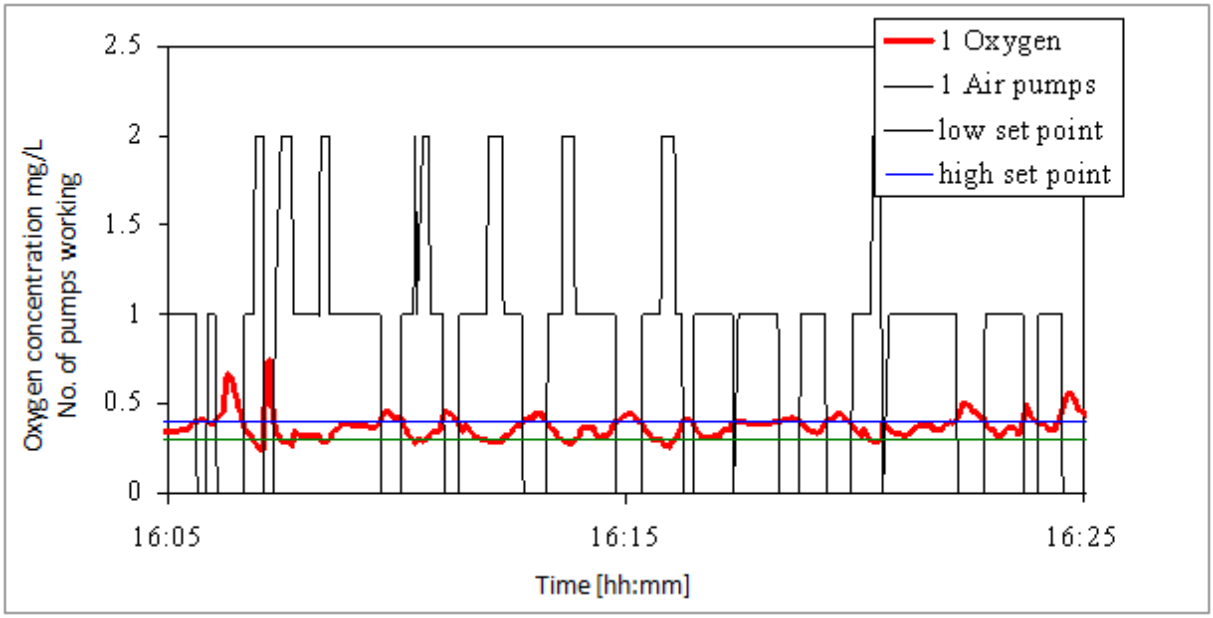


Figure 9 Oxygen control in set up 1

3.2.2 Sampling

Sludge samples (10 ml) were taken out of the reactors during the experiments with a glass pipette. During first three experiments (Electron acceptors I and II, digested sludge), samples were taken more frequently, ***Table 4*****.** Results for third experiment (Triclosan) showed that degradation occurs much slower in digested sludge than in activated sludge. Thus, in experiments four to seven (Molasses, Ultrasound, Oxygen concentration I and II) sampling frequency was lower, ***Table 4***.

3.3 Extraction procedure

The extraction was performed shortly after sampling (approximately 30 min). 10 ml sludge samples were diluted with 990 ml tap water in 1L volumetric flask. The same day the liquid–liquid extraction (LLE) was performed by addition of 10 mL toluene (residue grade, Merck, Darmstadt, Germany), an aliquot of 100 µL internal standard (IS) solution (containing 100 ng D15 musk xylene). The same amount of IS was used for all the samples. Deuterated musk xylene was chosen as IS as it gives an undisturbed signal and does not undergo any reaction itself [71]. The organic phase was separated from the aqueous phase by settling and subsequently the organic extracts were frozen overnight at -20°C to remove the residual water. The extract was concentrated to a volume of 1 ml by evaporating the toluene in a fume hood at 50°C with a gentle flow of N_2.

3.4 Instrumentation

The resulting extracts were analysed by gas chromatography with mass spectrometric detection (GC-MS), equipped with splitless injector and A200s auto sampler. Samples (1 µl) were injected in the splitless mode with a temperature of 240°C in the injector with 1.5 min splitless. The GC separation was performed with a Rxi-5 Sil MS fused-silica capillary column (Restek, USA) with length 12m, 0.18 mm inner diameter, 0.18 µm film thickness and deactivated (no film) pre-column with 1 m length, 0.53 µm inner diameter and temperature program as follows: 90°C [1minute] -> 50°C/min -> 135°C -> 10°C/min -> 200° C -> 40°C/min [5 minutes] -> 260°C with helium as a carrier gas. The gas carrier was operated with flow programming: the initial flow 1.3 [ml/min] and final flow 0.7 [ml/min]. The higher initial flow took care of fast transfer of sample from injector to the column, the decrease of flow provided better separation in the column. The GC interface temperature was 250°C to transfer all compounds from the GC into the MS. The ion source was operated at 160°C and mass fragments were determined in the selected monitoring ion mode with 31-61 ms dwell time for the respective mass fragments, sees ***Table 6***. The mass spectrometer's multiplier was operated with 450 V detection voltage, ionization was performed with 150 µA emission and 70 eV electron energy. The chromatogram of the first mass fragment was used for quantification and the second (and third) for verification, see ***Table 6***. A technical nonylphenols is a mixture of more than 100 isomers, which spectrum can be detected on three mass fragments (121, 135 and 149). Final concentration was calculated as a sum of three mass fragments.

3.5 Chemicals

Toluene (residue grade), acetone and ethyl acetate (analytical grade) were purchased from Merck (Darmstadt, Germany). Triclosan, AHTN, Nonylphenols, PAHs and the internal standard ($Mx\text{-}D_{15}$) were purchased from Ehrenstorfer (Augsburg, Germany). International Fragrances and Flavours provided OTNE, pure HHCB and HHCB lactone. DEHP was purchased from Riedel-de Haën.

Table 6 Masses fragments, retention times and dwell time per mass for organic compounds analyzed in this study.

Compounds	First mass [amu]	Second mass [amu]	Third mass [amu]	Retention time [min]	Dwell time per mass [sec]
OTNE	191	234		3.55	0.031
Nonylphenol 1	121	-		3.84-4.22	0.031
Nonylphenol 2	135	-		3.94-4.13	0.031
Nonylphenol 3	149	-		4.00-4.32	0.031
Mx-D15	294	312	-	4.87	0.033
HHCB & AHTN	243	258		4.92/5.02	0.033
Triclosan	288	290		7.22	0.043
HHCB - lactone	257	272		7.59	0.043
Triclosan methyl	302	304		7.24	0.043
DEHP	149	167	279	9.55	0.048
Acenaphtylene	152	76		2.45	0.031
Acenaphthene	154	76		2.60	0.031
Fluorene	166	82		3.12	0.031
Phenanthrene	178	152		4.50	0.033
Anthracene	178	152		4.57	0.033
Fluoranthene	202	101		6.75	0.043
Pyrene	202	101		7.16	0.043
Benzo(a)anthracene	228	114	-	9.29	0.048
Chrysene	228	114		9.33	0.048
Benzo(b)fluoranthene	252	250		10.25	0.061
Benzo(k)fluoranthene	252	250		10.25	0.061
Benzo(a)pyrene	252	250		10.55	0.061
Dibenzo(a,h)anthracene	276	138		12.18	0.061
Benzo(ghi)perylene	278	138		12.22	0.061
Indeno(1,2,3-cd)pyrene	276	137		12.66	0.061

4. Results and discussion

The presentation of the results in this chapter was performed on the seven representative compounds, which are used as markers for the respective processes. However, behaviour of all the compounds included in this study has been discussed. The presentation of the results was divided into three parts, in three subchapters. Firstly, results from the two experiments in activated sludge (Electron acceptor regimes I and II) was presented and discussed. Secondly, three experiments in digested sludge (Triclosan, Molasses and Ultrasound) were introduced and finally a presentation and discussion of the results from the two last experiments performed at Ejby Mølle WWTP was given. Concentration levels of the organic micro-pollutants in the activated and digested sludge were firstly presented. The focus of this chapter was to examine and discuss the removal of organic micro-pollutants in different systems and environments.

4.1 Concentration levels of the organic micro-pollutants in the activated and digested sludge

Table 7 shows the range of the starting concentrations of the organic micro-pollutants found in the activated sludge from the first degradation experiment (Electron acceptor regimes I) and in the digested sludge from the fifth and sixth degradation experiments (Molasses and Oxygen concentration I and II). There are also concentrations of regulated organic micro-pollutants (PAHs, DEHP and nonylphenols) from the analyse report from Ejby Mølle WWTP. PAHs and nonylphenols were not analysed in the degradation experiments in the activated sludge.

The concentration of the compounds in the sludge depends on the: concentration of the compound in the WWTP' influent, the degradation of the compound during treatment and the loss of sludge weight during stabilization. Most of the compounds were in higher concentrations in the digested sludge than in the activated sludge. The starting concentrations found in the digested sludge from Ejby Mølle WWTP were generally lower than those from Frederikshavn, and were in a similar range to concentrations found in the activated sludge from Aalborg East WWTP. Triclosan-methyl was only found in the activated sludge from Aalborg WWTP and in very low concentration in the digested sludge from Ejby Mølle WWTP. The highest concentration in each type of sludge was found for DEHP and nonylphenols.

Table 7 Starting concentration of organic micro-pollutants in the activated and digested sludge found in this study and from the analyse report from Ejby Mølle WWTP

Compound	Concentration			
	1.Electron acceptor regimes I Activated sludge (Aalborg)	**Analyse report- Ejby Mølle** Digested sludge	**5.Molasses** Digested sludge (Frederikshavn)	**6.Oxygen concentration I and II** Digested sludge (Ejby Mølle)
	µg/g TS			
OTNE	8-9	-	28-30	13-20
HHCB	20-30		60-70	30-35
AHTN	1-1,2		1- 1,5	1-1,2
HHCB-lac	0,6-0,8		1	0,3-0,5
TCS	**2-3** (elevated level)		8-9	2,5-3
TCS-meth	0,3-0,4		*	0,02-0,04
TCPP	-		*	0,3-1
TiBP			100-180	0,9-0,8
TnBP			4-5	1,8-2
DEHP	35-55	26	150-200	45-60
Acenaphtylene	-	-	0,2-0,4	0,03
Acenaphthene		0,072	3-5	0,15-0,18
Fluorene		0,17	3-4	0,17-0,22
Phenanthrene		0,42	3-5	0,4-0,7
Anthracene		-	3,4-4	0,6-0,9
Fluoranthene		0,32	1	0,3-0,8
Pyrene		0,43	2,5-3	0,4-0,8
Benzo(a)anthracene		-	0,8-1	*
Chrysene		-	1,5-2	0,2-0,4
Benzo(b)fluoranthene		0,29	1 - 2	0,1-0,4
Benzo(k)fluoranthene				
Benzo(a)pyrene		0,14	0,5-1,2	0,2-0,7
Dibenzo(a,h)anthracene		-	4-5	*
Benzo(ghi)perylene		0,16	0,1-0,2	*
Indeno(1,2,3-cd)pyrene		0,22	1-1,5	*
Sum of nonylphenols		19	≈200-300	≈130-150

- No data; compound was not analysed

* below the limit of quantification

The Ejby Mølle analyse report's results were compared with own data from the degradation experiments performed at the Ejby Mølle WWTP's laboratory. The concentration of the regulated organic micro-pollutants found in the sixth and seventh degradation experiments (Oxygen concentration I and II) are very similar to those found in the analyse report, except for nonylphenols, which concentration is one order of magnitude greater than the concentration from the analyse report. It is possible that these discrepancies between nonylphenols' concentrations occurred as different methods were used for determination of the compounds concentration in the analyse report and in the experiments performed in this study. Nevertheless, resemblance of the rest of the compounds' concentrations from the experiments performed in this study to the analyse report from the plant, gave a validation of own data.

4.2 Processes in the activated sludge

4.2.1 Influence of the different electron acceptors on the elimination of the organic micro-pollutants in the activated sludge.

As the biodegradation pathways of organic contaminants rely on the presence or absence of the appropriate electron acceptors [76], the organic micro-pollutants' behaviour was examined in the presence of oxygen, reductive environment and under anoxic conditions. For instance, degradation of PAHs requires oxygen as the current knowledge suggests that benzene is recalcitrant under all anaerobic conditions. Further degradation however can occur under anaerobic conditions [19, 40]. On the other side, aromatic polychlorinated compounds are more probable to degrade in the absence of oxygen i.e. during reductive dehalogenation [76].

Degradation of the compounds can be described by a first order degradation reaction. The first order degradation rate *k* can be calculated from equation:

$$C = C_0 \cdot \mathrm{e}^{-\mathrm{kt}}$$

Thus

$$\frac{C_0}{C} = e^{kt} \Rightarrow ln\left[\frac{C_0}{C}\right] = k \cdot t$$

This gives the first order degradation reaction:

$$k = \frac{ln\left[\frac{C_0}{C}\right]}{t}$$

where C_0 is the initial compound concentration at the time $t = 0$, and C is the compound concentration at the time t.

The half-life can be determined using the equation:

$$T_{1/2} = \frac{ln\left[\frac{C_0}{\frac{C_0}{2}}\right]}{k} = \frac{\ln 2}{k}$$

The fast degradation is described by a high k value and low $T_{1/2}$.

The most rapid elimination for most of the examined organic micro-pollutants, e.g. triclosan, in the activated sludge was observed in the presence of oxygen. *Figure 10* shows the concentration of triclosan from the first aeration experiment (Electron acceptor regimes I), while in *Figure 11* ln C/C_0 of triclosan is plotted for kinetic assessment.

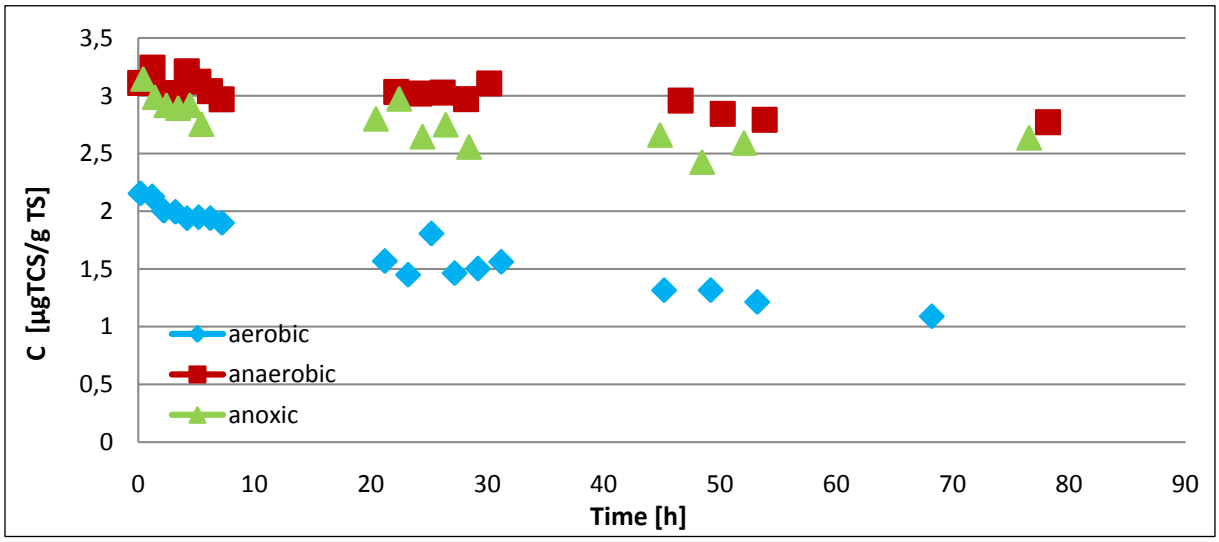


Figure 10 Concentration of triclosan under aerobic, anaerobic and anoxic conditions during first aeration experiment (Electron acceptor regimes I) in the activated sludge (triclosan was spiked to the experiment). TSC = triclosan, TS = total solid

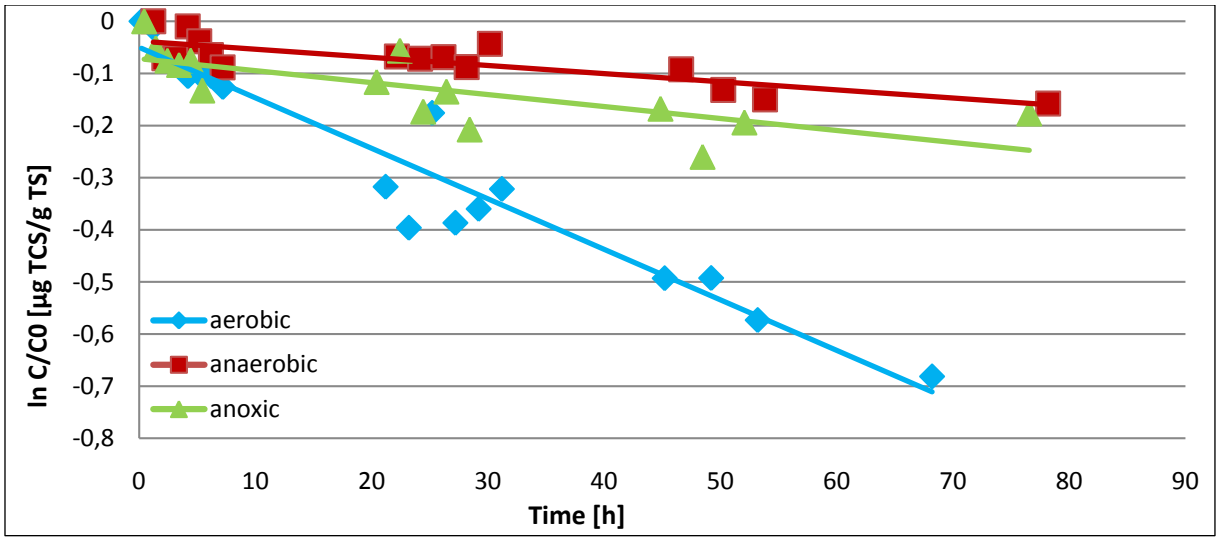


Figure 11 Ln C/C_0 of triclosan under aerobic, anaerobic and anoxic conditions during first aeration experiment (Electron acceptor regimes I) in the activated sludge (triclosan was spiked to the experiment)

In the ***Table 8***, first order degradation rates *k* [d^{-1}] and half-lives $T_{1/2}$ *[d]* are summarized. The *k* values found under the aerobic conditions where 50%, 50-70%, 70% and 80% greater than those found in the reductive environment for HHCB,OTNE, DEHP and triclosan, respectively and 80-90% greater than those found under anoxic conditions for these compounds. Only for AHTN, the optimal removal was observed under the anaerobic conditions. In this case, *k* values were 67% and 75% greater than those found under aerobic and anoxic conditions, respectively.

Table 8 Comparison of aerobic, anoxic and anaerobic treatment of organic micro-pollutants. First order degradation rates k and half-lives $T_{1/2}$ for organic micro-pollutants in the first and second degradation experiment (Electron acceptor regimes I and II) in the activated sludge

Compound	**1. Experiment** **Electron acceptor regimes I** Activated sludge (Aalborg) Elevated level of triclosan 100 µg/L						**2. Experiment** **Electron acceptor regimes II** Activated sludge (Aalborg) Elevated level of OTNE 100 µg/L			
	AEROBIC		**ANOXIC**		**ANAEROBIC**		**AEROBIC**		**ANAEROBIC**	
	k [d^{-1}]	**$T_{1/2}$** [d]	**k** [d^{-1}]	**$T_{1/2}$** [d]	**k** [d^{-1}]	**$T_{1/2}$** [d]	**k** [d^{-1}]	**$T_{1/2}$** [d]	**k** [d^{-1}]	**$T_{1/2}$** [d]
OTNE	0,71	1	0,06	11	0,18	4	0,56	1	0,24	3
HHCB	0,14	5	0,03	22	0,06	12	0,07	9,3	-	-
AHTN	0,03	21	0,03	6,4	0,11	22	-	-	0,13	5
Triclosan	0,23	3	0,04	18	0,06	12,6	0,06	11	-	-
TCS-methyl	-	-	-	-	-	-	-	-	-	-
HHCB-lact	-	-	-	-	-	-	-	-	-	-
DEHP	0,12	6	0,01	96	0,02	29	-	-	-	-

-Concentration was stable/increasing, thus it was impossible to determine k and $T_{1/2}$ values.

These results are in a good accordance with previous findings suggesting that the availability of oxygen is a major regulator of DEHP degradation [2], while under anaerobic conditions this compound is believed to be persistent [19]. These results show a faster degradation in the presence of oxygen for triclosan and OTNE as well. However, there are no comparison data on OTNE and triclosan in the sludge. Moreover, in one study, high oxygen concentrations resulted in the fastest removal processes of triclosan and biodegradation became the dominant removal mechanism [46]. This indicates that a certain change in environmental conditions could possibly influence the removal mechanisms of triclosan. For instance, an increase of oxygen levels could enhance the role of biodegradation.

4.2.2 Elimination pathways of the organic micro-pollutants during the degradation experiments in the activated sludge

Since the presence of oxygen seemed to favour the elimination of most of the compounds, further experiments were performed to gain more insight into the aerobic processes. In general, elimination behaviour of the organic micro-pollutants examined in this study during aeration of the activated sludge can be divided in three groups; compounds with high removal rates (OTNE), compounds with intermediate removal rates (triclosan, DEHP and HHCB) and finally those with very low or no removal at all (AHTN, HHCB-lactone and TCS-methyl). For a demonstration the concentrations of triclosan, OTNE and AHTN are shown in *Figure 12* while ln C/C_0 of these three compounds is presented in *Figure 13*.

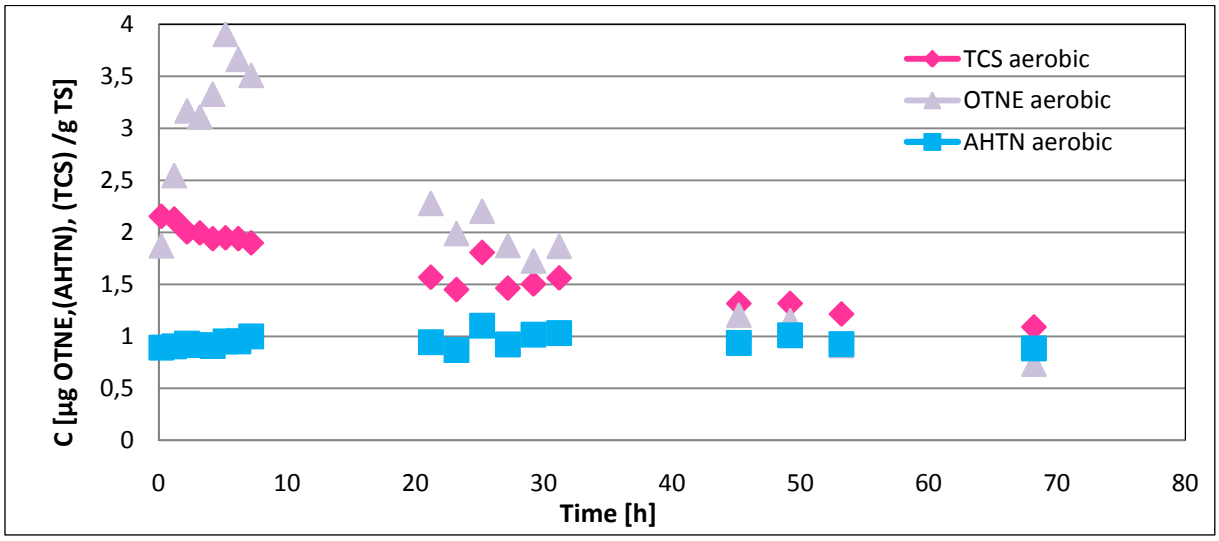


Figure 12 Comparison of AHTN, OTNE and triclosan during first aeration experiment (Electron acceptor regimes I) in the activated sludge

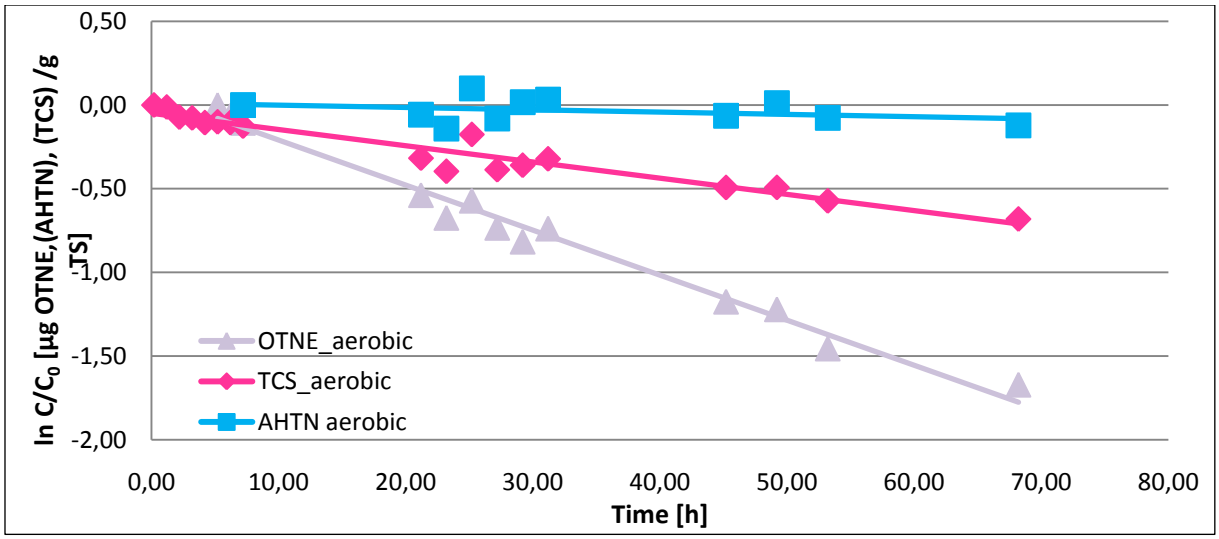


Figure 13 Comparison of AHTN, OTNE and triclosan ln C/C_0 during the first aeration experiment (Electron acceptor regimes I) in the activated sludge

The highest degradation rate *k* and half-life $T_{1/2}$ was found for the fragrance OTNE, while intermediate values for triclosan, DEHP and HHCB were found. The fragrance AHTN had the lowest degradation rate and the transformation products HHCB-lactone and TCS-methyl were not removed at all in aerobic environment. After 70 hours OTNE was removed by 83%, triclosan by 49%, DEHP by 35%, HHCB by 32% and AHTN by 8%.

The removal rates observed during experiments in the activated sludge may be a result of biodegradation but also transformations to unknown metabolites. AHTN has a high *n*-octanol–water partition coefficient K_{ow} and was possibly not available for microorganisms. Therefore, the first order degradation rate was very low in aerobic conditions. Nevertheless, the first order degradation rate *k* for AHTN in anaerobic conditions was comparable with values for DEHP and HHCB in the aerobic conditions, ***Table 8***. After 70 h AHTN was removed by 32% which indicates a potential for the biodegradation of AHTN under the anaerobic conditions. As HHCB also has a high log K_{ow}, it could also be sorbed onto the particles. However, it cannot be ruled out that transformation of the HHCB to HHCB-lactone and further to number of secondary metabolites, had contributed to the relatively high first order degradation rate of HHCB in comparison to AHTN. Though, the concentration of HHCB-lactone did not increase significant during the experiment.

In ***Table 9***, first order degradation rates *k* [L g^{-1} TVS d^{-1}] and half-lives $T_{1/2}$ [d L^{-1} g^{-1} TVS] are summarized. Total volatile solids (TVS) - the sludge's biomass, determine the microbial activity of the sludge. The *k* and $T_{1/2}$ values were normalised to the sludge's biomass of the respective sludge sample, thus both experiments in the activated sludge could be compared.

Table 9 First order degradation rates k and half-lives $T_{1/2}$ for organic micro-pollutants in the first and second degradation experiment in the activated sludge

Compound	Experiment 1 Electron acceptor regimes I Activated sludge (Aalborg) Elevated level of triclosan 100 µg/L						Experiment 2 Electron acceptor regimes II Activated sludge (Aalborg) Elevated level of OTNE 100 µg/L			
	AEROBIC		ANOXIC		ANAEROBIC		AEROBIC		ANAEROBIC	
	k [L g^{-1} TVS d^{-1}]	$T_{1/2}$ [d L^{-1} g^{-1} TVS]	k [L g^{-1} TVS d^{-1}]	$T_{1/2}$ [d L^{-1} g^{-1} TVS]	k [L g^{-1} TVS d^{-1}]	$T_{1/2}$ [d L^{-1} g^{-1} TVS]	k [L g^{-1} TVS d^{-1}]	$T_{1/2}$ [d L^{-1} g^{-1} TVS]	k [L g^{-1} TVS d^{-1}]	$T_{1/2}$ [d L^{-1} g^{-1} TVS]
OTNE	0,070	9,7	0,007	101	0,019	35	0,084	8,3	0,037	19
HHCB	0,016	44	0,004	187	0,007	104	0,011	62	-	-
AHTN	0,004	187	0,003	202	0,012	58	-	-	0,020	35
Triclosan	0,026	27	0,004	164	0,006	114	0,009	74	-	-
TCS-methyl	-	-	0,004	154	-	-	-	-	-	-
HHCB-lact	-	-	0,004	187	0,005	131	-	-	-	-
DEHP	0,013	54	0,001	874	0,003	262	0,002	385	-	-

-Concentration was stable/increasing, thus it was impossible to determine k and $T_{1/2}$ values.

4.3 Processes in the digested sludge

4.3.1 Elimination pathways of the organic micro-pollutants during the degradation experiments in the digested sludge performed at university's laboratory

During three experiments in digested sludge (Triclosan, Molasses and Ultrasound), different technologies, such as addition of aerated sludge, molasses and ultrasonic treatment were applied in order to enhance the removal of the organic micro-pollutants. Influence of these technologies will be discussed in following subchapters (4.3.2, 4.3.3, 4.3.4). The behaviour trends of the respective compounds are similar in all three experiments. Namely, the highest (lowest) degradation rates were observed for the same compounds in all of the discussed experiments. Therefore, the focus of this chapter will be to discuss the elimination pathways of the respective compounds. Overall, there are four groups of compounds regarding the elimination behaviour; compounds with very fast removal rates (light PAHs, dibenzo(a,h)anthracene, TiBP), compounds with relatively high removal rates (OTNE, DEHP, TnBP), compounds with intermediate removal rates (triclosan, HHCB, some of the heavy PAHs) and finally those with low removal rates (AHTN, HHCB-lactone and TCS-methyl, some of the heavy PAHs nonylphenols). For a demonstration the concentrations of triclosan, acenaphthene, DEHP, triclosan and AHTN are shown in *Figure 14* while ln C/C_0 of these three compounds is

presented in *Figure 15*.

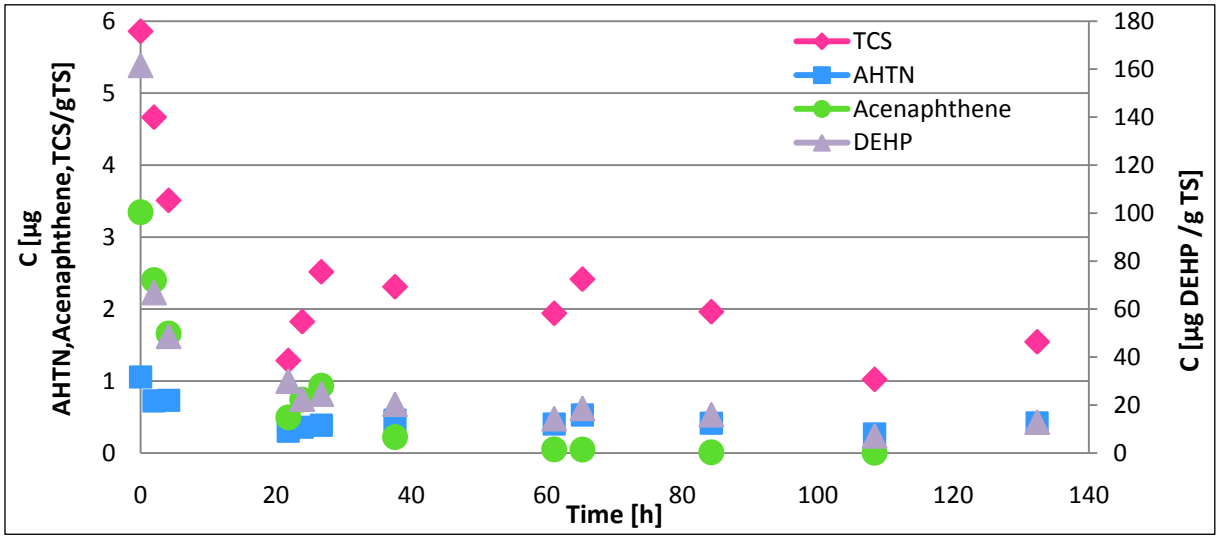


Figure 14 Comparison of AHTN, DEHP, triclosan and acenaphthene removal in the reactor I from the fifth degradation experiment (Ultrasound)

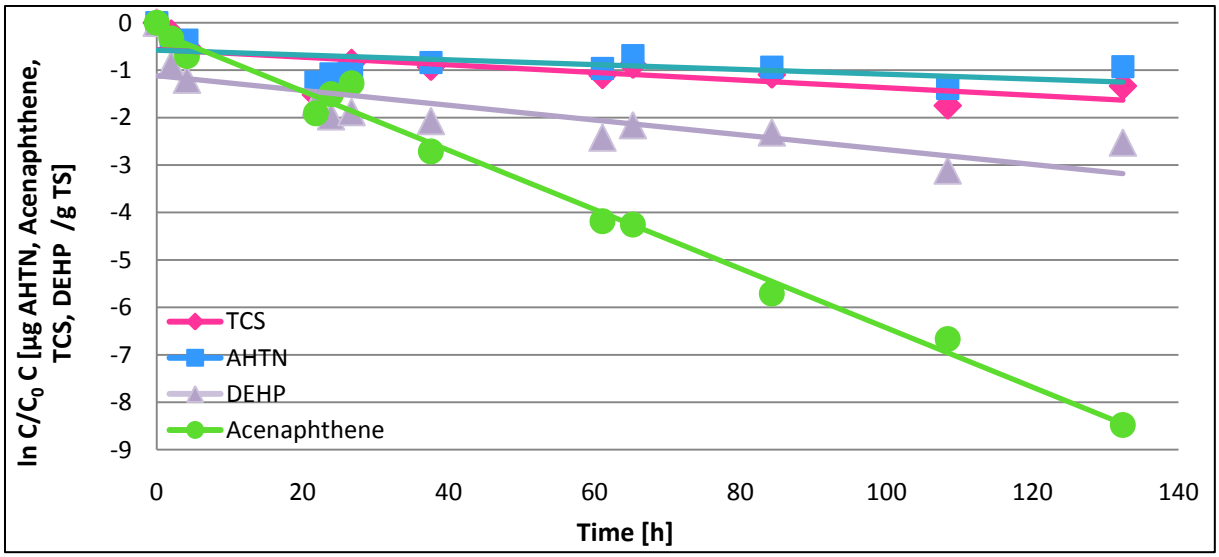


Figure 15 Comparison of ln C/C_0 of AHTN, DEHP, triclosan and acenaphthene removal in the reactor I from the fifth degradation experiment (Ultrasound)

The degradation rates, *k* and half-lives, $T_{1/2}$ from these three experiments are presented in ***Table 10*** - *Table* ***12*** below. Fast removals were observed for DEHP, OTNE and TnBP. Reaction rates *k* were from 0,14 to 0,23 and from 0,42 to 0,57 for these compounds in the degradation experiment: Triclosan, Ultrasound and Molasses, respectively. High reaction rates, *k* from 0,16 to 0,144, were observed for

triclosan as well in the degradation experiments: Triclosan and Ultrasound, while *k* in the Molasses experiment was in rather low range. The rapid elimination was also found for low molecular PAHs (acenaphthene, acenephthylene, fluorene, phenanthrene and anthracene), some of the heavy PAHs (fluoranthene and Dibenzo(a,h)anthracene) and TiBP, *Table **11***,*Table **12***. Reaction rates for the low PAHs were from 0,576 to 2,1 and from 0,47 to 0,646 in the degradation experiments: Molasses and Ultrasound, respectively. For fluoranthene and dibenzo(a,h)anthracene elimination rates were respectively 0,58 and 0,984 in the degradation experiments: Molasses and Ultrasound, respectively. Elimination rates for TiBP were 1,4 and 0,720 in the experiments: Molasses and Ultrasound, respectively. Relatively low elimination rates were found for HHCB, while very low or no elimination was observed for AHTN, HHCB-lactone and the nonylphenol isomers. Very low reaction rates for AHTN, HHCB and HHCB-lactone were found in the Triclosan experiment, when comparing to the Molasses and Ultrasound experiments. Elimination rates were in range from 0,034 to 0,077 and in from 0,11 to 0,15 for these compounds experiments: Triclosan, Molasses and Ultrasound, respectively. In the fourth experiment (Molasses) the reaction rate for HHCB was found to be relatively high, *Table **11***. The transformation product triclosan-methyl was detected only in the degradation experiment: Triclosan, *Table **10***.

Table 10 First order degradation rates k and half-lives $T_{1/2}$ for organic micro-pollutants in the third degradation experiment (triclosan)

Compound	**Experiment 3: Triclosan** (Ejby Mølle WWTP) Elevated level of triclosan (100 µg/L) **AEROBIC**	
	k [d^{-1}]	$T_{1/2}$ [d]
OTNE	0,214	3,0
HHCB	0,070	10
AHTN	0,077	9,0
Triclosan	0,160	4,3
Triclosan-methyl	0,840	1,0
HHCB-Lactone	0,034	21
DEHP	0,140	5,0

Table 11 Influence of activated sludge and molasses addition on the processes in the digested sludge. First order degradation rates k and half-lives $T_{1/2}$ for organic micro-pollutants in the fourth degradation experiment (Molasses)

	Experiment 4: Molasses Digested sludge (Frederikshavn) Elevated level of DEHP (100 µg/L) **AEROBIC**					
	Reactor I		**Reactor II**		**Reactor III**	
Addition of aerobic sludge	-		+		+	
Addition of molasses	-		-		+	
Compound	**k** [d^{-1}]	**$T_{1/2}$** [d]	**k** [d^{-1}]	**$T_{1/2}$** [d]	**k** [d^{-1}]	**$T_{1/2}$** [d]
TiBP	1,4	0,5	1,2	0,6	1,2	0,6
TnBP	0,570	1,2	0,473	1,5	0,514	1,3
OTNE	0,420	1,6	0,290	2,4	0,300	2,3
NP 149(1,2,3)	0,184	3,8	0,120	5,8	0,154	4,5
NP 135(1,2,3,4)	0,254	2,7	0,193	3,6	0,244	2,8
NP 121(1,2,3)	0,230	3,0	0,180	3,8	0,156	4,4
HHCB	0,266	2,6	0,180	3,9	0,206	3,4
AHTN	0,120	5,7	0,204	3,4	0,180	3,9
Triclosan	0,194	3,6	0,200	3,4	0,257	2,7
HHCB-Lactone	0,140	5,0	0,140	5,0	0,150	4,7
DEHP	0,420	1,6	0,330	2,0	0,330	2,0
Acenaphtylene	2,1	0,3	1,1	0,6	1,1	0,7
Acenaphthene	1,5	0,5	1,1	0,6	1,1	0,6
Fluorene	0,840	0,8	0,750	0,9	0,760	0,9
Phenanthrene	0,630	1,0	0,806	0,9	0,600	1,2
Anthracene	0,576	1,2	0,370	2,0	0,530	1,3
Fluoranthene	0,580	1,2	0,434	1,6	0,463	1,5
Pyrene	0,264	2,6	0,180	4,0	0,194	3,6
Benzo(a)anthracene	0,264	2,6	0,230	0,3	0,197	3,5
Chrysene	0,220	3,0	0,180	3,8	0,210	3,3
Benzo(b)fluoranthene / Benzo(k)fluoranthene	0,266	2,4	0,190	3,7	0,190	3,7
Benzo(a)pyrene	0,346	2,0	0,127	5,4	0,170	4,0
Dibenzo(a,h)anthracene	0,510	1,4	0,570	1,2	0,540	1,3
Benzo(ghi)perylene	0,276	2,5	0,283	2,5	0,377	1,8
Indeno(1,2,3-cd)pyrene	0,570	1,2	0,430	1,6	0,443	1,6

Table 12 Influence of activated sludge addition and ultrasonic treatment on the processes in the digested sludge. First order degradation rates k and half-lives $T_{1/2}$ for organic micro-pollutants in the fifth degradation experiment (Ultrasound)

	Experiment 5: Ultrasound Digested sludge (Frederikshavn) AEROBIC					
	Reactor I		**Reactor II**		**Reactor III**	
Aerobic sludge addition	-		+		+	
Ultrasound applied	-		-		+	
Compound	**k** [d^{-1}]	**$T_{1/2}$** [d]	**k** [d^{-1}]	**$T_{1/2}$** [d]	**k** [d^{-1}]	**$T_{1/2}$** [d]
TiBP	0,720	1,0	0,674	1,0	0,660	1,0
TnBP	0,185	3,8	0,074	9,3	0,074	9,3
OTNE	0,230	4,0	0,127	7,0	0,113	9,0
NP 149(1,2,3)	0,093	8,0	0,080	9,0	0,060	14
NP 135(1,2,3,4)	0,076	20	0,037	31	0,047	22
NP 121(1,2,3)	0,086	8,0	0,010	72	0,026	47
HHCB	0,150	4,6	0,060	12	0,046	15
AHTN	0,115	6,0	-	-	-	-
Triclosan	0,106	4,0	0,113	6,7	0,144	7,0
HHCB-Lactone	0,110	6,3	0,030	24	0,022	32
DEHP	0,160	4,4	0,120	6,0	0,048	14
Acenaphtylene	0,520	1,3	0,185	3,8	0,187	3,7
Acenaphthene	0,520	1,3	0,460	1,5	0,410	1,7
Fluorene	0,470	1,5	0,370	2,0	0,374	2,0
Phenanthrene	0,646	1,0	0,600	1,2	0,367	2,0
Anthracene	0,620	1,0	0,680	1,0	0,523	1,3
Fluoranthene	0,140	5,0	0,100	6,7	0,110	6,4
Pyrene	0,120	5,7	0,065	10	0,060	12
Benzo(a)anthracene	0,134	5,2	0,120	6,0	0,084	8,3
Chrysene	0,185	3,8	0,086	8,0	0,070	10
Benzo(b)fluoranthene	0,118	6,0	0,065	11	0,060	12
Benzo(k)fluoranthene						
Benzo(a)pyrene	0,197	3,5	0,053	13	0,010	58
Dibenzo(a,h)anthracene	0,984	0,7	0,810	1,0	0,760	1,0
Benzo(ghi)perylene	0,574	1,2	0,290	2,4	0,270	2,6
Indeno(1,2,3-cd)pyrene	0,120	5,8	0,160	4,4	0,160	4,4

-Concentration was stable/increasing, thus it was impossible to determine k and $T_{1/2}$ values.

The general high elimination rates for OTNE and DEHP in all of the three experiments (Triclosan, Molasses, and Ultrasound) indicates the presence of microbial species in the digested sludge that are able to mineralize or transform these compounds and required enzymes. The fast removal for OTNE is in good accordance with the results from the comparison experiments in the activated sludge (Electron acceptor regimes I and II), chapter 4.2. This also holds true for HHCB, AHTN and HHCB-

lactone. Only the removal rates for triclosan in these three experiments were somewhat slower in comparison to the results from the two first experiments (Electron acceptor regimes I and II). It cannot be ruled out however, that a lower oxygen supply leads to smaller oxygen concentrations in the digested sludge and subsequently decreased reaction rates for triclosan. The rapid elimination of light PAHs is expected to be partly due to the abiotic losses such as volatilization, see Henry's constant K_H, *Table* ***3***. Biodegradation however cannot be excluded, since this is well established for light PAHs [74, 19]. The heavy PAHs were rather persistent during these three experiments. It is well known that the biodegradation of heavy PAHs in the sludge can be limited by the low bioavailability resulting from their strong sorption to the organic particles [74]. Moreover, due to the rather low concentrations of heavy PAHs, they would be more likely degraded through co-metabolism rather than serve as a primary substrate [19]. Therefore, microorganisms that cooperate in this process might have needed longer exposure to the compound and thereby more time to adapt. The alkylphosphates TnBP and TiBP were efficiently removed during the aeration of the digested sludge. Elimination of the straight chained TnBP could be related to the biodegradation, however slower degradation of the branch chained TiBP was not expected. It is more possible that volatilization contributed to the removal of TiBP.

4.3.2 Influence of adding activated sludge from the aeration basin in order to introduce additional aerobic bacteria to the processes

Aeration of the digested sludge was applied with the intention to initiate aerobic degradation of the organic micro-pollutants. However, the consortia established in the digester might need to adapt to the new conditions and consequently the system might show an extended lag phase. To avoid this situation and minimize the adaption time, activated sludge from an aeration tank (100 ml) was added. It was expected that the aerobic bacteria would take advantage over the anaerobic microorganisms and start off the aerobic biodegradation. Surprisingly, addition of the aerated activated sludge considerably decreased the removal efficiency for the majority of the compounds. *Table* ***11*** and *Table* ***12*** show degradation rates *k* and half-lives $T_{1/2}$ calculated in two experiments (Molasses and Ultrasound), where the aerobic sludge was added to reactor II. As a demonstration *Figure 16* shows the concentration of HHCB in two reactors, with and without addition of the aerobic sludge.

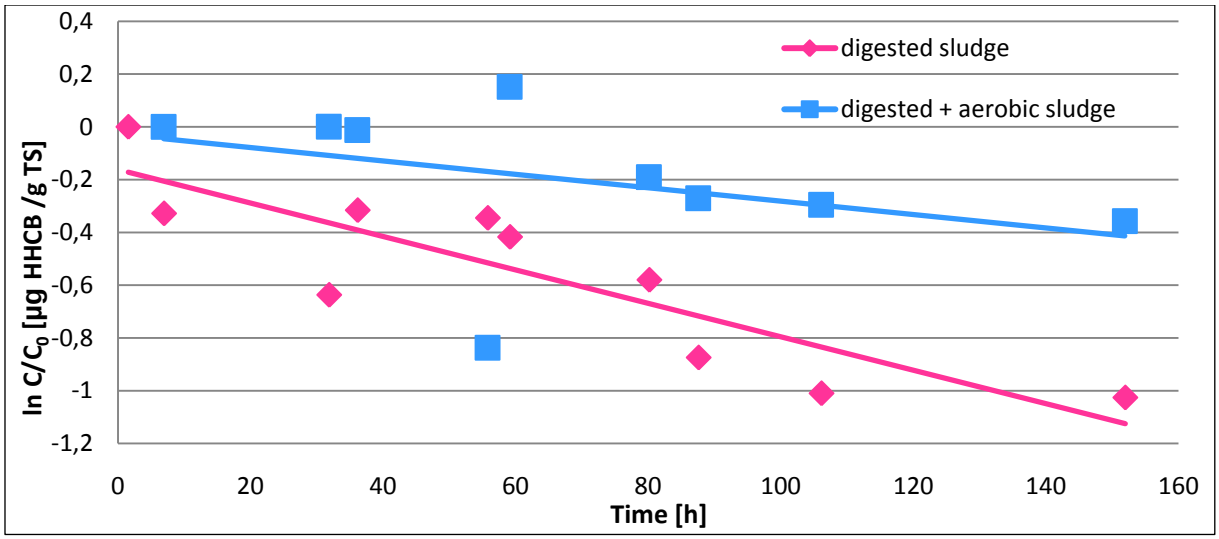


Figure 16 Comparison of ln C/C0 of HHCB in to reactors: I) digested sludge, and II) digested sludge +sludge from aeration tank

A higher degradation rate with aerobic sludge was only observed for indeno(1,2,3-cd)pyrene and triclosan in both degradation experiments, where aerobic sludge was added (Molasses and Ultrasound);*Table* ***11***, *Table* ***12***. For some compounds a none to slight increase of the biodegradation rate was observed as a result of the aerobic sludge addition. Considering the accuracy of the measurements it is more realistic to conclude no significant effect of the aerated sludge addition to digested sludge on the organic micro-pollutants removal.

4.3.3 The influence of molasses addition to enhance degradation/ co-metabolism

At low concentrations of organic micro-pollutants, insufficient energy and carbon may be available for growth and maintenance. Molasses was added to the digested sludge as a supplementary organic carbon source. An extra amount of food in the reactor might cause a faster growth of microorganisms and increase the biodegradation rate. Moreover, due to generally low concentrations of the organic micro-pollutants, degradation is more likely to occur through the co-metabolisms. This means that the organic compound would serve as a secondary substrate. In this case molasses would be served as the primary food source. However, no effect of the molasses addition was observed on the elimination rate for the majority of organic compounds. *Figure 17* shows a comparison between ln C/C_0 of HHCB in reactor I with digested sludge and reactor II, where molasses was added to the digested sludge. *Table* ***11*** shows the reaction rates *k* and half-lives $T_{1/2}$

found in these two reactors.

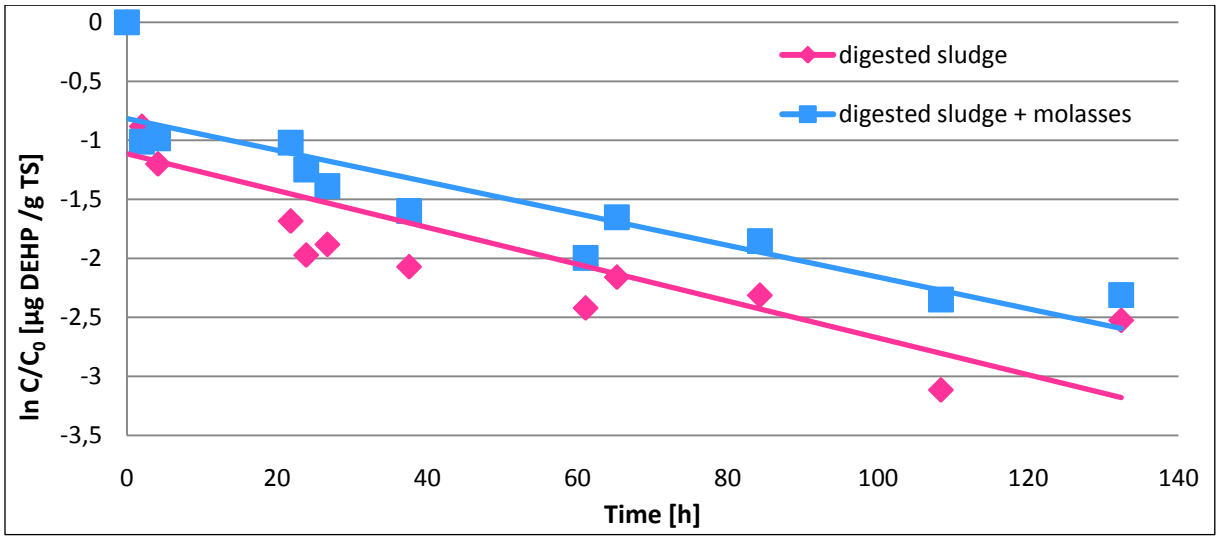


Figure 17 Comparison of DEHP ln C/C_0 in two reactors: I) digested sludge and II) digested sludge + molasses

No effect of molasses addition could be observed for most of the compounds. Therefore, it was concluded that the microorganisms in the digested sludge probably had a sufficient amount of food. Thus, no extra sources of carbon were needed.

It is important to mention that molasses was added to the reactor, with elevated level of DEHP. Spiking the compound to the sludge already provided a supplementary carbon source; hence the influence of the molasses addition might not be apparent. Therefore, it would be interesting to investigate the effect of molasses addition in the natural concentration of the digested sludge.

4.3.4 The influence of ultrasound on the processes in the digested sludge

It has been published that ultrasonic treatment of the digested sludge before the aeration increase microbial activity in sludge [81, 82]. A change in the structure of the sludge flocs and consequently release of the intracellular organic matter into the aqueous phase is expected to accelerate the transport of the oxygen and nutrients. As ultrasound was supposed to damage cells, aerobic sludge was added in order to provide new microorganisms to perform utilization of organic compounds.

The ultrasound disintegration had some positive effect on the elimination of triclosan, TiBP and indeno(1,2,3-cd)pyrene. In this case, the degradation rates increased by 36%, 10% and 33% for

these compounds, respectively; *Table* ***12***. No effect of the ultrasound treatment was observed on the elimination rates of fluorene, anthracene, fluoranthene and nonylphenols. Generally a negative effect of the ultrasound treatment of the digested sludge was observed. The elimination rates of most of the compounds were significantly lower in the reactor with ultrasound in comparison to the reactor with digested sludge, *Table* ***12***. *Figure 18* illustrates the comparison of DEHP ln C/C_0 in the two reactors, with and without ultrasonic treatment.

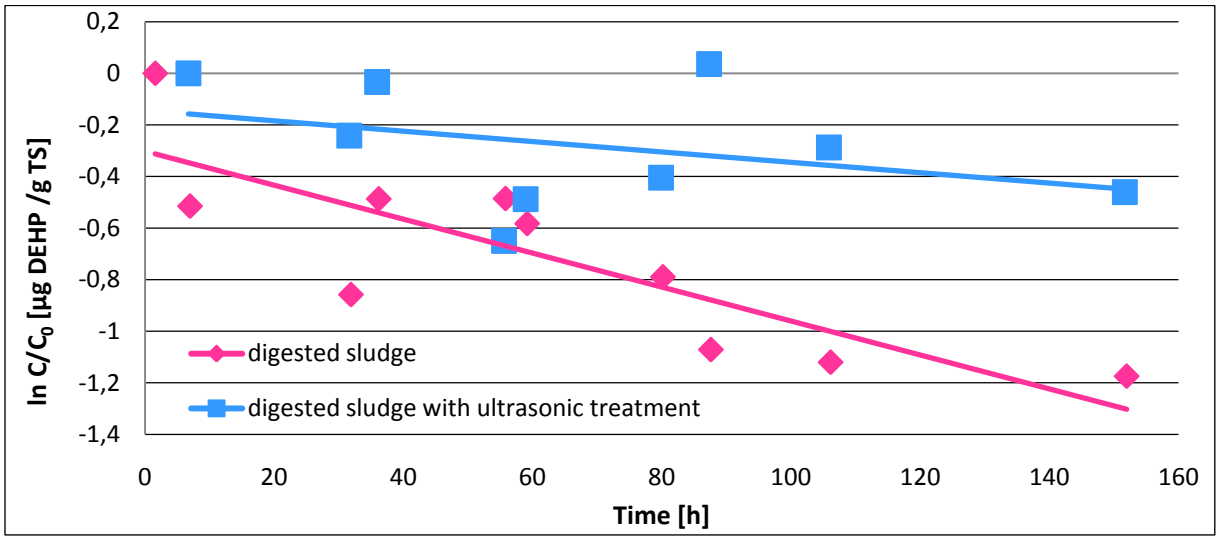


Figure 18 Influence of the ultrasound treatment on the processes in the digested sludge; ln C/C0 of DEHP versus time [h]

It is highly probable that after ultrasonic disintegration the microbial activity in the sludge was decreased and the amount of the new microorganisms from the aerated sludge was insufficient. It is also possible that these aerobic bacteria might need longer adaption time in the new environment. However, this is not necessarily the case in the full scale treatment system, where flow through the reactor would constantly provide it with new microorganisms. The ultrasound treatment potential cannot be excluded based only on the batch experiment. Considering the ultrasonic treatment it could be useful to control the soluble chemical oxygen demand (SCOD) and specific oxygen uptake (SOUR) in the sludge to avoid the damage of cells and reduction of the microbial activity. It is also crucial to select appropriate parameters of the ultrasound (frequency, density, exposure time). One of the reasons to consider ultrasonic treatment is that the organic matter solubilization induced by the ultrasonic action, can lead to an increase of the bioavailability of some micro-pollutants to the sludge [83]. It was reported, that ultrasonic treatment of the raw sewage sludge significantly enhanced the transfer of phenanthrene and naphthalene to the aqueous phase, what increased their

bioavailability [83].

During the third and fourth degradation experiments (Triclosan and Molasses), the primary increase of concentrations for most of the compounds was measured. Only the concentrations of the lightest PAHs as well as OTNE, TiBP, and TnBP decreased during the experiments. Several reasons could explain this situation. Firstly, due to the relatively high K_{ow} of the organic micro-pollutants examined in this study, these compounds could potentially be sorbed on the organic particles and slowly be released during aeration treatment of the sludge, while the sludge flocs would be disintegrated. For instance, several studies demonstrated a strong binding of heavy PAHs in environments with high organic matter content, such as sewage sludge, that also reduces their bioavailability [74]. Secondly, the diffusion from non-extractable to extractable fraction could take place within aeration, and thus increase of concentrations for these compounds. However, this would not explain that concentration of other compounds with the high K_{ow} were decreasing. More possibly a loss of water due to evaporation caused volatilization of light PAHs, while an increase in other, less volatile compounds. It is possible that abiotic losses, such as volatilization can occur and be prevalent during aeration, particularly for light PAHs [74]. The increase of TCPP concentrations during the experiments also supported this explanation. TCPP is known not to be biodegradable, thus rather stable during the degradation experiments. Moreover it is not considered volatile. Consequently, TCPP was used as a reference in order to correct the measured concentration. From the fifth degradation experiment (Molasses), the water evaporation was controlled. The weights of the reactors were checked after every sampling and tap water was added if any loss of the water was observed. The extent of the volatilization was also minimised by a reduction of airflow during aeration. *Figure 19* shows comparison of TCPP ln C/C_0 in the third degradation experiment (Triclosan), where water evaporation was not controlled, and in the fifth degradation experiment (Molasses), where the loss of water was taken into account. Increase of TCPP concentration was the result of water evaporation in the third degradation experiment (Triclosan). The concentration of TCPP compound is more stable in the fifth degradation experiment, after the water evaporation was controlled, *Figure 19*.

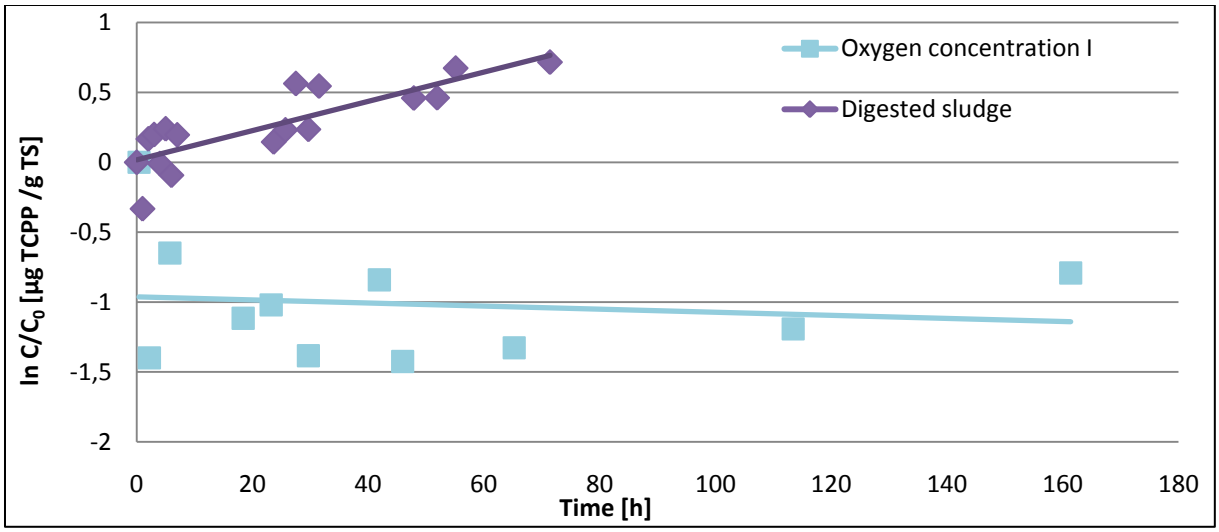


Figure 19 Comparison of TCPP ln C/C_0 in the third and fifth degradation experiment (Triclosan and Molasses), without and with correction of water evaporation, respectively.

4.3.5 Variability of the compounds' degradation rates within the degradation experiments performed at the university's laboratory

There were five different experiments performed in the digested sludge: Triclosan, Molasses, Ultrasound and Oxygen concentration I and II. The first three experiments the *k* and $T_{1/2}$ values can be compared as they are normalised to the biomass (TVS) of the respective sludge sample. Variability between the degradation rates for a single compound between performed experiments can be observed. In *Table* ***13***, ***Table 14*** and ***Table 15*** the first order degradation rates *k* and half-lives $T_{1/2}$ for the examined organic micro-pollutants in these experiments are presented.

Table 13 First order degradation rates k and half-lives $T_{1/2}$ for organic micro-pollutants in the third degradation experiment (Triclosan)

Compound	**Experiment 4 Triclosan** Elevated level of triclosan (100 µg/L) **AEROBIC**	
	k [L g^{-1} TVS d^{-1}]	**$T_{1/2}$** [d L^{-1} g^{-1} TVS]
OTNE	0,008	89
HHCB	0,003	275
AHTN	0,003	250
Triclosan	0,006	119
Triclosan-methyl	0,030	23
HHCB-Lactone	0,001	569
DEHP	0,005	137

Table 14 Influence of activated sludge and molasses addition on the processes in the digested sludge. First order degradation rates k and half-lives $T_{1/2}$ for organic micro-pollutants in the fourth degradation experiment (Molasses)

	Experiment 4: Molasses Digested sludge (Frederishavn) Elevated level of DEHP (100 µg/L) **AEROBIC**					
	Reactor I		**Reactor II**		**Reactor III**	
Addition of aerobic sludge	-		+		+	
Addition of molasses	-		-		+	
Compounds	**k** [L g^{-1} TVS d^{-1}]	**$T_{1/2}$** [d L^{-1} g^{-1} TVS]	**k** [L g^{-1} TVS d^{-1}]	**$T_{1/2}$** [d L^{-1} g^{-1} TVS]	**k** [L g^{-1} TVS d^{-1}]	**$T_{1/2}$** [d L^{-1} g^{-1} TVS]
TiBP	0,094	7,4	0,084	8,3	0,082	8,4
TnBP	0,040	18	0,033	21	0,036	19
OTNE	0,029	24	0,020	34	0,020	33
NP 149(1,2,3)	0,013	53	0,008	85	0,010	65
NP 135(1,2,3,4)	0,014	50	0,009	74	0,010	59
NP 121(1,2,3)	0,013	53	0,008	85	0,01	68
HHCB	0,018	38	0,012	56	0,014	48
MX	0,019	37	0,013	54	0,015	47
AHTN	0,008	82	0,014	49	0,012	55,5
Triclosan	0,013	52	0,014	50	0,018	39
HHCB-Lactone	0,010	72	0,010	72	0,010	67
EHDPP	0,016	42	0,012	58	0,014	50
DEHP	0,029	24	0,023	30	0,022	30
Acenaphtylene	0,100	6,7	0,079	9	0,079	9
Acenaphthene	0,148	5	0,076	9	0,074	9
Fluorene	0,058	12	0,052	13	0,053	13
Phenanthrene	0,044	16	0,056	12	0,040	17
Anthracene	0,040	17	0,026	27	0,037	19
Fluoranthene	0,040	17	0,030	23	0,032	22
Pyrene	0,018	38	0,013	55	0,013	51
Benzo(a)anthracene	0,018	38	0,015	45	0,014	51
Chrysene	0,015	55	0,013	55	0,014	48
Benzo(b)fluoranthene	0,018	38	0,013	54	0,013	53
Benzo(k)fluoranthene						
Benzo(a)pyrene	0,022	32	0,012	62	0,015	49
Dibenzo(a,h)anthracene	0,035	20	0,039	18	0,037	19
Benzo(ghi)perylene	0,019	36	0,020	35	0,026	27
Indeno(1,2,3-cd)pyrene	0,039	18	0,030	23	0,031	23

Table 15 Influence of activated sludge addition and ultrasonic treatment on the processes in the digested sludge. First order degradation rates k and half-lives $T_{1/2}$ for organic micro-pollutants in the fifth degradation experiment (Ultrasound)

	Experiment 5: Ultrasound Digested sludge (Frederikshavn) AEROBIC					
	Reactor I		**Reactor II**		**Reactor III**	
Aerobic sludge addition	-		+		+	
Ultrasound applied	-		-		+	
Compounds	**k** [L g^{-1} TVS d^{-1}]	**$T_{1/2}$** [d L^{-1} g^{-1} TVS]	**k** [L g^{-1} TVS d^{-1}]	**$T_{1/2}$** [d L^{-1} g^{-1} TVS]	**k** [L g^{-1} TVS d^{-1}]	**$T_{1/2}$** [d L^{-1} g^{-1} TVS]
TiBP	0,047	15	0,047	15	0,052	13
TnBP	0,012	57	0,005	132	0,006	119
OTNE	0,015	46	0,009	78	0,009	78
NP 149(1,2,3)	0,006	116	0,006	129	0,003	183
NP 135(1,2,3,4)	0,005	139	0,003	446	0,005	279
NP 121(1,2,3)	0,006	126	0,001	1027	0,002	595
HHCB	0,010	70	0,004	164	0,004	193
AHTN	0,008	91	-	-	-	-
Triclosan	0,007	100	0,008	87	0,011	61
HHCB-Lactone	0,007	95	0,002	342	0,002	408
EHMC	0,035	20	0,002	316	0,002	334
DEHP	0,010	66	0,008	84	0,004	184
Acenaphtylene	0,034	20	0,013	53	0,015	47
Acenaphthene	0,034	20	0,032	21	0,032	22
Fluorene	0,031	23	0,026	27	0,029	24
Phenanthrene	0,043	16	0,042	16	0,029	24
Anthracene	0,041	17	0,048	15	0,041	17
Fluoranthene	0,009	76	0,007	96	0,008	82
Pyrene	0,008	86	0,005	152	0,005	153
Benzo(a)anthracene	0,009	78	0,008	84	0,007	105
Chrysene	0,012	57	0,006	114	0,005	127
Benzo(b)fluoranthene	0,008	89	0,005	152	0,005	153
Benzo(k)fluoranthene						
Benzo(a)pyrene	0,013	53	0,004	187	0,001	735
Dibenzo(a,h)anthracene	0,065	11	0,057	12	0,06	12
Benzo(ghi)perylene	0,038	18	0,02	34	0,021	33
Indeno(1,2,3-cd)pyrene	0,008	88	0,011	62	0,012	56

-Concentration was stable/increasing, thus it was impossible to determine k and $T_{1/2}$ values.

There are a number of reasons for the differences between *k* and $T_{1/2}$ values. One possible explanation is that a different degree of biodegradation in two separate studies contributed to the removal of the compound. The degree of biodegradation is highly dependent on the initial compound's concentrations, incubation time, primary substrate concentrations and microbial inoculum sources [39], that vary between biodegradation experiments. Even though the incubation

time was similar in the performed experiments (around 7 days, except degradation experiment: Triclosan, with 3 days duration), the initial compounds concentrations varied vastly between two sludge samples from the same WWTP. Moreover, the experiments using the higher initial concentrations are supplying the compounds as the primary substrate, while the trace concentrations favour more compounds' biodegradation as a secondary substrate through cometabolism [39]. Differences in the biodegradation mechanisms could also have contributed to the discrepancies between removal rates in respective experiments. Moreover, similar microbial communities are never found in two sludge samples, even from the same plant. The composition of the WWTPs' sludges differs significantly as the result of different wastewater sources. Due to the diversity of the microbial community in the sludge samples, different abilities to degrade compounds are obvious.

4.4 Degradation experiments performed at Ejby Mølle WWTP

Two degradation experiments were performed at Ejby Mølle WWTP's laboratory: oxygen optimisation I and II. During these experiments the water loss was controlled and the weight of the sample was corrected to the initial weight, in the same fashion as in previous experiments, see chapter 3.2. The concentration of TCPP was rather stable during the experiment and thus it was not necessary to use the compound as a reference, *Figure 20*.

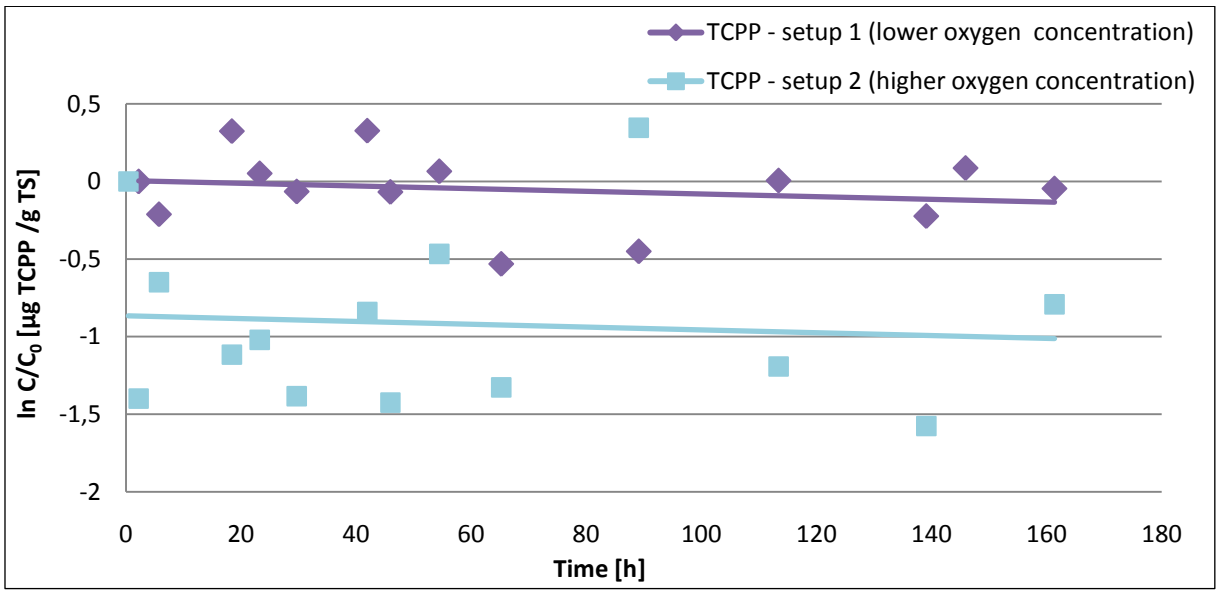


Figure 20 Ln C/C_0 of TCPP in the sixth degradation experiment (Oxygen concentration I) in two setups: 1) higher oxygen C, 2) lower oxygen C

4.4.1 Elimination pathways of the organic micro-pollutants during the degradation experiments in the digested sludge performed at Ejby Mølle WWTP

Here the elimination pathways of the analysed compounds were presented and discussed. Only the results from the sixth experiment (Oxygen concentration I) were presented. In ***Table 16*** the degradation rates *k* and half-lives $T_{1/2}$ for the compounds in the sixth degradation experiment are presented.

Table 16 Comparison of a treatment of organic micro-pollutants in higher and lower oxygen concentration. First order degradation rates k and half-lives $T_{1/2}$ for organic micro-pollutants in the sixth degradation experiment (Oxygen concentration I)

Compound	**Experiment 6: Oxygen concentration I** Digested sludge (Ejby Mølle) **AEROBIC**			
	Setup II Higher oxygen concentration (2 - 4 mg/L)		**Setup I Lower oxygen concentration** (0,2 – 0,4 mg/L)	
	k [d^{-1}]	**$T_{1/2}$** [d]	**k** [d^{-1}]	**$T_{1/2}$** [d]
TiBP	-	-	0,002	413
TnBP	0,084	8,3	0,067	10
OTNE	0,048	14	0,058	12
NP 149(1,2,3)	0,010	307	-	-
NP 135(1,2,3)	0,014	263	-	-
NP 121(1,2,3)	0,017	41	0,012	58
HHCB	0,038	18	0,050	14
AHTN	0,040	17	0,040	17
Triclosan	0,060	12	0,030	24
HHCB-Lactone	0,012	58	0,034	21
Triclosan-methyl	0,010	72	0,300	2,3
DEHP	-	-	-	-
Acenaphtylene	0,110	6,3	0,080	8,8
Acenaphthene	0,264	2,6	0,182	3,8
Fluorene	0,127	5,5	0,055	13
Phenanthrene	0,092	7,5	0,076	10
Anthracene	0,034	21	0,362	2,0
Fluoranthene	0,055	13	0,062	11
Pyrene	0,062	11	0,067	10
Chrysene	0,005	144	0,090	7,6
Benzo(b)fluoranthene	0,017	61	0,120	5,8
Benzo(k)fluoranthene				
Benzo(a)pyrene	0,040	17	0,120	6

-Concentration was stable/increasing, thus it was impossible to determine *k* and $T_{1/2}$ values.

Overall, there are three groups of compounds regarding the elimination behaviour; compounds with very fast removal rates (light PAHs), compounds with relatively high removal rates (OTNE, triclosan, TnBP), compounds with intermediate removal rates (AHTN,HHCB) and finally compounds with very low removal rates (TiBP, nonylphenol isomers).

The highest removal rate was observed for the lightest PAHs (acenaphthene, acenaphthylene, fluorene and phenanthrene). The reaction rates were from 0,264 to 0,92 and from 0,182 to 0,55 in setup 1 and setup 2, respectively. The high elimination rate was also observed for OTNE, TnBP and triclosan in the setup 1, *Table 16*. The degradation rates were from 0,084 to 0,048 and from 0,067 to 0,058 in setup 1 and setup 2, respectively. Heavy PAHs fluoranthene and pyrene were also removed relatively fast in both setups, and reaction rates were 0,055 and 0,062 for these compounds, respectively. Intermediate elimination rates were found for fragrances AHTN, HHCB, while very low or no elimination was observed for nonylphenol isomers and TiBP. Reaction rates for AHTN and HHCB varied between 0,030 and 0,040, while for nonylphenol isomers between 0,01 and 0,017, in both setups. The concentration of DEHP was increasing during the experiment, thus k and T1/2 values were not found. Since the results from the previous experiments in the digested sludge suggest a relatively high removal rate for DEHP, ***Table 10*** - *Table* ***12***, the increase of the compound concentration might be due possible contaminations (e.g. contamination from electrodes' plastic wires, aeration tubes and contamination from the laboratory that is intended for sludge examination).

Like in previous experiments in the digested sludge, the high removal of light PAHs is mainly expected to be due to the volatilization, partly biodegradation. The lower removal rate of the branched alkylphosphate TiBP in comparison to TnBP was expected since the branched compounds are supposed to degrade slower that the straight chained ones. The relatively high removal rates for OTNE, TnBP and triclosan are also in good accordance with the results from the previous degradation experiments in digested sludge, suggesting a presence of the microorganisms that are able to perform mineralization or transformation for these compounds. The relatively low elimination rate observed for AHTN, HHCB, or no elimination rate for HHCB-lactone and nonylphenol isomers is also in agreement with the results from previous experiments, ***Table 10*** - *Table* ***12***.

As some operation problems occurred during the seventh degradation experiment (Oxygen concentration II) results from the sixth experiment (Oxygen concentration I) were only presented. It was not possible to measure total volatile solids (TVS) at the plant laboratory, hence first order degradation rates k and half-lives $T_{1/2}$ of the compounds are only presented in $days^{-1}$ and days, respectively. Some of the heavy PAHs (benzo(a)anthracene, dibenzo(a,h)anthracene,

benzo(ghi)perylene and indeno(1,2,3-cd)pyrene) were not separated well enough on the GC column, thus it was impossible to determine the signal of these compounds and calculate the concentrations. Therefore they were not included in the discussion.

4.4.2 Influence of oxygen concentration on the processes in the digested sludge

Two setups with different oxygen levels were applied to determine the influence of oxygen concentration on the removal of the micro-pollutants. Oxygen concentration in setup 1 was 10 times smaller than in setup 2, *Table 4*. Higher oxygen concentration influenced positively the removal of TnBP, nonylphenols, triclosan and the lightest PAHs. The degradation rates in setup 2 (higher oxygen concentration) were 20%, 30%, 50% and 10-58% higher than in setup 1 for these compounds, respectively, ***Table 16***. On the other hand HHCB, HHCB-lactone, triclosan-methyl and heavier PAHs (anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene) were removed faster in the setup 1 (lower oxygen concentration). This time reaction rates were 24%, 65%, 96% and 67-94% higher for HHCB, HHCB-lactone, triclosan-methyl and heavy PAHs in the setup 1, respectively. No significant differences in the elimination rates between two setups were observed for OTNE, AHTN, fluoranthene and pyrene, ***Table 16***. *Figure 21* shows the comparison of acenaphthene ln C/C0 in the setups with lower and higher oxygen concentration.

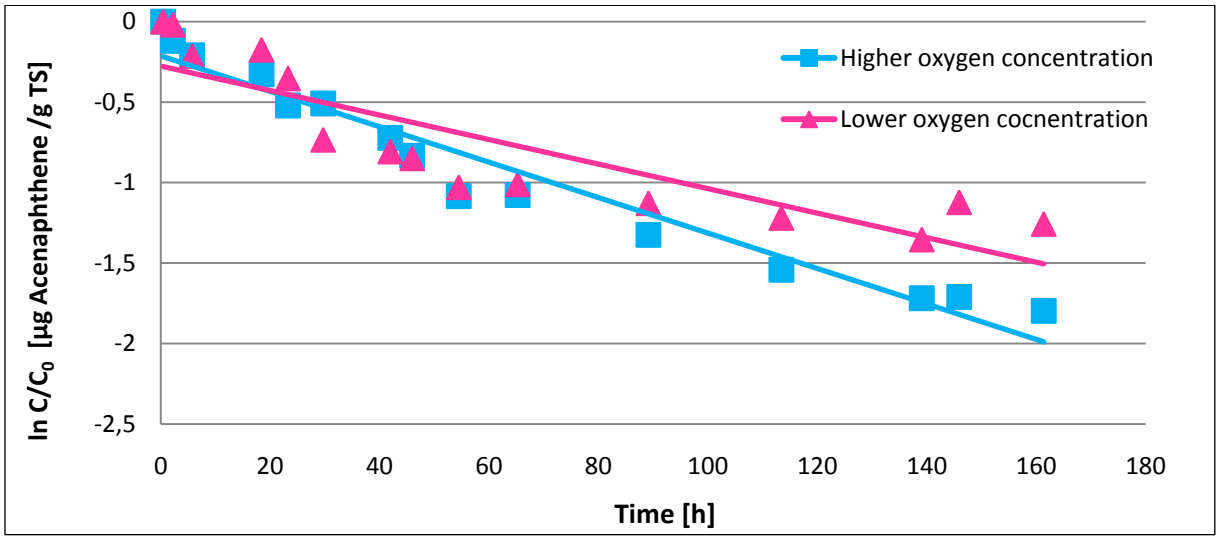


Figure 21 Comparison of acenaphthene ln C/C_0 during incubation with lower and higher oxygen concentration in the sixth degradation experiment (Oxygen concentration I)

Faster removal of triclosan in higher oxygen concentration is in accordance with the results from previous experiments, where after decreasing the supply of oxygen, the reaction rate for triclosan was lower. This indicates that oxygen is an important parameter in the removal of this compound from the sludge. The alkylphosphate TnBP was also removed faster in setup 2. It is possible that microorganisms needs higher oxygen concentration to use these compounds as a primary or/and secondary food source. A formation of triclosan-methyl could also contribute to the faster removal of triclosan in setup 2, as reaction rate for this transformation product was lower in this setup that in the setup 1. Faster elimination of the light PAHs in setup 2 can be partly explained by a bigger extent of the volatilization in more aerated sludge. A higher degradation rate for nonylphenols was observed in setup 2 than in setup 1. It is possible that the formation of nonylphenols from NP1EO and NP1EC was more significant in setup 1 than in setup 2, and hence decreased the reaction rate of nonylphenols. No significant differences between removal rates in setup 1 and 2 (lower and higher oxygen concentration) for AHTN, OTNE, fluoranthene and pyrene were observed. Possibly the removals of these compounds are not subject to changes of the oxygen concentration within in applied range.

4.4.3 Reduction of the solids volume during aeration of the digested sludge

The aerobic post-treatment of the Ejby Mølle digested sludge was partly intended to decrease the total solids (TS) in the sludge and hence the sludge volume. Sludge from Ejby Mølle is currently disposed through composting and landfills (chapter 1.7.2.). However this disposal is costly, due to the amount of sludge. Therefore, a reduction of the sludge amount is of great interest as it would save the money for the sludge disposal.

During the experiments performed at Ejby Mølle WWTP the total solids were measured in the beginning and in the end of the experiment. Thus it was possible to calculate the reduction of the solids after the aeration of the sludge. Total solids measurements and reduction are presented in the *Table **17***.

Table 17 Total solids measurements in the first and last day of the experiment Oxygen concentration I, performed at Ejby Mølle' laboratory

	TOTAL SOLIDS [%]	
	Experiment 6: Oxygen concentration I	
	Setup I Lower oxygen concentration (0.2 - 0.4 mg/L)	**Setup II Higher oxygen concentration** (2 - 4 mg/L)
Day 1	3,76	3,75
Day 7	3,52	3,58
Reduction [%]	6,5	4,5

Sludge volume was reduced by 6,5 and 4,5 % in the setup 1 and setup 2, respectively. By means of the daily production of the sludge at Ejby Mølle WWTPs a reduction of the sludge volume per day could be calculated. The production of the sludge at Ejby Mølle is 65 t/d (wet sludge), which equals around 25 t/d of (dry solids). If the reduction of the sludge solids is assumed to be 5%, the volume of the sludge would be reduced by 1,5 t per day. This is an estimation and variations are expected in reduction of the sludge solids during the aerobic post-treatment of sludge.

4.5 Full-scale considerations

Laboratory scale biodegradation studies performed in controlled experimental conditions provide a good first step in understanding the mechanisms and processes behind the degradation the organic micro-pollutants. Moreover, they give insight into the compounds behaviour in different environmental and operational conditions. This knowledge can be used for enhancement of organic micro-pollutants removal in the full-scale systems. For example, after the first degradation experiment in the digested sludge in this study it was discovered that a longer retention time is needed, as the elimination rates for the organic compounds are significantly lower than those found in the activated sludge. Moreover, the fastest removal rates were found in aerobic conditions for almost all of the organic micro-pollutants.

Before extrapolation of the results from laboratory-scale experiments to the natural environment one should be aware that some differences are expected. For instance, providing a constant supply of organic pollutants by feeding with fresh sludge, can lead to better biodegradation and subsequently increase the removal rates [9]. A rather negative effect of the ultrasound was

found in this study. However, it cannot be excluded that ultrasonic treatment may increase the microbial activity in the sludge in the full scale, as the flow through the reactor would constantly provide new microorganisms with the fresh sludge. Moreover, discrepancies in the incubation and adaption times are likely between laboratory and full-scale experiments.

4.6 Process control, oxygen uptake

4.6.1 Test on possible performance of an existing tank, calculation of the oxygen consumption

There were two approaches towards the design of the aeration reactor. In the first approach all the calculations were made under the assumption that the size of the aeration tanks is known and equals the size of the existing tank at Ejby Mølle WWTP. In the second approach the target concentrations of the regulated organic micro-pollutants were known, and the aim was to design the tank with enough capacity to fulfil the Danish criteria for concentrations of these compounds. Firstly, the oxygen consumption was calculated for the existing tank based on the results from the experiment: oxygen concentration I.

During the experiments performed at Ejby Mølle WWTP oxygen concentration, pH and redox potential were constantly monitored. *Figure 22* shows monitored data from the setup 1 (lower oxygen concentration) within a short period of the experiment.

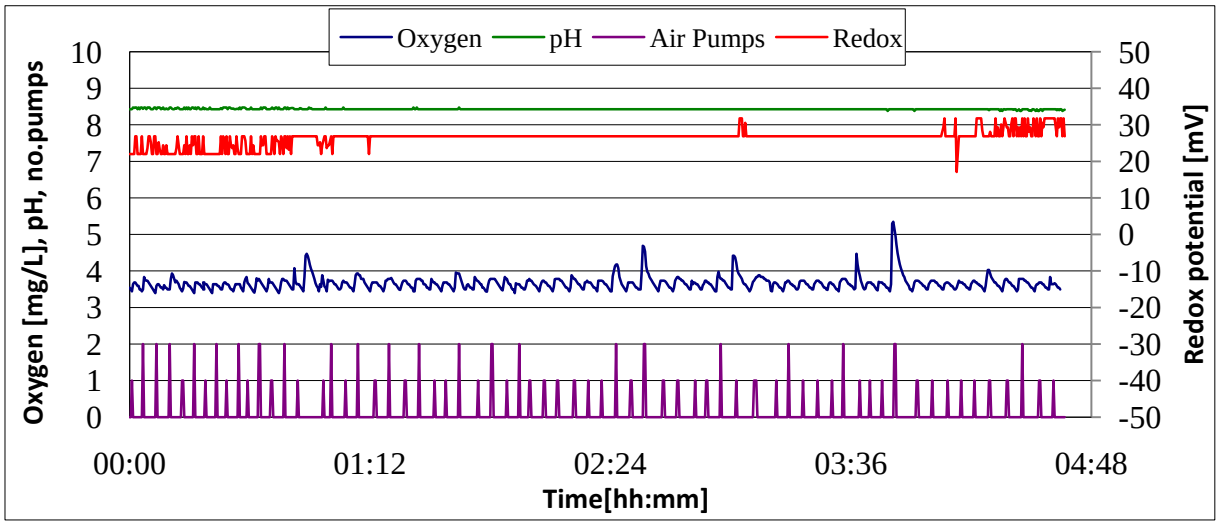


Figure 22 Constant measurement of oxygen, pH, redox potential and number of pumps running within a short period of the sixth experiment (oxygen concentration I).

Constant measurements of the oxygen concentration were used to calculate the oxygen consumption in the reactor. The oxygen consumption was calculated from the oxygen concentration during short periods when none of the pumps were running. During these periods the oxygen concentration was decreasing as the bacteria used the oxygen. Some of the longer periods without aeration were chosen from the beginning until the end of the experiments. The oxygen consumption was calculated as a slope of the oxygen concentration plotted against the time. As a result an oxygen consumption curve within the experimental period was obtained, *Figure 23.* Subsequently it was possible to determine the maximum and the total oxygen consumption from the curve. The maximum oxygen consumption expresses the highest demand for oxygen in the sludge and can be used to estimate the necessary capacity for the full scale aeration system. The total oxygen consumption is the related to the total oxygen needed per litre of sludge.

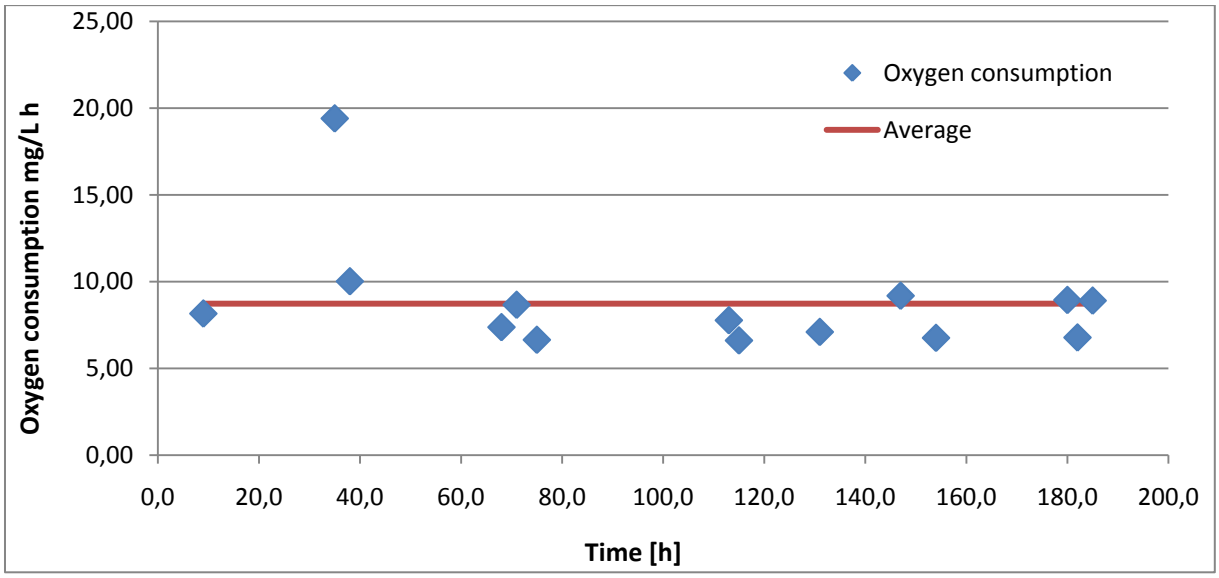


Figure 23 Oxygen consumption curve from the setup 1 during the oxygen concentration I

The oxygen consumption in the sixth experiment (Oxygen concentration I) was rather stable at 10 mg/L·h. Thus, the mean of the all measurement points 8,7 mg O_2/L h, was chosen as the maximum oxygen consumption per litre of the sludge in one hour. The area under the oxygen consumption curve was determined to calculate the total oxygen consumption in the sludge within the experiment time. The total and the maximum oxygen concentrations are presented in the *Table **18***.

Table 18 Measurements of the oxygen uptake during the experiment performed at Ejby Mølle WWTP

TEST ON POSSIBLE PERFORMANCE OF AN EXISTING TANK **Measurements of oxygen uptake in the tank**		
Tank size	1900	m^3
Flow to the tank	400	m^3/d
Retention time	4	d
Maximum oxygen consumption in the experiment	21,8	mg O_2/2,5L sludge h
	8,7	g O_2/m^3 h
Maximum oxygen consumption in the tank	**16,6**	kg O_2/h
Total oxygen consumption = Area under the curve, *Figure 23*	1,68	kg O_2/L sludge
Ammonia nitrogen concentration in the digested sludge	700	mg NH_4-N/L
Flow of the ammonia nitrogen to the tank	11,7	kg NH_4-N/h
Oxygen demand for the nitrification in the digested sludge	4,38	kg O_2/kg N
Oxygen demand for the nitrification in the tank per hour	**51,1**	kg O_2/h

During the two experiments performed at Ejby Mølle nitrification did not take place as the pH was too high (stable at around 8,5), see *Figure 22* and during nitrification pH tends to be rather depressed [9]. At this point it was difficult to predict whether the nitrification would occur or not in the full scale. Therefore, all the calculations were performed under the assumption that nitrification would not occur and the maximum and total oxygen consumptions were calculated only to cover the oxygen demand for the oxidation of the organic micro-pollutants and total organic carbon (TOC).

4.6.2 Assumption 1: Size of the aeration tank is known: 1900 m^3

The size of the existing tank and daily flow were used to measure the retention time. By knowing the maximum oxygen consumption and size of the tank it was possible to calculate the maximum oxygen consumption in the tank per hour, see *Table **18***. It is important to note that these concentrations are highly depended on whether the nitrification would take place or not. If nitrification works, then the

oxygen consumption will significantly increase. *Table **18*** shows the calculation of oxygen demand in the tank for nitrification only. It can be seen that the demand of oxygen for the nitrification is far bigger that the oxygen demand measured in the aeration process.

However, it was observed afterwards that the aeration of the sludge by mixing alone is significant. This means that the oxygen demand measured from the slope of the oxygen curve while not aerating is underestimated as oxygen was supplied continuously by mixing.

As the retention time was previously calculated, it was possible to determine the concentration of the organic micro-pollutants after available retention time in the tank and the elimination rates by using the starting concentrations of the compounds in the digested sludge and the first order degradation rates, see ***Table 16***. All the calculations are presented in ***Table 19.*** As it was not possible to determine first order degradation rates for DEHP, indeno(1,2,3-cd)pyrene and benzo(ghi)perylene in the experiments performed at Ejby Mølle (Oxygen concentration I and II), reaction rates from the fourth or fifth degradation experiment (Molasses and Ultrasound) were used to calculate concentrations after retention time and elimination rates for these compounds.

Table 19 Calculation of the organic micro-pollutants concentration and eliminations after retention time in the 1900 m^3 aeration tank

TEST ON POSSIBLE PERFORMANCE OF AN EXISTING TANK **ASSUMPTION 1** **Calculation of the organic-micro pollutants concentration after retention time**						
Regulated and Emerging Compounds	**Starting concentration C_0 [µg/g TS]**	**Concentration after Retention Time $C=C_0 \cdot e^{-kt}$ [µg/g TS]**		**Reduction [%]**		**Danish cut-off values [µg/g TS]**
		Setup II High O_2 C	Setup I Low O_2 C	Setup II High O_2 C	Setup I Low O_2 C	
DEHP	**50***	**27**		**47**		**50**
NP	**140**	**135**	**153**	**4**	**-9**	**10**
Sum of PAHs	**3,45**	**2,5**	**2,4**	**27**	**32**	**3**
Acenaphthene	0,17	0,08	0,15	65	52	-
Fluorene	0,20	0,42	0,48	40	20	
Phenanthrene	0,60	0,12	0,16	31	26	
Fluoranthene	0,60	0,48	0,47	20	22	
Pyrene	0,60	0,47	0,46	22	24	
Benzo(b,k)fluoranthene	0,40	0,36	0,25	7	38	
Benzo(a)pyrene	0,50	0,42	0,31	15	38	
Benzo(ghi)perylene	0,16**	0,12		67		
Indeno(123-cd)pyrene	0,22**	0,14		38		
Triclosan	3,0	2,4	2,67	21	11	
Triclosan-methyl	0,03	0,03	0,01	4	70	
OTNE	16	13	13	17	21	
AHTN	1,1	0,93	0,93	15	15	
HHCB	33	28	27	14	18	
HHCB-lactone	0,40	0,38	0,35	5	13	

Most of the starting concentrations and k values are taken from the 6th and 7th degradation experiments (Oxygen concentration I and II).

* DEHP starting concentration and k values were taken from the 5th degradation experiment (Ultrasound)

**Benzo(ghi)perylene and Indeno(123-cd) starting concentrations were taken from the analyse report from Ejby Mølle WWTP, and k values from the 4th and 5th degradation experiment (Molasses and Ultrasound)

Concentrations of the regulated compounds (DEHP, nonylphenols and sum of nine PAHs) after the retention time were compared to the Danish cut-off values. After the available retention time all the compounds would be below the limit except nonylphenol isomers, which has a concentration far above the cut-off value. The reason is an especially low elimination rate and a high concentration of the compounds. The minus next to the reduction for nonylphenol isomers in setup 1 means, that the concentration of this compound was increasing. The fact that under the aerobic conditions nonylphenol polyethoxylate (NPnEO) is degraded to nonylphenol mono-ethoxylate (NP1EO) and NP1EO is further degraded to nonylphenol under the anaerobic conditions [84], explains the high concentration of nonylphenols in the anaerobically digested sludge. The reason for the increase of nonylphenol isomers concentration during aeration of the digested sludge is that under the aerobic conditions further transformation of NPnEO to NP1EO and NP1EO to nonylphenols could have occurred [84]. Moreover, the formation of nonylphenols from NP1EO might be more significant in the lower oxygen concentration - setup 1 than in the higher oxygen concentration - setup 2, and hence decreased the reaction rate of nonylphenols in setup 1.

4.7 Outline reactor design based on the degradation kinetics of single compounds

4.7.1 Assumption 2: The target concentrations of the compounds are known

In this approach the aim was to design the tank with enough capacity to fulfil the Danish criteria for concentrations of regulated compounds, based on the degradation kinetics of the respective compounds.

Almost all compounds, except nonylphenol isomers, would be below the Danish cut-off values after the treatment in the existing tank. Thus, the focus in the second assumption was to design a tank with capacity big enough to perform successful treatment of nonylphenol isomers and fulfil the Danish criteria. The starting and target concentration of nonylphenol isomers and the degradation rate were used to calculate reduction of the compound and required retention time. Subsequently, by knowing the daily flow of the sludge the size of the tank was computed. All the calculations from the two assumptions are presented and compared in *Table **20*** .

Table 20 Comparison of an existing tank and tank designed in this study

	ASSUMPTION 1: KNOWN SIZE OF THE TANK 1900 m³	**ASSUMPTION 2: KNOWN TARGET CONCENTRATIONS**	**Unit**
Target concentration of nonylphenol isomers	135	10	µg/g TS
Reduction	4	93	%
Retention time $T = \ln[C_0/C]/k$	4	275	d
Flow to the tank	400		m^3/d
Tank size	1900	109 960	m^3
Maximum oxygen consumption	16,6	960,2	kg O_2/h

To perform a treatment of nonylphenols and fulfil the Danish criteria a long retention time would be required due to high starting concentration of nonylphenol isomers and their low degradation rate. Moreover, an almost sixty times bigger capacity, than the capacity of an existing tank, would be necessary. Consequently, the oxygen consumption as well as electricity consumption would increase significantly. The retention time and the tank size are certainly impracticable and thus other solutions are needed in order to bring down the concentration of nonylphenols. Answers should be rather searched in the mechanisms behind the degradation of the compounds. Deeper insight into environmental factors that influence the behaviour and removal of these compounds from the sludge is necessary. Important environmental factors that determine whether or not biotransformation takes place include pH, temperature, concentration and redox condition [40]. Knowledge of environmental factors and crucial parameters that favour biotransformation of a particular organic contaminant could help to achieve a desired biotransformation.

It is important to mention that the degradation rate of nonylphenol calculated in the degradation experiments in this study might be underestimated, as formation of nonylphenols from nonylphenol mono-ethoxylate (NP1EO) might have occurred. Since the nonylphenol could be generated, the degradation rate might be reduced. As a demonstration *Figure 24* shows the concentrations of

nonylphenols (NP) and nonylphenol polyethoxylates (NPnEO) in the sludge under the aerobic conditions.

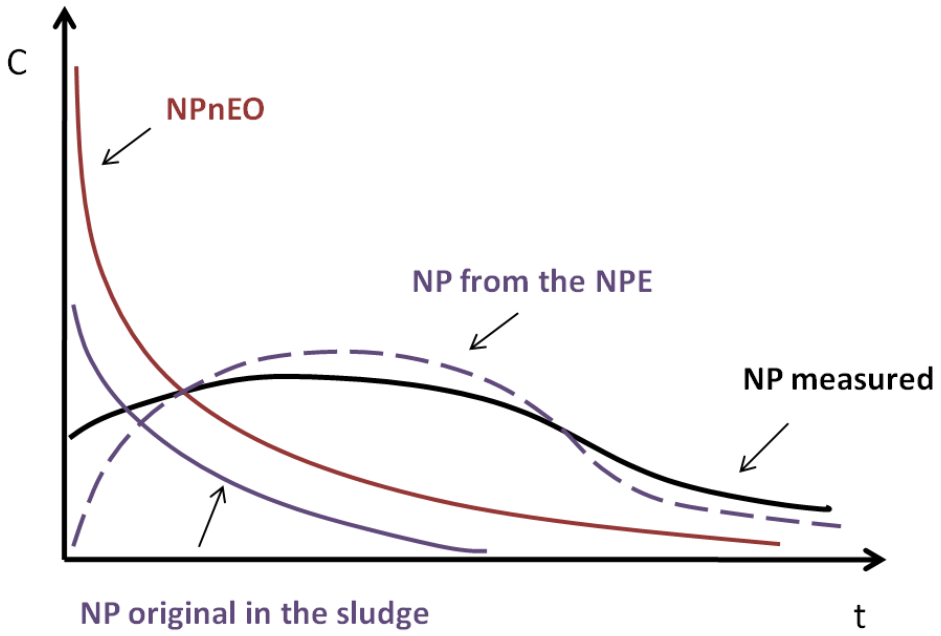


Figure 24 Expected concentration of nonylphenol (NP) and nonylphenol polyethoxylates (NPE) in the sludge under the aerobic conditions in the sludge

Nonylphenols (NP) are formed in the sludge from the nonylphenol polyethoxylates (NPnEO). Thus, the concentration of nonylphenols is increasing and consequently slowing down the compound's removal. *Figure 24* shows the measured concentration of nonylphenols and the concentration of the original nonylphenols found in the sludge - if no formation of nonylphenols would occur.

5. Conclusions

- The most rapid elimination for most of the examined organic micro-pollutants in activated sludge was observed under the aerobic conditions. The *k* values found under the aerobic conditions where 50%, 50-70%, 70% and 80% greater than those found in the reductive environment for HHCB,OTNE, DEHP and triclosan, respectively and 80-90% greater than those found under anoxic conditions for these compounds.
- The highest removal rate in the activated sludge is expected for OTNE; relatively high for DEHP, triclosan and HHCB; very low for AHTN, while no removal is expected for the transformation products HHCB-lactone and triclosan-methyl.
- The highest removal rate in the digested sludge is expected for light PAHs, some of the heavy PAHs (anthracene, dibenzo(a,h)anthracene), TnBP, OTNE and DEHP; intermediate removal rate is expected for triclosan, HHCB and some of the heavy PAHs; low or no removal at all is expected for AHTN, HHCB-lactone and nonylphenols.
- Only for AHTN, the optimal removal was observed under the anaerobic conditions. In this case, *k* values were 67% and 75% greater than those found under aerobic and anoxic conditions, respectively, in activated sludge.
- Abiotic losses, such as volatilization can contribute to the removal of light PAHs and reduce the role of biodegradation
- Aerobic sludge addition to the digested sludge was observed, which significantly decreased the reaction rates for most of the compounds. However the positive effect cannot be ruled out in the full scale system
- In general no effect of the molasses addition to digested sludge was observed. A sufficient amount of food for microorganisms in the sludge was assumed to be the reason.
- The ultrasonic treatment significantly decreased reaction rates for most of the organic compounds. However, a positive effect is expected in the full scale system, as constant flow through the reactor will provide with new microorganisms
- Higher oxygen concentration in the process significantly enhanced the elimination of triclosan and nonylphenols and light PAHs. Removal rates were 30%, 50% and 10-58% higher than in lower oxygen concentration for these compounds, respectively. It cannot be excluded that volatilization contributed to the removal of light PAHs
- For HHCB, HHCB-lactone, triclosan-methyl and heavy PAHs reaction rates were 24%, 65%, 96% and 67-94% higher in lower oxygen concentration than in higher oxygen concentration, respectively.

- After the available retention time during the aerobic treatment in the existing tank all the compounds would be below the limit except nonylphenol isomers. The concentration of the nonylphenol isomers is far above the Danish cut-off value.

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