KATI KLEIN

Studies and treatment of inhibitory and
recalcitrant wastewater

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UNIVERSITAS TARTUENSIS
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DISSERTATIONES TECHNOLOGIAE CIRCUMIECTORUM
UNIVERSITATIS TARTUENSIS

31

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Studies and treatment of inhibitory and recalcitrant wastewater

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Dissertation was accepted for the commencement of the degree of *Doctor philosophiae* in environmental technology at the University of Tartu on 25th of March 2021, by the Scientific Council on Environmental Technology, Faculty of Science and Technology, University of Tartu.

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Commencement: Room 1020, Chemicum, 14A, Ravila Street, Tartu,
                      on April 29th in 2021, at 12.15

University of Tartu Press
www.tyk.ee

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I   **Klein, K**, Mandel, A, Lilleoja, H, Salmar, S, Tenno, T. **(2020)** Assessment of enhanced biological phosphorus removal process inhibition. *Springer Nature Applied Sciences,* 2:1489; 10.1007/s42452-020-03281-1

II   **Klein, K**, Tenno, T. **(2019)** Estimating the impact of inhibitory substances on activated sludge denitrification process. *Water Practice and Technology*, 14(4):863-871; 10.2166/wpt.2019.069.

III   **Klein, K**, Kattel, E, Goi, A, Kivi, A, Dulova, N, Saluste, A, Zekker, I, Trapido, M, Tenno, T. **(2017)** Combined treatment of pyrogenic wastewater from oil shale retorting. *Oil Shale*, 34(1): 82−96; 10.3176/oil. 2017.1.06.

IV   Klauson, D, **Klein, K**, Kivi, A, Kattel, E, Viisimaa, M, Dulova, N, Velling, S, Trapido, M, Tenno, T. **(2015).** Combined methods for the treatment of a typical hardwood soaking basin wastewater from plywood industry. *International Journal of Environmental Science and Technology*, 12(11): 3575−3586; 10.1007/s13762-015-0777-2.

V   Klauson, D, Kivi, A, Kattel, E, **Klein, K**, Viisimaa, M, Bolobajev, J, Velling, S, Goi, A, Tenno, T, Trapido, M. **(2015)**. Combined processes for wastewater purification: treatment of a typical landfill leachate with a combination of chemical and biological oxidation processes. *Journal of Chemical Technology and Biotechnology*, 90(8): 1927−1536; 10.1002/ jctb.4484.

VI   Kattel, E, Kivi, A, **Klein, K**, Tenno, T, Dulova, N, Trapido, M. **(2016)**. Hazardous waste landfill leachate treatment by combined chemical and biological techniques*. Desalination and Water Treatment,* 57(28): 13236−13245; 10.1080/19443994.2015.1057539.

VII   **Klein, K**, Kivi, A, Dulova, N, Zekker, I, Mölder, E, Tenno, T, Trapido, M, Tenno, T. **(2017)**. A pilot study of three-stage biological-chemical treatment of landfill leachate applying continuous ferric sludge reuse in Fenton-like process. *Clean Technologies and Environmental Policy*, 19(2): 541−551; 10.1007/s10098-016-1245-5.

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Author`s contribution:

Ref. I   Author performed ca 80% of the experimental work and wrote the manuscript

Ref. II   Author performed the experimental work and wrote the manuscript

Ref. III   Author performed ca 80% of the experimental work and wrote the manuscript

Ref. IV   Author performed ca 50% of the experimental work and participated in the writing of the manuscript

Ref. V   Author performed ca 50% of the experimental work and participated in the writing of the manuscript

Ref. VI   Author performed ca 50% of the experimental work and participated in the writing of the manuscript

Ref. VII   Author performed the experimental work and wrote the manuscript

LIST OF ABBVERIATIONS AND ACRONYMES

3,5-DCP	3,5-dichlorophenol
AOB	ammonia-oxidizing bacteria
AOP	advanced oxidation process
BOD_7	a 7-day biochemical oxygen demand
BOD_7/COD	biodegradability (ratio of a 7-day biochemical oxygen demand and chemical oxygen demand)
COD	chemical oxygen demand
DO	concentration of dissolved oxygen
DOC	dissolved organic carbon
EBPR	enhanced biological phosphorous removal
FA	fulvic acid
GAO	glycogen accumulating organisms
HA	humic acid
HRT	hydraulic retention time
MLSS	mixed liquor suspended solids
NOB	nitrite oxidizing bacteria
PAH	polyaromatic hydrocarbon
PAO	polyphosphate accumulating microorganism
PBDE	polybrominated diphenyl ester
PE	population equivalent
PHA	polyhydroxyalkanoates
PHB	poly-β-hydroxybutyrate
PHV	polyhydroxyvalerate
POP	persistent organic pollutants
SBR	sequencing batch reactor
SRT	solids retention time
SVI	sludge volume index
TOC	total organic carbon
TN	total nitrogen
TS	total solids
VFA	volatile fatty acids
VOC	volatile organic carbon
WAS	waste activated sludge
WER	water exchange ratio
WWTP	wastewater treatment plant

1. INTRODUCTION

Challenging targets set for water protection demands application of cost-efficient wastewater treatment technologies. Activated sludge process is widely used for wastewater treatment due to its high efficiency, flexibility and low cost. However, pollutants present in the influents of industrial regions may be inhibitory or non-degradable for activated sludge microorganisms. Consequently, the efficiency of wastewater treatment decreases and pollutants may be streamed to water bodies. Application of physical and chemical technologies are necessary for removal of recalcitrant organic compounds, however, they are generally expensive to install and operate.

There are several approaches available for reducing the inhibition of wastewater, but there is lack of reliable methods to estimate the impact of wastewater on all main activated sludge processes individually. Reliable standard methods for estimating the inhibition are available for activated sludge oxygen uptake and nitrification processes, but yet so far, there are no reliable and easily applicable methods for denitrification and enhanced biological phosphorous removal (EBPR) processes. Estimating the inhibition and identifying the origin of inhibitory wastewater are essential to ensure high treatment efficiency, especially in municipal wastewater treatment plants, which are influenced by industries.

In this thesis, inhibition tests for denitrification and EBPR processes were developed to estimate the inhibition on every wastewater treatment processes individually (**Papers I and II**). Comparisons of studied wastewater treatment processes and developed inhibition tests were made by using 3,5-dichlorophenol as a model inhibitor. Additionally, effluents from the shale oil and wood industries (**Papers III and IV**), leachates from the municipal and hazardous landfills (**Papers V and VI**) were used as samples of recalcitrant and inhibitory wastewater with different origin and properties.

Since activated sludge process is known to be inefficient for the degradation of recalcitrant organic compounds, combined biological and chemical processes were tested for all studied wastewater samples (**Papers III–VII**). The order of applied processes was chosen based on the results from the inhibition and biodegradability studies. Activated sludge treatment was applied to remove biodegradable organic compounds and nutrients, chemical treatment for degradation of recalcitrant organic fraction, to increase biodegradability and to reduce inhibition. The Fenton process was chosen for the chemical treatment because it is efficient for inhibitory and recalcitrant effluents. However, the application of the classical Fenton process is limited due to the high cost of chemicals and the production of hazardous ferric sludge. In this thesis, an efficient and applicable Fenton sludge reuse system without external sludge regeneration step tested in a laboratory-scale pilot plant, is presented (**Paper VII**).

Three significant problems of inhibitory and recalcitrant wastewater treatment were solved with this thesis. Firstly, optimal conditions for inhibition tests

were found to estimate the impact of wastewater or inhibitory substance on wastewater treatment processes for which no reliable standard methods were found – activated sludge EBPR and denitrification. Inhibition tests could be applied to estimate the feasibility of activated sludge processes to treat a certain wastewater and to identify the origin of inhibitory wastewater. Secondly, combined activated sludge and physical-chemical treatment of complex wastewater was tested considering results from preliminary inhibition and biodegradability studies, which increased the treatment efficiency and reduced cost of complex wastewater treatment. Thirdly, operation the Fenton process was modified to reduce the production of hazardous ferric waste sludge and consumption for chemicals.

2. THE AIM OF THE STUDY

The main aim of the thesis was to give solutions for cost-efficient treatment of inhibitory and recalcitrant wastewater. The specific aims were:

1) To develop easily applicable and reliable methods to estimate the inhibition of wastewater or hazardous substance on activated sludge denitrification and enhanced biological phosphorus removal (EBPR) processes (**Papers I and II**);
2) To develop readily applicable and cost-efficient schemes for combined biological and chemical treatment of recalcitrant and inhibitory wastewater (**Papers III, IV, V, VI**);
3) To increase the applicability of the Fenton process by reducing the production of ferric sludge and consumption for chemicals (**Paper VII**).

3. LITERATURE REVIEW

3.1 Recalcitrant and inhibitory wastewater

Cost-efficient treatment of recalcitrant and inhibitory wastewater, which is mainly originating from industries, is a challenging task compared to the treatment of municipal wastewater. Municipal wastewater is generally treated by application of activated sludge technology because of its reliability, flexibility, easy operation and low cost compared to physical and chemical wastewater treatment methods. However, biological methods, which typically use activated sludge technology, are not always suitable for the treatment of complex effluents because of the toxic and bio-refractory characteristics, fluctuations in pollutant loads and hydraulic surges of the influent (Seetha, Bhargava, et al., 2010). Additionally, hazardous substances present in wastewater may inhibit the whole treatment process or any specific metabolic pathway of activated sludge microorganisms (Hernandez-Martinez, Ortiz-Alvarez, et al., 2018; Sibag, Choi, et al., 2015; Zhao, Xin, et al., 2020). As a result, a wide range of persistent pollutants and elevated loads of nutrients remain in the effluent of the wastewater treatment plant (WWTP) and accumulate in receiving water bodies and bottom sediments, causing severe environmental problems. Thus, it is essential to characterize the impact of wastewater on activated sludge processes and to find feasible methods for the treatment of inhibitory and recalcitrant wastewater.

Effluents from the shale oil and wood industries (**Papers III and IV**), leachate from the municipal and hazardous landfills (**Papers V and VI**) were used as relevant samples of recalcitrant and inhibitory wastewater with different origin and properties produced in Estonia. Following chapters describe main characteristics of chosen wastewater types.

3.1.1 Municipal landfill leachate

At landfill sites, hazardous and concentrated wastewater called leachate is formed, which can be produced until 30–50 years after the closure of a landfill (Ngo, Guo, et al., 2008). Municipal landfill leachate contains more than 200 individual organic compounds, including biodegradable organic matter as well as biorefractory substrates. Landfill leachate is a source of many types of persistent organic pollutants (POPs), such as humic substances (HA), including fulvic acids (FA), pharmaceuticals, phthalates, polyaromatic hydrocarbons (PAHs), polybrominated diphenyl esters (PBDEs), pesticides and inorganic pollutants, e.g. ammonium (NH_4-N) and heavy metals. Toxicity of landfill leachate is mainly caused by high concentrations of hazardous substances or NH_4-N. (Silva, Silva, et al., 2013; Deng and Englehardt, 2006; Paxéus, 2000; Kindsigo and Kallas, 2008; Kang, Shin, et al., 2002; Öman and Junestedt, 2008).

Treatment of landfill leachate is a site-specific and depends on the characteristics of the leachate. The concentration of pollutants and the proportion of recalcitrant organic compounds demanding application of advanced treatment methods increase with the age of landfill (Kang, Shin, et al., 2002). Different physico-chemical processes are used for the treatment of complex wastewater as well as landfill leachate, e.g. flocculation, activated carbon adsorption, chemical oxidation, membrane filtration and nanofiltration. (Gotvajn, Tisler, et al., 2009; Anfruns, Gabarró, et al., 2013; Primo, Rueda, et al., 2008; Silva, Fonseca, et al., 2016)

3.1.2 Wood industry's wastewater

Plywood is a widely used wood-based product, which manufacturing is an important part of woodworking industry. One stages of the plywood production is wood soaking, which is often carried out at elevated temperatures. During the wood soaking process, wastewater containing recalcitrant organic substances, e.g, lignin, water-soluble wood and bark matter (i.e. extractives). The extractives are a range of organics with diverse functions and structure, such as terpenes, resin acids, triglycerides, fatty acids, phenolic compounds. Extractives possess a certain degree of toxicity as one of their function is to protect trees from pathogens. Wastewater containing wood extractives have been reported to have pronounced toxicological and hormonal effects (Lehtinen, Mattsson, et al., 1999). Lignin and its derivates are mainly causing the brownish colour of the wood industry`s wastewater. These substances are resistant to microbial degradation and can also cause toxic effects in aquatic environments (Leiviskä, Rämö, et al., 2009). Presence of tannin and lignin in wastewater has shown a decrease in respiration rate of activated sludge and a toxicity to *Daphnia magna test* (Libralato, Avezzù, et al., 2011). Due to the characteristics of the extractives (e.g. lignin has the ability to adsorb other pollutants in the wastewater), the insufficiently treated wastewater from wood industry affects the quality of effluent water bodies.

3.1.3 Petrochemical wastewater

Shale oil, which is produced from oil shale by thermal treatment, is mainly used to fulfill energy requirements. During the thermal treatment, the organic part of oil shale is decomposed into four main components: shale oil (25.5–53.5%), retort gas (4.1–18%), semicoke (23.5–65.8%) and pyrogenic wastewater (4.6–5%) (Ots, Poobus, et al., 2011; Reinik, Irha, et al., 2015; Gerasimov and Volkov, 2015).

The pyrogenic wastewater contains high concentrations of volatile and recalcitrant organic compounds including phenols, PAHs, carboxylic acids, ketones, and inorganic compounds: NH_4-N and sulphates (Joa, Panova, et al., 2009; Kekisheva, Smirnov, et al., 2007). Concentrations of chemical oxygen demand (COD), phenols and NH_4-N in the pyrogenic wastewater may reach up to 40 000, 14 000 and 700 mg/l, respectively (Kekisheva, Smirnov, et al., 2007).

Phenols, as the main cause of toxicity, can be effectively removed from pyrogenic wastewater by applying dephenolation process when gaseous heat carrier technology (e.g. Kiviter process) is used (Kekisheva, Smirnov, et al., 2007; Kamenev, Munter, et al., 2003). The separation of phenols is not feasible for solid heat carrier technology (e.g. Galoter process) because the total yield of water-soluble phenols is three times smaller than that in gaseous heat carrier technology (Kamenev, Munter, et al., 2003). Thus, an efficient treatment process for wastewater with high content of hazardous and potentially inhibitory substances is required for the solid heat carrier technology.

Semicoke is a hazardous solid waste material, which is landfilled. Leachate of semicoke landfill contains different harmful substances, including phenols, polycyclic aromatic hydrocarbons (PAHs) and oil products (Kavitha and Palanivelu, 2004). Semicoke landfill leachate is substantially less concentrated compared to the pyrogenic wastewater.

The presence of aromatic compounds in the influent wastewater of activated sludge plant may reduce the affinity of bacterial cells for carbon source and change the structure of cell envelope and thereby inhibit biological wastewater treatment processes (Chen, Yao, et al., 2009).

3.2 Biodegradability and inhibition of wastewater

3.2.1 Biodegradability

Knowledge of biodegradability characteristics of influent wastewater is important for the design and operation of biological removal of nutrients. Biodegradability influences the dynamics of the activated sludge process, e.g., the oxygen demand (Płuciennik-Koropczuk and Myszograj, 2019).

The ratio of BOD and COD is a common biodegradability indicator in wastewater treatment. However, the interpretation of BOD/COD value is mainly illustrative and could be misleading when wastewater contains organics from different fractions: non-degradable, slowly degradable or biodegradable organic fractions. In order to use the biodegradability data for operational decisions of WWTP, detailed biodegradability data is needed and the organic content of wastewater should be fractionated based on the degradation duration.

In practice, good wastewater characteristics are obtained on the basis of COD fractionation in accordance with the manual "Methods for Wastewater Characterization in Activated Sludge Modelling" (IWA Publishing, 2004). Additionally, modification has been made to the standard that increases the accuracy of determining the COD fraction has been proposed. However, this method requires wastewater characteristics to be in high compliance with the assumptions of the method and may be incorrect for wastewater with a large share of industrial wastewater (Płuciennik-Koropczuk and Myszograj, 2019).

3.2.2 Inhibition

Municipal WWTPs are influenced by several industries producing different types of wastewater. The composition of industrial wastewater is often unknown, fluctuating and containing substances, which are potentially inhibitory to activated sludge processes. Hazardous substances present in the influent of WWTP may inhibit whole treatment process or specific metabolic pathway of activated sludge microorganisms (V. J. Inglezakis, Malamis, et al., 2017). The inhibitory effect of wastewater on activated sludge processes may be the main obstacle to the effective application of biological treatment.

It has been shown that wastewater treatment processes could be inhibited by different hazardous substances present in wastewater, such as heavy metals, cyanides, phenols, nanoparticles, different polar and non-polar organic substances etc. (Çeçen, Semerci, et al., 2010; Kim, Cho, et al., 2011; Sibag, Choi, et al., 2015; V. J. Inglezakis, Malamis, et al., 2017; Hernandez-Martinez, Ortiz-Alvarez, et al., 2018; Su, Zheng, et al., 2015). In order to determine the origin of inhibitors or to show the suitability of activated sludge treatment for a specific wastewater it is necessary to estimate the impact of studied wastewater on activated sludge treatment processes. There are different methods available for monitoring the quality of influent of the wastewater treatment plant. ISO standard tests are used for activated sludge processes, for example test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation (ISO 8192) and a method for assessing the inhibition of nitrification of activated sludge microorganisms by chemicals and wastewaters (ISO 9509). Additionally, methods based on bioluminescent bacteria indicate the toxicity of wastewater. Among these, *in vitro* bioassays with luminescent bacterium *Vibrio fisheri* have been applied to evaluate the acute toxicity. However, it has been found that *V. fischeri*, which is based on marine microorganisms, exhibits higher sensitivity to many toxicants found in wastewater resulting in a threat to over-estimate the inhibition for activated sludge processes. (Stasinakis, Mamais, et al., 2008) Nevertheless, there are no reliable and easily applicable methods available for measuring the impact of wastewater or hazardous compound on activated sludge denitrification and enhanced biological phosphorus removal (EBPR) processes. Batch tests are used to show the impact of hazardous substances on various activated sludge processes (Ochoa-Herrera, Banihani, et al., 2009; Inglezakis, Kudarova, et al., 2017), but the set-up of experiments is often varying and there is lack of information about the calculation of results and validation parameters of the method.

The inhibition on denitrification of activated sludge could be assessed by techniques that require advanced instrumentation (gas chromatography, mass spectrometry, atomic emission spectrometry) (Sun, Fan, et al., 2016). Mostly, nitrogen isotope techniques and acetylene inhibition method are used (Lin, Tay, et al., 2009). Inhibition on the EBPR process has been mainly performed by methods that require setup and operation of reactor for pre-adaption of the inoculum until steady-state conditions are achieved (Katsou, Alvarino, et al.,

2016; Welles, Lopez-Vazquez, et al., 2014; Wang, Ren, et al., 2011). Results of the EBPR process inhibition study depend on the difference of applied operating conditions of activated sludge acclimatization system, such as temperature and organic carbon type. Differences in the acclimatization procedure may affect the physical characteristics and microbial composition of the acclimated sludge (Wu and Rodgers, 2010). More advanced approaches for estimating the efficiency and inhibition of biological phosphorus removal process are based on fluorescence in situ hybridization (FISH) and Raman–FISH (Katsou, Alvarino, et al., 2016; Fernando, Jon, et al., 2019). Thus, inhibition of the EBPR and denitrification processes have been measured, but there are lack of reliable and easily-applicable methods.

3.3 Enhanced biological phosphorous removal (EBPR) process

Phosphorous (P) is a key driver of eutrophication which makes its efficient removal an essential task. In the EBPR process, which is conventionally applied for P removal, P is accumulated to waste activated sludge (WAS) by poly-phosphate accumulating microorganisms (PAOs). PAOs consume energy gained from hydrolysis of polyphosphate and glycogen degradation to take up volatile fatty acids (VFAs) under anaerobic conditions, which are converted to polyhydroxyalkanoates (PHAs). During the degradation of poly-phosphates ortho-phosphates are released from bacterial cells and the concentration of phosphorous increases in liquid phase (phosphorous release). PHAs, commonly poly-β-hydroxybutyrate (PHB), polyhydroxyvalerate (PHV) and poly-β-hydroxy-2-methylvalerate (PH2MV), are biopolyesters synthesized as intracellular storage reserves of carbon and energy (Oehmen, Lemos, et al., 2007). The exact type of produced PHA depends on the composition of VFAs in the wastewater and inoculum. PHB is stored when the most abundant VFA is acetate, PHV and PH2MV when propionate is the abundant VFA (Oehmen, Zeng, et al., 2005). Furthermore, higher amounts of PHV are stored when glycogen- accumulating organisms (GAOs) are present in the inoculum (Saunders, Oehmen, et al., 2003). In following aerobic process, PHAs are respired to provide energy for cell metabolism and excessive poly-P is accumulated to microbial cells (phosphorous uptake).

PAO-s are considered resistant against various environmental disturbances, however they are still vulnerable to hazardous substances and prone to process inhibition because of the location at the beginning of the conventional WWTP and the complex composition of sewage, which could contain inhibitors. Reported substances inhibiting the EBPR processes are pharmaceuticals (doxycycline, tetracycline, diclofenac), heavy metals (copper, tin, silver, chromium), salts, H_2S, nanomaterials (Katsou, Alvarino, et al., 2016; Tsai and Chen, 2011; Welles, Lopez-Vazquez, et al., 2014; Chen, Liu, et al., 2014; Wang, Ren, et al., 2011; Rubio-Rincón, Lopez-Vazquez, et al., 2017; Zheng, Sun, et al., 2014).

Since the demand for P as a fertilizer is globally increasing while its reserves are decreasing, efficiently treated P-enriched biomass can be further used as a fertilizer directly or indirectly as an alternative to the mineral fertilizer.

3.4 Nitrogen removal

Nitrogen removal in activated sludge treatment plant is conventionally performed via nitrification and denitrification processes. In the nitrification, ammonium is oxidized to nitrite in the nitritation process and subsequently converted to nitrate in the nitratation process with O_2 as a terminal electron acceptor. These processes are performed by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), respectively. Nitrification process is sensitive to various toxic substances mainly because of the low growth rate of nitrifying microorganisms (Philips, S. Laanbroek, H.J. Verstraete, 2002; Haandel and Lubbe, 2012). Substances inhibiting nitrification process are for example copper, arsenic, cadmium, lead, phenols, chloroform, cyanide, etc. (Svenskt Vatten, 2012).

In the following denitrification process, nitrate is reduced stepwise to nitrite (NO_2), nitric oxide (NO), nitrous oxide (N_2O) and molecular nitrogen (N_2). Each oxidized nitrogen form is catalysed respectively by NO_3, NO_2, NO or N_2O reductase enzymes. Organisms containing at least two or three of these specific enzymes and producing NO or N_2O will be referred to as „denitrifiers". (Philips, S. Laanbroek, H.J. Verstraete, 2002; Chen and Strous, 2013; Lin, Tay, et al., 2009)

Contrary to nitrification, denitrification process is accomplished by a relatively wide range of microorganisms (prokaryotic microorganisms, chemo-organo-heterotrophs, chemo-litho-autotrophs). The growth rate of anoxic denitrifying organisms is comparable with aerobic heterotrophic organisms and is much greater than the growth rate of nitrifiers. (Gray, 2004; Stasinakis, Mamais, et al., 2008) Thus, denitrifying organisms should be more resistant to toxic substances present in the influent when compared to nitrifiers. However, denitrifiers are sometimes more susceptible to the inhibitory effect of inhibitory substances (e.g. copper) than nitrifiers. Furthermore, denitrification process might be more vulnerable to inhibitory substances in raw wastewater because the denitrification tank is conventionally located before nitrification tank. However, incomplete denitrification is not always the result of inhibition. Denitrification efficiency could be affected also by following aspects: (a) imbalances in electron donor supply; (b) reactive radicals produced in biological processes; (c) low/high pH value; (d) presence of oxygen; (e) unsuitable temperature (Oh and Silverstein, 1999; Ochoa-Herrera, Banihani, et al., 2009; Sun, Fan, et al., 2016).

3.5 Advanced oxidation processes (AOP-s)

Advanced oxidation processes (AOPs) are chemical methods that are proven to be efficient for treatment of different types of wastewater. The AOP-s are continuously improved to provide an energy-efficient and environmentally friendly wastewater treatment technology. AOP-s are applied for different objectives: to mineralize organic pollutants, to diminish toxicity and/or to increase biodegradability of wastewater. Many different AOP-s have been applied for wastewater treatment, from which the Fenton and Fenton-like processes are few examples of applied technologies (Kavitha and Palanivelu, 2004; Ballesteros Martín, Casas López, et al., 2010; Fernández, Castro, et al., 2011; Campo, Romero, et al., 2014).

The efficiency of AOPs is based on the generation of highly reactive hydroxyl radicals that react quickly and non-selectively with the majority of organic contaminants. A hydroxyl radical can be generated by the combination of ozone and/or hydrogen peroxide with activators, e.g. transition metals (most commonly Fe^{2+}), semiconductors (e.g. TiO_2), ultraviolet and ultrasonic irradiation. (Dükkancı, Vinatoru, et al., 2014; Thomas, Dionysiou, et al., 2021).

Although AOPs can oxidize complex organic pollutants, the process efficiency is determined by the quantities of added chemicals. Chemical consumption limits the applicability of the process due to the high cost of chemicals – more concentrated wastewater requires higher dose and thereby leads to unreasonable treatment costs.

3.5.1 Fenton process

Application of the Fenton reaction in wastewater treatment processes are known to be very effective in the complete degradation of many hazardous organic pollutants to harmless compounds, e.g.CO_2, water and inorganic salts. The Fenton reaction causes the dissociation of the hydrogen peroxide and the formation of highly reactive hydroxyl radicals that destroy the organic pollutants. The Fenton`s reaction is given in Eq. 1.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-} \qquad \text{(Eq. 1)}$$

As a promising AOP, the Fenton process has been widely studied for treatment of various types of wastewater: landfill leachate (Singh and Tang, 2013; Silva, Silva, et al., 2013; Chen, Liu, et al., 2014), effluents from leather (Sekaran, Karthikeyan, et al., 2013), textile (Karthikeyan, Titus, et al., 2011) and meat industries (Páramo-Vargas, Granados, et al., 2015), etc. Different Fenton-like processes have been developed to increase the cost-efficiency of wastewater treatment, e.g. photo-Fenton (Rahim Pouran, Abdul Aziz, et al., 2015), electro-Fenton (Gökkuş, 2016) and nano-Fenton (Li, Ali, et al., 2014). However, it must be considered that high removal efficiencies in the Fenton and Fenton-like processes reported in the literature were often obtained either at elevated

reagent consumption, or with high solar flux, which are not cost-efficient solutions.

The most important operating parameters in the conventional Fenton process and several Fenton-like processes are pH and weight ratio (w/w/w) of COD/H_2O_2/Fe^{2+} (Deng and Englehardt, 2006). The optimal pH for the Fenton reaction is generally in the range of 2–3 (He, Tian, et al., 2015). The dose of hydrogen peroxide and catalyst is highly dependent on the characteristics of the wastewater and increases with the content of organic substances present in the wastewater (Deng and Englehardt, 2006). It is recommended that the most efficient performance of Fenton's reagent for wastewater treatment is achieved by applying a H_2O_2/Fe^{2+} molar ratio of 10/1 (Dulova and Trapido, 2011), which is approximately H_2O_2/Fe^{2+} of 6/1 (w/w). A low concentration of catalyst is not sufficient to catalyse the degradation of hydrogen peroxide and overdosing of the catalyst causes its reaction with hydroxyl radicals (Neyens and Baeyens, 2003).

The main drawbacks for the application of the Fenton process are the treatment cost and formation of large amounts of ferric sludge. The ferric waste sludge produced in the Fenton process is a substantial limiting factor for application of the process because the sludge requires further treatment and disposal. Ferric sludge contains significant amounts of water and subsequent dewatering is needed to reduce its volume. Furthermore, different organic pollutants, heavy metals and other substances from treated wastewater make it a concentrated hazardous waste. It is possible to reuse the ferric sludge to reduce the secondary pollution of the process. Different approaches have been applied for the ferric sludge reuse, e.g. dissolving the sludge in sulphuric acid followed by the addition of hydroxylamine forming hydroxylamine hydrochloride which reduces ferric ion to ferrous ion (Kavitha and Palanivelu, 2004) and electro-chemical reduction (Kishimoto, Kitamura, et al., 2013).

In general, the decomposition of H_2O_2 in ferric sludge catalysed Fenton process could be catalysed only by Fe^{3+} originated from the reused sludge during the oxidation phase initiated at acidic pH values as presented in Eqs. (2)–(5) (Bautista, Mohedano, et al., 2008):

$$Fe(OH)_3 + 3H^+ \rightarrow Fe^{3+} + 3H_2O \qquad\qquad (Eq.\ 2)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^+ \qquad\qquad (Eq.\ 3)$$

$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + H^+ + O_2 \qquad\qquad (Eq.\ 4)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^- \qquad\qquad (Eq.\ 5)$$
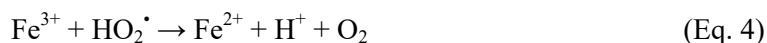
The above-mentioned Fenton-like processes catalyzed by the regenerated ferric sludge have shown high efficiency in the removal of organic pollutants, but separate ferric sludge regeneration process increases the overall cost of waste-water treatment.

3.6 Combined chemical and biological treatment of wastewater

Biological processes have proven to be sustainable, relatively cheap and efficient methods for removal of degradable organic substances, nitrogen and phosphorous from the wastewater. However, they are not always effective, mainly due to toxic and persistent pollutants in the wastewater (Reemtsma, Putschew, et al., 1999; Ren, 2004) and hydraulic and shock loads created by peak volumes and concentrations (Seetha, Bhargava, et al., 2010; Pophali, Kaul, et al., 2003).

Physical and chemical treatment methods have shown higher efficiencies in the treatment of high-strength wastewater compared to the biological treatment. However, application of single-stage treatment for complex wastewater containing high concentration of different substances is often insufficient. Thus, different combinations of physical, chemical and biological processes have been studied for purification of high-strength wastewater. Among other proposed combinations, the integration of AOPs and aerobic biological systems has proven to be cost-efficient and promising for the treatment of wastewaters with different characteristics. (Wu, Zhou, et al., 2011; Guo, Abbas, et al., 2010; Silva, Fonseca, et al., 2016) Combining biological treatment with physico-chemical techniques is a solution to improve the overall feasibility of wastewater treatment and reduce the total pollution load. The selection of physical and chemical methods for the treatment of inhibitory and recalcitrant wastewater is wide. Coagulation and flocculation methods are widely used to remove suspended solids from wastewater (Dulov, Dulova, et al., 2011; Gotvajn, Tisler, et al., 2009). Stripping processes (e.g. air stripping, batch distillation) have been studied for removal of NH_4-N as well as VOCs from wastewater due to the relatively low cost (Gotvajn, Tisler, et al., 2009; Ozyonar, Karagozoglu, et al., 2012).

A combined system consisting of individual physicochemical and biological treatment stages would be the solution for obtaining optimal treatment conditions, both in terms of performance and cost. Combined treatment technologies can increase the process performance. Biological treatment is still the base of the combined process, but physical and chemical treatment can be used as pre-treatment and/or post-treatment. The Fenton and Fenton-like processes are often combined with other treatment methods, commonly with activated sludge treatment or coagulation for three main reasons: (1) removing easily biodegradable organic fraction by activated sludge, which makes the recalcitrant fraction as the COD is the sole target of the Fenton process; (2) converting recalcitrant compounds to biodegradable substances for biological post-treatment; (3) reducing the toxicity of the wastewater for biological post-treatment. (Li, Zhou, et al., 2009; Moradi and Ghanbari, 2014; Ballesteros Martín, Casas López, et al., 2010; He, Tian, et al., 2015)

4. MATERIALS AND METHODS

4.1 Analytical methods

Standard methods for examination of water and wastewater were used in order to determine BOD_7, COD, PO_4-P, TS, MLSS (APHA 1985). The seed in BOD_7 measurements of pyrogenic wastewater were obtained from Kohtla-Järve WWTP, Estonia, because receives effluents from the semicoke thermal processing area and semicoke landfill, it was proposed to adapt its sludge sample to higher concentrations of hazardous substances. pH was measured with a digital pH meter (Evikon E6115 or Schott 840), electrical conductivity by digital EC meter (HANNA Instruments HI9032) and the concentration of dissolved oxygen with Marvet Junior 2000 portative oxygen analyser (Elke Sensor, Estonia). NO_3-N and NO_2-N were determined by spectrophotometric methods according to SFS 5752 and SFS 3029, respectively. NH_4-N was determined using the HACH-Lange spectrophotometric method with Nessler reagent (ISO 7150-1) or in the case of low concentrations according to ISO 7150–1 (ISO 1984). TN in unfiltered samples was measured with peroxodisulphate method (ISO 2003). DOC was measured with Analytik Jena Multi N/C 3000 TOC analyser and TOC was measured from unfiltered samples using the HACH-Lange cuvette test according to EN 1484 (EN, 1997). The concentrations of anions (F^-, Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, PO_4^{3-}) and acetate were measured using an ion chromatograph Metrohm 930 Compact IC Flex equipped with a column Metrosep A Supp 5 100/0.4 (Metrohm, Switzerland). The extraction of HS was performed from pre-acidified (pH 2.0 ± 0.1) leachate samples filtered through the XAD-8 (Superlite DAX-8) resin column, with subsequent HS separation with 0.1mol/l NaOH solution. HS concentration in the solution was measured by an Analytik Jena Multi N/C UV HS analyser. Total and ferrous iron concentrations were determined using the phenanthroline method (APHA 2012). The concentrations of lignin and tannins were measured spectrophotometrically using the tyrosine method with a HACH-Lange cuvette test (APHA 2012). The concentration of phenols was measured using a sodium nitrite method with HACH-Lange cuvette tests (APHA 2012). The H_2O_2 concentration was measured using oxidation– reduction titration with 0.01 M $Na_2S_2O_3$ under strongly acidic conditions and 10 % KI solution as the indicator. The samples were mixed with 5 ml of 30 % H_2SO_4, 5 ml of 10 % KI solution and 2 drops of saturated $(NH_4)_6Mo_7O_{24} \times 4H_2O$. Starch was added to change the colour of the solution from yellow to blue. Titration with 0.01 M of $Na_2S_2O_3$ was performed until the solution was colourless. Given analytical methods were used in **Papers I–VII**.

The results of all the analyses were expressed as the mean with standard deviations of at least 3 parallel replicates.

4.2 Study on biodegradability and inhibition

Zahn-Wellens test with the duration of 28 days was used to assess the biodegradability of raw and treated wastewater samples (ISO 9888) (**Papers III–VI**). Additionally to the standard procedures, the sample was also fractionated by dividing it into three fractions: easily biodegradable COD (degrades during the first 7 days), slowly biodegradable COD (degrades in 7–28 days), and recalcitrant COD (the residual COD).

Tests on the inhibition of oxygen consumption and nitrification rate by activated sludge in the of raw and treated wastewater samples were performed according to ISO standards 8192 and 9509, respectively (ISO 8192; ISO 9509) (**Papers I–VI**).

Activated sludge used in the biodegradability and inhibition tests originated from the conventional municipal WWTP (100 000 PE, negligible industrial influence), however, sludge from the municipal WWTP substantially influenced by the industrial inflow was used for phenolic samples (hazardous landfill leachate and pyrogenic wastewater) because it was proposed to adapt to higher concentrations of phenolic substances.

The acute toxicity of untreated and treated wastewater was tested using a *Daphnia magna Straus* test (DAPHTOXKIT F™ MAGNA, MicroBioTest Inc, Belgium) performed according to ISO 6341 standard at the Tallinn University of Technology, Department of Chemical Engineering.

4.2.1 Set-up of the EBPR process inhibition test

The EBPR process inhibition test was developed and tested in the **Paper I**. A returned sludge from the conventional municipal WWTP with an effective EBPR process was continuously aerated at an ambient temperature until the test began (not longer than 24h). The activated sludge was washed by applying centrifugation at 1000 RMP for 2 min in 250 ml tubes. Inhibition experiments were performed in 1000 ml Pyrex bottles, which contained 100 ml of 2 g/l sodium-acetate solution, 1 ml phosphate buffer, 1 ml $MgSO_4 \times 7H_2O$, 1 ml $CaCl_2 \times 7H_2O$, 1 ml $FeCl_3 \times 6H_2O$, 2 ml acidic microelements, 2 ml basic microelements, 550 ml municipal wastewater (if not stated otherwise), a toxicant (3,5-dichlorophenol) and 300 ml of activated sludge mixture (MLSS value in the test was 4 ± 0.8 g/l). The municipal wastewater used in the tests was taken from the same WWTP as sludge. Recipes of acidic and basic microelements were taken from Zhang et al. (2009). K_2HPO_4 was added in order to avoid phosphorus limitation for the EBPR process. Wastewater and sludge were both kept at ambient temperature to avoid temperature shock.

Experiment consisted of 1 h anaerobic followed by 6 h of aerobic phase, continuous stirring was applied during the experiment. Concentration of dissolved oxygen and value of pH were monitored during the whole experiment. pH was regulated with a 4 M HCl solution (if necessary) for keeping it below 8. At first, samples were collected and centrifuged immediately after the addition

of inoculum (starting point). Test bottles were purged with N_2 to achieve anaerobic environment and with air during aerobic phases. Samples were taken after 1.5, 2.5, 4 and 6 h under aerobic conditions. PO_4-P, COD were analyzed from liquid phase and PO_4-P, PHAs from solid phase. Concentrations of DOC and acetate were measured from the liquid phase to ensure sufficiency of VFAs. Experiments were performed at the ambient temperature.

The percent of EBPR inhibition was calculated as following:

$$R_p = \frac{(C1 - Cx)}{MLSS} \times \Delta t \qquad \text{(Eq. 6)}$$

$$I_p = \frac{(Rp,0 - Rp,x)}{Rp,0} \times 100\% \qquad \text{(Eq. 7)}$$

R_p – phosphorous release/uptake rate, $mgPO_4$-P/gMLSS×h
c_1 – concentration of PO_4-P at the end of anaerobic cycle, mg/l
c_x – total concentration of PO_4-P at the beginning of anaerobic cycle /chosen point of aerobic cycle, mg/l
MLSS – concentration of mixed liquid suspended solids, g/l
Δt – duration of chosen period, h
I_p – inhibition of phosphorous release/uptake process, %
$R_{p,0}$ – phosphorous release/uptake rate in the control vessel, $mgPO_4$-P/gMLSS×h
$R_{p,x}$ – phosphorous release/uptake rate in the test vessel at chosen inhibitor concentration, $mgPO_4$-P/gMLSS×h

The inhibition tests of the EBPR process were done in seven parallel replicates for the control of the developed method; in three parallel replicates when synthetic wastewater was used as a carbon source.

4.2.2 Model inhibitor of inhibition tests

3,5-dichlorophenol (3,5-DCP) was used as a model inhibitor to develop and test EBPR and denitrification inhibition tests (**Papers I, II**). Chlorophenols and their derivates are persistent and highly toxic environmental pollutants of industrial origin. 3,5-dichlorophenols are formed via dehalogenation of poly-chlorophenols (Villemur, 2013; Arora and Bae, 2014). Developing and testing new inhibition tests for denitrification and EBPR processes were done with a 3,5-dichlorophenol (3,5-DCP) solution (1 g/L) as a reference compound. This solution is used as a reference compound in ISO Standards: ISO 9509 "Toxicity test for assessing the inhibition of nitrification of activated sludge micro-organisms" and ISO 8192 "Test for inhibition of oxygen consumption by activated sludge". Respective standards declare that 9.3±3.7 mg/l of 3,5-DCP inhibits 50% of total respiration (ISO 8192) and 5.6±3.0 mg/l of 3,5-DCP

inhibits 50% of nitrification rate (ISO 9509). Based on tests performed during this study, 7.2±2.4 mg/l of 3,5-DCP inhibited 50% of oxygen uptake rate. The average oxygen uptake rate in blank sample was 7.3 mgO$_2$/gMLSS×h. 2.9±0.7 mg/l of 3,5-DCP inhibited 50% of nitrification rate with the average nitrification rate of blank sample 2.5 mgNO$_3$-N/gMLSS×h. Consequently, the inoculum used in this study was considered suitable when compared with values and uncertainties given in the respective ISO standards.

4.2.3 Analysis of polyhydroxyalkanoates (PHA)

PHA-s were analysed for the EBPR inhibition test in the **Paper I**. PHAs were analyzed by the method proposed by Oehmen et al., 2005. Samples were centrifuged and immediately frozen at −20 °C until the final measurements. Frozen samples were lyophilized during 20 hours in a Christ Alpha 1–2 freeze dryer operated at −49 °C and 0.7 mbar connected with a Vacuubrand RZ 2.5 vacuum pump. Lyophilized sample was weighted into a glass tube, where 2 ml of acidic methanol (10% H$_2$SO$_4$ v/v) and 2 ml of chloroform were added. The tubes were sealed and incubated at 105 °C for 2 hours for derivatization and subsequently cooled. 2 ml of derivatized sample was mixed with 1 ml of milli-Q water and a vortex was applied to facilitate the separation of two phases. Traces of water from chloroform phase were removed with Na$_2$SO$_4$. The sample was extracted into a gas chromatograph vial and analyzed immediately. 2 µl of sample was injected in an Agilent Technologies 7890A gas chromatograph equipped with 5975C inert MSD system with Triple-Axis Detector, G4513A Autosampler and HP-5MS column (30 m, 0.25 mm internal diameter, 0.25 µm film thickness, Agilent Technologies), helium was used as a carrier gas. A co-polymer of poly(3- hydroxybutyric acid-co-3-hydroxyvaleric acid), natural origin, 88:12 wt, Aldrich was used as a standard. Standards were processed in the same way as the samples, after being dissolved into a chloroform solution. The production of PHAs was calculated as concentration difference between the starting point and the end of anaerobic period. The PHA consumption was calculated as concentration difference between the end of anaerobic period and the end of aerobic period.

4.2.4 Set-up of the denitrification inhibition test

The denitrification process inhibition test was developed and tested in the **Paper II**. Two different matrixes were used as toxicants to develop and control the method. Firstly, tests were performed with 3,5-dichlorophenol (3,5-DCP) solution (0–12.5 mg/l). Secondly, test was controlled with highly inhibitory wastewater from shale oil industry.

Activated sludge samples (returned sludge) taken from a municipal WWTP (100 000 PE) were used as an inoculum unless stated otherwise. Firstly, activated sludge was washed with tap water at least three times by applying

settling period and subsequent decanting. 500 ml activated sludge mixture contained 1 ml phosphate buffer, 1 ml $MgSO_4 \times 7H_2O$, 1 ml $CaCl_2 \times 7H_2O$, 1 ml $FeCl_3 \times 6H2O$, 50 ml $NaHCO_3$ solution (2.52 g/L), 2 ml acidic microelements, 2 ml basic microelements. Recipes of acidic and basic microelements were taken from Zheng, et al., 2009.

Experiments were performed in 300 ml biochemical oxygen demand (BOD) bottles. 200 mg/l Na-acetate, 30 mg/l NO_3-N and 6 different concentrations of toxicant were added to the bottles. Seventh bottle was a blank control and it did not contain toxicant. Finally, 50 ml of activated sludge mixture was added into each bottle. Continuous stirring was applied during the experiment. First samples (starting point) were collected and centrifuged immediately after the addition of inoculum. Bottles were purged with N_2 for 20–30 minutes and sealed air tightly. Concentration of dissolved oxygen and value of pH were monitored during the process. Following samples were taken after 1 hour and 3 hours under anoxic conditions. NH_4-N, NO_3-N, NO_2-N, DOC and pH were analyzed from the centrifuged samples. DOC was measured to ensure that inhibiton was not resulted from carbon deficiency. Experiments were performed at the ambient temperature.

The percent of denitrification inhibition was calculated as following:

$$R_d = \frac{(C1,N - C3.N)}{MLSS} \times \Delta t \tag{Eq. 8}$$

$$I_d = \frac{(Rd,0 - Rd.x)}{Rd,0} \times 100\% \tag{Eq. 9}$$

R_d – denitrification rate (mgNO_x-N/gMLSS×h)
$R_{d,0}$ – denitrification rate in the control vessel (mgNO_x-N/gMLSS×h)
$R_{d,x}$ – denitrification rate in the test vessel (mgNO_x-N/gMLSS×h)
$c_{1,N}$ – total concentration of nitrate after one hour of test period (mg/l)
$c_{3,N}$ – total concentration of nitrate after three hours of test period (mg/l)
MLSS – concentration of mixed liquid suspended solids (g/l)
Δt – duration of test (h)
I_d – inhibition of denitrification (%)

Inhibition tests were done in seven parallel replicates during the control of the developed method; in three parallel replicates when the method was used after the control.

4.3 Characterisation of the model toxicant and wastewater samples

Wastewater samples from wood (**paper IV**) and shale oil (**paper III**) industries and leachates from municipal (**paper V**) and hazardous landfills (**paper VI**) were used in this study. The main parameters of used samples are shown in Table 1.

4.3.1 Plywood industry

Samples of wood industry`s wastewater were taken from the hardwood soaking basin of the local plywood industry (main parameters are shown in Table 1). Wastewater characteristics varied accordingly to the retention time in the soaking basin. Wastewater contained high concentration of total suspended solids; almost 50 % of COD was not dissolved. Besides organics, nitrogen, phosphorus and phenols need to be removed from the wastewater. Concentrations of pollutants in the wastewater may vary significantly in case of various samples, which can be explained by the differences in wood amounts soaked in the water, the composition of the soaked wood, i.e. relative abundance of different wood species, periodic additions of freshwater to compensate evaporation losses and residence time.

Table 1. The main characteristics of raw wastewater samples used for biodegradability, inhibition and treatment studies. Detailed characteristics are given in respective papers (**Papers II–VII**).

Para-meter	Unit	Plywood industry (Paper IV, S3)	Pyrogenic waste-water (Paper III, S2)	Pyrogenic waste-water (Paper II)	Hazardous waste landfill leachate (Paper VI, L2)	Municipal landfill leachate (Paper V, S4)	Municipal landfill leachate (Paper VII)
COD	mgO_2/l	6830±250	45 400±380	11 050±340	850 ± 47	6790 ± 45	11 800 ± 4000
BOD_7	mgO_2/l	3530±275	34 900±6500	6425±380	330 ± 30	4700 ± 300	6390 ± 2765
BOD_7/COD	–	0.51	0.77	0.58	0.39	0.69	0.57
pH		5.05	8.48	6.0	9.3	7.6	7.7
NH_4-N	mg/l	50±0.8	1650 ± 50	390 ± 5	3.5	840±40	1170 ± 330
Phenols	mg/l	94±6	1050 ± 35	470 ± 2	18	33 ± 3	72 ± 31

± Standard deviation of all parameter values, n≥3
* S2-S4 and L2 correspond to sample labels used in respective papers (all samples were not included to the thesis)

4.3.2 Shale-oil industry

Three different samples originated from the shale-oil industry. Firstly, the pyrogenic wastewater from oil shale thermal treatment (modified Galoter process, Estonia) (**Paper III**). Secondly the pyrogenic wastewater for the denitrification inhibition test (Kiviter process, Estonia) (**Paper II**). Thirdly, the leachate from the semicoke landfill (**Paper VI**). The main parameters of studied samples are shown in Table 1.

The volume of pyrogenic wastewater production in the modified Galoter process was 10–20 m^3/d; it is characterized by a high concentration of organic compounds and ammonium, as well as a high toxicity to *Daphnia magna* indicator organism – EC_{50}=0.34%. Raw wastewater samples were taken respectively in May 2012 and February 2014.

Wastewater samples from the Kiviter process were taken in September 2016. The characteristics of the wastewater were similar to the sample collected from the modified Galoter process: high content of organic compounds and NH_4-N.

The chemical composition of samples collected from a semicoke landfill area varied significantly, but generally, the semicoke landfill leachate can be characterized by high concentrations of organic material, medium BOD_7/COD and a low concentration of nitrogen.

4.3.3 Municipal landfill leachate

Leachate samples were obtained from the landfill specialized in municipal non-hazardous waste management and recycling, located near Tallinn, Estonia. The landfill started operating in 2003. The composition of the leachate was typical for a medium-aged landfill. Samples were taken in October 2012-October 2013 for the analysis of characteristics and preliminary studies (**Paper V**) and in July 2014–February 2015 for the pilot study (**Paper VII**).

All collected wastewater samples were stored at 4 °C.

4.4 Set-up of the biological treatment

The laboratory-scale biological treatment of studied samples (**Papers III–VI**) was carried out by simulating a typical plug-flow or SBR activated sludge processes. The activated sludge used for treatment of pyrogenic wastewater (**Paper III**) and leachate of hazardous landfill (**Paper VI**) was obtained from a municipal WWTP, which received effluents from a semicoke thermal processing area and semicoke landfill. The sludge from this WWTP was proposed to be largely adapted to higher concentrations of hazardous substances. The activated sludge used for treatment of municipal landfill leachate (**Paper V**) and wastewater from plywood industry (**Paper IV**) originated from municipal WWTP which received negligible proportion of industrial wastewater. Applied HRT

values were for pyrogenic wastewater 18 days, for landfill leachate 13–14 days and 3 days for hazardous landfill leachate and wastewater from plywood industry. Aerobic biological treatment for Fenton post-treated effluents were performed at lower HRT values: 1–2 days for municipal and hazardous landfill leachates and pyrogenic wastewater and 5 hours for wastewater from plywood industry. pH was regulated in the biological treatment when necessary to ensure bacterial activity in the activated sludge. If necessary, phosphorous (K_2HPO_4) was added to the influent during the primary and secondary biological treatment steps to ensure that the minimum PO_4–P concentration will be at least 0.5% of the COD value. During the operating periods, MLSS and COD were measured daily. The effluent was stored at 4 °C to avoid any further decomposition.

4.5 Set-up of physical and chemical treatments

4.4.1 Fenton treatment

The Fenton treatment experiments were performed in a batch mode for **Papers III–VII**. Wastewater samples were treated in a cylindrical glass reactors with a permanent agitation speed for a period of 24 h. The pH of samples was adjusted to 3, unless specified otherwise. After addition and complete dissolution of the activator ($FeSO_4 \times 7H_2O$) the Fenton reaction was initiated by adding H_2O_2. Concentrations of H_2O_2 and Fe^{2+} were always calculated according to the COD value (COD/H_2O_2/Fe^{2+} w/w/w). The oxidation process was stopped by the addition of NaOH (10 M) to adjust the pH to approximately 8.5-9 under mechanical stirring. This process was followed by a 24-h settling period of ferric-hydroxy complexes. Finally, the supernatant was collected for chemical analysis.

4.5.2 Air stripping, coagulation, flocculation and batch distillation

Air stripping of pyrogenic wastewater was carried out at 50 °C at an air flow rate of 0.4 l/min for 48 h. The wastewater volume was 50 l (**Paper III**).

The coagulation of the pyrogenic wastewater (**Paper III**) was performed at an 8% $Al_2(SO_4)_3$ dose of 0.16 ml/l followed by flocculation by adding 1.5 ml/l of 0.1% Zetag 4105 (anionic poly- electrolyte). The wastewater volume in each batch was 4 l.

Batch distillation (**Paper III**) was carried out by boiling the solution in a stainless steel vessel at 100°C by keeping the pH at 11 until the wastewater volume had decreased 15%. This process simulated the single stage steam distillation without the injection of direct steam.

The chemical pre-treatment (Fenton, photocatalytic oxidation, ozonation and coagulation) studies described in the **Papers III, IV, V** were performed by Tallinn University of Technology and were not the part of this thesis.

4.6 Set-up and operation of pilot plant

The treatment of landfill leachate was studied by using a following combined biological and chemical three-stage pilot plant: an activated sludge pre-treatment (BIO1), a chemical treatment (CHEM) and an activated sludge post-treatment (BIO2) with active volumes of 16, 1.6 and 4 l, respectively (Figure 1), (**Paper VII**). Besides the three main reactors, there were an influent and effluent tank and two buffer tanks before and after the chemical treatment tank to enable individual control of different treatment stages.

An automated control system (Unitronics®, Israel) was used for operating the pilot plant. The pH value in the main reactors was controlled by signal of optical pH/ORP/temperature sensor (Ponsel, France). Dissolved oxygen concentration was controlled using the Oxylog system (Elke Sensor Ltd., Estonia). During the biological treatment stages, aerobic and anaerobic conditions were controlled by DO concentration-based intermittent aeration. Two compressors with flow rates of 300 and 400 l/h were installed in the first biological reactor; a compressor with a flow rate of 100 l/h was installed in the second biological reactor. To achieve better solubility of oxygen, air diffusers were added to the biological reactors. Mechanical mixing was added to three main reactors. Eleven peristaltic pumps were installed for the chemical dosing and pumping of influents and effluents.

Biological pre- and post-treatments of the leachate were carried out in sequencing batch reactors (SBRs) operated at three 8-hour cycles per day with three feeding pulses per cycle. A settling period (30 min) was applied, after which the supernatant was decanted (45 s). Aeration was performed intermittently (1 h anoxic/1 h aeration) with the ratio of aerobic and anoxic phases of 1/1. The concentration of dissolved oxygen (DO) was kept between 0.7 and 2.5 mg/l during the aeration phase.

Biological treatments were performed with an activated sludge originating from a WWTP treating municipal landfill leachate in Torma, Estonia. Owing to the high- strength nature of the leachate, the reactor was operated with long hydraulic retention time (HRT = 23–28 days), low-rate sludge loading (F/M ratio = 0.028 $gBOD_5/(gMLSS×d)$) and a high sludge retention time (SRT = 30 days) during the start-up period. As the inflow of the second biological reactor had previously passed biological and chemical treatments, a prior adaption period was not required. After achieving stability (operational period), HRT in the first biological reactor was kept between 12 and 16 days with a F/M ratio of 0.05 $gBOD_5/(gMLSS×d)$ and a SRT of 21 days. In the second biological treatment reactor, HRT was kept at 6–8 days, F/M ratio at 0.01 $gBOD_5/(gMLSS×d)$ and SRT at 96 days. Biologically pre-treated supernatant was collected in the first buffer tank, after which it was pumped into the chemical treatment reactor. Values of COD, BOD_7, NH_4–N, NO_3–N, NO_2–N and pH were measured twice a week and values of lignin and tannins and phenols once a month.

## 4.6.1 Set-up of the Fenton/Fenton-like treatment

During the continuous operation of the chemical oxidation reactor of the pilot study, emphasis was placed on the modification of the Fenton-like process. The aim was optimization of the amount and mode of fresh ferrous iron (Fe^{2+}) catalyst addition with continuous ferric sludge reuse to increase biodegradability and reduce the COD value of the biologically pre-treated landfill leachate.

The main differences from the conventional operational method were a SBR-mode operation with a water exchange ratio (WER) of 50 % (V = 1.6 l) and 100 % (V = 0.8 l) and continuous ferric sludge reuse up to 12 times after the first reaction. The WER of 50 % means that half of the treated wastewater was decanted in each cycle. The Fenton/Fenton- like reaction was performed during 6–8 h of chemical oxidation followed by a settling period of 9–10 h. Ferric sludge was not removed from the reactor between cycles, and any chemical or thermal regeneration procedure was not applied. Following treatment cycles contained the addition of biologically treated landfill leachate to the reactor containing ferric sludge, the adjustment of the ferric sludge-wastewater suspension pH to 3 and the addition of supplementary catalyst (if applicable) and oxidant.

The Fenton-like process was performed applying a COD/H_2O_2 w/w of 1/4, with an intermittent or cyclic addition of the fresh Fe^{2+} catalyst at two H_2O_2/Fe^{2+} w/w of 40/1 and 20/1. The cyclic addition of the fresh catalyst meant that ferrous salt was supplementary added to each ferric sludge-catalysed Fenton-like treatment cycle. The intermittent addition of the Fe^{2+} catalyst meant that ferrous salt was added to every second Fenton-like treatment cycle. Lower Fe^{2+} catalyst doses were used in the case of Fenton- like trials as compared to the Fenton process usually conducted at a H_2O_2/Fe^{2+} w/w of 6:1 (Dulova and Trapido, 2011), since when applying ferric sludge reuse hydrogen peroxide degradation was additionally catalysed by ferric ion redissolved from the sludge.

The biologically and chemically treated supernatant was collected in the second buffer tank, which functioned as an inflow to the second biological reactor. Values of BOD_7 and COD were measured after every treatment, values of NH_4–N, NO_3 –N, NO_2–N and pH were monitored twice a week and values of lignin and tannins and phenols once a month. The efficacy of the reused ferric sludge-catalysed Fenton-like process was calculated involving cycles 2–13.
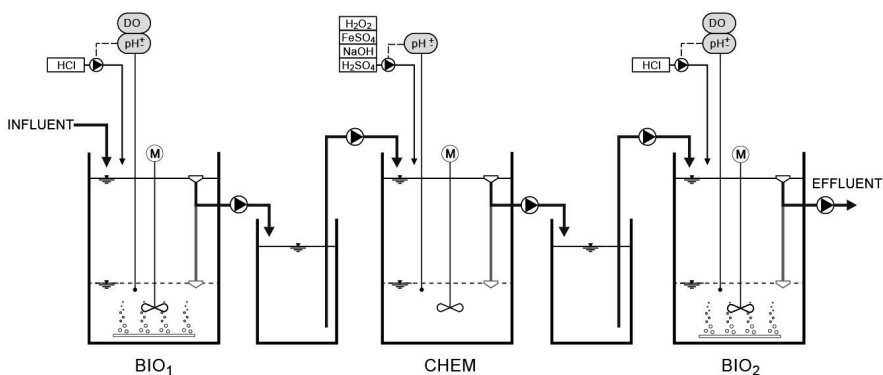
Figure 1. The technological scheme of the BIO_1-CHEM-BIO_2 pilot plant, (**Paper VII**)

4.7 The evaluation of reagent consumption of the Fenton and Fenton-like processes

In order to assess the economic feasibility of the Fenton and Fenton-like different schemes applied in the pilot-study, cost calculations were performed. The data obtained from the laboratory experiments allowed to make approximate calculations of operating costs (comprised of the chemicals required for the Fenton and Fenton-like process). Chemical demands were calculated on the basis of the chemical consumption per 1 m^3 of wastewater, where we took into account the mean initial COD value of the influent for each operational strategy and the COD/ H_2O_2/Fe^{2+} w/w/w ratio. The reagent consumption was presented as kilograms of ferrous sulphate ($FeSO_4 \times 7H_2O$) and litres of 50 % H_2O_2 per 1 m^3 of treated wastewater. In the cost calculations, prices of ferrous salt and hydrogen peroxide were ascribed as 0.6 €/kg and 1.1 €/l, respectively. The ferric sludge utilisation was considered based on the assumption that it is a hazardous waste because it may contain hazardous substances from wastewater and should be incinerated separately. The cost of incineration (0.51 €/kg) was taken based on a price offer received after describing properties of the ferric waste sludge. Energy consumption of the chemical treatment was not taken into account, as it would be highly dependent on the used equipment.

Additionally, the cost of biological treatment was calculated to demonstrate the contrast between the costs of the Fenton, Fenton-like and activated sludge treatment. Generally, 0.8–1.0 kW h (average 0.9) of energy is consumed for aeration that removes 1 kg of COD (Villano, Scardala, et al., 2013), which makes up a total of 40–65 % (average ca. 53 %) of the total energy consumed in the activated sludge process (Fernández, Castro, et al., 2011; Liu, Li, et al., 2011). Accordingly, 1.7 kW h of energy is consumed per 1 kg of COD removed during the aerobic treatment stage. Calculations were performed separately for both biological treatment tanks based on the current cost (year 2017) of electricity of 0.125 €/(kW h) in Estonia (fixed charge, network charges included).

5. RESULTS AND DISCUSSION

5.1 Biodegradability of wastewater

Biodegradability is an important wastewater parameter which influences the performance of the WWTP and is thereby an indicator for optimizing the operation wastewater treatment and choosing feasible technological set-up. In addition to the conventional BOD/COD ratio, the biodegradability of studied samples was also measured by a Zahn-Wellens biodegradability test. The test gives a biodegradability of wastewater sample calculated based on the difference of COD or DOC value at the beginning and end of the test period (ISO 9888). To achieve applicable information about the feasibility of activated sludge treatment and maximal potential removal efficiency of organic compounds, the interpretation of test results was improved. Based on the degradation curve during the test period of 28 days, results were fractionated to easily degradable COD (degrades during the first 7 days), slowly degradable COD (degrades in 7-28 days) and recalcitrant COD (residual COD of the test).

Table 2 shows that biodegradability values of all studied samples were at least 85% or higher, which means that all samples are inherently biodegradable (>70%). Regardless of high biodegradability values, recalcitrant COD values of studied samples remained between 120 – 2100 mg/l after 28 days of a test period, 4.6 – 16.7% from the initial COD value (Table 2, Figure 2). Furthermore, hazardous landfill leachate and wastewater from wood industry showed high fraction of slowly biodegradable organic compounds (9–12% from the initial COD value) which indicates the requirement for application of longer HRT value in the activated sludge process. High concentrations of recalcitrant and/or slowly biodegradable COD of wastewater samples from the plywood, shale oil industries and municipal landfill leachate indicated the need for combined biological and advanced treatment. However, the Zahn-Wellens biodegradability value is not influenced by the toxicity of wastewater due to the dilution procedure prescribed by the standard and samples may be inhibitory to activated sludge processes even at high biodegradability values.

Table 2. COD fractions from the Zahn-Wellens biodegradability test and BOD_7/COD ratios of studied samples

COD fraction	Unit	Plywood industry (Paper IV, S3)	Municipal landfill leachate (Paper V, S4)	Hazardous landfill leachate (Paper VI, L2)	Pyrogenic wastewater (Paper III, S2)
Initial	mgO_2/l	4200	5920	800	41 400
Easily degradable	mgO_2/l	3500	5000	590	39 400
Slowly biodegradable	mgO_2/l	380	220	90	0
Recalcitrant	mgO_2/l	370	700	120	1900
Biodegradability (Zahn-Wellens method)	%	93	88.1	85	96
BOD_7/COD	−	0.51	0.69	0.39	0.77

*S2-S4 and L2 correspond to sample labels used in respective papers (all samples were not included to the thesis)
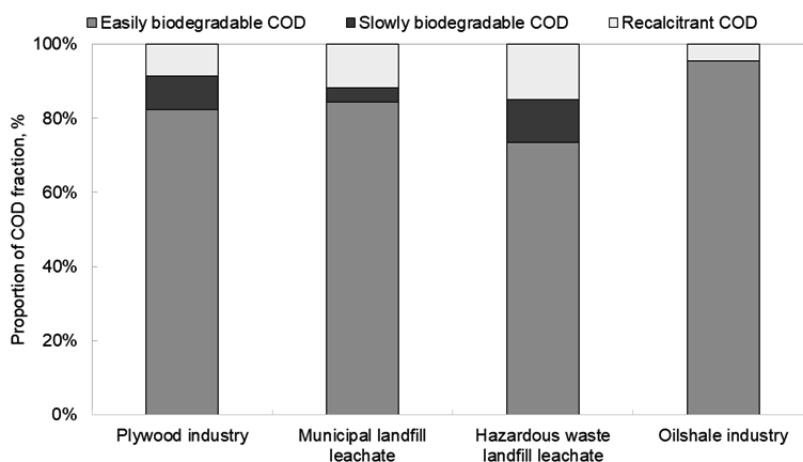
Figure 2. Proportion of easily, slowly and recalcitrant COD fractions of studied samples

5.2 Inhibition of wastewater on activated sludge processes

The impact of wastewater on activated sludge oxygen uptake (ISO 8192) and nitrification rates (ISO 9509) were studied in order to assess the inhibitory effect of wastewater on activated sludge microorganisms and its treatability with an activated sludge process.

Oxygen uptake rate inhibition test showed that hazardous waste landfill leachate and wastewater from the plywood industry had a negligible (Figure 4 in **Paper VI** and Figure 1 in **Paper IV**, respectively) and pyrogenic wastewater

strong negative effect (Chapter 3.1 in **Paper III**) on the process. 10 % of hazardous waste landfill leachate (**Paper VI**) and 8% of plywood industry`s wastewater (**Paper IV**) caused a 10% decrease in the oxygen consumption ($IC_{50}>20\%$). Already 3.4% of pyrogenic wastewater inhibited 50% of oxygen uptake rate. Municipal landfill leachate (Figure 1 in **Paper V**) did not show any negative effect on activated sludge oxygen uptake rate.

Tests indicated that the inhibitory effect of samples was higher on the nitrification process as expected. The most important reason behind this is that the growth rate of nitrifying microorganisms is low, which makes the process sensitive to toxic substances in the influent (Philips, S. Laanbroek, H.J. Verstraete, 2002; Haandel and Lubbe, 2012). 16% of hazardous waste landfill leachate (Figure 5 in **Paper VI**) and 17% of wastewater from the plywood industry (Figure 1 in **Paper IV**) caused a 50% decrease in the nitrification rate. 6% of municipal landfill leachate (Figure 1 in **Paper V**) and already 0.7% of pyrogenic wastewater (Chapter 3.1 in **Paper III**) inhibited 50% on nitrification rate. Thus, all samples showed a negative effect on the nitrification process but the highest were the inhibitory characteristics of pyrogenic wastewater and municipal landfill leachate.

Based on results achieved from inhibition studies, hazardous and municipal landfill leachates (**Papers VI and V**, respectively) and wastewater from the plywood industry (**Paper IV**) were suitable for the application of direct aerobic biological treatment. However, inhibitory characteristics of municipal landfill leachate ($IC_{50}=6$ % for nitrification process, **Paper V**) should be considered during the set-up of the nitrification process. Additionally, a pre-treatment of raw pyrogenic wastewater (**Paper III**) by activated sludge cannot be applied regardless of the biodegradability value of 96% and BOD_7/COD ratio of 0.77 because the wastewater was highly toxic to activated sludge nitrification and oxygen uptake rates. The biodegradability test also showed a high biodegradability value for the pyrogenic wastewater, but it was not applicable for toxic and concentrated wastewater because of the dilution procedure prescribed by the standard (ISO 9888).

During the studies with industrial wastewater, ISO 8192 was used for assessing the inhibition of oxygen consumption by activated sludge, ISO 9509 for assessing the inhibition on nitrification of activated sludge microorganisms, ISO 9888 for evaluating the aerobic biodegradability of organic compounds. However, there were no reliable and easily applicable methods available for measuring the impact of inhibitor on activated sludge denitrification and EBPR processes, which both locate before nitrification in conventional A2O (Anaerobic/Anoxic/Oxic) process and are thereby more susceptible for inhibition. Although nitrification is considered to be the most sensitive activated sludge process, which can be the indicator for toxicity, inhibition may be selective for different processes. Thus, it was decided to develop easily applicable laboratory methods for EBPR and denitrification to assess the impact of inhibitor on all activated sludge processes separately.

5.2.1 Estimating the impact of inhibitor on enhanced biological phosphorous removal (EBPR) process

The biological phosphorus removal process tank is the first process unit in an EBPR WWTP, in which return sludge is mixed with a fresh wastewater. The concentrations of organic compounds and inhibitors of the process are the highest in this step when compared to other wastewater treatment processes. It should be considered that both processes, the anaerobic release and aerobic uptake of phosphorus, may be inhibited.

Several studies have demonstrated the impact of hazardous compounds on the biological phosphorus removal process, but used methods require set-up and operation of acclimatization reactor for the pre-adaption of inoculum until steady-state conditions will be achieved (1.5-18 months) (Welles, Lopez-Vazquez, et al., 2014; Wang, Ren, et al., 2011; Katsou, Alvarino, et al., 2016). The major drawback of the acclimatization reactor method is that the inhibition depends on the applied operating conditions, such as temperature and organic carbon type. Different acclimatization procedure may affect the physical characteristics and microbial composition of the acclimated sludge (Wu and Rodgers, 2010). Since the aim of this study was to develop and test a feasible and reliable method for the evaluation of activated sludge biological phosphorus removal process, the developed method does not include the sludge acclimatization system (**Paper I**).

Biological phosphorus release and uptake tests were firstly performed with a non-acclimated sludge from municipal WWTP and synthetic wastewater (acetate) as a carbon source (Figure 3). Mean orthophosphate release and uptake rates for synthetic wastewater were 2.5 $mgPO_4$-P/gMLSS×h and 1.1 $mgPO_4$-P/gMLSS×h, respectively. Additionally, the concentration of PO_4-P was always higher in the effluent than in the influent which indicated that the test conditions were not favorable for the inoculum. Increase of MLSS value up to 5.2 mg/l and applying preceding 1-cycle acclimatization did not enhance the process. However, several studies show that the metabolism of PAO-s depend on the carbon source (Oehmen, Lemos, et al., 2007; Rey-Martínez, Badia-Fabregat, et al., 2019; Saunders, Oehmen, et al., 2003). Thus, influent of the same WWTP was used instead of the synthetic wastewater to test the process with a real carbon source (Figure 3). Results showed an efficient P-release and uptake, the mean release rate was 8.7 $mgPO_4$-P/gMLSS×h and the mean uptake rate was 2.65 $mgPO_4$-P/gMLSS×h (Figure 3). Batch-tests with municipal wastewater as an influent were suitable probably because the versatility of carbon sources in wastewater allowed other PAOs to proliferate in coexistence or competition with *Ca. Accumulibacter* (Rubio-Rincón, Welles, et al., 2019). Additionally, municipal wastewater contains different VFA-s, micro- and macro-elements required for the cell metabolism.
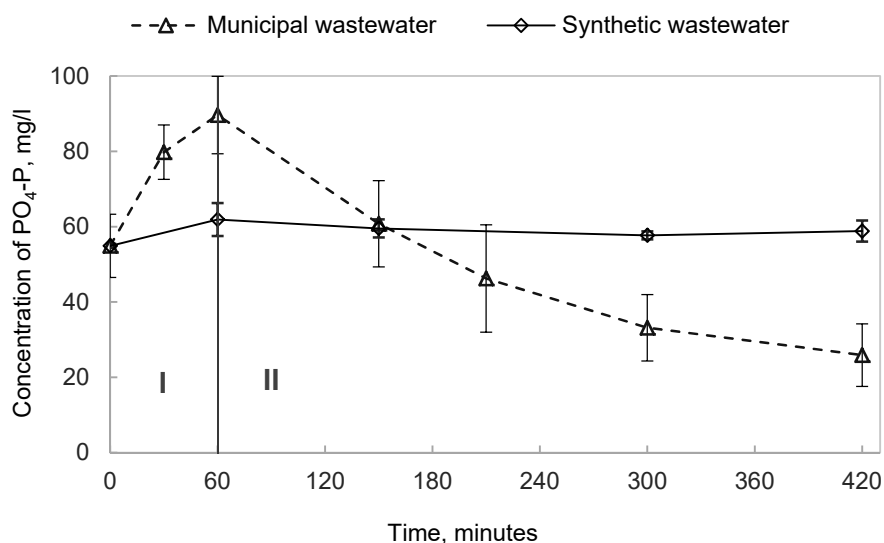
Figure 3. The release and uptake of phosphorus for EBPR batch tests with a synthetic and municipal wastewater as a carbon source (I – anaerobic phase, II—aerobic phase). Error bars represent the standard deviation of parallel tests (municipal wastewater: n=7, synthetic wastewater: n=3), (**Paper I**)

Addition of 3,5-DCP to the test substantially reduced the anaerobic orthophosphate release and PHA synthesis efficiencies. 58% less PO_4-P was released, 63.8% less PHB and 50.6% less PHV were synthesized under inhibitor concentration of 10 mg/l (Figures 2 and 3 in **Paper I**). Inhibitor may damage the cell membrane and destroy enzymes, which reduces poly-P degradation, leading to insufficient adenosine triphosphate (ATP) required for the synthesis of PHAs (Wang, Ren, et al., 2011; Seviour, Mino, et al., 2003).

In aerobic cycle, lower inhibitor concentrations (up to 4 mg/l) reduced the phosphate uptake at the beginning of the aeration phase followed by subsequent uptake, which could be caused by the decomposition of phenol under aeration and/or sludge adaption (Figure 4) (Klein, Kattel, et al., 2017; Arora and Bae, 2014). The PHAs degradation rate decreased with an increase in inhibitor concentration. We found that the PHB and PHV degradation was inhibited by 50% under 3,5-DCP concentrations of 4.0 mg/l and 4.5 mg/l, respectively. 100% of PHB and 79% of PHV consumptions were inhibited under 3,5-DCP concentration of 10 mg/l (Figure 3 in **Paper I**). 3,5-DCP had a significantly higher inhibitory effect under aerobic than anaerobic conditions. Higher sensitivity of aerobic process compared to anaerobic process is also reported in previous studies (Wu and Rodgers, 2010; Wang, Ren, et al., 2011). Reduction in poly-P degradation leads to insufficient energy necessary for the synthesis of PHAs, which are further used in aerobic cycle for phosphorous uptake (Wang, Ren, et al., 2011; Seviour, Mino, et al., 2003). Because of that, the inhibition of

the ortho-phosphate release causes inhibition of the EBPR process. Based on these results, it is advisable to estimate inhibition on the EBPR process based on the reduction of aerobic phosphate uptake rate which considers the potential reduction in anaerobic PHA synthesis.

It was found that the selection of suitable aeration duration was an important aspect of the test. Since the addition of toxicant reduced the phosphate uptake during the first 90 minutes which was followed by subsequent uptake, it is recommended to perform the test with aerobic period duration of 6 hours. Inhibition reduced with the duration of aeration period, where the maximum inhibitory concentration (IC_{50}) values for 1.5, 2.5, 4 and 6 hours of aeration were 1.6, 2.6, 3.1 and 3.9 mg/l, respectively. Achieved IC_{50} values were 2.4 times higher when the aerobic phase was 6 h instead of 1.5 h. Phosphorus uptake was completely under inhibitor concentration of 3 mg/l when calculations were made with values achieved after 1.5 hours of aeration and under 6.5 mg/l when 6 hours of aeration was considered. In practice, longer application of hydraulic retention time in aerobic basin could diminish the inhibition of EBPR process. The effect of 3,5-DCP on the EBPR and other WWTP processes is shown in Figure 5.
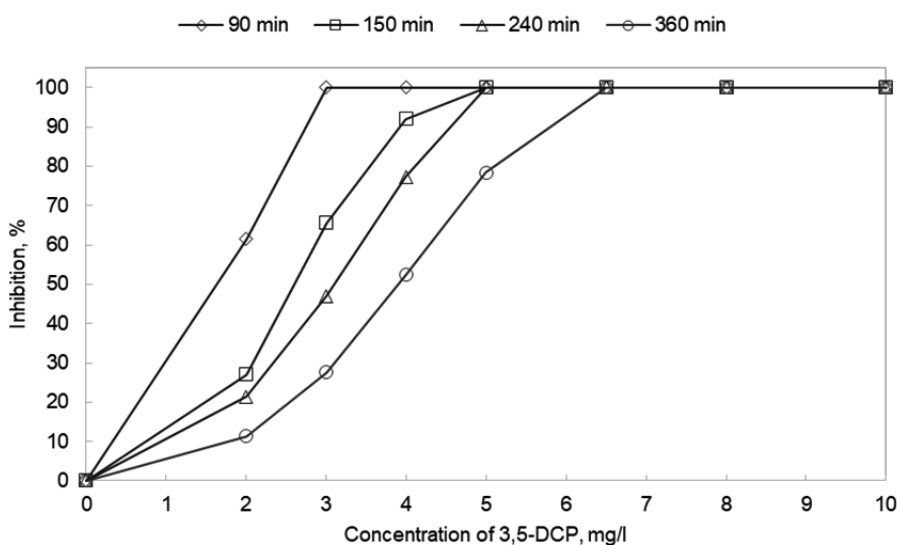
Figure 4. Inhibition curves of aerobic phosphorus uptake under 3,5-dichlorophenol concentrations from 0 – 10 mg/l when phosphorus uptake rates were calculated from 0 – 90 minutes, 0 – 150 minutes, 0 –240 minutes and 0 – 360 minutes of aeration (n = 7), (**Paper I**)

Suitable conditions for easily applicable EBPR inhibition test without previous sludge adaption were found in **Paper I**. It was found that influent of municipal WWTP was suitable as a carbon source resulting in efficient phosphorus release and uptake. It is advisable to estimate inhibition on the EBPR process based on the aerobic phosphate uptake rate during 6 hours of aeration duration which considers the energy deficiency from inhibition of anaerobic phosphorus release and PHA synthesis and the slower process in slightly inhibited samples at the beginning of aeration phase. In practice, longer hydraulic retention time in aerobic basin could diminish the EBPR process inhibition.

5.2.2 Estimating the impact of inhibitor on activated sludge denitrification process

Inhibitors are subjected to denitrification before aerobic process in WWTP-s, where pre-denitrification is applied. Denitrification is thereby more exposed to inhibitors in raw wastewater. In some cases, denitrification has been reported to be more susceptible to inhibitors (e.g. copper) when compared to nitrification (Sun, Fan, et al., 2016). There is no reliable and easily applicable method available for measuring the impact of wastewater or hazardous compound on activated sludge denitrification process. However, it is essential to detect the inhibitory characteristics of the influent and the origin of inhibitory wastewater. Batch tests have been used in some studies where effect of hazardous substances on activated sludge denitrification processes have been studied (Ochoa-Herrera, Banihani, et al., 2009; V J Inglezakis, Malamis, et al., 2017), but there is lack of applicable information like calculation procedure or main validation parameters. Thus, a method for evaluation of inhibition on the activated sludge denitrification process was developed and controlled (**Paper II**). Detailed description of the experiment set-up is given in Chapter 3.2.3 "Set-up of the denitrification inhibition test" and **Paper II**.

Addition of 3,5-DCP resulted in a decrease of the denitrification rate. Already 5.5 mg/L of 3,5-DCP inhibited 50% of denitrification rate, which shows that the 3,5-DCP was inhibitory to denitrification process and was a suitable model compound for control of the method (Figure 2 in **Paper II**). Effect of 3,5-DCP on denitrification and other WWTP processes is presented in Figure 5.

In order to avoid NO_2-N accumulation and underestimation of inhibition, it is necessary to estimate the impact of inhibitor on denitrification process based on measurement of NO_2-N and NO_3-N. Inhibition of NO or N_2O reductase and accumulation of NO_2-N (partial denitrification) could be unnoticed if inhibition is assessed only by NO_3-N concentrations. In this study, lower denitrification process rate in the control vessel and lower IC_{50} value were achieved when the concentration of NO_2-N was not considered (Table 3; Figure 2 in **Paper II**). As a result, denitrification inhibition could be underestimated without considering the accumulation of NO_2-N.

Table 3. Denitrification inhibition test results when inhibition calculations include concentration of NO_3-N and sum of NO_3-N and NO_2-N (NO_x-N) concentrations. 3,5-DCP was used as an inhibitor (**Paper II**).

Parameter	Unit	Considering NO_3-N	Considering NO_x-N
The mean denitrification rate in the control vessel	mg NO_x-N/gMLSS×h	2.5	1.9
IC_{50}	%	11.5	5.5

Selection of data points is important for calculating the denitrification rates of the process. The denitrification rate was unstable during the first hour of the test period (1.2 ± 1 mgNO_x-N/gMLSS×h), which led to a lower mean denitrification rate value and higher standard deviation when calculated from 0–3 hours of denitrification duration compared to 1–3 hours of respective period (Table 4). In this study, the first hour was considered to be an additional adaption phase for denitrifying microorganisms which caused higher uncertainty of the test. In order to achieve higher accuracy and better repeatability of the method, all inhibition calculations were made from 1–3 hours of the test period.

Table 4. Denitrification rates calculated based on NO_x-N values at 0–1 hours, 0–3 hours and 1–3 hours of test period (n=7), (**Paper II**)

Parameter	Unit	0–1 hours	0–3 hours	1–3 hours
The mean denitrification rate	mg NO_x^--N/gMLSS×h	1.2	1.6	1.9
Standard deviation	mg NO_x^--N/gMLSS×h	1.0	1.0	0.8

Finally, the method was tested with an inhibitory wastewater from shale oil industry (Table 1) using activated sludge originating from region of oil shale industries (200 000 PE, proportion of industrial inflow was 70%) and region without significant influence of industrial wastewater (100 000 PE, proportion of industrial inflow was 10%).

Already $2.6\pm0.7\%$ of phenolic wastewater inhibited 50% of denitrification rate (Figure 3 in **Paper II**). Denitrification was 1.7-fold more sensitive to phenolic wastewater compared to oxygen uptake process according to the IC_{50} value. Although the acclimated sludge should have been more efficient with higher content of phenols, studied wastewater showed the same IC_{50} value for both inoculums. Reason behind this result may be that used wastewater from oil shale industry was highly toxic for any kind of activated sludge and less harmful phenolic solution would have shown different effect. Additionally, activated sludge samples may have been taken during the period when sludge resistance had decreased because of any disturbance.

5.2.3 The reliability of denitrification and EBPR process inhibition tests

The main validation parameters were calculated to compare achieved results with existing standard inhibition methods (Table 5). Results showed that high variation of activated sludge based experiments is common.

The main aspects which can lead to higher uncertainty of activated sludge based tests are following:
1. activated sludge taken from WWTP during different seasons has different activity;
2. uncertainties from measurements of process parameters;
3. change of the inoculum environment (transportation from WWTP to laboratory, temperature difference, sludge washing).

Table 5. Validation parameters of developed methods for assessing the inhibition on denitrification and EBPR processes and ISO 8192 (oxygen uptake) and ISO 9509 (nitrification) standard methods (**Papers I, II**)

Validation characteristic	Unit	Denitri-fication	EBPR	Nitrification	Oxygen uptake
Average IC_{50} value	mg/l	5.5	3.9	5.6	9.3
Standard deviation	mg/l	2.2	1	3	3.7
95.4% confidence level, k=2	mg/l	1.2…9.8	2…5.9	0.7…9.6	7.1…11.3
Relative standard deviation	%	40	25	54	40

Furthermore, in order to have representative results, biomass adaption should be considered at the time of inoculum selection. Activated sludge is able to acclimate with inhibitory compounds and acclimated cultures can tolerate higher concentrations of inhibitory compound compared to non-acclimated inoculum (Lin, Tay, et al., 2009).

5.2.4 The inhibition of different activated sludge treatment processes

3,5-DCP is a toxic reference compound which has been used for estimating the reliability of different activated sludge tests (ISO 9509; ISO 8192). The impact of 3,5-DCP on following activated sludge processes was analyzed: biological phosphate uptake and release, denitrification, nitrification, oxygen uptake. Results of the tests are shown in Figure 5 and Table 5.

The inhibition of wastewater treatment processes performed by heterotrophs are similar, but no negative impact of the inhibitor was detected for the phosphorus release before the 3,5-DCP concentration of 3 mg/l. Aerobic phosphate uptake was the most sensitive activated sludge process at inhibitor

concentration above 4.8 mg/l. Higher sensitivity of aerobic metabolism of PAO-s has been explained by greater biomass growth and enzymatic activity under aerobic conditions (Wu and Rodgers, 2010). Additionally, the inhibition of aerobic process can be higher compared to anaerobic due to the inhibition of PHA synthesis. This demonstrates the importance of inhibition tracking of the EBPR process to ensure high biological phosphorus removal efficiency. Nitrification, which is often considered as an indicator process for inhibition, was the most sensitive process at lower inhibitor concentrations which coincides with the results reported by Inglezakis et al., 2017.
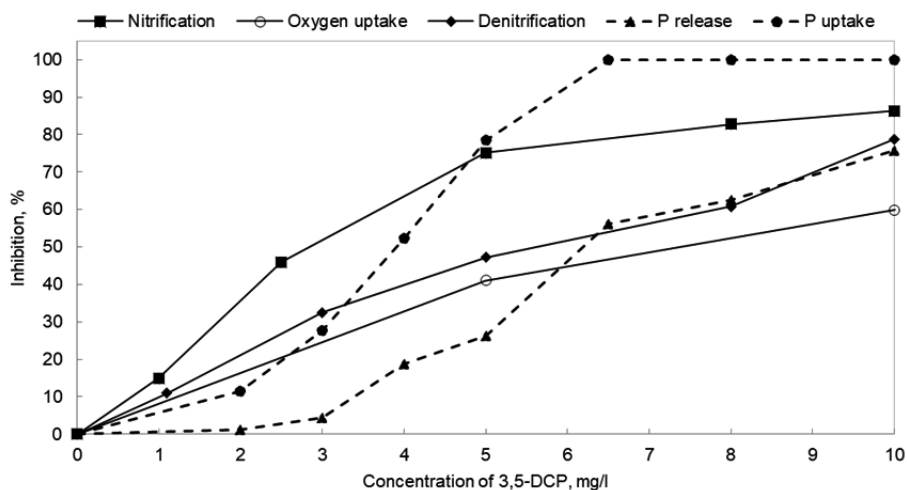
Figure 5. Inhibition curves of nitrification, denitrification, oxygen uptake, phosphate release and phosphate uptake processes under 3,5-dichlorophenol concentrations from $0 – 10$ mgL^{-1}, (**Paper I**)

Inhibition tests are feasible tools for estimating the inhibition and identifying the origin of flows causing inhibition of activated sludge treatment processes (inhibition tracking) with the aim to reduce flows of hazardous substances to WWTPs and thereby increase the treatment efficiency. Furthermore, studies of wastewater characteristics – concentrations of pollutants, biodegradability fractions and inhibition are valuable for selection of cost-efficient treatment technologies. In the frame on this thesis, results from biodegradability and inhibition studies were used to determine the applicability and sequence of combined biological (BIO) and physical-chemical (PHYS-CHEM) treatment of studied samples from shale oil and wood industries and leachates from municipal and hazardous waste landfills.

5.3 Physical and chemical pre-treatment of wastewater (PHYS-CHEM)

Wastewater from the shale-oil industry (**Paper III**) showed high inhibition on activated sludge oxygen uptake (Chapter 4.2) and the biological pre-treatment of wastewater was not applicable. Therefore, a pre-treatment scheme was required to remove the inhibitory characteristics of the wastewater. Since the Fenton treatment of raw wastewater has very high chemical consumption, other physical-chemical treatment methods were studied.

The scheme consisted of air-stripping followed by coagulation-flocculation and batch distillation at 100°C and pH value of 11. Air stripping and batch distillation was used in order to remove dissolved and/or colloidal volatile components. Based on additional inhibition tests the applied scheme removed the inhibitory effect of the wastewater on activated sludge oxygen consumption (Table 6). Removal of inhibition on oxygen uptake rate was most probably caused by substantial decrease in phenols concentration (46%). Furthermore, COD fractioning of the pre-treated wastewater showed high proportion of easily degradable COD and further activated sludge treatment was applicable to remove easily degradable organic compounds (Table 7). The sample was still strongly inhibitory on activated sludge nitrification rate after the physical and chemical pre-treatment (IC_{50} value of 1.2%) and was not suitable for discharge to WWTP with nitrification process.

Table 6. Parameters of pyrogenic wastewater after air-stripping, coagulation-flocculation and batch distillation, (**Paper III**)

Parameter	Unit	Initial value	Value	Change
COD	mgO_2/l	45 400±380	23 300±720	↓ 49%
BOD_7	mgO_2/l	34 900±6500	16 300±813	↓ 53%
NH_4-N	mg/l	1650 ± 50	140±20	↓ 92%
Phenols	mg/l	1050 ± 35	560±20	↓ 46%
Inhibition on oxygen uptake rate (IC_{50})	%	3.4	> 20	–
Inhibition on nitrification rate (IC_{50})	%	0.7	1.2±0.1	–

Table 7. COD fractions of wastewater from shale oil industry after physical and chemical pre-treatment (**Paper III**)

COD fraction	Unit	Raw wastewater	Pre-treater wastewater
Initial	mgO_2/l	41 400	22 330
Easily degradable	mgO_2/l	39 400	20 000
Slowly biodegradable	mgO_2/l	0	310
Recalcitrant	mgO_2/l	1900	1900
Biodegradability (Zahn-Wellens method)	%	96	90

5.4 Biological treatment of recalcitrant wastewater (BIO)

Biological treatment is a cost-effective approach for removing readily bio-degradable organic substances from the raw wastewater if the wastewater does not have substantial inhibitory effect on activated sludge processes, especially on the oxygen uptake. Due to the high biodegradability values and moderate or low inhibitory effect of municipal and hazardous landfill leachates (**Papers V and VI**, respectively), wastewater from plywood industry (**Paper IV**) and physico-chemically pre-treated pyrogenic wastewater (**Paper III**), aerobic biological treatment was found technologically feasible without significant inhibition problems emerging. Longer HRT values were applied for samples containing slowly biodegradable fraction to achieve higher COD removal efficiency of the processes. The HRT value was not increased for the hazardous waste landfill leachate which contained 12% of slowly biodegradable COD, but respective concentration was negligible, 90 mgO_2/L.

COD values achieved with biological treatments (Table 8) were always higher than respective value of recalcitrant COD from the Zahn-Wellens biodegradability tests (Table 2). Results achieved by application of the tests were lower most probably because the test had more favourable conditions for microorganisms (e.g. longer hydraulic retention time, up to 28 days).

Wastewater from the plywood industry (**Paper IV**) showed moderate inhibitory effect on the nitrification process and 88% of TN was removed by the biological pre-treatment (Table 8). Municipal landfill leachate (**Paper VI**) was inhibitory to the nitrification process (IC_{50}=6%) and relatively low NH_4-N removal efficiency (39%) was achieved during the shorter study period (**Paper VI**). However, total nitrification (>99% NH_4-N removal efficiency) was achieved in spite of inhibitory characteristics of the studied wastewater when adaption period with a low organic loading was applied for the continuous-flow pilot study (Table 8, **Paper VII**). The long-term pilot study also demonstrated that steadily high removal efficiencies of organics and ammonium could be achieved after sufficient adaption of the inoculum.

Preliminary biological treatment can be effective for removing easily bio-degradable organic compounds from a non-inhibitory wastewater, but applica-tion of advanced treatment methods is required when wastewater contains substantial amount of slowly degradable or recalcitrant organics. In this study, all samples were subjected to following advanced treatment to degrade recalcitrant compounds. The main benefit of combining biological and chemical treatment schemes is that biological treatment stage decreases the consumption of reagents for subsequent chemical treatment by the elimination of biodegradable COD and removes nutrients from the wastewater. Furthermore, a preceding biological oxidation also avoids solids accumulation during chemical treatment. Thus, biological treatment should be performed before chemical treatment if possible.

Table 8. Removal efficiencies and values of parameters of biologically pre-treated samples from municipal and hazardous waste landfills (**Papers V–VII**), plywood and shale oil industries (**Papers III, IV**)

Para-meter	Unit	Municipal landfill leachate (Paper VI, S4)		Municipal landfill leachate, pilot study (Paper VII)		Hazardous waste landfill leachate (Paper V, L2)		Wastewater from plywood industry (Paper IV , S3)		Pre-treated pyrogenic wastewater (Paper III, S2)	
		Value	Change	Value	Change	Value	Change	Value	Change	Value	Change
COD	mgO$_2$/l	1840±90	↓73%	1490±260	↓86%	278±7	↓67%	1490±120	↓78%	4460±315	↓81%
BOD$_7$	mgO$_2$/l	330±30	↓93%	60±45	↓99%	19±3	↓94%	300±40	↓92%	310±15	↓98%
BOD$_7$/COD	–	0.18	–	0.04	–	0.07	–	0.2	–	0.07	–
NH$_4$-N	mg/l	510±10	39%	2 ±2.3	↓>99%	<l.d^a	↓>99%	n.a.	n.a.	44±1	↓69%
TN	mg/l	n.a.	n.a.	n.a.	–	n.a.	n.a.	8.5±0.07	↓88%	n.a.	n.a.
Phenols	mg/l	15.8	52%	14±4	↓83%	3.4±0.05	↓81%	15.4±0.5	↓84%	28	↓95%

* S2-S4 and L2 correspond to sample labels used in respective papers (all samples were not included to the thesis)

[a] below the limit of detection

n.a. – not analys

5.5 Fenton and Fenton-like treatment of recalcitrant wastewater

5.5.1 Chemical post-treatment of recalcitrant wastewater (BIO-CHEM)

Fenton treatment was chosen after activated sludge pre-treatment due to the highly efficient performance of towards composite wastewater parameters, such as COD, phenols, lignin and tannins, coupled with simplicity of the Fenton treatment instrumental set-up. In general, up to 85% of COD, >99% of phenols and 92% of lignin and tannins were removed by the Fenton process. BOD_7 was removed up to 66%, however lower removal efficiency (26%, effluent BOD_7/ COD= 0.07) was achieved for hazardous landfill leachate with low influent values of pollutants (Table 9). BOD_7 value increased 67% for pyrogenic wastewater leaving effluent BOD_7 value 550 mgO$_2$/l.

Table 9. Removal efficiencies and values of parameters in the effluent of the Fenton process at chosen optimal chemical dose ratios for treatment of recalcitrant wastewater (**Papers III–VI**)

Para-meter	Unit	Municipal landfill leachate COD/H$_2$O$_2$/Fe^{2+} w/w/w 1/4/0.8 (Paper VI, S4)		Hazardous waste landfill leachate COD/H$_2$O$_2$/Fe^{2+} w/w/w 1/1/0.2 (Paper V, L2)		Wastewater from plywood industry COD/H$_2$O$_2$/Fe^{2+} w/w/w 1/2/0.4 (Paper IV, S3)		Pre-treated pyrogenic wastewater COD/H$_2$O$_2$/Fe^{2+} w/w/w 1/4/0.8 (Paper III, S2)	
		Value	Change	Value	Change	Value	Change	Value	Change
COD	mgO$_2$/l	550±30	↓70%	191±18	↓31%	220±30	↓85%	1050±15	↓76%
BOD$_7$	mgO$_2$/l	213±13	↓35%	14±2	↓26%	102±10	↓66%	510±30	↑65%
BOD$_7$/ COD	–	0.39	–	0.09	–	0.46	–	0.49	–
NH$_4$-N	mg/l	500±20	–	<l.d[a]	–	n.a.	–	44±0.7	–
Phenols	mg/l	2.4	↓85%	1.06	↓69%	n.a.	–	0.37	↓>99%
Lignin& tannins	mg/l	n.a.	–	n.a.	–	5.1±0.4	↓92%	n.a.	–

[*]S2–S4 and L2 correspond to sample labels used in respective papers (all samples were not included to the thesis)
[a]below the limit of detection
n.a. – not analysed

Biodegradability fractions of the Fenton-treated wastewater should be determined to choose a feasible technological scheme (BIO-CHEM or BIO-CHEM-BIO) of wastewater containing substantial proportions of biodegradable and recalcitrant organics. For the biologically pre-treated wastewater from plywood industry (**Paper IV**, sample S3, characteristics are given in Table 1), the Fenton post-treatment increased the biodegradability of effluent and decreased the

recalcitrant COD fraction (Figure 6). $COD/H_2O_2/Fe^{2+}$ (w/w/w) of 1/2/0.4 increased biodegradability 11%, reduced slowly biodegradable fraction by 47% and recalcitrant COD by 52%. This implies that the treatment of wastewater from plywood industry with ratios ($COD/H_2O_2/Fe^{2+}$, w/w/w) of 1/0.5/0.1 and 1/2/0.4 could be efficiently combined with subsequent biological treatment, suggesting lowest possible residual COD to be around 180 and 90 mg/L, respectively.
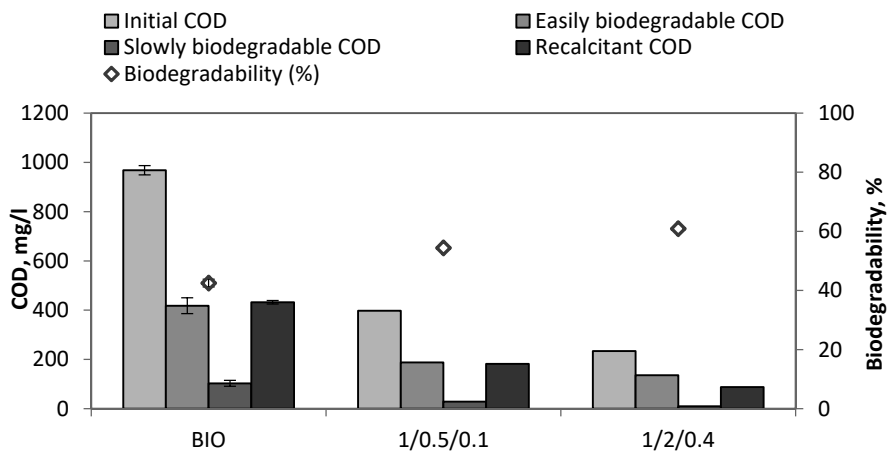
Figure 6. Changes in wastewater biodegradability fractions after biological pre-treatment and Fenton post-treatment with $COD/H_2O_2/Fe^{2+}$ dose ratios of 1/0.5/0.1 and 1/2/0.4 (**Paper IV**, sample S3).

For the wastewater from the shale oil industry (**Paper III**, Sample S2, characteristics are given in Table 8), $COD/H_2O_2/Fe^{2+}$ w/w/w 1/0.5/0.1 was insufficient for the degradation of organic compounds (achieved COD removal efficiency was 9%). Increase of $COD/H_2O_2/Fe^{2+}$ dose up to 1/4/0.8 w/w/w removed COD 76% and phenols >99%, leaving residual COD and total phenols concentrations of 1050 mgO_2/l and 0.37 mg/L, respectively. The subsequent increase of $COD/H_2O_2/Fe^{2+}$ w/w/w from 1/4/0.8 to 1/6/1.2 yielded no significant improvement in the removal efficiencies (Figure 7). However, higher concentration of the Fenton`s reagent decreased the biodegradability value of treated wastewater by 15%. Moreover, the use of higher reagent doses decreased the concentration of the easily biodegradable fraction and fractions of slowly degradable and re-calcitrant COD remained higher compared to lower doses of reagents. It means that higher chemical dose was less efficient in increasing the biodegradability of wastewater.
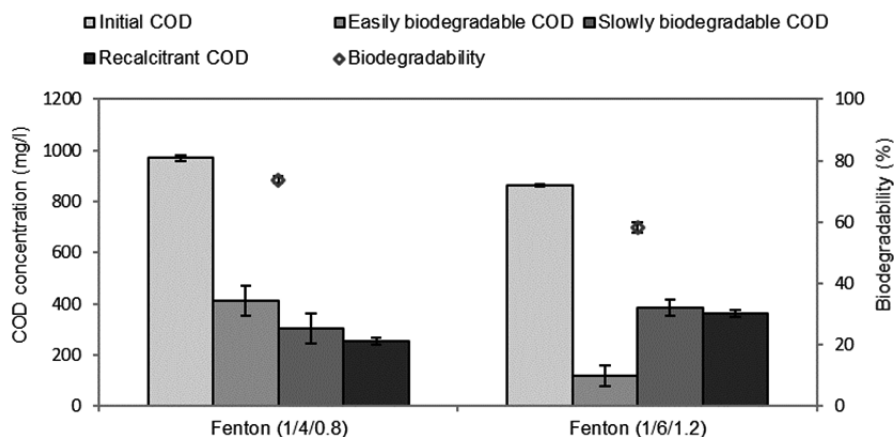
Figure 7. Zahn-Wellens biodegradability and COD fractions of pre-treated wastewater samples from shale-oil industry treated by the Fenton process, the $COD/H_2O_2/Fe^{2+}$ w/w/w 1/4/0.8 and 1/6/1.2, (n=3), (**Paper III**)

As the hydroxyl radicals abundantly produced in Fenton`s reaction are strong oxidisers reacting with all types of organic pollutants indiscriminately, it is highly important that the biological treatment removes the easily biodegradable COD fraction beforehand and leaves the recalcitrant fraction of COD as the sole target for Fenton process. The efficiency of the Fenton process depends largely on the right chemical dosage, which in turn depends on the characteristics of the wastewater. The obtained results indicated that the Fenton treatment was efficient in removing recalcitrant organic substances and increasing the biodegradability of wastewater. However, $COD/H_2O_2/Fe^{2+}$ w/w/w 1/6/1.2 was found to be less efficient in increasing the biodegradability of biologically pre-treated pyrogenic wastewater than $COD/H_2O_2/Fe^{2+}$ w/w/w 1/4/0.8. It means that higher dose of chemicals does not lead to higher efficiency of the process and it is necessary to optimize the dose ratio of $COD/H_2O_2/Fe^{2+}$ respectively to characteristics of the treated wastewater and technological set-up of treatment. The Fenton treatment was a powerful polishing step which improved the overall treatment efficiency of biological-chemical treatment.

5.5.2 The reuse of the Fenton catalyst

The Fenton process was found to be effective in removing the inhibitory characteristics of industrial wastewater and also for treatment of different types of wastewater. However, the Fenton process was not found feasible for practical applications because of its major drawbacks: high chemical consumption, intensive foaming and formation of ferric sludge. High chemical consumption significantly increased the treatment costs; intensive foaming required bigger volume

for the treatment tank and the produced ferric sludge needs further treatment which additionally increases complexity and operational costs of the wastewater treatment process.

In order to reduce the secondary pollution caused by production of ferric sludge in the Fenton process, reuse of the sludge during 12 cycles at constant chemicals concentrations was considered at a laboratory-scale pilot reactor operated as a SBR for treatment of municipal landfill leachate (**Paper VII**).

The continuous loss of catalyst during the sludge reuse cycles was observed during the ferric sludge reuse cycles. Thus, fresh Fe^{2+} was added to the process to replace the lost iron and to promote the ferric sludge catalysed Fenton-like system. In addition, we applied two different operational strategies to identify the impact of process operation on the wastewater treatment efficiency:

<u>Strategy 1:</u> Reused sludge catalysed Fenton-like experiments with cyclic addition of supplementary Fe^{2+} iron (fresh catalyst was added to every cycle) at a $COD/H_2O_2/Fe^{2+}$ w/w/w of 1/4/0.1 and 1/4/0.2 at WER (water exchange ratio) of 50%.

The results (Figure 8, series B and C) showed that the average COD removal efficiency in the series with a lower catalyst dose (series B) was 62% and the ratio of BOD_7/COD increased from 0.17 to 0.37. BOD_7 concentration in the effluent was 37% higher than in the influent.

A twofold higher dose of catalyst (Figure 8, series C) increased COD removal by 8% and value of BOD_7 by 20% more than in series B; the BOD_7/COD ratio increased from 0.09 to 0.32.

<u>Strategy 2</u>: Reused sludge catalysed Fenton-like experiments with intermittent addition of supplementary Fe^{2+} iron (fresh catalyst was added to every second cycle) at a $COD/H_2O_2/Fe^{2+}$ w/w/w of 1/4/0.4 at a WER of 50% and 100% were performed.

The BOD_7/COD value increased from 0.04 to 0.31 (Figure 8, series D). The mean BOD_7 concentration in the effluent was 178 mg/L, which was 87% higher than in the influent. The mean COD removal efficiency was 60%, with mean COD value in the effluent 564 mg/L.

The results with WER of 100% showed that the achieved mean COD removal efficiency was only 43%, which was 17% lower than in the process with WER of 50% and 32% lower than in the conventional Fenton process (Figure 8, series E).
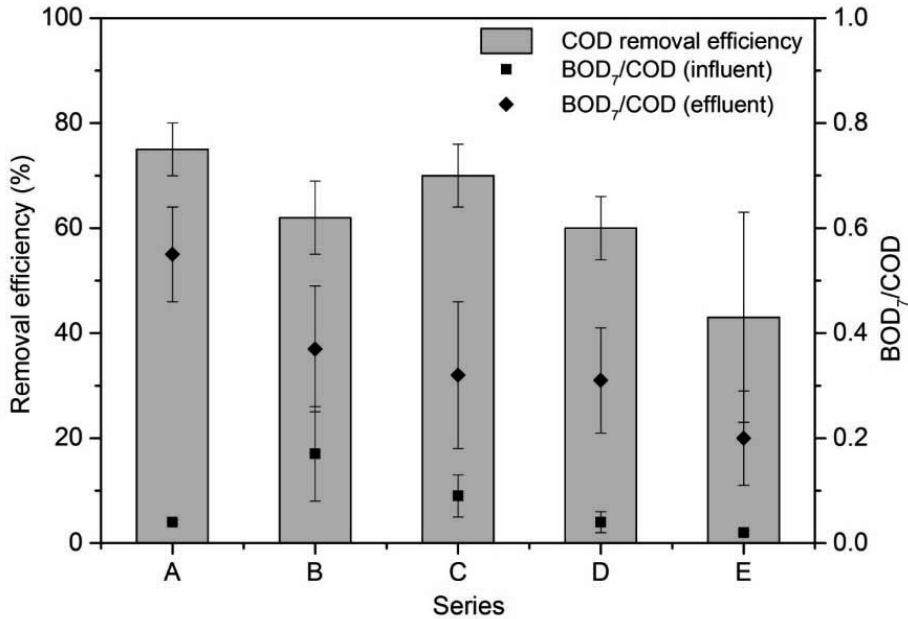
Figure 8. Effectiveness of the Fenton-like treatment expressed by COD removal efficiency and BOD_7/COD improvement at a COD/H_2O_2 w/w of 1/4. A: The conventional Fenton process, $H_2O_2/Fe^{2+} = 5/1$ (w/w); B: The Fenton-like process with sludge reuse, WER of 50% and a cyclic addition of the catalyst $H_2O_2/Fe^{2+} = 40/1$ (w/w); C: The Fenton-like process with sludge reuse, WER of 50% and a cyclic addition of the catalyst $H_2O_2/Fe^{2+} = 20/1$ (w/w); D: The Fenton-like process with sludge reuse, WER of 50% and intermittent addition of the catalyst $H_2O_2/Fe^{2+} = 10/1$ (w/w); E: The Fenton-like process with sludge reuse, WER of 100% and intermittent addition of the catalyst $H_2O_2/Fe^{2+} = 10/1$ (w/w), (**Paper VII**)

It could be concluded that the degradation of recalcitrant pollutants requires doubled oxidation cycle and are removed more effectively when WER is reduced to 50%. Furthermore, intermittent addition of catalyst is feasible when it is necessary to increase the biodegradability (e.g. following biological oxidation is applied) and cyclic addition of catalyst should be used when higher COD removal is required (e.g. following biological oxidation is not applied). Since the ferric sludge was reused up to 12 times and a reduction in removal efficiency was not noticed, it would be possible to reuse ferric sludge more than 12 times.

In the conventional Fenton-process, an intense foaming takes place due to the CO_2 produced from the carbonate species at an acidic pH and by the organic foaming agents present in the leachate (Deng and Englehardt, 2006) and by O_2 originating from the decomposition of H_2O_2. Reuse of ferric sludge substantially reduced the foaming due to a lower reaction rate. Thus, over-dimensioning of chemical treatment reactors is not required when the ferric-sludge catalysed Fenton process is applied.

5.5.3 Ferric sludge

Amounts and characteristics of the ferric sludge are important for the waste-water treatment process as well as for sludge utilization. Thus, volume and concentration of total suspended solids (TSS) of the ferric sludge were measured during the sludge reuse cycles (**Paper VII**).

Although the volume of ferric sludge decreased 2.3 ± 0.9 fold, the concentration of total suspended solids increased 2.4 ± 0.3 fold. Additionally, the volume in millilitres occupied by 1 gram of a ferric sludge suspension after six hours of settling (SVI) decreased 4.2 ± 1.4 fold (from 10.5 ± 2.6 mL/g to 2.6 ± 0.5 mL/g). The substantial reduction of the volume was a result of better settleability of the sludge. Settleability is an important factor to avoid ferric sludge flow to effluent or following treatment step. Additionally, a low SVI value increases the cost-efficiency of possible sludge utilization.

However, the average amount of sludge produced when treating 1 L of biologically pre-treated landfill leachate was 9.8 ± 3.5 g for the first treatment in the series and 8.6 ± 2.3 g when sludge reuse was applied. Thus, already 12-cycle ferric sludge reuse leads to 13.7-fold smaller production of potentially hazardous waste sludge.

5.6 Final biological purification (BIO-CHEM-BIO)

The Fenton and Fenton-like treatments increased the biodegradability of wastewater (Chapter 5.5.1), which needed a final purification step before the discharge. Final purification by activated sludge process was considered feasible for municipal landfill leachate and wastewater samples from the plywood and shale-oil industries.

Secondary biological treatment was less loaded compared to the biological pre-treatment and high BOD_7 and COD removal efficiencies were achieved. Up 99.7% and 94% of BOD_7 and COD were removed, respectively. Additionally, up to >99% of NH_4-N was removed in the biological post-treatment process.

Table 10. Removal efficiencies and parameters` values in the effluent of the final biological purification (**Papers III–VI**)

Para-meter	Unit	Municipal landfill leachate (Paper VI, S4)		Municipal landfill leachate, pilot study (Paper VII)		Wastewater from plywood industry (Paper IV, S3)		Pre-treated pyrogenic wastewater (Paper III, S2)	
		Value	Change	Value	Change	Value	Change	Value	Change
COD	mgO$_2$/l	400±30	↓27%	290±14	↓49%	90±3	↓59%	845±34	↓20%
BOD$_7$	mgO$_2$/l	19±9	↓91%	10±8	↓94%	10±1	↓90%	63±23	↓88%
BOD$_7$/COD	–	0.09	–	0.03	–	0.09	–	0.14	–
NH$_4$-N	mg/l	370±30	↓26%	0.8±2.8	↓98%	n.a.	–	0.16±0.02	↓>99%
Phenols	mg/l	<l.d[a]	↓>99%	3.0±0.6	↓6%	n.a.	–	<l.d[a]	–
Lignin and tannins	mg/l	n.a.	–	6.5±0.9	↓23%	n.a.	–	n.a.	–

[a]below the limit of detection
n.a. – not analysed

More than 94% of organic substances expressed as COD, BOD$_7$, phenols and lignin & tannins were removed in the three-stage treatment strategy. Activated sludge removed mainly easily degradable organic compounds and the Fenton or Fenton-like treatment removed recalcitrant organic compounds and increased the biodegradability regardless of the operational parameters.

5.7 Cost estimation of the Fenton, Fenton-like and activated sludge processes

The combined treatment proved to be a cost-efficient solution for treatment of wastewater with inhibitory and recalcitrant characteristics based on this study. The cost estimations about Fenton, Fenton-like and activated sludge treatments were performed based on results achieved from the pilot study of the three-stage biological-chemical treatment of landfill leachate applying continuous ferric sludge reuse in Fenton-like process (**Paper VII**). The pilot-plant was conti-nuously operated during 8 months and wastewater of the same origin (municipal landfill leachate) was used as an influent. Thus, it was a reliable source of data for cost comparisons.

The main sources of costs and respective estimations of the different treat-ment opportunities are presented in Table 11. Activated sludge treatment is known to be a cost-efficient technology, where electricity for aeration is the biggest source for expenditure (Fernández, Castro, et al., 2011; Liu, Li, et al., 2011). The Fenton-treatment is known to be a costly method for treatment of wastewater because of a high consumption of reagents (iron salt and H$_2$O$_2$) and utilisation of potentially hazardous ferric waste sludge (Mahtab, Farooqi, et al., 2021). When comparing the Fenton-like process that was developed during the

pilot study to the conventional Fenton process, the main benefits came from a substantial decrease of the ferrous salt (catalyst) consumption and reduction of ferric sludge formation. The consumption of the ferric salt was reduced 71…86%. This resulted a 25…36% lower treatment costs for the chemical treatment and less secondary pollution caused by formation of ferric sludge.

Table 11. The cost estimation of the Fenton, Fenton-like and activated sludge treatments.

	Energy demand (kW h/m^3)	H$_2$O$_2$ (L/m^3)	H$_2$O$_2$ (€/m^3)	FeSO$_4$ ×7H$_2$O (kg/m^3)	FeSO$_4$ ×7H$_2$O (€/m^3)	Ferric sludge incineration (€/m^3)	Treatment cost (€/m^3)
BIO$_1$	18.2	–*	–	–	–	–	2.3
Fenton** (1/4/0.8)	–	77.8	86.9	42.5	25.9	5	117.8
Series A	–	10.3	11.5	5.6	3.4	5	19.9
Series B	–	12.1	13.5	0.8	0.5	0.4	14.4
Series C	–	12.1	13.5	1.6	1.0	0.4	14.9
Series D	–	12.1	13.5	1.6	1.0	0.4	14.9
Series E	–	10.3	11.5	1.4	0.9	0.4	12.8
BIO$_2$	0.3	–	–	–	–	–	0.04

Series A-E are technological options related to the ferric sludge reuse (see details in Figure 8)
* – not determined
** – theoretical chemical consumption, i.e. the demand for chemicals required to remove the same amount of COD removed in the BIO$_1$ reactor (10.7 kgCOD/m^3)

The proposed combined technological scheme for purification of inhibitory and recalcitrant wastewater by combined biological Fenton-like processes proved promising option to reduce the amount of generated solid sludge waste as well as the overall cost of treatment, making ferric sludge catalysed Fenton-like process applicable for industrial scale use.

6. CONCLUSIONS

Inhibition and biodegradability studies

- Dividing the COD value to easily, slowly and recalcitrant fractions provides valuable data for improving the design and operation of WWTP compared to the biodegradability value. This is especially relevant for complex wastewater in which characteristics are not in compliance with a typical municipal wastewater.

- Inhibition tests are tools to reduce flows of hazardous substances to WWTPs and thereby allows to enhance the treatment efficiency and the quality of waste activated sludge. Inhibition tests performed for all main wastewater treatment processes can be used for estimating the inhibition trends and identifying the origin of flows, which are responsible for causing inhibition of activated sludge treatment processes (inhibition tracking).

- In this thesis, reliable and easily applicable tests for activated sludge enhanced biological phosphorus removal (EBPR) and denitrification processes were developed and related to the standard tests for oxygen consumption and nitrification. Achieved validation parameters were similar to existing methods. It was demonstrated that aerobic phosphorous uptake was highly sensitive process (IC_{50} value for 3,5-DCP was lower than for nitrification process) and inhibition on the EBPR process should be considered to ensure efficient biological removal of phosphorus.

Combined biological and chemical (BIO-CHEM) treatment of wastewater

- Activated sludge treatment is a cost-effective approach for removing readily degradable organic fraction from wastewater if there is no inhibitory effect on biological processes, especially on the oxygen uptake. Up to 86% of COD, 99% BOD_7 and 95% of phenols were removed with activated sludge treatment, which substantially reduced the load to following chemical treatment process. However, the COD values achieved from laboratory-scale biological treatments were always higher than the respective value from the Zahn-Wellens test because the Zahn-Wellens test has more favourable conditions for microorganisms.

- The total nitrification was achieved for the treatment of municipal landfill leachate (IC_{50}=5.9%) in the continuous pilot plant by applying an adaption period with low organic loading. Thus, steadily high removal efficiencies could be achieved after sufficient adaption of inoculum.

- Application of advanced treatment methods is required when wastewater contains substantial amount of slowly degradable or recalcitrant organic compounds. The main benefit of combining biological and chemical treatment schemes is that biological treatment stage decreases the consumption of reagents for subsequent chemical treatment by the elimination of biodegradable COD. Additionally, biological oxidation avoids solids accumulation during chemical treatment.

- The Fenton treatment reduced valued of COD and phenols up to 85 and 99%, respectively, removed inhibitory characteristics of wastewater and increased the biodegradability of studied samples. However, higher chemical dose was found to be less efficient in increasing biodegradability.
- An effective sludge reuse system without external regeneration process, with optimized addition of reagents dosage was developed in this study to increase the applicability of the Fenton process. It was found that the sludge reuse does not substantially reduce the process efficiency when water exchange ratio was reduced to 50%. Intermittent addition of catalyst is feasible when it is necessary to increase the biodegradability and cyclic addition of catalyst should be used when higher COD removal is required. Additionally, the reuse of ferric sludge substantially reduced the foaming due to a lower reaction rate and over-dimensioning of chemical treatment reactors was not required when the ferric-sludge catalysed Fenton-like process was applied. 12 cycles of ferric sludge reuse led to 13.7-folds smaller production of potentially hazardous waste sludge. When comparing the Fenton-like process that was developed during this thesis to the conventional Fenton process, the main benefits came from a substantial decrease of the ferrous salt consumption (71…86%) and reduction of the ferric sludge formation. This resulted a 25…36% lower treatment costs for the chemical treatment and less secondary pollution caused by the formation of ferric sludge.
- As the Fenton or Fenton-like treatment increased the biodegradability of wastewater, final biological purification was found feasible for the final purification. The combined treatment proved to be a cost-efficient solution for treatment of wastewater with inhibitory and recalcitrant characteristics. More than 94% of organic substances expressed as COD, BOD_7, phenols, lignin and tannins were removed in the three stage treatment strategy.

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SUMMARY IN ESTONIAN

Inhibeeriva ja raskestilaguneva
reovee töötlemise uuringud

Aina väljakutsuvamate veekaitsenõuete täitmine eeldab efektiivsemate aga ka kulutõhusate reoveepuhastamise tehnoloogiate rakendamist. Aktiivmudaprotsessi on reovee puhastamiseks laialdaselt kasutatud põhiliselt tulenevalt selle kõrgest efektiivsusest, madalast hinnast ning paindlikkusest. Küll aga võivad erinevad reovee koostises olevad saasteained olla bioloogiliselt mittelagunevad või pärssida aktiivmudaprotsesse ning seeläbi jõuavad keskkonnale ohtlikud saasteained suublasse. Raskestilagunevate ühendite eemaldamiseks reoveest on vajalik rakendada füüsikalis-keemilisi tehnoloogiaid, mille rajamise ja opereerimise hind võib aga olenevalt reovee omadustest olla väga kõrge. Näiteks on kõrge efektiivsus Fentoni protsessil, mille rakendamine on aga tulenevalt suurest kemikaalikulust ja ohtliku rauarikka jääkmuda tekkest limiteeritud.

Reovee inhibeerivate omaduste vähendamiseks on saadaval erinevaid tehnoloogiaid, kuid igale põhilisele aktiivmudaprotsessile puudusid rakendatavad ja usaldusväärsed metoodikad hindamaks reovee inhibeerivat mõju igale protsessile eraldi. Kasutusel on standardmetoodikad, et hinnata reovee või ohtliku ühendi mõju aktiivmuda hapnikutarbele ja nitrifikatsioonile, kuid ei leitud metoodikat hindamaks mõju bioloogilisele fosforiärastusele ja denitrifikatsioonile. Samal ajal on fosforiärastus ja denitrifikatsioon reoveepuhastis tavapäraselt enne aeroobseid protsesse, mistõttu on sinna suunatud ka kõrgem saasteainete koormus.

Käesoleva doktoritöö eesmärgiks oli lahendada kolm olulist probleemi raskestilaguneva ja inhibeeriva reovee puhastamisel. Esiteks, välja töötada kergesti rakendatavad ja usaldusväärsed metoodikad hindamaks reovee või ohtliku ühendi mõju aktiivmuda bioloogilisele tõhustatud fosforiärastuse ja denitrifikatsiooni protsessidele. Teiseks, testida aktiivmuda- ja füüsikalis-keemiliste protsesside kombinatsioone keeruliste omadustega reovee puhastamiseks ning kolmandaks, Fentoni protsessi rakendatavuse suurendamine vähendades rauarikka jääkmuda teket ja kemikaalikulu.

Töös leiti optimaalsed tingimused tõhustatud bioloogilise fosforiärastuse ning denitrifikatsiooni inhibitsioonitestidele. Testide valideerimise parameetrid olid sarnased olemasolevatele standardmetoodikatele. Inhibitsioonitestide võrdlemisel leiti, et 3,5-diklorofenooli kasutamisel inhibiitorina on fosfori sidumine tundlik protsess (IC_{50} väärtus oli madalam kui nitrifikatsioonil), mis rõhutab bioloogilise fosforiärastuse inhibitsiooni määramise olulisust protsessi efektiivsuse tagamisel.

Aktiivmudaprotsess oli kuluefektiivne lahendus kergestilaguneva orgaanika eemaldamiseks, kui reoveel ei olnud olulist inhibeerivat mõju aktiivmudale. Bioloogilise eeltöötlusega eemaldati kuni 86% KHT-st, 99% BHT_7-st ja 95% fenoolidest. Bioloogilise töötlusega saavutatud väljavoolu KHT väärtused olid aga alati kõrgemad kui jääk-KHT väärtused Zahn-Wellensi testis, mille puhul olid mikroorganismidele soodsamad tingimused (näiteks pikem hüdrauliline

viibeaeg). Kuigi prügila nõrgvesi oli inhibeeriv nitrifikatsioonile (IC_{50}=5,9%), siis eelneva aktiivmuda kohastumise perioodi rakendamisel saavutati täielik nitrifikatsioon. See näitab aktiivmuda kohanemise võimet inhibiitoriga ning stabiilse ja kõrge reoveepuhastuse efektiivsuse saavutamise võimalikkust piisava adaptsiooni rakendamisel.

Aktiivmudaprotsess ei ole üldjuhul efektiivne raskestilagunevate orgaaniliste ühendite lagundamisel, mistõttu kombineeriti käesolevas töös aktiivmudaprotsessi keemilise töötlusega ning skeemi testiti reoveeproovidega puidu- ja põlevkiviõli tööstustest ning olmeprügila ja ohtlike jäätmete (poolkoks) prügila nõrgveega. Protsesside rakendamise järjekord valiti tulenevalt biolagundatavuse ja inhibitsioonitestide tulemustest. Biolagundatavuse hindamiseks kasutati Zahn-Wellensi biolagundatavuse testi, mida täiustati orgaanilise aine jaotamisega kergesti-, aeglaselt- ja raskestilagunevaks fraktsiooniks. Bioloogilise töötluse eesmärk oli biolaguneva orgaanika ja toitainete eemaldamine, keemilise töötluse eesmärk aga raskestilagunevate ühendite lagundamine, inhibitsiooni vähendamine ja biolagundatavuse suurendamine. Keemilise töötlemise metoodikatest valiti Fentoni protsess tulenevalt selle efektiivsusest inhibeeriva ja raskestilaguneva reovee puhastamisel. Fentoni protsess vähendas KHT ja fenoolide väärtuseid vastavalt kuni 85% ja 99%, eemaldas reovee inhibeerivad omadused ning suurendas reovee biolagundatavust. Küll aga leiti, et kõrgem kemikaalide doos oli biolangudatavuse suurendamisel madalama efektiivsusega.

Fentoni protsessi rakendatavuse suurendamiseks testiti pilootseadmel Fentoni-sarnast protsessi, milles rakendati rauarikka jääkmuda taaskasutamist. 12 puhastustsükli läbiviimisel leiti, et rauarikka jääkmuda taaskasutamine ei vähenda olulisel määral reovee puhastamise efektiivsust kui veevahetust vähendada 50%-ni. Katalüsaatori (Fe^{2+}) lisamine igasse teise tsüklisse on mõistlik kui eesmärgiks on reovee biolagundatavuse suurendamine ning igasse tsüklisse, kui vajalik on kõrgem KHT ärastusefektiivsus. Fentoni jääkmuda taaskasutamise eelisteks on ka vähenenud vahutamine tulenevalt madalamast reaktsiooni kiirusest, mistõttu ei ole keemilise töötluse mahutit vajalik üle dimensioneerida. 12-tsükliline jääkmuda taaskasutamine vähendas potentsiaalselt ohtliku jääkmuda teket 13,7 korda. Selles töös kasutatud Fentoni-sarnase protsessi võrdlemisel klassikalise Fentoni protsessiga selgub, et protsessi rakendamise hind vähenes olenevalt opereerimisest 25…36%.

Kuna Fentoni ja Fentoni-sarnased protsessid suurendasid reovee biolagundatavust, rakendati reovee sekundaarset bioloogilist töötlust. Kombineeritud bioloogiline-keemiline-bioloogiline reovee puhastamine oli efektiivne lahendus inhibeeriva ja raskestilaguneva reovee puhastamiseks. Töötlusskeemi efektiivseks rakendamiseks on aga oluline läbi viia eeluuringud reovee biolagundatavuse ja inhibeerivate omaduste tuvastamiseks.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Assoc. Prof. Taavo Tenno for continuous support and assistance to my work during my academic studies in the University of Tartu. Also, I would like to thank all the co-authors and contributors of the scientific publications the PhD thesis is based on and all my colleagues in our research team for their support and advice.

But the most of all, I would like to thank my family and friends for their unlimited support and patience throughout my studies in the University of Tartu.

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