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Intensification of Activated Sludge Process – the Impact of Ozone and Activated Carbon

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Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for any academic degree.

Oliver Järvik

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OLIVER JÄRVIK

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Munter, R., Trapido, M., Kallas, J., Kamenev, S., Preis, S., Viiroja, A., Kamenev, I., Järvik, O., Kulik, N., Veressinina, J., Ozonation and AOPs for Oil Shale Industry's Wastewater Treatment: an Overview. Ozone News, 2009, 37(4), pp. 17-22.

THE AUTHOR'S CONTRIBUTION TO THE PUBLICATIONS

Article I: The author fulfilled the experiments and analyses, analysed the results and wrote the paper. The results were presented by the author at the conference on “Ozone & related oxidants in advanced treatment of water for human health and environment protection. Disinfection, elimination of persistent pollutants and control of by-products” in Brussels, Belgium, in 2008.

Article II: The author fulfilled the experiments and analyses, analysed the results and wrote the paper.

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INTRODUCTION

Two wastewater treatment technologies, namely activated sludge process and ozonation are widely studied and also widely applied. The activated sludge process that utilizes aerobic microorganisms to achieve purification is known for its ability to treat large amounts of wastewaters that may be of municipal or industrial origin. However, in the case of industrial wastewater containing recalcitrant compounds or chemicals toxic for microbial culture, ABO often results in inadequate effluent water quality in terms of COD, BOD and suspended solids, the latter of which is caused by poorly settling sludge. Beside that, certain difficulties arise concerning generation of secondary wastes – excess sludge and volatile organic compounds emission. Additionally, fluctuations or sudden increase of organic loading (shock loadings) may damage sludge population and interfere bioreactors normal operation.

Ozonation is used for removal of organics, for disinfection purposes, for partial oxidation of recalcitrant compounds, and for pre- or post-treatment to aid in other unit operations. Since in many cases ozonation efficiently reduces toxicity or increases biodegradability of wastewater, it is often used in combination with the activated sludge process, especially when the purification efficiency of the latter is inadequate. Naturally, ozonation alone could be used to treat these wastewaters but the price would be too high for practical application. The combination of the two processes is more economical because relatively small ozone doses are used. Ozonation and the activated sludge process are also combined to reduce excess sludge production. Due to the need to fulfill the requirements set on more stringent legislation the latter is expected to increase.

Activated carbon is known as an excellent adsorbent and its addition into the activated sludge process has also been shown to be useful in the purification of contaminated and toxic wastewaters.

The interest toward more efficient ozone utilization for the destruction of recalcitrant/toxic compounds and reduced equipment size created an idea to study a novel process where ozone and activated carbon are added straight into the biomass. Hence, there are less soluble compounds that would compete for ozone with target compounds, and there is no need to use multiple activated sludge units or recirculation of ozonated wastewater (van Leeuwen *et al.*, 2009). The addition of activated carbon possibly further improves the performance by acting as an adsorbent. It may be expected that ozone, which is known as a disinfectant, dosed directly to the activated sludge process, also influences sludge yield. However, a question – whether a process where ozonation, activated carbon addition and biochemical wastewater treatment are integrated into a single unit could be utilized in such a manner that both emission of soluble pollutants and excess sludge production are simultaneously reduced – has remained unanswered.

Therefore, the main objective of the work was to study the impact of ozone and activated carbon on the activated sludge process when they are dosed directly to the bioreactor treating oil shale phenolic wastewater and to establish application possibilities for this process. Improvement of biochemical purification of oil shale phenolic wastewater and simultaneous reduction of excess sludge generation was focused upon. The choice of the wastewater was based on the most well known environmental issue in Estonia and also future trends in the oil shale industry were considered. The effect was evaluated on the basis of the purification efficiency achieved in the studied processes and changes in activated sludge activity.

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LIST OF ABBREVIATIONS AND SYMBOLS IN ALPHABETICAL ORDER

ABO – activated sludge process
ABO/AC – activated sludge process with the introduction of activated carbon directly to the bioreactor
ABO/AC/O_3 – activated sludge process with the introduction of activated carbon and ozone directly to the bioreactor
ABO/intermittentO_3 – activated sludge process with intermittent dosing of ozone directly to the bioreactor
ABO/O_3 – activated sludge process with the introduction of ozone directly to the bioreactor
AS – activated sludge
AS/O3 – ozonation of activated sludge suspended in distilled water
AS+PW/O_3 – ozonation of activated sludge in phenolic wastewater
ATP – adenosine-5'-triphosphate concentration
BOD – biochemical oxygen demand
COD – chemical oxygen demand
CSTR – continuous stirred tank reactor
HRT – hydraulic retention time
MLSS – mixed liquor suspended solids
MLVSS – mixed liquor volatile suspended solids
O_3/AC – ozonation in the presence of activated carbon
OUR – oxygen uptake rate
PW – phenolic wastewater
SATP – specific adenosine-5'-triphosphate concentration
SCOD – soluble chemical oxygen demand
SOUR – specific oxygen uptake rate
SVI – sludge volume index
TCOD – total chemical oxygen demand

1. LITERATURE REVIEW

The main idea of the PhD work was to experimentally study the process where ozone and activated carbon are dosed directly to the activated sludge process with the main focus on the effect of ozone on the process. The literature review will therefore give a brief insight into the current knowledge and situation in the sphere of the activated sludge process, ozonation, their combinations and the effect of ozone on microbial population in the activated sludge process.

1.1. Activated sludge process

In this chapter an overview of the basic principles of the activated sludge process and parameters influencing the performance are described. The main attention is focused on the purification of phenolic wastewaters with a short insight into the problems related to the origin and purification of oil shale phenolic wastewaters in Estonia.

Activated sludge process is the most widely used biochemical wastewater treatment process. It has been applied efficiently both for municipal and industrial wastewater treatment. The growth of population and an adequate water supply has increased wastewater generation. The absolute minimum water requirement for a person in a day is 3 litres (Gleick, 1996) (equal to consumption in Mozambique) but in developed countries the daily water consumption per person is as high as ~600 litres (in USA in 2002) (/1/). Therefore the municipal wastewater can be considered essentially as 99.9% water (Gray, 2004). The widespread usage and popularity of the activated sludge process arise from the fact that it is one of the most effective methods for removing pollutants from large volumes of relatively dilute wastewater as well as from relatively strong wastewater with COD up to 4000 $mg \cdot L^{-1}$ (Grady, 1999).

1.1.1. Principles of the activated sludge process

In the activated sludge process wastewater purification is achieved through the growth of different prokaryotic and eukaryotic microorganisms that are mostly embedded into flocks. Pollutants in the activated sludge process are converted into soluble organic and inorganic compounds, into new biomass, and partly into carbon dioxide, H_2O, and ammonia-nitrogen. Pollutants are used to gain energy (ATP) and synthesize intermediate compounds needed in anabolic reactions – i.e. for growth, movement, maintenance, synthesis of macromolecules (Bitton, 2005). It means that organic contaminants are used both as an energy source and a carbon source. Because of their role in metabolism (the conversion of carbon source through the catabolic reactions into energy and intermediate compounds that are used in anabolic pathway for cell synthesis), organic pollutants are also called substrates. The removal on insoluble compounds is achieved as they are mostly entrapped into sludge flocks.

It has been pointed out that as a result of microbial action the quality of effluent water from the activated sludge process is high. This is only true when the conditions in the bioreactor favour the development of active pollutants consuming bacterial population as well as flock forming bacteria to develop flocculent biomass. As the process relies on bacteria, its performance is affected by the environmental factors. The most important factors are (Grady, 1999, Gray, 2004):

1. hydraulic retention time (HRT) (at least 5 hours, depending on the composition of wastewater);
2. solids retention time (SRT) (at least 3 days, usually 5 to 15 days);
3. MLSS concentration (usually in the range of 2 to 5 $g \cdot L^{-1}$);
4. dissolved oxygen concentration (usually at least 2 $mg \cdot L^{-1}$);
5. nutrients concentration (expressed as the ratio of BOD:N:P, although micronutrients are also needed);
6. temperature (affecting the biochemical reaction rates, maximum 35° to 40°C).

As these factors are for some reason changed or the composition of wastewater changes (pH, biodegradability, nutrients, refractory compounds), the effluent water quality usually deteriorates.

The rate of the change of biomass concentration in a sample/reactor (dX/dt) is proportional to the rate of substrate uptake/removal (dS/dt) by microorganisms:

$$\frac{dX}{dt} = Y\frac{dS}{dt} \Leftrightarrow \mu = Y\nu \ , \tag{1.1}$$

where ν is the specific rate of substrate uptake (substrate concentration·time^{-1}), X is biomass concentration (mass of biomass per volume of liquid), Y is the observed yield coefficient (mass of biomass formed per unit of substrate removed), and μ is the specific growth rate (time^{-1}). The exact composition of real wastewaters is usually unknown. Therefore, the substrate concentration S available for micro-organisms is usually measured as a soluble COD, which is a measure of the electrons available in a compound (Grady, 1999). The amount of biomass formed can also be expressed and measured as COD. Consequently, sludge yield can be expressed on the basis of COD. Nevertheless, it is possible to establish the relationship between COD and commonly used mass of suspended solids (SS) or mass of volatile suspended solids (VSS). For example, COD of one gram of ash-free SS and VSS, with assumed empirical formula of $C_5H_7O_2N$, is 1.20 and 1.42 $mg \cdot L^{-1}$, respectively (Grady, 1999).

The specific substrate uptake and specific growth rate are given by the Michaelis-Menten equation and the Monod (empirical) equation, respectively (Bitton, 2005):

$$v = \frac{v_{\max} S}{K_S + S} \text{ and} \tag{1.2}$$

$$\mu = \frac{\mu_{\max} S}{K_S + S}, \tag{1.3}$$

where v_{max} is the maximum specific substrate uptake, μ_{max} is the maximum specific growth rate, K_S is the half-saturation coefficient, K_M is the half-saturation coefficient of substrate and S is the substrate concentration.

As a result of substrate/pollutant removal, the observed yield varies greatly. It has been found to range from 0.43 to 0.59 mg biomass COD formed per mg substrate COD removed for different species of aerobic bacteria grown on glucose. Naturally, the yield coefficient for a certain species is not constant and its value is affected by the environmental conditions (pH, temperature) and growth substrate (Grady, 1999).

In biochemical wastewater treatment processes under steady-state conditions (defined system parameters) the sludge is habitated by the organisms whose presence is favoured by the conditions, developing its mixed liquor biota food chain (Gerardi, 2008). The observed sludge yield in this system depends on the type of wastewater and micro-organisms and in the activated sludge process treating municipal wastewater is usually within the range of 0.48-0.72 mg biomass COD formed per mg substrate COD removed. In the case of industrial wastewaters containing xenobiotic compounds the values are usually found to range from 0.20 to 0.60 mg biomass COD formed per mg substrate COD removed (Grady, 1999). Generation of considerable amount of new biomass, as indicated by the values of the observed yield coefficients, is considered to be the main drawback of the activated sludge process, because excess sludge must be disposed of securely, without adversely affecting environment.

The removal of soluble substrates is mostly carried out by Archae and Bacteria. Under normal operating conditions of the activated sludge process the microbial community contains also protozoa, rotifers and nematodes that are essential for turbidity reduction, although filamentous bacteria act also as sieves for turbidity reduction (Grady, 1999). Fungi may under certain conditions (low pH, insufficient oxygen and/or nitrogen, toxicity) compete for the substrate with bacteria and cause similar operational problems such as proliferation of filamentous bacteria. Organisms that form the sludge are also bio-indicators of the conditions within the reactor, meaning that any change in these conditions lasting for a certain time will cause easily observable changes in the biota and in effluent quality parameters. The changes in the biota may be caused by changes in (Gerardi, 2008)

i. wastewater strength and composition;
ii. mode of operation;
iii. strength and composition of recycle streams.

1.1.2. Biochemical treatment of phenolic wastewaters

Although biochemical wastewater treatment processes are used efficiently and economically for wastewater treatment, their implementation is problematic in some cases. Main problems arise when wastewater contains toxic compounds or there are transient toxic load conditions. These conditions may be mainly seen when industrial wastewaters are concerned. The influence of toxic compounds on bacteria depends on the concentration and structure/properties of the specific compound.

Common contaminants in industrial wastewaters are phenolic compounds. Phenols are found in the pulp and paper industry, petrochemical, metal casting, resin production, pharmaceutical, oil-shale industry etc. wastewaters. The main source of phenolic wastewaters in Estonia is the oil-shale industry, causing severe local environmental problems and also negatively influencing the water quality in the Baltic Sea. There are two phenolic wastewater sources. Firstly, phenolic wastewater is generated in the chemical industry within the oil production process – oil-shale is heated in the absence of oxygen to a temperature at which kerogen forms gas, condensable oil, and solid residue. The oil is condensed and phenols in it are extracted with water, following the dephenolation of the water. Secondly, solid residue formed in the semi-coking process is dumped to the landfill, where the organics left in the semi-coke is leached out, forming leachate with high concentration of total phenols. Since 2007 the semi-coke is dumped to the new landfill where the leachate is gathered in basins with the volume approximately equal to the annual flow rate (VKG 2008) (the formation of leachate is diminished due to utilized landfilling technology). Before that the leachate was discharged directly to the Baltic Sea without any purification (Kamenev *et al.*, 1995).

The annual flow rate of dephenolated wastewater containing mainly dibasic phenols (resorcinols) with an average concentration of 106 $mg \cdot L^{-1}$ (Kamenev *et al.*, 2003) ranges from 388 333 to 484 000 m^3 with (VKG 2008). The leachate and dephenolated water are sent to the local wastewater treatment plant operated by Järve Biopuhastus LLC, where it is mixed with municipal and industrial wastewaters from other industries prior to purification in the activated sludge process (VKG 2008). Mixing of wastewaters is performed to reduce the (supposed) toxicity, as phenolic compounds are classified as toxic. As a result of dilution, almost total phenol destruction (~90%) is achieved in the activated sludge process.

As described by McFarland (1970), toxicity is a result of two preceding events – diffusion of a compound to the surface of bacteria, followed by the interaction between the compound and the site of action. Interaction at the site of action can be described by the octanol/water partition coefficient (K_{OW}), which for the most toxic compounds is found to range between 1 and 5 (phenol 1.45) (Heipieper *et al.*, 1994), the highest occupied and lowest unoccupied molecular orbital energy (E_{HOMO} and E_{LUMO}, respectively) and the hydrogen bond donor number (N_{hdon}) (Melagraki *et al.*, 2006).

Biodegradability of phenolic compounds

The impact of phenolic compounds on bacterial populations and activated sludge is widely studied. It is common knowledge that high loading rates and fluctuations in phenolic compound loads cause disturbances and the breakdown of activated sludge processes (Watanabe *et al.*, 1996, Kibret *et al.*, 2000). Lab-scale studies have shown that most of the substituted phenolic compounds are biodegradable (Tabak *et al.*, 1981), but the rate of biodegradation depends on the structure and concentration of the phenols. Concentration may be considered as the most important factor, because from a certain concentration on, phenols inhibit the growth of micro-organisms and thereby also concomitant biodegradation of phenols. The Andrews equation is most commonly used to describe the specific growth rate μ (h^{-1}) inhibition kinetics of microorganisms (Andrews, 1968):

$$\mu = \frac{\mu_{\max} S}{K_S + S + \left(S^2/K_i\right)}, \tag{1.4}$$

where K_S is the half-saturation constant ($g \cdot L^{-1}$), K_i is the substrate inhibition constant ($g \cdot L^{-1}$), S is the substrate concentration ($g \cdot L^{-1}$) and $\mu_{\max}$ is the maximum specific growth rate (h^{-1}).

As it may be expected, the substrate inhibition constant, as well as other constants, depend on the type of micro-organisms and the type of substrate. For example, for *P. putida* the inhibition constant in the case of phenol is in the range of 129 to 470 $mg \cdot L^{-1}$ and for *Acinetobacter* it ranges from 188-315 $mg \cdot L^{-1}$, depending on the substrate concentration (Hao *et al.*, 2002). However, the low inhibition constant value does not mean that wastewater with high initial inhibiting substrate concentration can not be degraded. For example, Yan *et al.* (2005) studied phenol degradation at initial concentrations up to 2000 $mg \cdot L^{-1}$, using pure culture of *Candida tropicalis* (K_i=207.9 $mg \cdot L^{-1}$) and found that for the highest initial concentration of approximately 66 hours was needed for complete phenol degradation with 5% starting inoculum. It was also noticed that as the initial substrate concentration increased, also the lag-time increased (time needed for adjustment to new conditions). Similar trends are usually seen if the activated sludge process is used for phenolic wastewater treatment. In the case of activated sludge, the inhibition constant of the phenol is found to vary from 142 to 1200 $mg \cdot L^{-1}$ (Vázquez-Rodríguez *et al.*, 2006). Nevertheless, the results of phenol degradation with acclimated activated sludge suggest that the highest phenol concentration in the influent that does not cause disturbances is close to 200 $mg \cdot L^{-1}$, although in that case the feed has to be steady and continuous (Galil *et al.*, 1988). Higher concentrations or sudden changes in concentrations are found to cause deflocculation (Galil *et al.*, 1998) or in severe cases, may even cause total poisoning of the activated sludges (Kahru *et al.*, 2000). For example, Galil *et al.* (1998) showed that wastewater containing phenol with an initial concentration of 1500 mg·L^{-1} inhibited OUR by 92%. Despite the initial OUR inhibition, total phenol removal after the lag-phase of ~200 hours was achieved within 370 to 430 hours.

Higher tolerance of acclimated activated sludge against shock loadings of toxic phenolic compounds is stressed in several articles (Galil *et al.*, 1988, Kahru *et al.*, 1996). Also, microbial granules (Jiang *et al.*, 2002) and cells embedded in alginate (Keweloh *et al.*, 1989; Heipieper *et al.*, 1991) show improved tolerance against shock loadings as compared to free cells. This phenomenon is assumed to be a result of binding of phenols by cells on the exterior of the flock particles or by the media surrounding bacteria that avoids inhibition of internal bacteria (Galil *et al.*, 1998, Jiang *et al.*, 2002).

Biodegradability and toxicity of oil shale phenols found in leachate and dephenolated water

Toxicity detection methods give relatively contradictory results. Tests relying on luminescence inhibition of photobacteria (Vibrio fischeri, Photobacterium phosphoreum) show that monobasic phenols are approximately 10 times more toxic than resorcinols (Kahru *et al.*, 2000). On the other hand, the usage of higher organisms (*Daphnia magna*) shows opposite results, namely resorcinols are approximately two times more toxic than monobasic phenols (Trapido and Veressinina, 1999). Despite the contradictory results it is accepted that resorcinols are toxic and hazardous if discharged to the nature. Kahru et al. (2000) found that 5-methylresorcinol, the resorcinol found in oil-shale phenolic wastewaters with the highest concentrations, may be classified as toxic (L(E)C_{50} 1 to 10 mg·L^{-1}). Resorcinol and 2,5-dimethylresorcinol are classified as highly toxic (L(E)$C_{50} \leq 1$ mg·L^{-1}). Accordingly, the biodegradation tests with acclimated and nonacclimated sludge showed that 2,5-dimethylresorcinol is slowly biodegradable (detoxification is slow), whereas 5-methylresorcinol degradation was fast (detoxification is fast) (Kahru *et al.*, 2000).

The measurements made in the 1990s showed that the removal of mono-basic phenols, xylenols and cresols in the activated sludge process was relatively high, resorcinols on the other hand were found to be relatively resistant to biodegradation (Preis *et al.*, 1994). Therefore resorcinol concentration in biochemically treated effluent was in the range of 35 to 40 mg·L^{-1} (Munter *et al.*, 1994). By now, the situation has improved, as according to the data from Järve Biopuhastus LLC, an average phenol concentration in the influent entering the activated sludge process is in the range of 8 to 24 mg·L^{-1} (an average of 2010, higher during summer period, lower during winter period). In the effluent water the concentration of phenols ranges from 0.03 to 2.2 mg·L^{-1}. According to Kamenev *et al.*, (2003), during the period from 2000 to 2002 the typical concentration of total phenols in the influent wastewater was 18 mg·L^{-1} while the phenol removal efficiency in the activated sludge process was ~93%, giving total phenol concentration in the effluent water of 1.2 mg·L^{-1}. The toxicity measurements have shown that because of the residual resorcinol concentration this effluent water is still toxic (Kahru and Põllumaa, 2006). According to the Estonian legislation the maximum permissible concentration of monobasic and dibasic phenols in the effluent water is 0.1 and 15 mg·L^{-1}, respectively. But as suggested by the Helsinki Committee the maximum permissible concentration for total phenols should not exceed 0.5 mg·L^{-1} and additionally the mixing or diluting of different wastewaters to comply with the established limit value for the effluent should not be allowed (HELCOM 2002).

The leachate of newly formed semi-coke has been found to be acutely toxic. The toxicity is caused by the phenols and also high TOC and pH (Otsa and Tang, 2003). This is also the reason why semi-coke is classified as a hazardous waste. As the concentration of leachable compounds is reduced, the toxicity is also reduced. As shown by Otsa and Tang (2003), the leachate of the 10-year old semi-coke is not toxic. Kahru and Põllumaa (2006) on the other hand found that the leachate of an old semi-coke is much less toxic than of that of new semi-coke but classified still as toxic. The Finnish company ”Vesi-Hydro” has also studied the bio-oxidation of leachate. According to their findings, the wastewater can be treated biochemically only when the amount of leachate in the total stream is below 7%. Nevertheless, total phenol concentration in that case in the effluent water is still 9 to 10 mg·L^{-1} (Kamenev *et al.*, 1995).

1.2. Ozone in wastewater treatment

In this section a brief overview of the behaviour of ozone in “pure” water and wastewater, including decomposition into hydroxyl radicals, is given. Additionally, ozone applications are described.

1.2.1. Aqueous chemistry of ozone

Ozone is a gas that is unstable under the conditions used in water and wastewater treatment. Its half-life is measured to range from seconds to several hours and is affected by the water matrix. When ozone is introduced into water it may remain as molecular O_3 or decompose. The decomposition of ozone in water may be initiated by the presence of impurities or by water itself (OH^-). As shown by Staehelin and Hoigne (1982), the presence of OH-anions (at pH values higher than 8) initiates ozone decomposition that is accelerated as pH is increased (Staehelin and Hoigne, 1985). In “pure” water the decomposition of ozone can only be initiated by OH-anions or by photolysis. H_30^+ does not initiate ozone decomposition as the half life of ozone at low pH values is measured to be within days (e.g. at pH = 2 $t_{1/2}$ is > 55.6 hours). (Staehelin and Hoigne, 1982) In this decomposition process primary and secondary radicals (superoxide anion and hydroperoxyl radical) are formed, initiating radical-type chain reaction yielding more highly reactive radicals. Most important of these radicals is hydroxyl (•OH) radical that under strongly alkaline conditions is converted to its conjugated base, oxyl anion radical ($•O^-$) (Buxton *et al.*, 1988). OH radicals are powerful non-selective oxidants that have reaction rates with organic compounds typically in the range of 10^8 to 10^9 $M^{-1}s^{-1}$ (Glaze and Kang, 1989). The mechanism of ozone decomposition in "pure" water is clearly explained by Staehelin and Hoigne (1985).

Oxidation by molecular ozone is considered to be the main pathway under acidic conditions ($pH<4$) as OH ions does not contribute to ozone self-decomposition (Masschelein, 2000), whereas at pH values greater than 9 the formation of OH radicals prevails. Naturally, the real pathways are not that simple – in real water samples containing soluble organic and inorganic compounds the decomposition of ozone proceeds by the two competitive pathways which may be difficult to distinguish. These are radical reactions and direct reactions with molecular ozone (electrophilic reaction and cycloaddition). Radical reaction pathway consists of three steps: initiation, propagation and inhibition (Staehelin and Hoigne, 1985). Radical reactions may be initiated, promoted, or inhibited by different soluble compounds. Examples of initiators and promoters are hydrogen peroxide, fulvic acids (Xiong *et al.*, 1992), humic acids (Xiong *et al.*, 1992, Masten and Davies, 1994), methanol, phosphate ion (Staehelin and Hoigne, 1985), and natural organic matter (NOM) (Beltran, 2004). The concentration of radical scavengers in natural or wastewaters is high. Scavengers are compounds that either remove, or convert radicals to unreactive radicals that terminate the chain reaction. Therefore radical-type chain reactions under these conditions are hindered unless initiators are present/produced providing constantly high concentration to assure initiation and propagation steps. Well known radical scavengers are bicarbonate ion, carbonate ion, compounds containing alkyl groups, *t*-butyl alcohol, acetic acid (Staehelin and Hoigne, 1985), p-clorobenzoate, natural organic matter (Beltran, 2004) etc.

In brief, as stated by Rice (1997), the effect of ozone in water is usually a result of its direct reactions with solutes, its decomposition into secondary radicals following subsequent reactions of formed radicals with solutes. Most importantly, the reaction pathways are determined by the water composition.

Ozone reactions in wastewater

Ozone is quite a selective oxidant. In the case of simple oxidizable ions, the reaction rate is determined by the reduction potential ($E°=+2.07$ V). If chemical reactions between ozone and organic molecules are considered, kinetic factors will usually determine whether ozone will oxidize a pollutant within reasonable time or not (Gogate and Pandit, 2004). The target molecule, in order to react with ozone, should contain unsaturated bonds (alkenes, aromatics). Electrophilic addition reactions occur at negatively charged atoms (N, P, O, or nucleophilic carbons). In aromatic compounds the position of the ozone attack depends on the substituting groups. The groups that tend to release electrons (-OH, $-NH_2$, -NHR, $-NR_2$, -OR, -NHCOR, -alkyl) activate the aromatic ring, while groups that attract electrons ($-NO_2$, -CN, -COOH, -CHO, -X) deactivate the aromatic ring (Beltran 2004). The carbons at the ortho- and para- positions are subjected to electrophilic attack when the substituting group has the tendency to release electrons. The carbon at the meta- position is attacked when the substituting group attracts electrons. Generally, aromatics substituted with electrons donating group tend to react faster with ozone (Masten and Davies, 1994). Depending on the nature of the organic compound, the reaction rate with ozone varies significantly (Table 1).

Wastewater ozonation is a chemisorption process and due to ozone's low solubility in water, the rate of its mass transfer is controlled by the liquid film mass transfer coefficient. In case wastewater contains contaminants that have high reaction rates with ozone, the mass transfer is increased (Danckwerts, 1970). As proposed by Zhou and Smith (2000), usually two different kinetic regimes for ozone absorption can be seen. These are slow and instantaneous regimes. In the case of slow regime ozone dissolves in the liquid phase where it is consumed. If wastewater is concentrated, reactions usually take place in the proximity of the liquid-gas interface and ozone is instantaneously consumed, meaning that dissolved ozone concentration in the liquid phase is zero. This enhanced ozone consumption is accounted into the mass transfer equation through the addition of the enhancement factor.

Table 1. Reaction rates of ozone and hydroxyl radicals with some organic molecules (Hoigne and Bader, 1983, Acero et al., 2001, Mill et al., 2000, Teramoto et al., 1981, Huang et al., 2009, Malik et al., 2001)

Solute	k_{ozone}, $M^{-1}s^{-1}$	$k_{OH\ radicals}$, $M^{-1}s^{-1}$
Acetic acid	$3\cdot10^{-5}$	$4.1\cdot10^{8}$
MTBE	0,14	$1.6\cdot10^{9}$ to $3.9\cdot10^{9}$
Toluene	14	$7.8\cdot10^{9}$
Resorcinol	$1.8\cdot10^{5}$	$1.0\cdot10^{10}$
Phenol	$1.3\cdot10^{3}$	$6.6\cdot10^{9}$

Enhancement of the ozonation process

In case reaction rates between ozone and target molecules are low, ozonation is inefficient, and more reactive nonselective oxidants, like hydroxyl radicals need to be generated in order to achieve destruction of contaminants within reasonable time. For example, the reaction rate of MTBE with hydroxyl radicals is in the range of $1.6\cdot10^9$ to $3.9\cdot10^9$ $M^{-1}s^{-1}$ (von Gunten *et al.*, 2001), showing the importance of advanced oxidation processes (AOPs) where hydroxyl radicals are constantly generated. It must be noted that in order to generate hydroxyl radicals, ozone is not always required. OH radicals may be generated in the liquid phase (homogeneous catalysis – O_3/H_2O_2, O_3/UV, H_2O_2/UV, Fenton, photo-Fenton) and on the surface of catalyst (heterogeneous catalysis – Fenton, Fenton-like, photo assisted Fenton, photocatalysis). As the reaction rates between contaminants and hydroxyl radicals are usually high (Table 1), the destruction of contaminants during heterogeneous AOP is diffusion limited. This is an important factor to be considered as the half-life of the OH radical is about 10^{-9} s, meaning that the reaction can only take place within a distance of approximately few Å from the location it was generated (Jensen and Csizmadia, 2001). In the case of homogeneous catalysis involving ozone, the amount of the OH radicals generated depends on the ozone absorption process efficiency (Andreozzi *et al.*, 1999).

To improve the efficiency of the ozonation process the addition of activated carbon (AC) may be used. Ozonation in the presence of AC is considered to be one of the AOPs. Due to the influence of AC, the process is sometimes called (heterogeneous) catalytic ozonation (Beltrán *et al.*, 2002), although the exact mechanism has remained unclear. Some claim that AC can only be described as an initiator or a promotor of radical type ozone decomposition reactions because its surface properties are modified during the ozonation process (Alvárez *et al.*, 2006). Nevertheless, ozonation in the presence of AC is still widely studied mainly because:

- ozone decomposes into hydroxyl radicals (Legube and Karpel Vel Leitner, 1999; Alvárez *et al.*, 2006) and mineralization of organic contaminants is improved (Lei *et al.*, 2007);
- the amount of ozone consumed for the removal of contaminants is reduced (Kasprzyk-Hordern *et al.*, 2003);
- the negative impact of radical scavengers is reduced (Kasprzyk-Hordern *et al.*, 2003);
- prolonged saturation time of AC (Legube and Karpel Vel Leitner, 1999).

Under these considerations it is evident that the process is highly complex as it involves simultaneous adsorption-desorption processes, surface chemistry (reactions between ozone and AC) and reactions in the liquid phase that are all interrelated and should be considered. The complexity is confirmed by the fact that regardless of the pH the activation energy of ozone decomposition is always smaller in a heterogeneous process (Beltrán *et al.*, 2002). However, the results suggest that the effect of AC on purification efficiency is more pronounced at pH values greater than 6, making it possible to achieve a remarkable synergetic effect in terms of COD removal (Lei *et al.*, 2007). According to the experimental results ozonation in the presence of AC is applicable for purification of pharmaceutical wastewaters (Lei, *et al.*, 2007), landfill leachate (Kaptijn, 1997), phenolic wastewaters (Lin and Wang 2003), real coloured wastewaters (Faria *et al.*, 2005) etc.

1.2.2. Ozone applications in wastewater treatment

Ozonation is a method that has been applied both for drinking and wastewater treatment – i.e. for disinfection and for oxidation. In wastewater treatment ozone is used to achieve direct oxidation of contaminants or in combination with other processes.

In early ozone applications ozone was used for treatment (disinfection) of drinking water. Ozone was applied for drinking water treatment at the beginning of the 20th century in the USA (Rice, 1999), France (Paulouë and Langlais, 1999), and Germany (Böhme, 1999), at the beginning of the 1930s in Japan (Matsumoto and Watanabe, 1999), 1946 in Switzerland (Geering, 1999), 1954 in Canada (Larocque, 1999). Currently, ozonation is mainly used to meet regulations for the inactivation of viruses and protozoa and in some cases also for the removal of some specific contaminants (pesticides, micro-pollutants) (Masten and Davies, 1994). It is an excellent method as other disinfectants, such as silver, hydrogen peroxide, chlorine, and chlorine dioxide, in some cases fail (von Gunten, 2003). The evaluation of disinfection efficiency is based on an estimation of disinfectant exposure, also known as the CT-factor. CT-factor calculations are usually performed by measuring residual disinfectant concentration at the outlet and multiplying the value with the contact time (the time needed for the 10% of the applied tracer to travel through the reactor). A target CT-factor value in ozonation is usually 1.5 to 2 $mgO_3 \cdot min \cdot L^{-1}$. Under these conditions the main micro-organisms are removed, although pH and temperature may significantly influence the necessary CT-factor value (von Gunten and Laplanche, 2000).

The announcement of pollution problems associated with the development of industry in the middle of the 20th century broadened the field of ozone applications. Initially ozone in wastewater treatment utilities was applied mainly for disinfection and odour control (Rice, 1999, Larocque, 1999, Oneby *et al.*, 2010) (mainly in municipal wastewater treatment plants). Other common uses were micro-flocculation of suspended solids, flotation of suspended solids, oxidation of organics prior to filtration (Rice, 1999), and colour removal (Lowndes, 1999). Later, as the impact of different discharges on receiving water bodies became evident and more stringent legislation was adopted, ozone was also applied for treatment of industrial and other hazardous wastewaters: for pharmaceutical process water, pulp and paper water (Larocque, 1999, Masten and Davies, 1994), cyanide destruction in electroplating wastewaters, oily wastewater (Rice 1999), textile industry wastewater, and landfill leachate treatment (Rice, 1997, Böhme, 1999). The main purpose is to simultaneously remove toxicity, colour and to improve the biodegradability of the wastewater. Additionally, effluent water quality is improved as dissolved oxygen concentration is increased (Lowndes, 1999). The amount of ozone used for the removal of one gram of DOC during the wastewater treatment ranges from 0.5 to 1.5 g of ozone. As a result of its selectivity, ozone doses in most wastewater treatment processes remain relatively low – usual doses applied for wastewater treatment are between 2 and 50 $g \cdot m^{-3}$. Doses applied for drinking water treatment range approximately from 0.5 to 1.2 $g \cdot m^{-3}$ (Böhme, 1999).

By the end of the 1990s there weres over 110 ozone installations meant for municipal water, wastewater and industrial wastewater treatment in Canada (Larocque, 1999) and more than 600 in Germany (Böhme, 1999). In the USA currently only seven wastewater treatment plants use ozone for final disinfection (Oneby et al., 2010) compared to 45 plants at the beginning of the 1980s (Rice, 1999). The decline in ozone use in the USA was mainly caused by the change in the disinfection policy in 1976, meaning that effluent disinfection was not required unless the receiving waters are used for aquaculture or there may be direct human contact with the effluent water (Rice, 1999).

1.3. Activated sludge processes combined with ozonation

An overview of possible reasons for combining ozone with the activated sludge process is given, followed by the description of the processes and the results obtained when ozone is applied in the recirculation line or straight into the activated sludge process. The effect of ozone on activated sludge, including the effect on filamentous bacteria, on sludge yield and sludge viability is described.

1.3.1. Principles

Wastewater purification by means of ozone or by other methodologies (e.g. AOPs) as a research topic has become more popular than methodologies for detection of trace concentration of different emerging contaminants and the knowledge about their behaviour and impact on nature has improved. These emerging contaminants are endocrine disrupting chemicals, pharmaceuticals, and personal care products (Oneby et al., 2010). Due to their properties, they are also called persistent organic pollutants as they usually pass conventional biochemical purification processes, meaning that for their removal some other oxidation process must be used. In some cases the oxidation reactions may produce undesired by-products. The removal of these products is usually carried out in the subsequent biochemical filtration step (von Gunten, 2003). It is estimated that only bromoorganic compounds that are formed during the ozonation of bromide and organics containing water are not biodegraded in this step (von Gunten, 2003). This shows that the combination of ozonation for partial oxidation with biochemical wastewater treatment is a beneficial and practical application that has also received considerable attention. The possible ozone application fields are (Ried *et al.*, 2009):

- effluent water quality improvement, i.e. post-treatment (disinfection, colour removal, destruction of POPs, COD removal);
- sludge treatment in the recirculation line (foam reduction, disintegration

of sludge, enhancement of dewaterability);
- treatment of exhaust air (removal of odour);
- treatment of highly contaminated streams, i.e. pre-treatment (COD removal, colour removal, biodegradability improvement, degradation of toxic substances).

Although wastewater ozonation is usually considered to be a relatively expensive method that requires high ozone doses, ozone doses are remarkably reduced if ozonation is combined with biochemical wastewater treatment. Naturally, the dose depends on the water matrix and several effects can be achieved simultaneously (like COD and colour removal). The latter is one of the main reasons why ozone is (re-)gaining its popularity (Burns et al., 2007). Typical ozone doses applied in wastewater treatment are shown in Figure 1.

The activated sludge process combined with pre- and post-ozonation has been extensively studied. These processes are usually arranged in two ways: serial treatment or recycle treatment (Jeworski and Heinzle, 2000). Pre-ozonation has commonly been found to significantly improve the biodegradability of domestic and different industrial wastewaters (Qian *et al.*, 1994, Scott and Ollis, 1995, Shin and Lim, 1996, Perkowski *et al.*, 1996, Beltran *et al.*, 1999, Turan-Ertas, 2001), thereby improving the purification efficiency achieved in the biochemical purification step, although in the case of sludge acclimated to some specific wastewater, an adverse effect may be seen (Hu and Yu, 1994). Pre-ozonation is considered to be less effective by means of destruction of target contaminants as biodegradable compounds compete for the available ozone (Aparicio et al., 2007). Post-ozonation on the other hand is usually meant for destruction of compounds that have already passed the biochemical wastewater treatment – therefore the amount of competitive contaminants is reduced. As a result of destruction of refractory organics, the biodegradability is increased, meaning that recalcitrant compounds decomposed into more biodegradable intermediates should be removed in the subsequent biochemical process (van Leeuwen *et al.*, 2009). The repetitive biochemical degradation-ozonation process enables high purification efficiency to be achieved at relatively low specific ozone consumption, although the literature survey carried out by Jeworski and Heinzle (2000) showed that the ozone doses needed for 1 mol of carbon removal range from 0.02 to 1.4 molO_3.

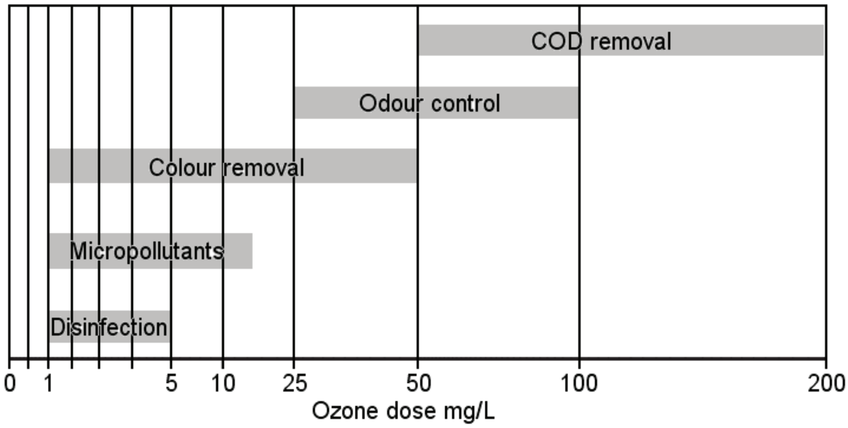

Figure 1. Common ozone doses used in wastewater treatment (Ried et al., 2009)

1.3.1. Ozone in the activated sludge process

Considering the ideas presented previously, a process where ozonation and the activated sludge process are integrated into a single unit was proposed by van Leeuwen *et al.* (2001). The aim of the proposed process is to improve effluent water quality by oxidizing recalcitrant organic compounds into biodegradable intermediates that are then degraded in the same reactor. Although the idea is simple, the implementation is rather complicated as ozone in this integrated process may influence both contaminants and activated sludge.

There are several papers available where the effect of ozone on pure cultures is evaluated. Caravelli *et al.* (2006) described the effect of ozone and chlorine on *Acinetobacter anitratus*, Hunt and Mariñas (1999) evaluated chemical and inactivation kinetics on *Escherichia coli*. The results confirmed the positive effect of ozone on these processes no matter if the purpose is to improve settling properties or to inactivate bacteria. In real applications it is usually not reasonable to assume that there is a pure culture in the reactor, especially in the case of wastewater treatment. Therefore, from a practical point of view, it would make sense to study the effect of ozone on a mixture of bacterial populations – like real activated sludge samples.

As described earlier, wastewater ozonation is a chemisorption process, where the absorption is enhanced by ozone reactive contaminants. It has also been found that particulate matter, like activated sludge in the suspension, may further improve the absorption process as this matter may react with ozone and produce solubilized compounds, although distinguishing the reactions of ozone with sludge and ozone with contaminants is rather difficult (Beenackers and van Swaaji, 1993). As stated by Cesbron *et al.* (2003), activated sludge ozonation is a complicated process and the effect of ozone is mainly determined by wastewater composition and the size distribution of sludge particles (flocks). Even though ozone induced activated sludge lysis may be masked by the soluble fraction in the wastewater (Cesbron *et al.*, 2003), ozone is successfully applied to activated sludge for the control of filamentous bulking and for excess sludge reduction. There are also reports about ozone's positive effect on process performance and purification efficiency when ozone is dosed directly to the activated sludge process (van Leeuwen *et al.*, 2009, Kamenev *et al.*, 2008, Kamiya and Hirotsuji, 1998). This process has not been extensively studied possibly because of the complexity of the ozonation reactions in the integrated process and a firm belief in ozone's strong cell lytic activity.

The effect of hydroxyl radicals on micro-organisms

In oxidation (purification) processes OH radicals play an important role as they are the strongest oxidants found in water (Staehelin and Hoigne, 1985). The effect of OH radicals in the disinfection process is usually considered to be negligible as the ratio of the concentrations of OH radicals to ozone is in the range of 10^{-6} to 10^{-9} (von Gunten, 2003). As suggested by Ireland *et al.* (1993), OH radicals may cause inactivation of bacteria (*Escherichia coli*) only if their concentration is constantly high. In case OH radicals are present, the CT-factor is approximately 10^4 times higher compared to ozonation (Cho *et al.,* 2003). The efficiency of OH radicals is again greatly reduced in the presence of radical scavengers and contaminants that compete for the oxidant, meaning that hydroxyl radicals are relatively inefficient for disinfection purposes.

Excess sludge reduction

Excess activated sludge is considered to be a secondary solid waste. Excess sludge generation is expected to increase as Urban Waste Water Treatment Directive 91/271/EEC requires that most of EU population is served by wastewater treatment facilities. Sewage Sludge Directive 86/278/EEC on the other hand restricts the usage of excess sludge in agricultural applications (Fabiyi *et al.*, 2007). As the disposal of wastes became a critical issue, disintegration technologies for excess sludge reduction within a system were developed. Among other possible candidates such as ultrasonic disintegration (Lajapathi Rai *et al.*, 2004), mechanical, thermal, alkaline and acidic treatment, chlorination, addition of metabolic uncouplers, encouraging growth of predation, and varying operational conditions (concentration of O_2, sludge retention time) (Wei *et al.*, 2003, Liu and Tay, 2001), ozonation stood out as a potential technology with the highest disintegration efficiency (Müller, 2000). All disintegration methods are based on the principle of lysis-cryptic growth. The term "cryptic growth" is used in order to distinguish it from the growth on the substrate in wastewater (Mason *et al.*, 1986).

Sludge ozonation for excess sludge reduction is based on the idea of destruction of active bacteria and formation of soluble and dispersed micro-particles which are then consumed by bacteria. Sludge yield reduction is achieved, as only part of the substrate is used for (re-)growth and the other part is used for maintenance.

Ozone for sludge reduction is usually applied in the recirculation line as direct introduction is considered to be detrimental for the process performance. Therefore no information regarding the influence of direct ozone dosing on the activated sludge process in literature is found. The other common ozone application route for excess sludge reduction is excess sludge ozonation before anaerobic treatment. An activated sludge process coupled with ozonation of a small amount of activated sludge in the recirculation line was first proposed by Yasui and Shibata (1994). Their findings were applied to a full-scale plant which was operated successfully for 10 months with only minor reduction in effluent water quality (Yasui *et al.*, 1996). Based on the verification of the results on a pharmaceutical full-scale wastewater treatment plant, a process named Bioleader® was developed and according to Yasui *et al.*, (2003) about 30 units were installed in Japan until 2002. Another similar process named Biolysis® O was developed by Degremont (Pérez-Elvira *et al.*, 2006). Boehler and Siegrist (2007) reviewed several papers describing ozonation in the recirculation line and concluded that the optimal ozone dose is approximately 50 $mgO_3 \cdot gTSS^{-1}_{treated}$ in the aerobic tank, allowing 25-35% of sludge reduction. The study of Sievers *et al.* (2004) seems to confirm these results. Above that dose disintegration efficiency decreases. Ozone dose needed for 100% of TSS reduction seems to be above 175 $mg \cdot gTSS^{-1}$ (initial TSS in the activated sludge process) (Table 2).

Table 2. Sludge reduction in full-scale applications (Sievers et al., 2004, Chu et al., 2009)

Source	Wastewater type	Period	Ozone dose, $gO_3 \cdot gTSS^{-1}$	TSS reduction, %
Sakai *et al.*, 1997	Municipal	~3 months ~2 months ~3 months	0.133 0.148 0.178	36 77 100
Yasui *et al.*, 1996	Pharmaceutical	10 months	0.165	Up to 95
Vergine *et al.*, 2007	Industrial+ municipal	10 weeks	0.070	39
Sievers *et al.*, 2004	Municipal	-	0.395	Up to 35

Although disintegration technologies are excellent for excess sludge reduction, purification process efficiency in terms of effluent water quality is usually reduced. During long term ozonation an increase in inert COD was observed (Yasui *et al.*, 1996, Huysmans *et al.*, 2001, Deleris *et al.*, 2002, Boehler and Siegrist, 2007). Sakai *et al.* (1997) found that total nitrogen concentration, BOD, phosphorus, and suspended solids in the effluent of the ozonated reactor were slightly higher. Ammonia nitrogen concentration was not affected showing that nitrifying bacteria were not inhibited by ozone. As effluent water of normally operating activated sludge processes usually easily meets all the discharge limits, small deterioration does not permit implementation of the ozone disintegration technology. However, the evaluation of the cost of disintegration technologies has revealed that disintegration methods are economical only in the case of high disposal costs, although ozonation is considered potentially viable in case additional operational problems like foaming and bulking can be reduced (Boehler and Siegrist, 2007).

Control of filamentous bacteria

Main problems related to the proliferation of filamentous organisms that cause operational difficulties are foaming and sludge bulking. There are several articles describing the use of ozone for the control of filamentous bulking (Caravelli *et al*., 2006, Saayman *et al*., 1998, Saayman *et al*., 1996, van Leeuwen and Pretorius, 1988). Ozone serves as an excellent alternative for chlorine, which in wastewater may form trihalomethanes (Saby *et al*., 2002) and cause loss of nitrification and phosphorus removal. As shown by Saayman *et al.* (1996), dosing of ozone into a full-scale activated sludge process (with a dose of 0.36 to 1.42 $\mathrm{mg \cdot gMLSS^{-1} \cdot d^{-1}}$) improves sludge settling properties and phosphorus removal while nitrification and denitrification are not affected. Improvement of sludge settling properties was confirmed in another full-scale experiment conducted by Okouchi *et al.* (1996), whereas the rate of nitrification, as a result of prolonged sludge retention time in the ozonated reactor was increased. Okouchi *et al.* (1996) also showed that ozone is efficient for *Nocardia* caused foam suppression. The significant reduction of the number of filamentous bacteria (Deleris *et al.,* 2002) produces more compact flocks with improved settling properties.

The efficiency of ozone treatment against filamentous bacteria may be considered as a result of several effects:

- hydrophobic nature of cell walls of filamentous bacteria – their tendency to attach on gas bubbles making them more prone to ozone attack as their transport to the gas-water interface is increased;
- greater surface area of filamentous bacteria – increasing the possibility of filamentous bacteria to be attacked by ozone;
- length of filamentous bacteria – makes them more vulnerable as they stretch out from the flock surface.

The effect of ozone on sludge viability

As ozone and other oxidants may affect both flock-forming and filamentous micro-organisms, their dose has to be optimized to avoid reduction of the purification process efficiency by deactivating viable micro-organisms. There are several different approaches to evaluate the effect of an oxidant on sludge.

The most widely used method is the gravimetric measurement of total biomass in the process, expressed as MLSS or MLVSS. This method along with influent and effluent water quality measurement (for example COD) can be used to detect changes in sludge yield caused by the oxidant addition. Microscopic studies are also valuable to determine the changes in the sludge structure – namely changes in the amount of filamentous micro-organisms and flock structure. Many studies related to sludge reduction through oxidant addition have determined the effect of oxidant on activated sludge by measuring changes in soluble chemical oxygen demand (SCOD). These methods give necessary and valuable information about the overall performance but the lag-time is much too great for practical application.

Another important factor affected by the oxidant addition is the microbial metabolic activity or viability. There have been several attempts to evaluate the changes in viability in activated sludge or in certain bacteria populations. The most widely used methods are the plate count test (Yasui and Shibata, 1994, Hunt and Mariñas, 1999), respirometry (oxygen uptake rate OUR) (Paul and Debellefontaine, 2007, Deleris *et al.*, 2002, Kim *et al.*, 1994, Chu *et al.*, 2008, Yasui *et al.*, 1997, Yasui and Shibata, 1994), INT-dehydrogenase (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride) activity (DHA) (Kim *et al.*, 1994, Caravelli *et al.*, 2006), adenosine-5'-triphosphate (ATP) measurement (Gikas and Livingstone, 1997, Kim *et al.*, 1994, Archibald *et al.*, 2001, Arretxe *et al.*, 1997, Patterson *et al.*, 1970), measurement of inhibition of nitrification (Gernaey *et al.*, 1997), fluorescence microscopy (for example fluorescein diacetate measurement) (Jorgensen *et al.*, 1992, Seka *et al.*, 2003), and also direct assessment of viable micro-organisms by flow cytometry (Ziglio *et al.*, 2002, Prorot *et al.*, 2008). Among different methods OUR (and also inhibition of nitrification) and ATP measurements stand out as two least time-consuming methods and OUR is the only method that may be carried out with the instrumentation readily available in every laboratory where aerobic wastewater treatment is studied, i.e. no complicated instrumentation is needed.

During activated sludge ozonation, bacteria are inactivated by means of oxidation reactions. First, cell wall is destroyed, following oxidation of the released substances. The reactions occurring between the bacterial cell and ozone in pure cultures are described as follows: oxidation of lipids and sulfhydryl compounds, followed by decrease in culturability, and the leakage of nucleic acids from the cells (Komanapalli and Lau, 1996). The same reactions take place in the mixture of bacterial populations, although different bacteria may be altered differently. There is also some evidence that molecular ozone may diffuse through the cell membrane, react with cell constituents (Ishizaki *et al.*, 1987), and genetically alter the cytoplasmic constituents (Hamelin and Chung, 1974). It means that ozone does not cause instantaneous cell lysis but the cell is destroyed only after exposure to a certain threshold dose (White, 1999). As shown on *E. coli* by Hunt and Mariñas (1999), cell lysis takes place only when most of the cells are non-viable, suggesting that effluent water quality reduction in the activated sludge process can only take place after prolonged oxidation. It has to be mentioned that the results of activated sludge ozonation have given different results.

As shown by Chu *et al.* (2008), oxygen uptake rate was reduced by 80% at ozone doses of 20 $mgO_3 \cdot gTSS^{-1}$. Yan *et al.* (2009), measured culturability and DNA leakage from bacteria in activated sludge and showed that the same dose reduced culturability 10 times, while DNA was released noticeably only at higher doses. Yan *et al.*, (2009) also pointed out that mainly Gram-negative bacteria with a distinctive morphology of cocci that occur in tetrads, sheets or clusters are more resistant to ozonation and their DNA was not released even at doses higher than 60 $mgO_3 \cdot L^{-1}$. Caravelli *et al.* (2006) have reported that at an ozone dose of 18 mg $O_3 \cdot g^{-1}VSS$, the OUR of the sludge and filamentous bacteria were inhibited by 54 to 60% and by 87%, respectively. According to Dziurla *et al.* (2005), ozone doses leading to a decrease in the OUR range from 1 to 13.6 $mgO_3 \cdot g^{-1}COD_{sludge}$, depending on the sludge tested. Additionally, approximately 13 to 54 $mgO_3 \cdot g^{-1}COD_{sludge}$ (10 to 135 $mgO_3 \cdot g^{-1}MLSS$) was needed to reduce OUR by 50 %. Therefore, as ozone doses needed for sludge viability reduction vary greatly, it can be concluded that the viability reduction by ozone depends on the sludge structure. Intracellular ATP concentration measurements have shown that ozonation of sludge with doses up to 5 $mgO_3 \cdot g^{-1}TSS$ does not influence ATP concentration, while ozone dose of 10 and 20 $mgO_3 \cdot g^{-1}TSS$ will reduce ATP concentration by around 15% and 60%, respectively (Chu *et al.*, 2009).

Nitrifying micro-organisms need longer development time compared to heterotrophic bacteria. Therefore their population is considered to be more threatened by ozone attack. The results of different experiments have shown that contrary to all expectations the viability of nitrifying micro-organisms is not impaired. Di Iaconi *et al.* (2010) studied a combined process where biochemically treated wastewater was ozonated and recirculated back to the aerobic granular biomass system. Their findings showed that even in the presence of ozone, biomass still contained approximately 5% of ammonia oxidizing bacteria and nitrogen removal was not affected. It is also worth mentioning that neither were filamentous bacteria affected. Ozonation of activated sludge in a membrane bioreactor in the recirculation line for zero excess sludge generation showed that ammonia and total nitrogen removal efficiency was even slightly increased (Wang *et al.*, 2008). This improvement is usually attributed to ozonation that produces additional soluble organic carbon needed for denitrification (Boehler and Siegrist, 2004). The lack of influence of ozone on nitrifying bacteria is possibly caused by the overgrowth of nitrifying bacteria by the faster growing heterotrophs. It means that nitrifiers are partly protected in the sludge flock and as compared to heterotrophs, less exposed to ozone (Boehler and Siegrist, 2004).

Dosing of ozone directly into the activated sludge process

Fumitake *et al.* (2006) applied ozone with a dose of 6.7 $mgO_3 \cdot g^{-1}MLSS$ into the activated sludge process treating phenol as a bio-refractory compound and found that purification efficiency was improved through chemical oxidation while microbial activity was not inhibited. Van Leeuwen *et al.* (2009) studied the same integrated process with an ozone dose of 45 and 30 $mg \cdot L^{-1}$wastewater, where target contaminant was non-biodegradable azo dye, Orange II. Their findings showed that ozone dose of 30 $mg \cdot L^{-1}$wastewater did not inhibit bacteria in the activated sludge reactor, whereas at a higher dose purification efficiency deteriorated, suggesting that beneficial carbon consuming bacteria were inhibited. Sankaran *et al.* (2008) used ozone to affect strain of the *Rhizopus oligosporus* fungus carrying out corn-milling wastewater purification. The purification efficiency in terms of soluble COD was affected significantly at doses higher than 45 $mg \cdot L^{-1}$, whereas the highest efficiency was achieved at a dose of 57 $mg \cdot L^{-1}$. Kamenev *et al.* (2008) applied small ozone doses (2 $mgO_3 \cdot L^{-1}$) straight into the activated sludge process treating phenolic wastewater and found that as a result, the specific oxygen uptake was increased by 15-20%. Further increase in dose to 4 $mgO_3 \cdot L^{-1}$ lowered the oxygen uptake rate to the same level as in a conventional activated sludge process.

An effort for excess sludge reduction was made by Kamiya and Hirotsuji (1998) who compared intermittent and continuous ozonation of 2/3 of the suspension in a aerobic tank in a day. Their findings suggest that intermittent ozonation is more efficient by means of excess sludge reduction as ozone concentration in the gas phase is higher. Effluent water quality in terms of DOC was not affected at low ozone doses (5.3 $mgO_3 \cdot gMLSS^{-1} \cdot day^{-1}$) whereas at a higher dose (21.4 $mgO_3 \cdot gMLSS^{-1} \cdot day^{-1}$) slight reduction was measured.

Phosphate and ammonia-nitrogen removal are considered to be more affected by ozone addition. In the case of sludge solubilization, phosphorus is also solubilized, increasing its concentration in effluent water. The problems related to ammonia-nitrogen removal were described previously. The results of the study of van Leeuwen (1988a) showed that when ozone is dosed directly to the activated sludge process, nitrification is not affected even at a dose of 30 $mgO_3 \cdot g^{-1}MLSS \cdot d^{-1}$. Dosing of ozone directly to a pilot scale plant treating synthetic municipal wastewater showed that at an optimal dose of 6 $mgO_3 \cdot L^{-1}$ both ammonia-nitrogen and phosphate removal were improved (van Leeuwen, 1988b).

Based on the results of the described studies it can be concluded that ozone dosed directly to the aerobic tank at low doses does not inhibit the process but rather activates the sludge and improves purification efficiency. It is also noticeable that there is a great variability in optimal doses, ranging from 2 to 57 $mgO_3 \cdot L^{-1}$. This phenomenon is explained by the fact that if ozone is applied directly to the aerobic tank, ozone may attack simultaneously both the activated sludge and the contaminants in wastewater, meaning that there is a competition between soluble and particulate matter (Cesbron *et al.*, 2003). Additionally, the effect of ozone on activated sludge may depend on the properties of the sludge (Kamiya and Hirotsuji, 1998). The mode of ozone dosing also plays its role as during intermittent ozonation the ozone concentration applied in the influent gas phase is higher than that in the continuous mode.

1.4. Aims of the study

Resulting from the findings presented in the literature review it may be concluded that introduction of ozone straight into the activated sludge process is be beneficial for the effluent water quality improvement or for sludge yield reduction.

The aim of the research was to study the mechanism of the integrated process – the activated sludge process (ABO) with activated carbon (AC) addition and ozonation, i.e. ABO/AC/O_3 – and to establish application possibilities for this process. Focus was on the improvement of biochemical purification of oil shale phenolic wastewater and simultaneous reduction of excess sludge generation.

The objectives of the study were as follows:

- to evaluate the impact of ozone on the activated sludge process when ozone is introduced directly to the biomass;
- to detect the optimal ozone dose for excess sludge reduction and to evaluate its dependence on the properties of suspension of activated sludge and wastewater properties;
- to study the impact of activated carbon on the activated sludge process and ozonation (of phenolic wastewater);
- to study the mechanism of the integrated process;
- to develop the dynamic model of the integrated process.

2. EXPERIMENTAL STUDY

The experiments could be divided into three groups. First, the integrated process – ABO with AC addition and ozonation – in a continuous bioreactor was investigated to compare the integrated process with the conventional ABO along with the evaluation of the impact of AC and ozone on sludge settling properties.

Secondly, the binary combinations – ABO with direct introduction of ozone into the biomass and ABO with AC addition– in batch bioreactors were studied to compare the integrated processes with the conventional ABO along with the evaluation of the impact of ozone on the viability of the biomass (based on ATP and OUR measurements). The kinetics of pollutant removal was also evaluated in batch reactors.

Thirdly, the integrated process – ABO with direct introduction of ozone intermittently into the biomass – was studied in continuous reactors. The application possibilities and the optimal ozone dose for simultaneous effluent water quality improvement and sludge yield reduction were established.

In the following section the materials and methods used in the experiments are briefly described.

2.1. Experimental methods and procedures

The following processes were experimentally studied:

i. continuous activated sludge process with the introduction of activated carbon and ozone directly to the bioreactor (ABO/AC/O_3) (Paper I);
ii. batch activated sludge process with the introduction of activated carbon and ozone directly to the bioreactor (batch ABO/AC/O_3) (Paper I) and batch activated sludge process with the introduction of activated carbon directly to the bioreactor (batch ABO/AC) (Paper I);
iii. batch ozonation in the presence of activated carbon (O_3/AC) (Paper V);
iv. batch ozonation of activated sludge suspended in distilled water (AS/O_3) (Papers II, III and IV);
v. batch ozonation of ABO in phenolic wastewater (AS+PW/O_3) (Papers II, III and IV);
vi. continuous activated sludge process with intermittent dosing of ozone directly to the bioreactor (continuous ABO/intermittentO_3) (Paper IV).

Ozonation of wastewater and biochemically purified water in the presence of AC (O_3/AC) showed that no measurable synergetic effect in terms of COD or BOD removal was achieved (Paper V) as the efficiency of the integrated process is equal to the sum of the two sequential processes. Therefore the results of the batch ABO/AC/O_3 processes and batch ABO/AC processes (Paper I) were used to evaluate the effect of dosing of ozone directly to a batch ABO reactor – i.e. batch ABO/O_3 process (Paper III).

Generally, continuous processes can be used to evaluate the efficiency, while batch processes suit better to evaluate the kinetics.

2.1.1. Materials and methods

Wastewater

In all studies synthetic PW was used. Synthetic PW was used instead of real wastewater mainly because:

- the composition of industrial PW depends on the process parameters which vary in time, making it difficult to achieve steady-state conditions in lab-scale units;
- "ageing" of PW – i.e. as a result of oxidation, microbial action, and mass transfer phenomena the properties of PW change in time (during transportation and storage) – synthetic PW may be easily prepared in laboratory at any time needed.

The source of phenols was a product of VKG Oil AS marketed under the name of Rezol that is prepared by removing water soluble phenols from the process water in the dephenolation process. Therefore it may be expected that the composition of real and synthetic PW is relatively similar.

The composition of Rezol used in different studies was slightly different (Table 3), as a phenol fraction from different batches was used. Therefore the composition of PW was slightly changed (Table 4), as also shown in papers I and IV.

Table 3. The composition of Rezol (according to the laboratory of VKG Oil AS)

Compounds	Mass fraction, %	
	Continuous ABO/AC/O_3 (i), batch ABO/AC/O_3 (ii), O_3/AC (iii)	Batch ABO ozonation (v), continuous ABO/intermittentO_3 (vi)
monobasic phenols	1.25	0.06
catechol	-	0.05
3-methylcatechol	-	0.02
4-methylcatechol	-	0.07
resorcinol	5.5	2.7
2-methylresorcinol	2.5	1.0
4-methylresorcinol	1.8	2.4
5-methylresorcinol	49.1	40.4
2,5-dimethylresorcinol	8.0	7.0
5-ethylresorcinol	10.4	10.7
2-methyl-5-ethylresorcinol	1.1	-
4,5-dimethylresorcinol	7.5	7.6
unidentified compounds	12.85	28.0

Table 4. The composition and main parameters of synthetic phenolic wastewater used in the studies

Compound or parameter	Concentration, mg·L^{-1}	
	Continuous ABO/AC/O_3 (i), batch ABO/AC/O_3 (ii), O_3/AC (iii)	Batch ABO ozonation (v), continuous ABO/intermittentO_3 (vi)
Rezol	700	614
$(NH_4)_2SO_4$	365	205
NH_4Cl	156	90
Ethanol	66	90
Methanol	-	90
Isobutanol	300	-
Butyl acetate	265	200
Acetic acid	173	110
$FeCl_3$	0.4	0.4
$MgSO_4$	27	27
$CaCl_2$	124	124
KH_2PO_4	15	15
K_2HPO_4	39	39
Na_2HPO_4	32	32
$COD_{average}$	3110	2290
$BOD_{average}$	1450	1080

2.1.2. Experimental methods

ABO and integrated processes

Continuous ABO/AC/O_3 process (i) (with the ABO process for reference data) (Paper I) was conducted in a laboratory scale unit consisting of two continuous stirred tank reactors (CSTR) (aeration basin 7.5 L, clarifier 2.5 L) and supporting equipment (Figure 2). Both reactors operated in the same hydraulic conditions. Hydraulic retention time (HRT) was five days.

Activated sludge for batch ABO/AC/O_3 and batch ABO/AC processes (ii), batch ABO/O_3 process (iv) and batch AS ozonation process (v) was sampled from a continuous CSTR operating with HRT of two days and solids retention time (SRT) between 9 and 10 days. MLVSS/MLSS was approximately 0.80. Activated sludge used in the experiments was acclimated to wastewater.

Batch ABO/AC/O_3 and batch ABO/AC processes (ii) (Paper I) were studied in the reactors with the working volume of 1 L (without clarifier).

For batch AS ozonation (iv) (Papers II and IV), the AS was separated from the liquid phase and resuspended in distilled water (with necessary nutrients). Ozonation was carried out in the batch mode (described in the section of

Ozonation procedures) – after the certain ozone dose was transferred to the suspension (initial volume of 2 to 2.4 L), 300 to 400 mL of the ozonated suspension was decanted to one of the reactors with the working volume of 1 L (without clarifier).

Batch ABO ozonation process (v) (Papers II and IV) – ozonation was carried out similarly to the batch AS ozonation process (iv).

Continuous ABO/intermittentO_3 process (vi) (Paper IV) was studied in four identical lab-scaled CSTRs (Figure 3). Aeration basins had a volume of 2 litres and clarifiers of 0.3 litres. HRT was maintained for three days and SRT, depending on the applied ozone dose, ranged between 7 and 16.5 days. One of the CSTRs served as a control reactor (conventional ABO), while ozone was intermittently dosed (batch ozonation) at different doses to the other reactors – the suspension was transferred to the ozonation reactor, ozonation was carried out and ozonated suspension was decanted back to the CSTR. This methodology assured the transfer of a precise ozone dose.

All experiments were carried out at room temperature (20±1 °C). Dissolved oxygen concentration in ABO processes was maintained between 2-4 $mg \cdot L^{-1}$ to avoid rate limitation, using compressors (MaximaR or ELITE 799) and ceramic diffusers. Dissolved oxygen concentration was measured with a dissolved oxygen meter (Marvet Junior, Elke Sensor LLC, Estonia) mounted with an electrochemical sensor (HELOX, Elke Sensor LLC, Estonia) (batch processes – ii, iii, iv – and a continuous process with intermittent ozonation – v) or measured and recorded using a computer (i).

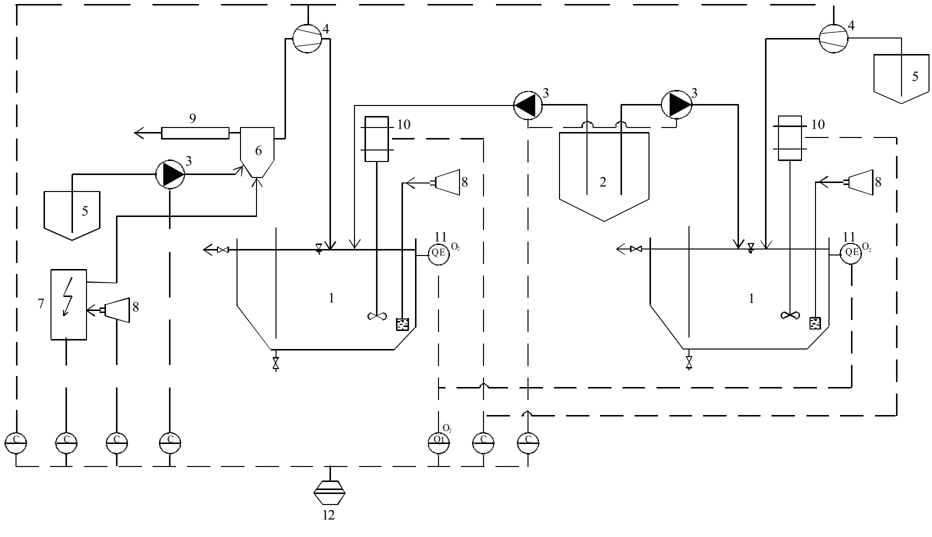

Figure 2. Experimental set-up for the continuous ABO/AC/O_3 process study
1 – aerobic bioreactors, 2 – wastewater container, 3 – peristaltic pumps, 4 – membrane pumps, 5 – distilled water containers, 6 – bubble column, 7 – ozone generator, 8 – air compressors, 9 – residual ozone destruction unit, 10 – agitators, 11 – dissolved oxygen probes, 12 – controlling PC

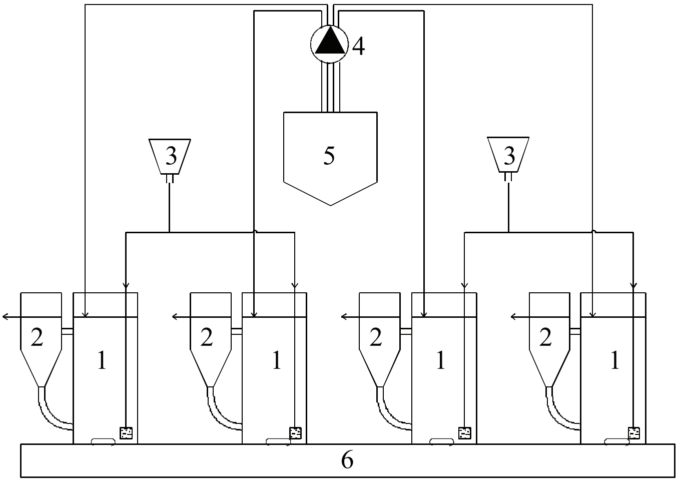

Figure 3. Lab-scaled CSTRs for the study of the continuous ABO/intermittentO_3 process 1 – aeration basins, 2 – clarifiers, 3 – air compressors, 4 – peristaltic pump (four channels), 5 – wastewater container, 6 – magnetic stirrer

Ozonation procedures

Dosing of ozone into continuous ABO/AC/O_3 (i) and into batch ABO/AC/O_3 processes (ii) was performed by saturating distilled water with ozone at room temperature (22±1 °C) and injecting the solution to the aeration basin. Ozone was generated from air using a Clear Water Tech. Inc. P-2000 ozone generator (San Luis Obispo, CA, USA) – i – or using a Trailigaz Labo LO ozone generator (Paris, France) – ii. Dissolved ozone concentration $C_{O3,liq}$, $mgO_3 \cdot L^{-1}$, in distilled water was measured daily using the indigo colorimetric method (Standard Method 4500). The flow rate of the water saturated with ozone L_{liq} ($L \cdot day^{-1}$), and daily wastewater flow rate L_{WW} ($L \cdot day^{-1}$) were also measured. The ozone dose was calculated as follows:

$$Dose\left[\frac{mgO_3}{L}\right] = \frac{C_{O3,liq} \cdot L_{liq}}{L_{WW}}. \tag{2.1}$$

Dosing of ozone during batch AS ozonation (iv), ABO ozonation (v), O_3/AC (iii) and intermittently into ABO processes (vi) was performed in a batch ozonation reactor – a cylindrical glass reactor with a conical bottom and a volume of 2.6 L. Ozone-air mixture was dispersed to the reactor using a slightly off-centered porous glass diffuser located at the lowest point of the reactor. The design allowed the sludge (iv, v, vi) or AC (iii) to be kept suspended. All ozonation experiments were carried out at room temperature (20±1 °C). Ozone was generated from compressed air with a Trailigaz Labo LO ozone generator (Paris, France). The transferred dose was calculated using the following equation:

$$Dose\left[\frac{mgO_3}{L_{sample}}\right]=\frac{\left(C^{i}_{O3,gas}-C^{o}_{O3,gas}\right)\cdot L_{gas}\cdot t}{V_{sample}}, \qquad (2.2)$$

where $C^{i}_{O3,gas}$ is ozone concentration in influent gas ($mgO_3 \cdot L^{-1}$), $C^{o}_{O3,gas}$ is ozone concentration in effluent gas ($mgO_3 \cdot L^{-1}$), L_{gas} is ozone-air gas mixture flow rate ($L \cdot min^{-1}$), t is ozonation time (min), and V_{sample} is the volume of the sample subjected to ozonation (L).

2.1.3. Analyses

Water parameters

To evaluate the performance and changes in reactors different parameters were monitored. COD and BOD_7 were determined according to Standard Methods (5220 D and 5210 B, respectively). pH was measured with a pH-meter (Mettler Toledo SevenEasy, Switzerland). Ammonia nitrogen was measured according to Standard Methods (4500-NH3 F). Turbidity and colour were measured according to the Attenuated Radiation Method and the APHA Platinum-Cobalt Standard Method, respectively, using a Hach DR/2010 spectrophotometer. Polysaccharide content was measured by the phenol-sulfuric acid method, using D-Glucose as a standard (using a spectrophotometer Heλios β) (DuBois *et al.* 1956).

The quantitative detection of oil shale phenols was carried out using a GC with a FID detector (Thermo Electron Corporation Focus GC) or the GC/MS system (Shimadzu). The phenol extraction technique and GC-FID parameters are described in the section of Materials and Methods of Paper I. For the analysis of resorcinols in GC/MS the sample preparation was identical to the GC-FID analysis, except the solvent was exchanged to the hexane-acetone mixture (1:1) before the analysis. 1 µL of sample was automatically (autosampler Shimadzu AOC-20i) injected to the injector. GC/MS (Shimadzu GC-2010) parameters were as follows: injector (operating at splitless mode, sampling time 1 minute) temperature 280 °C, initial oven temperature 60 °C (held for 2 minutes), the temperature was ramped to 160 °C at 10 °C/min, then to 290 °C at 20 °C/min and held for 3 minutes (total program time 21.5 minutes). The sample was separated in the Zebron ZB-5MS (Phenomenex) column (30m x 0.32 mm x 0.25 µm) and was carried to the mass spectrometer (Shimadzu GCMS-QP2010) by carrier gas (Helium 5.0, flow rate 1.9 $mL \cdot min^{-1}$). Interface temperature was set to 280 °C and ion source temperature to 200 °C. The samples were analysed in the full scan mode (m/z 40 to 350).

Toxicity

The acute toxicity of the effluents from the continuous activated sludge process with intermittent ozonation was estimated using *Daphnia magna* as described by Trapido and Veressinina (1999). Briefly, *Daphnia magna* less than 24 hours old were exposed to control water, wastewater and effluents (dilution series from 5% to 90%). The mortality after 24 hours of exposure was measured. The mortality in control water was always zero. The median effective concentration (EC50) values and 95% confidence limits were then calculated using the PROBIT computer program.

Activated sludge

Activated sludge concentration in the processes was measured as MLSS or/and MLVSS. The measurements were performed according to Standard Methods (2540 B and 2540 E, respectively). To determine the sludge volume index (SVI) the settled sludge volume was measured, as described in Standard Methods (2710C).

The results of the analyses were used to calculate the sludge yield and sludge age:

Sludge yield, Y – the mass of the biomass formed per unit of the substrate removed:

$$Y\left[\frac{g\Delta MLSS}{g\Delta SCOD}\right]=\frac{\Delta MLSS_{formed}\cdot V_{reactor}}{L_{WW}\cdot \Delta SCOD}, \tag{2.3}$$

where $\Delta MLSS_{formed}$ is the amount of MLSS formed in a day (gMLSS·day^{-1}), $V_{reactor}$ is the volume of the aeration basin (L), L_{WW} is daily wastewater flow rate (L·day^{-1}) and $\Delta SCOD$ is the amount of substrate as SCOD removed (mgO$_2$·L^{-1}).

Sludge age, Θ – the ratio of the mass of sludge in the aeration basin, $MLSS$, to the mass of sludge leaving the process daily, $MLSS_{out}$ (wasted sludge and effluent solids):

$$\Theta=\frac{MLSS\cdot V_{reactor}}{MLSS_{out}}. \tag{2.4}$$

OUR measurements

OUR measurement procedures are described precisely in Papers II and IV. Before OUR measurements each sample was saturated with oxygen (up to 5 to 7 mg·L^{-1}), pH of the samples was not adjusted. The measurement procedure was carried out in air tight vessels (volume of 100 mL), where dissolved oxygen (DO) concentration was measured by an electrochemical sensor (HELOX, Elke Sensor LLC, Estonia). DO concentration was measured using an oxygen analyzer Marvet Junior (supplied by Elke Sensor LLC, Estonia) and recorded either manually or automatically in MS Excel. Suspension in measuring vessels was stirred using a magnetic stirrer. During the first 2 minutes DO values in the samples were recorded to determine endogenous OUR (OUR_{end}), after that 1 mL of substrate was added. DO was further recorded to determine total OUR

(OUR_{tot}). Exogenous OUR (OUR_{ex}) for sludge activity determination was calculated as follows:

$$OUR_{ex} = OUR_{tot} - OUR_{end}\,, \tag{2.5}$$

where OUR is the dissolved oxygen concentration depletion in the measuring vessel in time ($mgO_2 \cdot L^{-1} \cdot min^{-1}$).

As OUR depends on the concentration of sludge, the results of OUR are expressed by means of specific OUR (SOUR):

$$SOUR\left[\frac{mgO_2}{gMLSS \cdot h}\right] = \frac{OUR}{MLSS}\,. \tag{2.6}$$

Determination of adenosine triphosphate (ATP)

The exact procedure is described in Paper II. Briefly, 2 ml of the samples (activate sludge suspension) was mixed with an equal volume of TCA/EDTA solution (TCA – trichloroacetic acid, EDTA – ethylenediaminetetraacetic acid). The fixed samples were frozen and analysed within four weeks. ATP concentration was determined using luciferin-luciferace reaction mixture (ViaLight®MDA Plus, Lonza, USA). Light emission was measured with a 1253 luminometer (ThermoLabsystem, Helsinki, Finland). Internal calibration with the known ATP concentration was used throughout the procedure.

The results of ATP measurements were expressed as specific ATP (SATP) similarly to SOUR:

$$SATP\left[\frac{mgATP}{gMLSS}\right] = \frac{ATP}{MLSS}\,. \tag{2.7}$$

Microscopy

The changes in sludge structure were monitored using a Nikon microscope Microphot-fx equipped with an Olympus C-5060 digital camera. For an average flock size evaluation, micrographs of a sample and calibrated standard scale were taken (total magnification of 100x, 200x and 400x) and analysed later by measuring the length and breadth of at least 40 randomly chosen flocks in each sample.

2.2. Results and discussion

2.2.1. Continuous ABO/AC/O_3 process (i)

The main aim of the study was to evaluate the performance of the combined process – ABO with AC treatment and ozonation (ABO/AC/O3) – and compare this with the ABO process. The performance was assessed by means of changes in COD, BOD, pH, SVI and MLSS concentration.

The experiments were carried out at the organic loading of the bioreactor at about 620 mgCOD·L^{-1}·day^{-1}. Ozone was dosed directly to the aerobic tank at an approximate dose of 2.45 mgO_3·L^{-1}, since previous studies (Kamenev *et al.*, 2008) showed that dosing of ozone directly to the activated sludge process with the dose of 2 mg/L increased the specific oxygen uptake rate by 15 to 20% (at loading of 2000 mgCOD·L^{-1}·day^{-1}). Activated carbon is used as an efficient aid against shock loadings in the activated sludge process (De Walle and Chian, 1977, De Jonge *et al.*, 1991), as well as a flock-forming backbone to improve flock formation and sludge settling properties (Hart Kwelle, 1992), and improves effluent water quality (Martin *et al.*, 2002).

The experiments started with the addition of activated carbon into a bioreactor that improved SVI in comparison to the reference reactor by 14% (Figure 3, Paper I). However, the results showed that up to the activated carbon dose of 1 g·L^{-1} no measurable effect neither on COD nor on BOD removal was observed: COD removal in ABO/AC and reference reactors was of 80%, and BOD removal of 96.5%. The bio-regeneration of AC was not observed. These results confirm the findings that activated carbon in the integrated process acts simply as an adsorbent (Xiaojian *et al.*, 1991, Sublette *et al.*, 1982, Bornhardt *et al.*, 1997) and once added to the bioreactor the AC is saturated. As well as AC particles are incorporated to the sludge flock as a backbone they are surrounded by the bio-film and mass transfer (adsorption-desorption) is strongly hindered (Widjaja *et al.*, 2004) and AC particles can not further act as adsorbents.

Since the ABO/AC and ABO processes were operating identically (in terms of effluent water quality), ozone was dosed directly to the ABO/AC process. Dosing of ozone improved effluent water quality almost immediately. The results of the ABO/AC/O_3 process compared to the conventional ABO process showed that ozone dosing improved COD removal by ~2.5% and a further decrease in SVI value by ~10% was achieved. BOD removal was not changed. The improved purification efficiency was attributed to increased biomass activity, as AS concentrations in both reactors were equal. However, after approximately a week of operation activated sludge concentration started to decrease, and both COD and BOD removal and also sludge settling properties deteriorated by 10%, 100% and 80%, respectively. The disruption of the process was assumed to be a result of decreased activity of living micro-organisms, which influenced also AS concentration in the reactor. Increase in COD and BOD in effluent water was a result of both reduced activity and increased organic loading caused by ozone induced cell lysis. The deterioration of sludge settling properties was an evidence of the destabilization of activated sludge flocks by ozone.

Conclusions – Continuous ABO/AC/O_3 process (i)

The comparison of the processes proved that the integrated process at an ozone dose of 2.45 mgO_3·L^{-1} and AC dose of 1 g L^{-1} is more effective than the conventional activated sludge process in COD removal. The analysis of the results showed that activated carbon added to the process acts only temporarily as an adsorbent. Therefore, the improved purification efficiency is a result of dosing of ozone straight to the activated sludge process. In the case of long-termed ozone dosing ozone detrimentally affected activated sludge. Thus, it may be concluded that ozonation should be carried out intermittently or only for a short period. It is also essential to evaluate the effect of ozone on the viability of activated sludge. As experiments were carried out only at single ozone dose (2.45 $mgO_3 \cdot L^{-1}$), optimization is necessary to maximize the purification efficiency.

Despite of the negative effects of the long termed ozonation on effluent water quality, it was shown that ozonation may be used to reduce the concentration of activated sludge in a bioreactor.

2.2.2. Kinetics – batch ABO/AC/O_3 and ABO/AC processes (ii)

The aim of the study of batch ABO/AC/O_3 and ABO/AC processes was to evaluate the influence of a small ozone dose on the pollutant removal kinetics during 24 hours. The target pollutants that were monitored were oil shale phenols (resorcinols). Additionally, changes in COD and BOD in time were measured. Ozone dose and activated carbon concentration added to the reactor were 0.57 $mgO_3 \cdot L^{-1}$ and 300 $mg \cdot L^{-1}$, respectively.

The batch experiments revealed that all processes (integrated and reference) were capable of reducing the concentrations of resorcinols below the maximum permissible concentration of 15 mg/L in effluent water (according to the Estonian Water Act /2/) in less than 12 hours (Table 3, Paper I). Final COD removal efficiency in the ABO/AC/O3 process was ~3% higher than in the ABO process (74% and 71%, respectively). COD removal efficiencies in ABO and ABO/AC processes differed by 1% (70% and 72%, respectively) (Figure 6, Paper I). This allowed estimating that ozone addition improved COD removal by 2%. BOD and resorcinol removal efficiencies in all processes were close to 100%.

Additionally, the results of ozonation of wastewater and biochemically purified effluent water in the presence of AC (iii) showed that there is no expected synergetic effect in terms of COD or BOD removal (Paper V) and the efficiency of the integrated process is equal to the sum of the two sequential processes. Therefore the results of the batch ABO/AC/O_3 processes and batch ABO/AC processes (Paper I) were used to evaluate the effect of ozone dosing directly to the batch ABO reactor – i.e. the batch ABO/O_3 process (Paper III). The dibasic oil shale phenol removal and COD removal kinetics are presented in Figure 4.

The final efficiencies of all processes were almost equal, showing that 24 hours was sufficient for degradation of resorcinols and other biochemically degradable compounds. However, the comparison of COD removal efficiencies shows that dosing both activated carbon and ozone straight into the bioreactor improves to some extent degradation of refractory compounds. The differences in process efficiencies were most evident during the first six hours, where in the integrated process the same purification degree as in the ABO process was achieved approximately two times faster. Based on the data, the processes may be ranked in order of the time needed to achieve the purification efficiency as follows: ABO > ABO/AC > ABO/O_3 > ABO/AC/O_3. This was mainly a result of the initial lag-phase in the ABO reactor, caused by the sudden addition of phenolic wastewater at the beginning of the experiments, which is in agreement with the findings of Kahru *et al.* (2000) who measured that resorcinols are relatively toxic to micro-organisms. The lag-phase was the most evident in the case of the 4-methylresorcinol, 2,5-dimethylresorcinol, and 4,5-dimethylresorcinol.

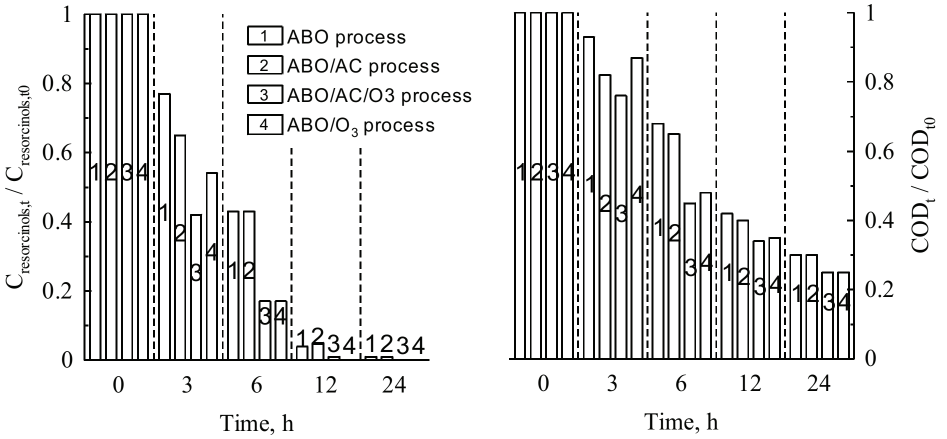

Figure 4. The kinetics of resorcinols (left) and COD removal (right) in ABO and integrated processes

The improved performance of the ABO/AC process over the conventional ABO process could be attributed to the adsorption of toxic compounds that stimulates bacterial activity (Morinaga *et al.* 2003). However, as the results of the experiments in the system with continuous wastewater inflow showed (ABO/AC/O_3 process in Section 2.2.2.), the addition of AC does not give a measurable effect in the purification efficiency.

Dosing of ozone straight into the ABO process further improves the rate of pollutant removal. As the ozone dose used in the experiments was low, the improved purification efficiency can not be related to the oxidation of pollutants by ozone. Therefore improvement in the purification efficiency is assumed to be caused by the increased activity of AS.

Conclusions – kinetics

The positive impact of small ozone doses on the ABO process was confirmed by the kinetics study. It was demonstrated that already a small ozone dose ($0.57\ mgO_3\cdot L^{-1}$) increases the rate of oil shale phenols, COD, and BOD removal, which would reduce the necessary size of the purification equipment by affecting the necessary HRT. In addition, final purification efficiencies in ABO/AC and ABO/AC/O_3 processes were also slightly higher. The improved performance was attributed to activated carbon addition and direct ozone introduction to the biomass, which enhance bioprocess stability against shock loadings and increase the activity of AS.

The conclusions drawn demonstrate again that in order to better understand the impact of ozone on the ABO process, it is essential to evaluate ozone induced changes in the activity of AS.

2.2.3. Batch AS ozonation (AS/O_3) process (iv) and batch ABO ozonation (AS+PW/O_3) process (v)

Ozone may influence the activated sludge in two ways: direct attack of activated sludge or oxidation of contaminants. In the first case the performance of the purification process probably deteriorates, while the opposite effect may be expected in the second case.

The following studies were carried out to evaluate the effect of ozone on sludge viability as well as on effluent water quality under different initial conditions. Namely, the amount of soluble compounds (evaluated as SCOD) in the liquid phase was varied:

- For batch AS ozonation (AS/O_3), AS was separated from the initial suspension (continuous reactor fed with synthetic phenolic wastewater). To separate AS centrifugation the following filtration and washing with distilled water was used. The obtained AS was re-suspended in distilled water containing necessary nutrients (Table 5), giving an initial SCOD of 200 to 245 $mgO_2\cdot L^{-1}$. Added nutrients do not react with ozone or the rate of reaction is very slow, therefore ozone is consumed only by AS (or by lysed cell material).
- In the case of ABO ozonation (AS+PW/O_3) two different suspensions with different initial SCOD values were used:
    1. AS in the initial suspension with an average SCOD of 560 $mgO_2\cdot L^{-1}$ (SCOD ranged from 444 to 647 $mgO_2\cdot L^{-1}$).
    2. half of the liquid phase of the initial suspension was replaced with distilled water. The obtained suspension had an average SCOD of 300 $mgO_2\cdot L^{-1}$ (ranged from 243 to 356 $mgO_2\cdot L^{-1}$).

The applied ozone doses ranged from 2.5 to 86 $mgO_3\cdot L^{-1}$.

Table 5. The composition of water used for re-suspension of AS

Compound	Concentration, mg·L^{-1}
$FeCl_3 \cdot 6H_2O$	0.6
$MgSO_4 \cdot 7H_2O$	56.0
$CaCl_2$	68.8
NH_4Cl	2.7
KH_2PO_4	21.3
K_2HPO_4	54.4
$Na_2HPO_4 \cdot 7H_2O$	83.5
Ethanol	0.08

Results – batch AS ozonation (AS/O_3) (iv)

Initial $SOUR_{end}$ (ozone dose is zero, ABO process as a reference) of the sludge varied between 6 and 9 $mgO_2 \cdot gMLSS^{-1} \cdot h^{-1}$. Depending on the applied ozone dose, the measured $SOUR_{end}$, without the addition of external substrate, increased linearly up to 1.8 times (at the highest ozone dose) compared to initial $SOUR_{end}$ (Figure 1, Paper II). It indicates that an increase in the measured $SOUR_{end}$ values is caused by the substrate generated during ozonation, i.e. ozone penetrates the cell wall and cell constituents are released to the surrounding environment where these are used by the viable sludge as a substrate. An increase in sludge-based solubilized substrate concentration was confirmed by the SCOD measurements and was found to be proportional to the transferred ozone dose (1.6 $gCOD \cdot gO_3^{-1}$) (Figure 4, Paper II). The soluble compounds formed during ozonation of AS (AS/O_3) appeared to resist further oxidation by ozone, as the increased SCOD did not influence the activity reduction (which was reduced by 61 to 72% at ozone dose of 86 $mgO_3 \cdot L^{-1}$). Sludge solubilization did not cause practically any changes in pH, although NO_3-N in the liquid phase was detected at higher ozone doses (Figure 7, Paper II). It is also important to note that the sludge based SCOD appears to be biodegradable, as shown by the increased $SOUR_{end}$ value.

To determine the relative changes in the amount of viable sludge, $SOUR_{ex}$ and SATP concentration were measured. As both methods are well known, they are widely used for viability detection in AS. However, the literature survey revealed that there is no information regarding the effect of ozone on changes in OUR_{ex} versus changes in ATP. Additionally, there are contradictory results regarding the correlation between OUR and ATP in AS. Therefore the applicability of the methods for viability detection during the AS and ABO process ozonation was evaluated.

Initial $SOUR_{ex}$ ranged from 68 to 84 $mgO_2 \cdot gMLSS^{-1} \cdot h^{-1}$ and SATP concentration ranged from 1.73 to 2.10 $mgATP \cdot gMLSS^{-1}$ (ABO process). During ozonation, $SOUR_{ex}$ as well as SATP progressively decreased, meaning that inactivation of the biomass started immediately after ozone addition. An ozone dose of 55 $mgO_3 \cdot L^{-1}$ lowered relative $SOUR_{ex}$ by 72% and relative SATP by 61% (Figure 1a, Paper IV and Figure 5, Paper II, respectively). Accordingly, the relationship was found to be linear between ATP and OUR (Figure 6, Paper II) and both methods seem to be applicable for viability detection, although ATP showed less variability. The higher scattering of the results of OUR measurements is probably caused by the mass transfer limitations, as oxygen and external substrate must diffuse into the flock surface before they are consumed. The results reveal that a relatively low ozone dose is needed to deactivate the sludge. According to the trends of the decrease in relative SATP and $SOUR_{ex}$, it appears that OUR is more sensitive to viability detection, although the difference is in the range of 95% confidence limit. This difference is explained by the fact that the metabolic level of the survived micro-organisms is low and they do not consume oxygen.

Results – batch ABO ozonation (AS+PW/O_3) (v)

In the experiments an average initial concentration of the activated sludge expressed as MLSS was of 2.09 (1.94 to 2.50) $g \cdot L^{-1}$ and as MLVSS was of 1.57 (1.45 to 1.86) $g \cdot L^{-1}$, giving the MLVSS/MLSS ratio of 0.75. An average initial COD of wastewater was as high as 525 $mgO_2 \cdot L^{-1}$.

Initial $SOUR_{end}$, $SOUR_{ex}$ and SATP values (in ABO process) in all replication experiments were almost constant. $SOUR_{ex}$ ranged from 51 to 54 $mgO_2 \cdot gMLSS^{-1} \cdot h^{-1}$, $SOUR_{end}$ from 11 to 16 $mgO_2 \cdot gMLSS^{-1} \cdot h^{-1}$ and SATP from 1.91 to 2.03 $mgATP \cdot gMLSS^{-1}$. Compared to the experiments of AS ozonation (AS/O_3), the behaviour of the measured parameters during ABO ozonation (AS+PW/O_3) is different. The larger the quantity of compounds that will deplete most of the ozone before it reacts with sludge (expressed as SCOD), the more ozone must be dosed for activity reduction. According to OUR and ATP measurements, ABO ozonation (AS+PW/O_3) up to the ozone dose of 86 $mgO_3 \cdot L^{-1}$ had only small effect on the viability of sludge (Figure Ia, Paper IV) – OUR was reduced by 13%, while ATP was not affected. When AS was in a suspension where SCOD was approximately two times lower compared to initial suspension, an ozone dose of 86 $mgO_3 \cdot L^{-1}$ reduced OUR by 35% (Figure Ia, Paper IV). The absence of remarkable OUR and ATP reduction indicates the occurrence of fast reactions between ozone and dissolved compounds in water, i.e. ozone is depleted before it can alter the viability of sludge.

During AS ozonation (AS/O_3) an increase in SCOD was detected, ABO ozonation (AS+PW/O_3) on the contrary lowered an average SCOD value by as much as 85 $mgO_2 \cdot L^{-1}$ (15.6%) (Figure 8a, Paper II). The results are in good agreement with the results obtained when phenolic wastewater was subjected to ozone treatment: ozone dose of 83 $mgO_3 \cdot L^{-1}$ reduced COD value by 87 $mgO_2 \cdot L^{-1}$. Ozonation also slightly reduced the pH of the suspension, which was also detected during phenolic wastewater ozonation, suggesting that by-products are formed. The concentration of NH_4^+-N and NO_3^--N did not change as the ozone dose was increased. The findings indicate that COD reduction is mainly achieved through the action of ozone on wastewater and soluble fraction in wastewater is not increased. It also shows that in order to reduce the excess sludge production, a relatively large ozone dose is needed.

Conclusions – batch AS (iv) and ABO ozonation (v)

The studies showed that the effect of ozone on activated sludge depends on the fraction of ozone reactive compounds in the liquid phase (effluent water, expressed as SCOD). The results clearly demonstrated that in the case of ozonation of AS in distilled water (AS/O_3) the activity inhibition was immediate and fast. Ozonation of AS in wastewater (AS+PW/O_3) caused the reduction of the rate of inhibition – the activity of AS was only slightly reduced up to the ozone dose of 86 $mgO_3 \cdot L^{-1}$, while SCOD reduction at the same dose was remarkable (~16%).

Changes in sludge activity were ascertained by both ATP and OUR measurements. Although OUR is more strongly affected by ozone (the sensitivity is higher), ATP may be considered slightly more accurate as the deviation between the parallel measurements is smaller.

The results of batch ozonation are useful for the evaluation of the possible impact of ozone on AS. However, batch experiments usually do not describe adequately processes in continuous units (they only show trends) and some changes are immeasurably low during batch processes.

Considering the results of the continuous ABO/AC/O_3 process, as well as the results obtained during batch AS and batch ABO ozonation experiments, further studies were conducted by intermittently dosing ozone at different doses (up to 70 $mgO_3 \cdot L^{-1}$) straight into the continuous ABO process.

2.2.4. Continuous ABO/intermittentO_3 process (vi)

The studies were carried out to evaluate the impact of intermittent ozonation on sludge yield and effluent water parameters in the ABO process and to establish application possibilities for the integrated process.

The main problem in the purification of oil shale phenolic wastewaters is the toxicity of the phenols that has caused several related problems, like high concentration of suspended solids (turbidity) and frequently also elevated COD of biochemically treated effluent water. Additionally, the effluent water is highly coloured. These problems were also seen in the current study and therefore, the following water parameters were determined: toxicity, turbidity, colour, total COD (TCOD), SCOD, concentration of resorcinols. As ozone influences AS, its properties, like OUR, ATP and also the concentration of MLSS for sludge yield evaluation were measured.

Effluent water quality

Intermittent ozonation, carried out daily, was found to cause several positive changes in effluent water. The most noticeable of those was the reduction of turbidity and colour. Turbidity reduction was also the main reason for remarkable decrease in total COD (TCOD, unfiltered sample), although the latter was also slightly affected by the changes in SCOD. An average TCOD in ABO processes was 598 $mg{\cdot}L^{-1}$, while the lowest and highest values were 525 $mg{\cdot}L^{-1}$ and 681 $mg{\cdot}L^{-1}$, respectively. The highest ozone dose introduced to the ABO process improved the effluent water quality in terms of TCOD by as much as 35.5±3.6% (TCOD of effluent water 385±8 $mg{\cdot}L^{-1}$), compared to the conventional ABO process (Figure 6a, Paper IV). At the same dose, the turbidity was reduced by 51±1% (Figure 4, Paper IV). The highest turbidity reduction was measured at an ozone dose of 30 $mgO_3{\cdot}L^{-1}{\cdot}day^{-1}$ by 55.6±2.8% compared to the ABO process (an average turbidity in the ABO process was 325±18 FAU), indicating that further increase in the ozone dose above 30 $mgO_3{\cdot}L^{-1}{\cdot}day^{-1}$ is not reasonable. The turbidity was mainly caused by the non-settleable dispersed small filamentous organisms (Figure 5), which is not unusual in the ABO processes treating phenolic wastewaters (Galil *et al.*, 1988). The identification of these organisms, carried out by a microscope showed that they are possibly *Haliscomenobacter hydrossis* often found in the activated sludge process used where phenolic wastewater is treated. Additionally, the small sludge flocks contributed to high turbidity as they were carried out by the effluent water leaving the reactor.

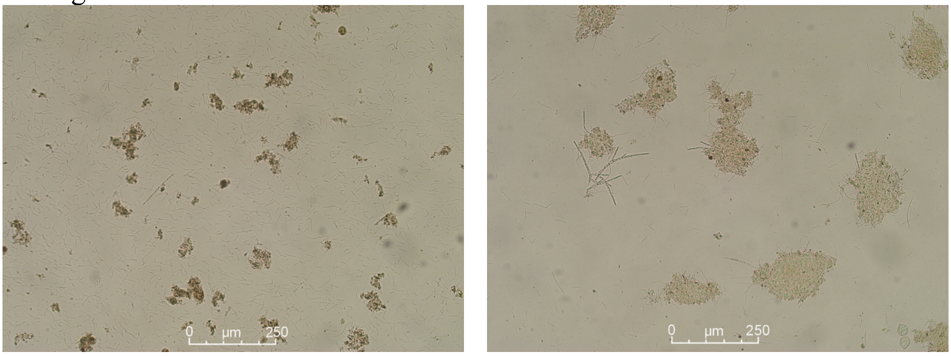

Figure 5. 100x micrographs of non-ozonated (left) and ozonated sludge (right, at dose of 70 $mgO_3{\cdot}L^{-1}{\cdot}day^{-1}$).

As a result of the intermittent ozonation, colour of the effluent water was reduced from initial value of 390±23 Pt-Co (in the ABO process) to 209±5 Pt-Co at the ozone dose of 70 $mgO_3{\cdot}L^{-1}{\cdot}day^{-1}$. These results are comparable with post-ozonation of the effluent from the ABO process and may be considered as an expected result, as colour forming compounds contain unsaturated bonds that are prone to ozone attack.

SCOD values in the ABO process were measured to range from 239 to

323 mg·L^{-1}. It is important to note that up to the ozone dose of 70 mgO$_3$·L^{-1}·day^{-1}, the SCOD removal efficiency was not affected detrimentally. The trends in changes of the SCOD suggest that intermittent ozonation even reduces SCOD by approximately 10%. As demonstrated by the batch AS and ABO ozonation experiments (Section 2.2.3.), the formation of SCOD depends on the water matrix, and in the case of ozonation of ABO suspension, ozone is assumed to be mostly depleted by the soluble compounds in the liquid phase (SCOD). Ozonation of soluble compounds was confirmed by measuring the concentrations of the target oil shale phenolic compounds in the effluent water. The results showed that the concentration of oil shale phenols in the effluent water was reduced as a result of ozone dosing straight into the bioreactor by 2.5 (0.87 mg·L^{-1}) and 4 times (0.53 mg·L^{-1}) at the ozone doses of 30 and 70 mgO$_3$·L^{-1}·day^{-1}, respectively, compared to the ABO process (2.16 mg·L^{-1}) (Figure 6). The comparison of the chromatograms and SCOD values of the effluents from different reactors also shows that even if the AS is ozonolyzed, the soluble cell content and dispersed matter released to the bulk solution are adhered to flocks or consumed by the viable AS. Therefore no measurable accumulation of refractory compounds could be detected.

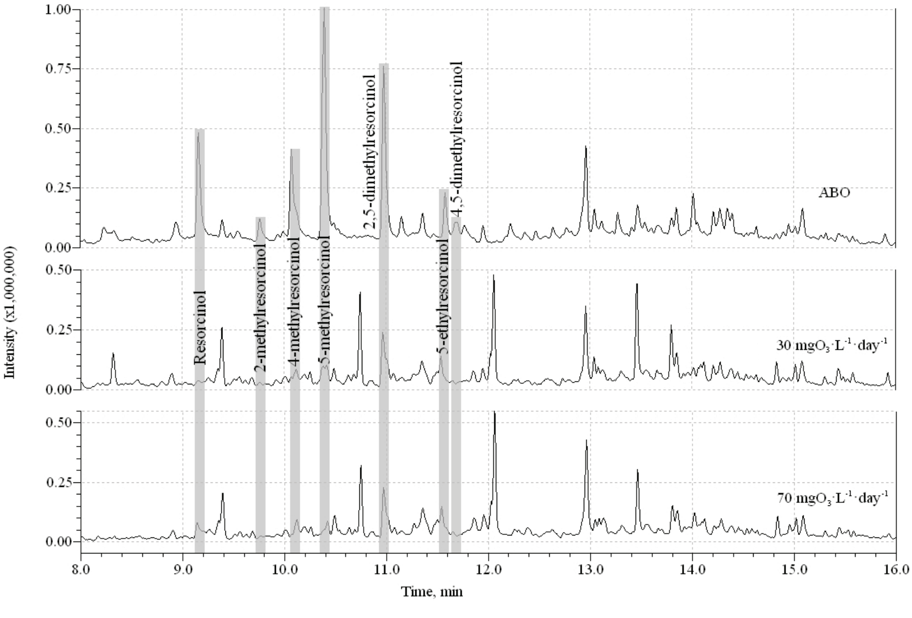

Figure 6. The comparison of the removal of resorcinols in ABO and in integrated processes (ozone doses 30 and 70 mgO$_3$·L^{-1}·day^{-1})

The reduction of pH during the ozonation of oil shale phenolic wastewater is well known, indicating the formation of more oxidized (readily biodegradable) compounds. No effect of different ozone doses on the pH of effluent water in the current study could be detected, showing that ozone oxidation by-products are rapidly consumed or the changes are too small to make any measurable difference.

Toxicity

Phenols are widely known for their high toxicity. Therefore their emission to the nature is prohibited or maximum permissible concentrations in the effluent waters strictly specified. The toxicities of different oil shale phenolic effluents are also ascertained (Kahru *et al.*, 2000, Trapido and Veressinina, 1999). The toxicity of these effluents may significantly affect the quality and also the ecology of the receiving waters. The toxicity of the phenolic wastewater (PW) expressed as a median effective concentration EC_{50} (and 95% confidence limit) presented in Figure 7 was measured to be 2.0% (with 95% confidence limit 1.6-2.6%) (Table 2, Paper IV). This was considered to be a result of relatively high concentration of phenols in the wastewater (the sum of the concentrations of detected resorcinols was of 487 $mg\cdot L^{-1}$) (Figure 8).

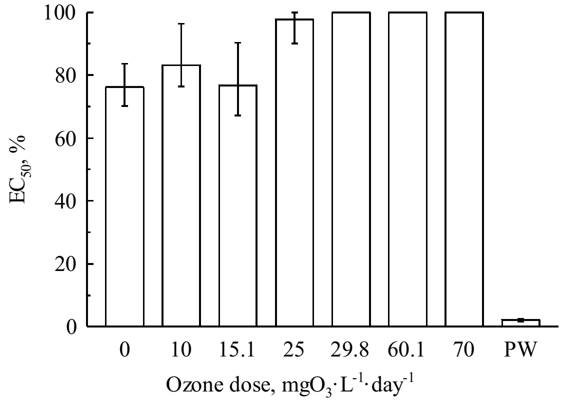

Figure 7. The detoxicification of the phenolic wastewater in the activated sludge process (ozone dose is 0) and in integrated processes (ozone doses ranging from 10 to 70 $mgO_3\cdot L^{-1}\cdot day^{-1}$)

As shown by Kahru *et al.* (2000), the activated sludge process is feasible for the detoxification of oil shale phenolic wastewaters. They also found 2,5-dimethylresorcinol and the mixture of resorcinols (2,5-dimethyl-, 5-methylresorcinol and resorcinol) to be most resistant to detoxification. The results of the study of kinetics (Paper I) as well as the current study are in accordance with these results, as the total concentration of detected resorcinols and the toxicity of the effluent from the conventional activated sludge process was reduced to 2.16 $mg\cdot L^{-1}$ (Figure 8) and 76.1% (70.2-83.6%), respectively. However, total detoxification was not achieved. Intermittent ozonation, at the ozone dose of 25 $mgO_3\cdot L^{-1}\cdot day^{-1}$, on the other hand allowed the effluent toxicity to be reduced to 97.8% (90.0-100). At the dose of 30 $mgO_3\cdot L^{-1}\cdot day^{-1}$, the effluent water was totally detoxified. This was probably a result of direct reactions between ozone and phenolic compounds. Trapido and Veressinina (1999) have also shown that in the case of ozonation of 5-methylresorcinol and resorcinol, the consumed ozone dose of approximately 25 $mgO_3 \cdot L^{-1}$ is sufficient for their total detoxification (at an initial concentration of 0.4 mM). Therefore in the case of purification of phenolic wastewater, dosing of ozone directly to the ABO process is useful not only for turbidity but also for toxicity reduction.

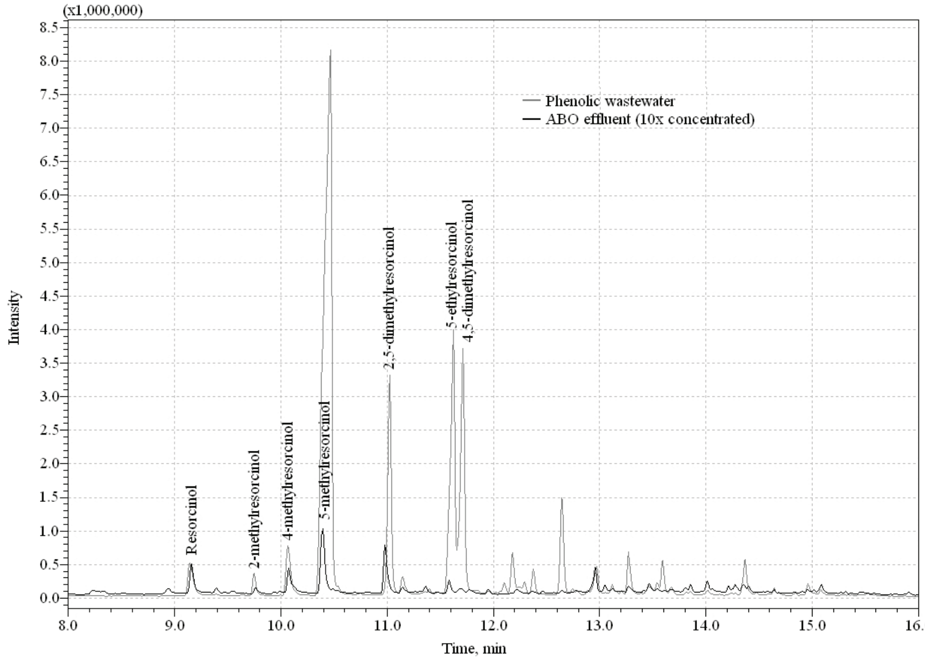

Figure 8. Chromatograms of phenolic wastewater and effluent from the conventional ABO process

Activated sludge properties

To determine the effect of intermittent ozonation on AS, the AS samples from the ABO process and from integrated processes were microscopically examined, and the viability of the sludge and sludge yield were measured.

Microscopic examination of AS from the ABO process showed that sludge flocks are mostly small (pinpoint flocks) with an average size of 44(±18)·81(±31) μm (Table 1, Paper IV). Additionally, the AS contained relatively few ciliates (protozoa) and rotifers (metazoa). Ozonation seemed to have a positive impact on sludge properties in terms of flock size (as can be seen in Figure 5) and the abundance of protozoa and metazoa. At an ozone dose of 10 $mgO_3 \cdot L^{-1} \cdot day^{-1}$, an average flock was measured to be with the length of 102±58 μm and breadth of 52±25 μm and there were noticeably more rotifers in the AS. As rotifers are used for toxicity testing (Toussaint *et al.*, 1995), the reduced toxicity possibly contributed to the increase in their number. The abundance of metazoa and protozoa was considered to be the main reason for effluent quality improvement in terms of turbidity reduction, as they feed on activated sludge flocks (bacteria) and non-soluble dispersed particles with the size smaller than 10 μm and rotifers produce the fecal pellets consisting of an undigested material, which forms the "backbone" of new flocks. This dispersed matter was generated during the ozonolysis of filamentous organisms with the initial length in the range of 20 to 70 μm. As the ozone dose was increased the size of the sludge flocks was also increased reaching up to 69(±18)·135(±35) μm at the ozone dose of 70 $mgO_3 \cdot L^{-1} \cdot day^{-1}$. Other factors that were considered to affect the turbidity reduction were oxygen and polysaccharide concentrations. During intermittent sludge ozonation oxygen concentration in the water phase temporarily increased over 30 $mg \cdot L^{-1}$. According to Gerardi (2002), in an ABO process the sludge settling properties are improved if oxygen limitation is avoided. Polysaccharides are considered essential to form a settling sludge flock (Zhang *et al.*, 1998). In the ABO process an average polysaccharide concentration in AS was found to be 23.0±1.6 $mgGlu \cdot g^{-1}MLSS$. When the ozone dose was increased, polysaccharide concentration was also increased, reaching up to 53.2±3.0 $mgGlu \cdot g^{-1}MLSS$ at the ozone dose of 70 $mgO_3 \cdot L^{-1} \cdot day^{-1}$.

The co-effect of ozonation and increased number of metazoa and protozoa caused changes in the sludge yield. In the ABO process an average sludge yield ranged from 0.16 to 0.22 $gMLSS \cdot g^{-1}COD$. As shown in Figure 9, the yield gradually decreased (by ~56%) up to the ozone dose of 30 $mgO_3 \cdot L^{-1} \cdot day^{-1}$ and then almost levelled off (Figure 3, Paper IV). In fact, investigations into bacterial predation have indicated that sludge yield is considerably lowered in the presence of large numbers of protozoa and metazoa (Mayhew and Stephenson, 1997). Despite the fact that the sludge yield was lowered (at the ozone dose of ~25 $mgO_3 \cdot L^{-1} \cdot day^{-1}$ by 44.3±2.8% and at the dose of 70 $mgO_3 \cdot L^{-1} \cdot day^{-1}$ by 53.4±4.1%), the amount of sludge in the integrated processes, compared to the ABO process, was actually slightly increased (at the ozone dose of ~25 $mgO_3 \cdot L^{-1} \cdot day^{-1}$ by 9.1±6.6% and at the dose of 70 $mgO_3 \cdot L^{-1} \cdot day^{-1}$ by 14.0±7.2%) (Figure 5, Paper IV). As a consequence, sludge age increased from ~7 days for non-ozonated sludge up to 16.5 days at the ozone dose of 70 $mgO_3 \cdot L^{-1} \cdot day^{-1}$. These results are in agreement with the knowledge that increased sludge age forms the basis for the development of rotifers, which unlike protozoans, are generally more abundant in processes with long sludge ages (Gray, 2004). Additionally, as the sludge age is increased, also the polysaccharide concentration is increased (Chu *et al.*, 2009).

The viability of AS was evaluated mainly by measuring $SOUR_{ex}$ (Figure 10). An average initial $SOUR_{ex}$ was of 39 $mgO_2 \cdot gMLSS^{-1} \cdot h^{-1}$. Contrary to the batch ABO and AS ozonation, an increase in the ozone dose up to ~20 $mgO_3 \cdot L^{-1} \cdot day^{-1}$ increased sludge activity by 20% compared to the control (ABO) sludge. Higher ozone doses (up to 70 $mg \cdot L^{-1} \cdot day^{-1}$) gradually reduced the $SOUR_{ex}$ to 60% of its initial value, indicating that destruction of active biomass was initiated. The difference between the batch and the continuous experiments is probably caused by the development of AS favoured by the environmental conditions in the integrated process. The batch experiments do not indicate these changes as the adaption of AS to changed environmental conditions takes some time.

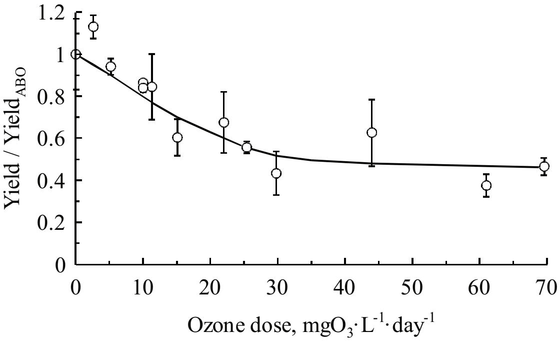

Figure 9. Changes in the relative sludge yield in integrated processes

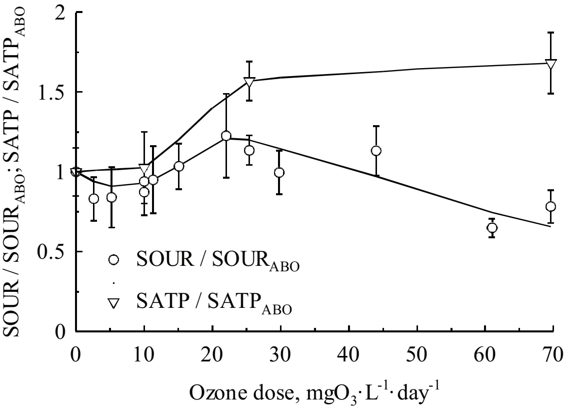

Figure 10. Ozone induced changes in the activity of activated sludge in integrated processes compared to the activity in tha activated sludge process

A major difference between the ozonation of batch ABO and the intermittent ozonation of continuous ABO processes was revealed as ATP concentration in several AS samples (non-ozonated, 10, 25 and 70 mgO$_3$·L^{-1}·day^{-1}) was measured. Compared to batch ozonation results, the measured SATP values were very low (ranging from 0.090 to 0.220 mgATP·g^{-1}MLVSS), suggesting that the actual number of viable micro-organisms is relatively low (during batch ozonation studies SATP in non-ozonated sludge samples ranged from 2.30 to 2.80 mgATP·g^{-1}MLVSS). Up to the ozone dose of 25 mgO$_3$·L^{-1}·day^{-1} the correlation between ATP and OUR measurements was good, as could be predicted by the results of the previous batch studies. As the ozone dose was further increased up to 70 mgO$_3$·L^{-1}·day^{-1}, remarkable deviation between the results of OUR and ATP measurements could be observed, namely the ATP concentration was equal or even slightly higher than at the ozone dose of 25 $mgO_3 \cdot L^{-1} \cdot day^{-1}$. A possible explanation for this is the spore formation by some of the micro-organisms to survive the oxidative stress caused by ozonation. Although with the concentration lower than respiring bacteria, spores still contain ATP, but they do not consume oxygen (Spencer and Powell, 1952, Venkateswaran *et al.*, 2003). Also, as stated in Gray (2004), the presence of large organisms, especially protozoa and rotifers, makes ATP measurements less useful as a measure of bacterial viability.

Conclusions – Continuous ABO/intermittentO₃ process (vi)

ABO with intermittent ozonation was proved to be a reasonable process for the purification of PW as it allowed simultaneous reduction of toxicity, SCOD, TCOD, colour of effluent water and sludge yield.

The optimal ozone dose for the current system was established to be approximately 30 $mgO_3 \cdot L^{-1} \cdot day^{-1}$. At this optimal ozone dose the AS showed the highest activity while the effluent water quality in terms of SCOD was the lowest and the sludge yield was lowered by ~56%. At this dose the effluent water was also non-toxic and the concentration of resorcinols was reduced to 0.87 $mg \cdot L^{-1}$ (2.16 $mg \cdot L^{-1}$ in ABO process). Higher ozone doses further improved the effluent water quality in terms of phenols, TCOD and colour removal, while SCOD was slightly increased and $SOUR_{ex}$ was reduced, indicating the destruction of the biomass and inhibition of bacterial activity.

The comparison of the results of batch and continuous studies showed that batch studies are useful but their usage is limited to chemical oxidation, as changes in biochemical purification processes depend on the development of AS.

The results demonstrate that the activated sludge process with intermittent ozonation could be a useful approach for simultaneous effluent water quality improvement and sludge yield reduction in the purification of phenolic wastewater. These results must be attributed to ozone that initiated the changes in the integrated process.

2.2.5. Modelling of the activated sludge process with intermittent dosing of ozone into a bioreactor

A dynamic model of the activated sludge process with intermittent ozonation was composed. The model consists of differential equations of biochemical reaction kinetics and mass balances. It enables ozone induced changes to be calculated in activated sludge concentration in a bioreactor and substrated concentration (SCOD) in the effluent water if ozone is dosed directly to the bioreactor. The biochemical reaction kinetics is described with the Michaelis-Menten equation.

Dynamic model

In order to describe the changes in effluent water quality (in terms of SCOD) and in activated sludge concentration (as MLSS) in CSTR (Figure 11) the dynamic model was composed.

The following assumptions were made:

- the volumetric flow rate of influent and effluent of the bioreactor are equal, $Q = Q_{in} = Q_{out}$;
- the activated sludge and the substrate are completely mixed;
- the concentration of the substrate in the CSTR is equal to the SCOD of the effluent water, S;
- oxygen concentration does not limit biochemical processes;
- biochemical reaction kinetics follows the Michaelis-Menten equation (1.2):

$$v = \frac{v_{\max} S}{K_M + S}$$

- the excess activated sludge was considered to be removed continuously (with an effluent stream) although small amounts were also removed periodically (sampling).

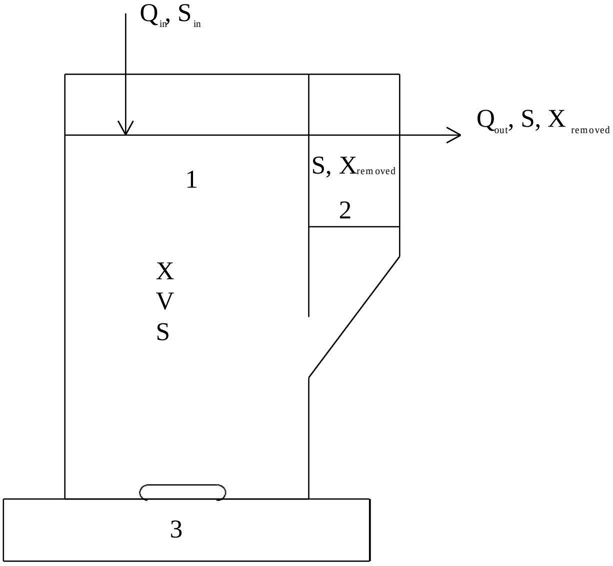

Figure 11. Activated sludge reactor
1 – aeration basin, 2 – clarifier, 3 – magnetic stirrer

The following independent parameters were taken into account:

- volume of the CSTR, V;
- biomass concentration expressed as MLSS, X;

- substrate concentration in PW expressed as SCOD of the PW, S_{in};

The model consists of the following differential equations:

Activated sludge mass balance

$$\frac{dX}{dt} = -X_{removed} + vY_{XS}X - k_d X \ , \tag{2.8}$$

where $vY_{XS}X$ is the amount of activated sludge generated in time.

Substrate mass balance in the CSTR

$$\frac{dS}{dt} = \frac{Q(S_{in} - S)}{V} - vX \ . \tag{2.9}$$

The influence of ozone on the activated sludge process was taken into account by an empirical ozone impact coefficient k, which was introduced to the Michaelis-Menten equation to express the ozone impact on the kinetics of the continuous ABO process with intermittent ozonation. The differential equations were modified accordingly:

$$\frac{dX}{dt} = -X_{removed} + (1+k)vY_{XS}X - k_d X \quad \text{and} \tag{2.10}$$

$$\frac{dS}{dt} = \frac{Q(S_{in} - S)}{V} - (1+k)vX \ . \tag{2.11}$$

Estimation of parameters and process simulation

As a starting point to estimate the required parameters the data obtained from the study of the kinetics of the ABO process (ii, Paper I) was used. The values of the initial parameters were evaluated to be in the range of:

- v_{max} = 0.04 to 0.15 h^{-1}.
- K_M = 460 to 1000 $mg \cdot L^{-1}$.

The range for Y_{XS} was obtained from the continuous ABO/intermittentO_3 process study (vi, Paper IV)

- Y_{XS} = 0.19 to 0.25.

The value of the biomass decay coefficient k_d was obtained from the literature (Grady, 1999):

- k_d = 0.0025 h^{-1}.

To estimate the values of the parameters in the model, the non-linear constrained global optimization method with random search developed by Palosaari *et al.* (1986) was used:

$$F = 100\sum_{i=1}^{n}\left(\left|\frac{S^{exp} - S^{calc}}{S^{exp}}\right| + \left|\frac{X^{exp} - X^{calc}}{X^{exp}}\right|\right), \tag{2.12}$$

where n is the number of experiments, the superscripts *exp* and *calc* denote experimental and calculated values, respectively.

The coefficients were evaluated so that the sum of the relative differences between the experimental data (the performance of the ABO process – data obtained during the continuous ABO/intermittentO_3 process study (vi, Paper IV)) and the simulated data was minimized.

The obtained values for the activated sludge process fed with PW as a substrate are as follows: $v_{max} = 0.0927$ h^{-1}, $K_M = 998$ mg·L^{-1}, and $Y_{XS} = 0.252$.

To estimate the coefficient of ozone impact the same optimization method and the data of the continuous ABO/intermittentO_3 process study (vi, Paper IV) were used. The effect of the ozone dose on the ozone impact coefficient is presented in Figure 12.

At ozone doses up to ~30 mgO_3·L^{-1}·day^{-1}, the ozone impact coefficient increases with an increasing ozone dose and then begins to decrease and becomes negative at an approximate ozone dose of 45 mgO_3·L^{-1}·day^{-1}. These results are in noticeable accordance with the experimental results. At low ozone doses the positive value of the ozone impact coefficient correlates with the improved purification efficiency (in terms of SCOD removal, Figure 6b, Paper IV) and increased activated sludge activity (expressed as $SOUR_{ex}$, Figure 2, Paper IV). At higher doses (more than ~30 mgO_3·L^{-1}·day^{-1}) the decrease in the value of the ozone impact coefficient also correlates with the decreased SCOD removal and decreased sludge activity. Therefore the ozone impact coefficient may be used to describe both promoting and inhibiting effects of ozone.

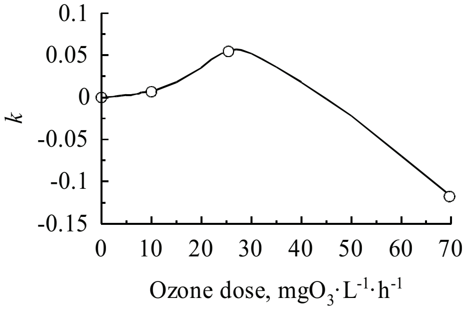

Figure 12. Influence of ozone doses on the ozone impact coefficient

For the simulation the differential equations must be solved using estimated parameters. In Table 6 the experimental and simulated concentrations of S (SCOD) and biomass X are presented. The concentrations were calculated as an average of the values measured and calculated during the steady state period.

The model was used to simulate the response of the activated sludge process on intermittent ozonation. The dynamic response of the concentration of the substrate (SCOD) to changed operational conditions is presented in Figure 13. As it can be seen, the introduction of the ozone impact coefficient into the model enables improved accuracy of the prediction of SCOD removal.

Table 6. Experimental and simulated concentrations of SCOD in the effluent water and biomass concentrations in the bioreactors

Ozone dose, $mgO_3 \cdot L^{-1} \cdot day^{-1}$	Experimental			Simulated	
	S_{in}, $mg \cdot L^{-1}$	S, $mg \cdot L^{-1}$	X, $g \cdot L^{-1}$	S, $mg \cdot L^{-1}$	X, $g \cdot L^{-1}$
0	2240	248	1.25	250	1.11
10	2240	241	1.24	238	1.12
25	2240	250	1.37	222	1.23
70	2240	264	1.42	255	1.21

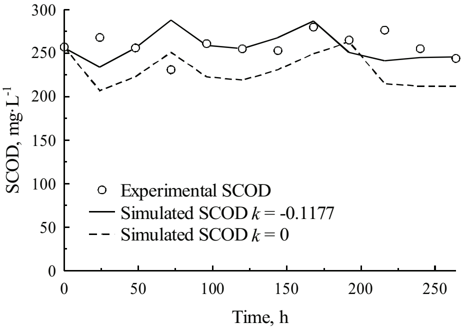

Figure 13. Changes in the substrate concentration caused by the intermittent ozonation of the activated sludge process (at the ozone dose of 70 $mgO_3 \cdot L^{-1} \cdot day^{-1}$)

3. CONCLUSIONS

A novel integrated process where ozone and activated carbon are dosed directly to the activated sludge process was studied. It was demonstrated that the ABO/AC/O_3 process is more effective than the conventional activated sludge process in terms of purification efficiency. The experimental results showed that the addition of activated carbon beneficially affects sludge settling properties while its effect on purification efficiency is only one-time based. Considering also the results that no apparent synergetic effect between ozone and activated carbon during ozonation of phenolic wastewater could be detected, the improvement in the performance of the ABO/AC/O_3 process was attributed to ozonation. It was demonstrated that already a small ozone dose (0.57 $mgO_3 \cdot L^{-1}$) increases the rate of oil shale phenols, COD, and BOD removal, which would reduce the necessary size of the purification equipment by affecting the necessary hydraulic retention time. The results of the ABO/AC/O_3 process study indicated that continuous ozone dosing eventually deteriorates the performance by possibly altering the sludge activity.

To better understand the effect of ozone within the activated sludge process sludge activity was measured. For the evaluation of the changes in activity on the course of ozonation, OUR and ATP measurements were confirmed to follow similar trends. The effect of ozone on activated sludge activity depends on the fraction of ozone reactive compounds in the liquid phase. The sludge activity was practically not inhibitied during the ozonation of activated sludge in the wastewater at the ozone doses up to 86 $mgO_3 \cdot L^{-1}$. These results confirm that it is not only possible but also beneficial to introduce ozone straight into the activated sludge process if improvement in effluent water quality in the treatment of oil shale phenolic wastewater is desired.

Beneficial effect of intermittent ozonation on the ABO process, the complexity and the nature of the integrated ABO/O_3 process were demonstrated by following changes in the activated sludge and in the effluent water quality. Most importantly it was shown that by integrating the activated sludge process and ozonation into a single unit, effluent water quality improvement in terms of toxicity, resorcinol concentration, SCOD, and TCOD removal, and simultaneous sludge yield reduction is attainable.

A dynamic model of the activated sludge process with intermittent ozonation was composed to simulate the integrated process. In the model the kinetics was described using the Michalis-Menten equation. To predict the effect of intermittent ozonation on the activated sludge process an empirical ozone impact coefficient was introduced into the equation. The model enables the dynamic response of the activated sludge process to changes to be predicted in operating conditions.

To conclude, the results presented in the thesis show that the integrated process – the activated sludge process with intermittent ozonation – could be used for the purification of wastewaters, including phenolic wastewaters, to improve sludge properties, detoxify the effluent water and to reduce the excess sludge generation without adversely affecting the quality of effluent water. Continuous ozone dosing may be recommended only for a short period of time.

Further research is currently in progress in order to evaluate the applicability of intermittent ozonation in municipal wastewater treatment plants for the removal of ozone reactive antibacterial contaminants and to evaluate its effect on the performance of the aerobic bioreactor. However, some research is still needed as there are unanswered questions regarding ozone induced changes in and the effect of long term ozonation on activated sludge (increased resistance to ozone).

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ABSTRACT

The main aim of the present thesis was to study an integrated process – aerobic bio-oxidation with activated carbon treatment and ozonation (ABO/AC/O_3) – in order to establish feasibility of the process, to evaluate the efficiency of the process in the treatment of the oil shale phenolic wastewater, and also to study the mechanism of the integrated process.

The idea was to combine the solution of two problems occurring in the biological purification of wastewater - effluent quality and the generation of excess sludge – into one integrated process. A novel part of the process is that both activated carbon and ozone are added straight into the biomass.

An integrated process where ozone and activated carbon are dosed directly to the activated sludge process was studied. It was demonstrated that the ABO/AC/O_3 process is superior over the conventional activated sludge process in terms of purification efficiency. The experimental results showed that the addition of activated carbon beneficially affects sludge settling properties. It was demonstrated that already a small ozone dose (0.57 $\mathrm{mgO_3 \cdot L^{-1}}$) increases the rate of oil shale phenols, COD, and BOD removal, which would reduce the necessary size of the purification equipment by affecting the necessary hydraulic retention time.

Also, the experiments to study the interactions between activated sludge and activated carbon and between activated carbon and ozone were carried out. The results indicated that activated carbon acts only as an adsorbent – an expected catalytic effect was not detected. Therefore, the focus shifted to an integrated process – activated sludge process with intermittent ozonation.

The impact of ozone on the activity of activated sludge and on the quality of effluent water was studied in batch experiments. The activity of activated sludge was characterized by specific oxygen uptake rate (SOUR) and also by specific adenosine-5′-triphosphate (SATP) concentration. The experiments indicated that although SATP measurements give the precise amount of the active biomass, both methods are convenient for the measurement of the activity of the ozonated activated sludge. It was established that the impact of ozone on activated sludge activity depends on the concentration of contaminants in the suspension – in the presence of contaminants sludge activity was not inhibited up to the ozone dose of 86 $\mathrm{mgO_3 \cdot L^{-1}}$, otherwise an immediate activity reduction was detected. Also, the introduction of ozone straight into the activated sludge process can be effectively used to improve the effluent water quality.

To evaluate the effect of ozone on activated sludge and the activated sludge process continuous activated sludge process with intermittent ozonation was studied. It was established that by integrating the activated sludge process and ozonation into a single unit, simultaneous effluent water quality improvement and sludge yield reduction is attainable. Most importantly, total detoxification of the wastewater was achieved. Ozone induced changes in operational conditions favoured the development and improved the properties of activated sludge.

A mathematical model of the activated sludge process with intermittent ozonation was composed to simulate the integrated process. In the model the kinetics of the biochemical reaction was described using the Michaelis-Menten equation. To take into account the influence of ozone on the process, the ozone impact coefficient was introduced. The model enables the dynamic response of the activated sludge process to intermittent ozonation to be predicted.

In conclusion, the integrated process – the activated sludge process with intermittent ozonation – could be used for the purification of wastewaters, including phenolic wastewaters, to improve sludge properties, detoxify the effluent water and to reduce the excess sludge generation without adversely affecting the quality of effluent water.

KOKKUVÕTE

Aktiivmudaprotsessi rakendatakse erinevate reovete töötlemisel, kusjuures see protsess on traditsiooniliselt töötlusskeemi põhiosa. Tööstuslike, biokeemiliselt raskesti lagundatavaid või mikroorganismidele toksilisi ühendeid sisaldavate reovete puhastamisel ei taga aktiivmudaprotsess alati KHT, BHT ja hõljuvainete osas piisavalt head heitvee kvaliteeti. Heitvee kvaliteediga seotud probleemid esinevad tihti näiteks fenoolsete reovete, sealhulgas ka põlevkivist saadava õli töötlemisel tekkiva fenoolse reovee biokeemilisel puhastamisel. Heitvee erinevate parameetrite normidele mittevastavuse üheks oluliseks põhjustajaks on tihti lisaks lagundamata saasteainetele ka heitveega reoveepuhastist välja kanduv halvasti settiv aktiivmuda, sest fenoolsete ühendite leidumine reovees põhjustab halvasti flokuleeruva aktiivmuda teket või aktiivmuda deflokuleerumist. Lisaks heitvee kvaliteediga seotud probleemidele, kaasneb reovee puhastamisega aktiivmudaprotsessis üldiselt märkimisväärne jääkaktiivmuda teke, mille edasine käitlemine on komplitseeritud.

Heitvee kvaliteediga seotud probleemide (KHT, BHT, biokeemiliselt raskesti lagundatavad/toksilised ühendid) lahendamiseks on võimalik kombineerida biopuhastust keemilise oksüdatsiooniga, näiteks osoonimisega. Osoonimine on ka üks efektiivsemaid meetodeid jääkaktiivmuda tekke vähendamiseks. Aktiivmudaprotsessi parendamiseks on pakutud välja ka meetod, kus aktiivmudaprotsessi lisatakse pulbrilist aktiivsütt. Aktiivsöe roll sellises protsessis on vähendada reovee toksilisust ning luua sellega paremad tingimused aktiivmudaprotsessi rakendamiseks. Lisaks on andmeid ka aktiivsöe katalüütilise toime kohta osoonimisel.

Võttes arvesse osooni ja aktiivsöe võimalikku koosmõju ja mõju aktiivmudaprotsessile, oli käesoleva töö peamiseks eesmärgiks uurida integreeritud protsessi – aktiivmudaprotsess koos osooni ja aktiivsöe lisamisega otse aerotanki (ABO/AC/O_3) – et selgitada protsessi rakendatavust nii heitvee kvaliteedi parandamisel kui ka jääkaktiivmuda tekke vähendamisel ning hinnata protsessi efektiivsust põlevkiviõlist eraldatud kahealuseliseid fenoole sisaldava reovee puhastamisel. Protsessi uudsus seisneb selles, et nii osooni kui ka aktiivsütt lisatakse otse aktiivmudasse.

Eksperimendid näitasid, et võrreldes traditsioonilise aktiivmudaprotsessiga on integreeritud ABO/AC/O_3 protsess stabiilsem ja efektiivsem. Ehkki üldine puhastusaste oli integreeritud protsessis parem vaid vähesel määral, näitasid tulemused, et juba madal osoonidoos (0,57 $mgO_3 \cdot l^{-1}$) kiirendab nii põlevkivifenoolide (resortsinoolid) kui ka KHT ja BHT ärastust. Samuti paranesid aktiivmuda omadused. Seega integreeritud protsessi rakendamisel oleks võimalik saavutada kokkuhoid väiksemate seadmete kasutamise teel, kuna konventsionaalse aktiivmudaprotsessiga võrdse puhastusefektiivsuse saavutamiseks vajalik reovee viibeaeg oleks lühem.

Integreeritud protsessi mehhanismi selgitamiseks uuriti aktiivmuda ja aktiivsöe ning aktiivsöe ja osooni koosmõjusid. Katsetulemused näitasid, et lisanditeta aktiivsöe kasutamine protsessis parandab nii aktiivmudaprotsessi kui ka osoonimise puhastusefektiivsust, kuid niinimetatud sünergeetilist efekti ei saavutatud – aktiivsüsi toimis adsorbendina. Seetõttu pöörati edasistes uuringutes põhitähelepanu integreeritud protsessile, kus osooni juhiti otse aktiivmudaprotsessi.

Integreeritud protsessi – aktiivmudaprotsess osooni juhtimisega otse aktiivmudasse – uuringud näitasid, et osooni mõju aktiivmuda aktiivsusele sõltub oluliselt vedelfaasis olevate saasteainete kontsentratsioonist. Saasteainete olemasolu korral ei toimunud aktiivmuda aktiivsuse inhibeerimist kuni osoonidoosini 86 $mgO_3 \cdot l^{-1}$ ja heitvee kvaliteet paranes. Samas ainult aktiivmuda (resuspendeeritud vette) osoonimine põhjustas kohese muda aktiivsuse vähenemise. Seega reageerib osoon eelkõige vees olevate saasteainetega ning seejärel atakeerib mikroorganisme aktiivmudas.

Järelikult on integreeritud protsessis otstarbekas osoonimist läbi viia selliselt, et osooni juhitakse aktiivmudasse perioodiliselt teatud ajavahemike tagant (aktiivmudaprotsess koos osooni perioodilise juhtimisega aktiivmudasse). Eksperimentidest selgus, et selle protsessiga on võimalik saavutada samaaegne heitvee kvaliteedi paranemine ja aktiivmuda tekke vähenemine. Oluliseks tulemuseks on see, et osooni mõjul saavutati täielik toksiliste ühendite eemaldamine reoveest. Lisaks peab märkima, et osooni poolt põhjustatud muutused protsessis soodustasid nii normaalse kooslusega aktiivmuda teket kui ka aktiivmuda omaduste paranemist.

Katsetulemuste põhjal koostati matemaatiline mudel, et kirjeldada osooni mõjul toimuvaid muutuseid aktiivmudaprotsessis. Biokeemiliste reaktsioonide kineetika kirjeldamiseks kasutati Michaelis-Menten´i võrrandit. Osooni mõju arvestamiseks lisati mudelisse empiiriline osooni mõju koefitisient. Saadud mudel võimaldab hinnata aktiivmudaprotsessi reaktsiooni osooni doseerimisel aktiivmudasse.

Kokkuvõtteks, integreeritud protsess – osooni lisamine otse aktiivmudasse – on kasutatav reovete, sealhulgas fenoolsete reovete, puhastamiseks, et parandada aktiivmuda omadusi, eemaldada veest toksilisi ühendeid ja vähendada jääkaktiivmuda teket ilma, et heitvee kvaliteet selle tulemusena halveneks.

APPENDIX I
PUBLICATIONS

PAPER I

Järvik, O., Kamenev, I., Viiroja, A., Kallas, J. 2010. Purification of Phenolic Wastewater Using Aerobic Bio-oxidation Combined with Activated Carbon Treatment and Ozonation. – Ozone: Science & Engineering, 32 (6), 417-423.

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Purification of Phenolic Wastewater Using Aerobic Bio-oxidation Combined with Activated Carbon Treatment and Ozonation

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The combined process – aerobic bio-oxidation with activated carbon addition and ozonation was studied (ABO/ AC/ O3). The performance of the process was compared with conventional aerobic bio-oxidation (ABO). The studies were carried out in two continuous and periodic reactors to evaluate the purification efficiencies (in terms of COD, BOD and resorcinols removal), excess sludge generation and stability of the bioreactors against shock loadings. The parameters in continuous combined process were as follows: organics loading was 620 mgCOD/ (day·L), activated carbon concentration 1g/ L and ozone dose 2.45 mgO$_3$*/ L (mg ozone per liter of treated water). In periodic reactors the combined process was studied at lower activated carbon concentration and ozone dose (0.3 g/ L and 0.57 mg/ L respectively).*

The results indicated that compared with conventional ABO, the co-effect of AC addition and short-termed (less than 1 week) or intermittent ozonation improved the removal of COD and BOD, while the longer period of ozonation resulted in reduction of excess sludge concentration in the bioreactor. The impact of AC and ozone on the ABO in the combined process did not concern only increased biomass activity, but AC and ozone improved also settleability of activated sludge and enhanced stability of the bioreactor to shock loadings.

Keywords Ozone, Activated carbon, Aerobic bio-oxidation, Combined process, resorcinols, Excess sludge, Phenolic wastewater, Oil shale industry

INTRODUCTION

Phenolic effluents from the Estonian oil-shale industry have for many years been the major environmental issue in Northern Estonia. The phenolic effluents are treated together with industrial and domestic wastewater at the aerobic bio-oxidation (ABO) plant, and after purification the water is discharged into the Gulf of Finland. The advantages of the ABO are its potentially high purification efficiency, the possibility of process control and relatively low cost.

However, in the case of industrial wastewater containing recalcitrant compounds or chemicals toxic for microbial culture, the ABO often results inadequate effluent water quality in terms of COD, BOD and suspended solids, the latter of which is caused by poorly settling sludge. There are also certain difficulties concerning generation of secondary waste – excess sludge. Additionally fluctuations or sudden increase of organic loading (shock loadings) may damage sludge population and interfere the normal bioreactor operation.

To overcome the effluent water quality problems, intensive studies on chemical treatment of different phenolic effluents were carried out during the 1990s in Tallinn University of Technology at the Department of Chemical Engineering. It was shown that among different purification methods molecular ozone and O_3/H_2O_2 are the most practical (Kamenev et al., 2003). Despite the high purification efficiency of the chemical oxidation processes, they are not applicable to large-scale applications, because of the high cost. Therefore it is advisable to combine chemical oxidation with aerobic bio-oxidation.

Treatment and disposal of excess sludge, which retains large amount of water (> 95%), may account for 25 to 65% of the total plant operation cost (Liu, 2003). Moreover, as the disposal and reuse of excess sludge, because of the potential risk to human health, is restricted and intensification of wastewater treatment due to more stringent wastewater treatment legislation increases sludge production (Liu, 2003; Wei et al., 2003), an innovative technology is needed for excess sludge reduction. The most appropriate way to solve this problem is to reduce the sludge production within the bioprocess rather than to post-process the sludge.

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Among different excess sludge reduction methods, sludge ozonation in recirculation system has received great attention as it is easily controllable and efficient method, allowing up to 100% sludge reduction (Yasui and Shibata, 1994; Perez-Elvira et al., 2006), and almost total inactivation of pathogenic microorganisms (Ahn et al., 2002).

The combination of chemical and biochemical oxidation could be a beneficial way to overcome the disadvantages of these two processes, namely high cost of chemical oxidation, excess sludge production and inadequate effluent water quality in biochemical oxidation process. The combined method, ABO with direct introduction of ozone containing water into bioreactor at low ozone dose (2–4 mg/ L), has been found to have several positive effects: increased biomass activity and improved COD reduction efficiency (Kamenev et al., 2003).

Additionally, recycled sludge ozonation improves significantly sludge settling properties (Kamiya and Hirotsuji, 1998; Deleris et al., 2002) and reduces bioreactor foaming (Boehler and Siegrist, 2007). On the other hand, excess biomass destruction by ozone could lead to accumulation of heavy metals in water phase in bioreactor (Neyens *et al.*, 2004), which may become detrimental to the performance of the bioprocess.

To improve the effluent water quality the addition of powdered activated carbon (AC) into bioreactor is used (originally implemented by DuPont in 1970s) (Sher et al., 2000). Due to its small particle size and large surface area, AC is used in ABO process as a flock forming backbone to improve sludge settling properties and as an adsorbent to reduce the concentration of toxic and recalcitrant compounds in bioreactor. As activated carbon adsorption is an effective and widely used technique for the removal of toxic heavy metals from wastewater (Selvi et al., 2001), AC addition to the ozonated sludge could be used to prevent upsetting the process performance by heavy metals.

The idea of the study was to evaluate the performance of the combined process – ABO with AC treatment and ozonation (ABO/ AC/ O3) – and compare it with ABO process. To assess processes stability to shock loadings, COD, BOD and phenolic compounds concentrations in periodic reactors were monitored. The impact of low ozone dose to activated sludge concentration was investigated in continuous reactors.

MATERIALS AND METHODS

In the experiments the synthetic resorcinols containing wastewater, corresponding by its composition to the oil-shale semicoking process effluent (Kamenev et al., 2003), was used. For the wastewater preparation resorcinols containing oil-shale processing product “Rezol” (Table 1) was obtained from the Estonian oil-shale industry (Viru Keemia Grupp Oil AS). “Rezol” was dissolved in water and necessary nutrients (N, P) solutions were added so that BOD:N:P ratio was approximately 100:5:1. The composition of wastewater is presented in Table 2. The main parameters of the synthetic wastewater were as follows: an average soluble chemical oxygen demand (COD) 3100 mg/ L and biochemical oxygen demand (BOD) 1300 mg/ L.

TABLE 1. Composition of Resorcinols Source “Rezol” (according to VKG Oil AS)

Compound	Mass fraction, %
Monobasic phenols	1.25
Resorcinol	5.5
4-methylresorcinol	1.8
5-methylresorcinol	49.1
2-methylresorcinol	2.5
2,5-dimethylresorcinol	8.0
5-ethylresorcinol	10.4
2-methyl-5-ethylresorcinol	1.1
4,5-dimethylresorcinol	7.5
5-propylresorcinol	1.2
Unidentified	11.65

TABLE 2. Composition of the Synthetic Wastewater

Compound	Concentration, mg/ L
“Rezol”	700
$(NH_4)_2SO_4$	400
$(NH_4)Cl$	150
Ethanol	78
Isobutanol	360
Butyl acetate	315
Acetic acid	207

Experiments were conducted in laboratory scale continuous and periodic units. The unit for continuous process study consisted of two reactors (working volume of 10 L) with automatically controlled supporting equipment. Organics loading was of 620 mgCOD/ (day·L), and both reactors operated at the same hydraulic conditions. The periodic unit used to estimate process kinetics consisted of 2 periodic reactors (1 L). Combined process in both continuous and periodic unit was carried out in one of the reactors, while the other operated as a reference reactor to acquire conventional ABO data. Activated sludge used in experiments was acclimated to wastewater. Units operated at 22 ± 1 °C and dissolved oxygen level in bioreactors was monitored and kept between 2 and 4 mg/ L.

For ozone introduction, distilled water was saturated with ozone and periodically pumped (continuous process) or injected via syringe (periodic process) to the reactor. Ozone was produced from air by Clear Water Tech. Inc. P-2000 ozone generator, San Luis Obispo, CA, USA (continuous process) or from compressed air by Trailigaz Labo LO ozone generator, Paris France (periodic process).

Analyses

The 7-day biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were analyzed according to

Standard Method 5210B and 5220D, respectively (APHA-AWWA-WEF, 1995). Ozone concentration in distilled water was measured by indigo colorimetric method as described in Standard Method 4500 (APHA-AWWA-WEF, 1995). Activated sludge concentration in bioreactors was determined as total suspended solids dried at 103–105 °C (Standard Methods, 1995). During continuous process study, when the sample for COD and BOD determination was taken, also the sludge concentration was measured. In periodic reactors the sludge concentration was measured at the beginning and at the end of the experiment.

Resorcinol concentrations in periodic reactors were measured by gas chromatography using Thermo Electron Corporation Focus GC system (Madison, WI, USA) with a flame ionization detector. Resorcinols were extracted with diethyl ether, which was then exchanged with chloroform. 2 μL of sample was manually injected to split-splitless injector (300 °C, splitless time 0.5 min). The sample was carried by carrier gas (N_2, flow rate 1 mL/min) to Restek Rtx-1 (fused silica) capillary column (30 m × 0.32 mm × 0.25 μm) where it was separated. Out of the 9 resorcinols presented in Table 1, 7 compounds concentration was measured (2-methyl-5-ethylresorcinol and 5-propylresorcinol were not detected as they were not available as pure compounds).

RESULTS AND DISCUSSION

The Study of ABO and ABO/AC/O3 Processes in Continuous Unit

In initial experiments, with organics loading of 620 mgCOD/(day·L), and at activated sludge (AS) concentration of 0.74 g/L, both reactors had almost identical efficiencies and conditions: COD was reduced by 80%, and BOD by 96.5%, pH was 7.2. In continuous combined process activated carbon (AC) concentration was 1 g/L and ozone dose 2.45 mgO$_3$/L (mg ozone per liter of treated water). During the study, two periods in combined process were detected:

- I period: increase in efficiency,
- II period: reduction of AS concentration and decrease in process efficiency.

The results of the continuous process study are presented in Figures 1 and 2. As can be seen in Figures 1 and 2, during the I period a 0.39 g/L increase in AS concentration in combined process was measured (AS concentration increased from 0.74 to 1.13 g/L). It was slightly smaller compared to reference reactor, where AS concentration at the end of the I period was 1.19 g/L. During the whole experiment period (13 days), AS concentration in ABO process constantly increased reaching finally up to 1.3 g/L. After the I period, biomass concentration in ABO/AC/O3 process started to drop. The experiment was stopped, when the AS concentration in combined process dropped to 0.5 g/L.

It is known that ozonation usually lowers the pH of wastewater by easily oxidizing unsaturated bonds and aromatics substituted with electron donor functional groups (as –OH) to products with carbonyl and carboxyl functional groups. As pH in the bioprocesses plays a crucial role by influencing the rate of biodegradation, it was steadily monitored. Introduction of ozone, despite of the low dose, to the bioreactor lowered the pH from 7.22 to 5.90 (6.60 at the end of the I period). The pH in the reference reactor remained practically constant, showing only small fluctuations (from 6.93 to 7.34) during the experiment time.

At the end of the I period both reactors showed slightly improved purification efficiencies compared to initial conditions: COD reduction efficiencies were 83.7% and 81.4% in combined and ABO processes, respectively, while BOD reduction efficiencies remained practically constant (slightly higher in ABO/AC/O3 process). It can be seen clearly from Figure 1 and Figure 2 that introduction of ozone almost immediately improved the combined process performance (COD and BOD of effluent water) compared to initial conditions (COD_0 and BOD_0 of effluent water) over the reference bioreactor.

This improvement in efficiency can be attributed to the ozone addition, as the AC was added to the bioreactor before the start of the experiment. The exact influence of small ozone dose to the activated sludge in combined process has still

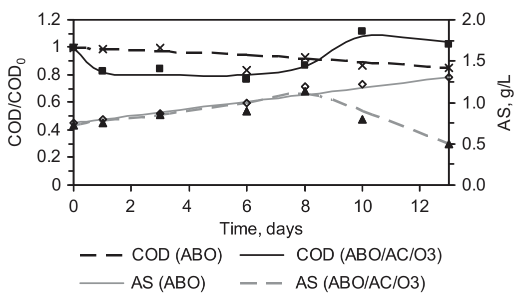

FIGURE 1. Changes in biomass concentration and COD in continuous reactors.

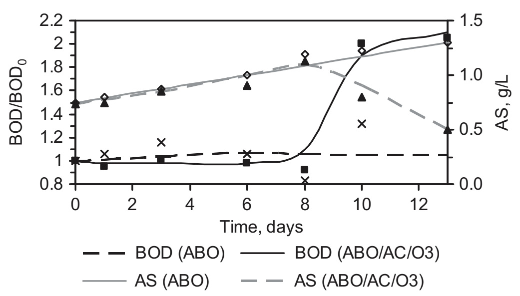

FIGURE 2. Changes in biomass concentration and BOD in continuous reactors.

remained unclear although it is determined that direct ozonation of activated sludge enhances biological activity (van Leeuwen et al., 2001).

Ozonation of suspended solids in bioreactor may lead to increase in effluent water COD. (Deleris et al., 2002) have presented that AS ozonation increases soluble COD fraction and slightly reduces total COD removal efficiency. This increase in COD is caused by ozone as it attacks and lyses microorganisms and produces soluble organic compounds (approximately 50% of the carbon obtained after ozonation is readily biodegradable (Deleris et al., 2000)). (Kamiya and Hirotsuji, 1998) have claimed that some part of the sludge may also be totally mineralized (and thereby not affecting water quality) although the influence of ozone depends on the properties of sludge.

In the present study the decrease in AS concentration (II period), caused by ozone introduction, increased the COD (Figure 1) and BOD (Figure 2) in effluent water compared to initial conditions by 10 (from 600 to 650 mg/ L) and 100% (from 45 to 90 mg/ L) respectively. This remarkable decrease in effluent water quality may be contributed to decreased AS concentration – part of it was lysed to soluble COD and BOD. Increase in BOD suggests that ozonation can greatly disturb the biodegradation of organic substances. It may have been caused by decreased pH (optimal pH for ABO process is 6.5 to 8.5) or decreased oxidation capacity (activity) of living microorganisms. It is also possible, as stated by (Kamiya and Hirotsuji, 1998), that the degradation of effluent water quality was caused by increased organic loading as a side effect of AS concentration reduction.

Figure 3 shows the sludge volume index (SVI) in combined and ABO process. Both reactors showed excellent settling properties. The initial SVI in combined process was lower because of the AC that was added to the bioreactor before the start of the experiment. Ozone is widely known to improve sludge settling characteristics in terms of SVI (sludge volume index) by controlling growth of filamentous bacteria, stabilizing surface charges and/ or changing sludge water content (Dziurla et al., 2005). Although the initial SVI was excellent, further improvement was achieved as ozone was directly introduced to the bioreactor. Later the deterioration of sludge settling properties occurred, which could be related to flock destabilization by ozone.

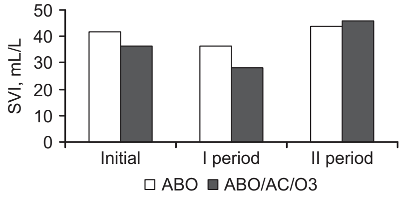

FIGURE 3. SVI in combined and reference process.

The Study of ABO and ABO/ AC/ O3 Processes in Periodic Unit

Research of ABO and the ABO/ AC/ O3 process in periodic reactors working parallel, was carried out to ascertain the kinetics of the processes. In periodic reactors the combined process was studied at lower AC concentration and ozone dose (0.3 g/ L and 0.57 mg/ L respectively). COD, BOD, and concentration of resorcinols were monitored within 24 hours. The sludge was withdrawn from continuous reactors. An average initial AS concentration in both reactors was 1.46 g/ L. At the startup of the periodic study, the wastewater was added to the bioreactor giving an average initial COD of 1250 mg/ L and BOD of 646 mg/ L.

The AS concentration during the experiments decreased in both process because of the dilution with distilled water (ABO) and distilled water saturated with ozone (ABO/ AC/ O3). Some part of the AS was probably lost because of the extensive foaming of both reactors (especially the reactor with ABO process), although to maintain the dissolved oxygen level above 2 mg/ L, the flow rate of air to the ABO/ AC/ O3 process and thereby also the foaming was usually lower (except first couple of hours, data not shown). Final biomass concentrations were 1.34 and 1.16 g/ L in ABO and combined process, respectively. This was possibly caused by the solubilization of the solids by ozone.

In a continuous process, the solubilization of AS led to reduced process efficiency in terms of soluble COD and BOD, in the periodic reactors, on the contrary, the COD and BOD removal efficiencies in combined process remained higher compared to ABO process. This finding suggests that short termed ozonation or small ozone doses actually increases biomass activity. Whether it is achieved by solubilizing only dead or non-active AS, is not known, but it seems that specifically this part of the sludge is firstly subjected to ozone attack. In fact, living bacteria incorporated into sludge flocks, when put under oxidative stress produce antioxidant enzymes that protect bacteria until these enzymes are depleted (Dziurla et al., 2005).

Figure 4 shows the changes in relative COD and BOD (compared to initial conditions: COD_0 and BOD_0) of effluent water (dilution because of the distilled water addition was taken into account). In combined process the same purification level was achieved approximately two times faster than in ABO process. Final COD removal efficiency in combined process was 3.1% higher than in ABO process (71.4%). Greatest difference in favor of ABO/ AC/ O3 process in efficiencies between the two processes was achieved 3 hours after the beginning of the experiment (28% and 12% in means of relative BOD and COD respectively).

According to the Estonian Water Act (1996), effluent water COD is usually limited to value of 250 mg/ L. In periodic reactors under conditions used this limit was not achieved. BOD maximum allowable limit of 15 mg/ L, on the other hand, in ABO/ AC/ O3 and ABO process was easily met (7 and 14 mg/ L respectively).

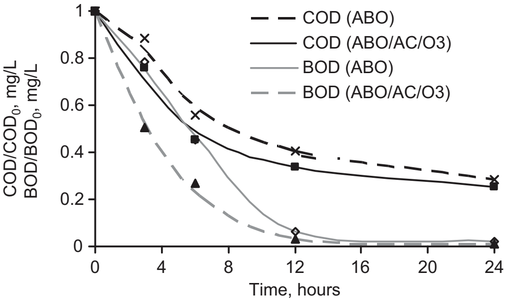

FIGURE 4. Evolution of the process parameters during the periodic processes.

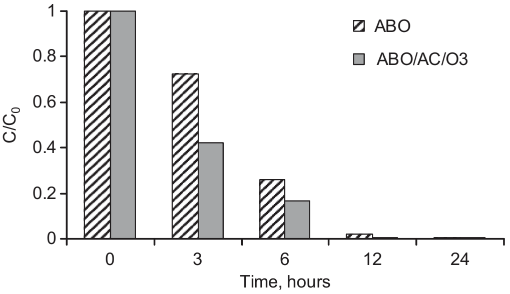

FIGURE 5. Evolution of concentrations of total resorcinols.

In the beginning of the periodic experiments, the pH in the bioreactors was 6.37. During the experiments the pH dropped and final values of 6.03 in ABO/ AS/ O3 process and 5.80 in ABO process were measured. The decrease in pH in periodic ABO process, possibly because of the oxidation of free ammonia-nitrogen to the nitrate-nitrogen, is nothing unusual. According to the data obtained during continuous process study, the change in pH in combined process should have been greater than in ABO process. Contrary results cannot be explained, but the adsorption to the fresh activated carbon may be responsible for these changes.

Initial concentrations of resorcinols in both reactors were equal. Resorcinols are considered to be relatively toxic for microorganisms – among the resorcinols present in "Rezol", resorcinol and 2,5-dimethylresorcinol are classified as very toxic. In addition, 2,5-dimethylresorcinol is also found to be slowly biodegradable (Kahru et al., 2000). One must also consider that phenolic compounds exhibit the additive toxicity, which means that the sum of toxicity of the individual compounds is lower than the toxicity of the mixture. Although the data presented by (Kahru et al., 2000) suggests that the biodegradation of resorcinols should be a slow process, the sludge adapted to the phenolic wastewater is well capable of rapidly reducing the concentration of the resorcinols (Figure 5).

According to the Estonian Water Act (1996), the maximum permissible concentration of dibasic phenols (including resorcinols) in wastewater effluent discharge is 15 mg/ L. In periodic processes this limit was achieved in less than 12 hours. It has to be noted that in combined process the concentration of resorcinols was lowered approximately 2 times faster than in ABO process. Sudden addition of the phenolic wastewater (shock loading) at the start up to the bioreactors caused a short lag phase in ABO process. It is especially evident in case of the 2,5-dimethylresorcinol, 4,5-dimethylresorcinol and 4-methylresorcinol, which concentration reduction during the first 3 hours was marginal (Table 3). After 24 hours the total concentration of the resorcinols in both processes was well below the permissible concentration (30% higher in ABO process).

To distinguish the impact of ozone and AC on ABO process it was needed to evaluate the performance of the combined process – ABO with AC addition (ABO/ AC). The conditions in reactors during the ABO/ AC process study were similar to those during the ABO/ AC/ O3 process study: initial AS concentration of 1.47 g/ L (did not change during the experiment), COD of 1153 mg/ L and, BOD of 638 mg/ L. AC concentration was 0.3 g/ L. For the comparison of the ABO, ABO/ AC and ABO/ AC/ O3 processes, the values of the COD, BOD and total resorcinols concentration (C) were divided by the initial concentrations. The results are presented in Figures 6, 7 and 8.

Both combined processes had improved performance over the ABO process. AC in bioreactor is known to stimulate bacterial activity by adsorbing toxic compounds (Morinaga et al., 2003), which is well illustrated in Figures 6, 7 and 8. Also it can be seen that further noticeable improvement was achieved as ozone was introduced into the bioreactor. It has to be noted that total final efficiencies (after 24 h of operation) of the processes in periodic reactors were almost identical, but the improved performance of the combined processes can be seen during the first 12 h.

CONCLUSIONS

In the present study, wastewater treatment using aerobic bio-oxidation and combined process – aerobic bio-oxidation with activated carbon addition and direct ozone introduction to biomass was examined. Treatment of the synthetic phenolic wastewater was conducted in laboratory scale continuous and periodic reactors. The results clearly showed the combined process improved performance over conventional aerobic bio-oxidation.

The results of the continuous study indicated that small ozone doses almost instantly increase COD and BOD removal efficiencies and this efficiency is maintained while pH in the reactor slightly lowers. The increase in SVI was detected as small ozone doses were directly introduced to the biomass.

TABLE 3. Evolution of Concentrations of Resorcinols

	0		3		6		12		24	
Time, hours	ABO	ABO/ AC/ O3	ABO	ABO/ AC/ O3	ABO	ABO/ AC/ O3	ABO	ABO/ AC/ O3	ABO	ABO/ AC/ O3
Resorcinol	8.78	8.78	6.27	3.79	2.59	2.23	0.36	0.16	0.05	0.04
2-methylresorcinol	5.14	5.14	4.15	3.33	2.40	1.51	0.97	0.26	0.04	0.04
4-methylresorcinol	8.38	8.38	7.04	5.09	2.41	1.80	0.69	0.15	0.04	0.05
5-methylresorcinol	115.51	115.51	85.04	42.49	28.83	17.76	0.63	0.35	0.27	0.20
2.5-dimethyl-resorcinol	16.44	16.44	16.16	12.66	8.07	4.97	0.88	0.25	0.25	0.16
5-ethylresorcinol	26.63	26.63	8.31	4.22	1.12	0.56	0.25	0.09	0.06	0.05
4.5-dimethyl-resorcinol	21.96	21.96	19.37	14.06	7.33	5.01	0.81	0.03	0.02	0.02
TOTAL	202.84	202.84	146.34	85.64	52.75	33.84	4.59	1.29	0.73	0.56

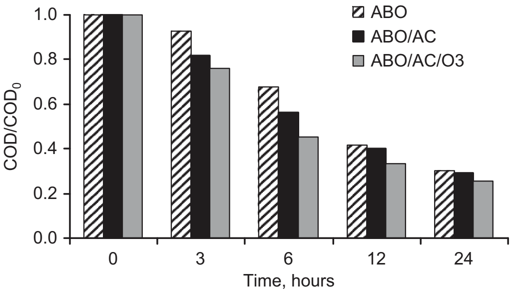

FIGURE 6. Comparison of the changes in COD in studied processes.

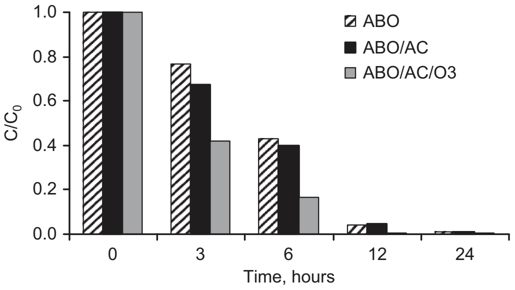

FIGURE 8. Comparison of the changes in total resorcinols concentrations in studied processes.

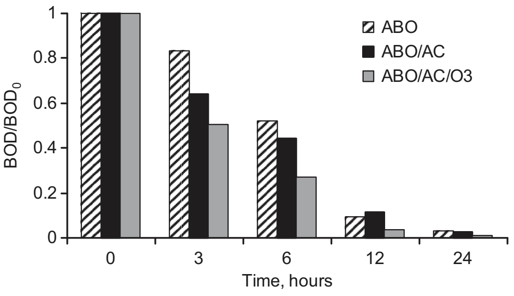

FIGURE 7. Comparison of the changes in BOD in studied processes.

It was also shown that the activated sludge concentration increase is lowered and the long termed biomass ozonation is well capable of reducing the sludge concentration in the bioreactor.

The longer biomass ozonation led to disturbance of the bioprocess and to formation of soluble COD and BOD that reduces effluent water quality. In addition, the deterioration of sludge settling properties occurred as an evidence of the destabilization of activated sludge flocks by ozone.

The periodic experiments revealed that all processes were capable of reducing the concentrations of resorcinols below the maximum permissible concentration of 15 mg/ L in effluent water in less than 12 hours. Final COD removal efficiency in ABO/ AC/ O3 process was 3.1% higher than in ABO process, BOD removal efficiencies were 99% and 98% in ABO/ AC/ O3 and ABO processes respectively and resorcinols removal efficiencies in both processes close to 100%. The COD, BOD and resorcinols removal kinetics shows that in combined process the same purification degree as in ABO process is achieved approximately two times faster. Also activated carbon addition and direct ozone introduction to biomass enhanced bioprocess stability against shock loadings.

The comparison of ABO, ABO/ AC and ABO/ AC/ O3 processes showed that AC addition could be used to enhance the purification efficiency of the ABO process. However, ozone introduction directly to the ABO/ AC process further improves the efficiency and increases the activity of biomass. Considering the promising results, further studies focus on optimization of the ozone dose as well as evaluation of the impact of ozone on sludge yield, and the results should also be controlled with real industrial wastewater.

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PAPER II

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Effect of Ozone on Viability of Activated Sludge Detected by Oxygen Uptake Rate (OUR) and Adenosine-5′-triphosphate (ATP) Measurement

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The current study focused on treatment of phenolic wastewater using an integrated process – dosing of ozone directly to activated sludge. The main goal was to analyze the effect of ozonation on viability of activated sludge in different systems – activated sludge in distilled water and activated sludge in wastewater. Two viability detection methods, oxygen uptake (OUR) rate and adenosine-5'-triphosphate measurement (ATP), were compared. The linear correlation between ATP and OUR measurements in studied range was found to be good ($r^2 = 0.90$). In case of ozonation of activated sludge in wastewater, ozone doses up to 42 $mgO_3 \cdot gMLVSS^{-1}$ did not influence the viability of sludge. In addition, contrary to ozonation of sludge in distilled water, soluble COD was reduced by 15.6% (at ozone dose of 42 $mgO_3 \cdot gMLVSS^{-1}$).

Keywords Ozone, Phenolic Wastewater, Activated Sludge, Viability, Adenosine-5′-triphosphate, Oxygen Uptake Rate, Oil Shale

INTRODUCTION

Industrial wastewaters often contain refractory organics and therefore the purification efficiency achieved by activated sludge process, as the most widely used wastewater treatment method, is usually inadequate. To improve the effluent water quality, combinations of ozonation and activated sludge processes were proposed. Lately, there have been several attempts to integrate ozonation and activated sludge process in order to improve treatment of wastewaters containing recalcitrant compounds. Van Leeuwen et al. (2009a, 2009b) studied an integrated process, where small ozone doses (up to 30 $mgO_3 \cdot L^{-1}$) were applied directly into the activated sludge process with the aim to improve effluent water quality by oxidizing recalcitrant organic compounds (methylene blue, Orange II).

Based on the results, it was concluded that there is apparent synergetic effect between chemical and biochemical oxidation i.e., the removal of pollutants in an integrated process is more effective than with subsequent biochemical and ozone oxidation. Kamenev et al. (2008) applied small ozone doses (2 mg/L) directly into the activated sludge process treating phenolic wastewater and found that it increased the specific oxygen uptake by about 15–20%. Therefore it was concluded that ozone at low doses does not inhibit the process but rather activates the sludge. There are also reports on the positive effect of ozone on sludge settling characteristics when ozone is applied directly into an aeration tank (van Leeuwen 1988a, 1988b) or into a recirculation line (Collignon et al., 1994; Kamenev et al., 2008).

On the other hand, in case of municipal wastewaters the purification of wastewater is achieved through generation of considerable amount of excess sludge that has to be disposed. Treatment and disposal of excess sludge, which retains large amounts of water (> 95%), may account for 25 to 65% of the wastewater treatment plant operation cost (Zhao and Kugel, 1996).

Moreover, the intensification of wastewater treatment by building more wastewater treatment plants due to more stringent wastewater treatment legislation (Urban Waste Water Treatment Directive 91/271/EEC) increases sludge production (Perez-Elvira et al., 2006). As the disposal and reuse of excess sludge is restricted because of the potential risk to human health (Wei et al., 2003), an innovative technology is needed for excess sludge reduction.

There is accepted understanding that the most appropriate way to solve excess sludge generation problem is to reduce sludge production within the bioprocess or at very

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source rather than to post-process the sludge (Paul and Debellefontaine, 2007). Among different excess sludge reduction methods (thermal, mechanical, chemical), sludge ozonation in the re-circulation system has received great attention as an efficient method, allowing up to 100% reduction of sludge production (Yasui and Shibata, 1994; Perez-Elvira et al., 2006; Huysmans et al,. 2001; Sakai et al., 1997) and almost total inactivation of pathogenic micro-organisms (Ahn et al., 2002). To reduce ozone dose needed for reduction of excess sludge generation, Kamiya and Hirotsuji (1998) developed a system where biological wastewater treatment is combined with intermittent ozonation.

It would be especially desirable to achieve the main goal of the wastewater treatment i.e., effluent water quality meets all standards, along with reduced excess sludge generation. Integration of biochemical oxidation (aerobic bio-oxidation) and chemical oxidation (ozonation) is believed to be one possible way to achieve this, although little is known about the actual effect and mechanism of ozone on biomass in integrated process.

The result of the ozonation and activated sludge integrated process depends strongly on ozone dose. Ozone dose has to be sufficient to improve the effluent water quality (in case of industrial wastewaters) and to reduce the excess sludge generation, but low enough in order not to disrupt aerobic bio-oxidation. To keep the process economically competitive/ feasible and not to hinder aerobic bio-oxidation, ozone dose has to be minimized and optimized. Considering these facts some questions have arisen: what is the most appropriate (simple and accurate) method for evaluation of the effect of ozone on activated sludge and how much ozone should be introduced to the bioreactor in order to achieve the desired goal?

Control and optimization of activated sludge ozonation in a recirculation line is usually based on measurement of mixed liquor volatile suspended solids (MLVSS) and mixed liquor suspended solids (MLSS) by keeping the ratio MLVSS/ MLSS constant i.e. via time consuming indirect methods. To assure that ozone does not inhibit aerobic bio-oxidation a fast and sensitive method for sludge viability detection is needed. A commonly used method for viability detection in activated sludge process has been oxygen uptake rate (OUR) measurement (Paul and Debellefontaine, 2007; Deleris et al., 2002; Kim et al., 1994; Chu et al., 2008; Yasui et al., 1997; Yasui and Shibata, 1994). OUR measurement is appropriate method for the viability analysis because it is directly correlated with the amount of viable (respiring) micro-organisms and with the biodegradation rate of organic compounds in the wastewater.

In addition to OUR measurement, several other methods for evaluation of sludge viability have been proposed, including plate count (Yasui and Shibata, 1994), live/ dead stain (fluorescence microscopy) (Seka et al., 2003), fluorescein diacetate (FDA) (Jorgensen et al., 1992), inhibition of nitrification (Gernaey et al., 1997), 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT) dehydrogenase activity (DHA) (Kim et al., 1994; Caravelli et al., 2006), and analysis of ATP (Gikas and Livingstone, 1997; Kim et al., 1994; Archibald et al., 2001; Arretxe et al., 1997; Patterson et al., 1970). Most of the above-mentioned methods have shortcomings. Although they may be sensitive, they only represent a portion of the micro-organisms in sludge (inhibition of nitrification), or underestimate total numbers, or they are time consuming (plate count, DHA), or their response time is not fast enough.

ATP is a high energy compound found in relatively constant amounts in every living cell and is instantly destroyed on the death of cells (Holm-Hansen and Booth, 1966). Therefore, the ATP content of activated sludge reflects its viable cells content (Patterson et al., 1970). As the ATP assay is rapid, simple, reliable, and measures all living cells, it can be effectively used for viability detection. Moreover, ATP can be easily extracted from the cells and used for the evaluation of the viable biomass of micro-organisms in pure culture (Kahru et al., 1982; Kahru and Vilu, 1983; Kahru and Vilu, 1988) as well as for quantification of viable microbial biomass in soil and activated sludges (Kahru et al., 1991).

The aim of the present work was to analyze and clarify the effect of ozonation on the viability of activated sludge and on performance of the activated sludge process by assessing changes in effluent water quality (as COD). For the viability detection two fast and simple methods, measurements of OUR and ATP, were used and compared.

MATERIALS AND METHODS

To evaluate the effect of ozone on activated sludge process, two different systems were subjected to batch ozonation:

1. Activated sludge in distilled water – designated here as AS;
2. Activated sludge in wastewater – designated here as AS+ WW.

Activated sludge in distilled water (with necessary nutrients; Table 1) was used as a system where ozone reacts only with activated sludge i.e., there is no competitive reactions between wastewater and ozone. The purpose of the second system, AS+ WW, was to evaluate the effect of ozone on viability of activated sludge and on activated sludge process performance when also competitive reactions between wastewater and ozone take place.

Wastewater

In the experiments a synthetic phenolic wastewater, corresponding by its composition to the oil-shale semi-coking process effluent after the extraction of phenols with butyl acetate (dephenolated process water) was used (Kamenev et al., 2003). The choice of wastewater was based on fact that the purification of the phenolic effluents from the Estonian oil

TABLE 1. Concentration of Added Compounds in Distilled Water Used for Re-suspending of Sludge

Compound	Concentration, $mg \cdot L^{-1}$
$FeCl_3 \cdot 6H_2O$	0.6
$MgSO_4 \cdot 7H_2O$	56.0
$CaCl_2$	68.8
NH_4Cl	2.7
KH_2PO_4	21.3
K_2HPO_4	54.4
$Na_2HPO_4 \cdot 7H_2O$	83.5
Ethanol	0.08

shale industry is still not adequate. Soluble chemical oxygen demand (COD) of the wastewater was of 3100 $mgO_2 \cdot L^{-1}$ and 7-day biochemical oxygen demand (BOD_7) of 1300 $mgO_2 \cdot L^{-1}$.

Activated Sludge

Activated sludge was sampled from a laboratory-scale, continuous stirred tank reactor (CSTR) (volume of 10 L), where it was previously adapted to the phenolic wastewater (approximately 2 years). Hydraulic retention time (HRT) was 2 days and solids retention time (SRT) was between 9 and 10 days. The reactor was kept at room temperature ($21 \pm 1^\circ C$), dissolved oxygen level was maintained between 2–4 $mg \cdot L^{-1}$, MLVSS/ MLSS was approximately 0.80.

For evaluation of the impact of ozone on activated sludge in distilled water, the suspension (2 L) taken from CSTR was separated to sludge and liquor by centrifugation ($2500 \cdot g$, 30 min) with subsequent filtration. Sludge was then re-suspended in 2 liters of distilled water, where necessary nutrients were added (Table 1), giving an initial COD of 200 to 245 $mgO_2 \cdot L^{-1}$. For evaluation of the impact of ozone on AS+ WW, the suspension was taken directly from laboratory scale CSTR. MLSS concentration in the experiment of AS ozonation varied from 1.20 to 1.45 $g \cdot L^{-1}$ and in AS+ WW ozonation from 1.40 to 2.40 $g \cdot L^{-1}$.

Ozonation of Activated Sludge

Batch ozonation experiments were carried out in a 2.6 L cylindrical glass reactor with a conical bottom. The ozone-air gas mixture, produced from compressed air by Labo LO (Trailigaz, France) ozone generator, was introduced to the reactor with the slightly off-centered diffuser locating at the lowest point of the reactor. Ozone concentration was approximately 25 $mgO_3 \cdot L^{-1}$, gas flow rate 0.5 $L \cdot min^{-1}$. The design allowed the gas to be used for suspension mixing.

During ozonation experiments, four ozone doses (~15, ~30, ~40 and ~70 $mgO_2 \cdot L^{-1}$) were transferred to the suspension (initial volume of 1.5 L) in and ozonation reactor. Then, 0.5 L out of 2 L was used as an initial or zero-dose sample. After transferring a certain dose, a sample was taken. Ozonation time, depending on the transferred ozone dose, ranged from ~70 to ~120 seconds.

Ozone concentration in the inlet gas was detected with ozone analyzer (Anseros GM-PRO, Tübingen, Germany), in the outlet gas by iodometry. The transferred ozone dose was calculated from ozonation time, gas flow rate, volume of suspension, and difference between ozone concentration in inlet and outlet gas stream. The ozone transfer efficiency was found to be above 94% in all cases. Ozonation was carried out at room temperature ($21 \pm 1^\circ C$). Experiments with activated sludge in distilled water were done in 4 replications. Experiments with activated sludge in wastewater were done in 3 replications.

OUR Measurement

After ozonation all samples of AS and AS+ WW, were aerated for an hour (dissolved oxygen level was maintained between 2 and 4 $mgO_2 \cdot L^{-1}$) in small batch reactors. Before viability measurement each sample was saturated with oxygen. OUR measurement was conducted without pH adjustment in an air tight vessel (100 mL), mounted with electrochemical DO electrode that was connected to DO meter (Marvet Junior MJ97, Estonia). Suspension in measuring vessel was stirred using a magnetic stirrer. DO values in all AS and AS+ WW samples were recorded for 2.5 min to determine endogenous OUR, after that 0.8 mL of synthetic phenolic wastewater was added (sludge was adapted to it). DO was further recorded for another 4 minto determine total OUR.

Determination of Adenosine Triphosphate (ATP)

Sample for ATP measurement was taken before OUR measurement. ATP was extracted from the samples essentially as described in Kahru et al. (1982). Briefly, 2 mL of the samples (activate sludge suspension) was mixed with an equal volume of TCA/ EDTA solution (10%/ 4 mM) (TCA – trichloroacetic acid). The fixed samples were frozen at $-18^\circ C$ and analyzed within 4 weeks. For ATP analysis samples were thawed, mixed, and MLSS was allowed to settle (~5 min). Prior to assay, samples were diluted 50-fold with Tris-EDTA buffer (0.1 M/ 2 mM, the pH of the buffer was adjusted to 7.75 with acetic acid). ATP concentration was determined using luciferin-luciferace reaction mixture (ViaLight® MDA Plus, Lonza, USA). Internal calibration with known ATP concentration was used throughout the procedure. Then, 100 μL of diluted sample was pipetted to a cuvette and background light emission was measured; 100 μL of luciferine-luciferase reaction mixture was added, sample was rapidly mixed and light emission was measured with a Model 1253 luminometer (ThermoLabsystem, Helsinki, Finland), subsequently 10 μL of ATP internal standard ($1.65 \cdot 10^{-6}$ $molATP \cdot mL^{-1}$, Sigma, USA) was added, sample was mixed and light emission was measured.

Analyses

COD, NH_4^+-N, NO_3^--N, MLSS, MLVSS, and pH were also determined in all samples. Determination of COD, and NH_4^+-N was performed according to Standard Methods (1995) (5220 D and 4500-NH_3 F, respectively). MLSS and MLVSS measurements were performed via porcelain dishes as described in Standard Methods (1995) (2540 B and 2540 E). NO_3^--N was determined by ion chromatography (Metrohm, Switzerland), pH was measured with a pH meter (Mettler Toledo SevenEasy, Switzerland).

RESULTS AND DISCUSSION

Ozonation of Activated Sludge in Distilled Water

The effect of ozonation on Our. The effect of ozone on respiratory activity of activated sludge was determined by measuring the maximum oxygen uptake rate (OUR) expressed in $mgO_2 \cdot L^{-1} \cdot h^{-1}$ of sludge treated with various ozone doses (15–70 $mgO_3 \cdot L^{-1}$). For better comparison of the OUR results, the specific oxygen uptake rate (SOUR), expressed in $mgO_2 \cdot gMLVSS^{-1} \cdot h^{-1}$, was calculated by dividing OUR by MLVSS, as it depends on the concentration of MLVSS.

For calculation of exogenous SOUR ($SOUR_{ex}$) both endogenous and total SOUR ($SOUR_{end}$ and $SOUR_{tot}$, respectively), were measured. $SOUR_{ex}$, used for evaluation of sludge viability, was calculated by subtracting $SOUR_{end}$ from $SOUR_{tot}$. $SOUR_{end}$ is defined as the oxygen consumption in the absence of external substrate (Dawes and Ribbons, 1962). $SOUR_{ex}$ is oxygen consumption caused by addition of external biodegradable substrate.

Initial $SOUR_{end}$ of the studied sludge varied between 8 and 12.3 $mgO_2 \cdot gMLVSS^{-1} \cdot h^{-1}$ (Fig. 1). Depending on the ozone dose applied, the measured $SOUR_{end}$, without the addition of external substrate, increased up to 1.8 times compared to initial $SOUR_{end}$ (Fig. 1). Ozone is known to be a strong cell lyses agent and thereby reduces the number of viable micro-organisms in activated sludge. If the number of viable micro-organisms is reduced, the $SOUR_{end}$ should also be reduced, as it depends on the concentration of oxygen consuming cells which use intracellular reserve material for maintenance purposes (Dawes and Ribbons, 1962).

Endogenous respiration defines the metabolism of the cell in the absence of an external substrate insofar as it is expressed by gas exchange. An increase in endogenous respiration, depicted in Figure 1, is only apparent, probably because of the viable micro-organisms use lysed sludge as a substrate, thereby seemingly increasing $SOUR_{end}$. The measured $SOUR_{end}$ is not actually “pure” $SOUR_{end}$, but the sum of respiration with oxygen using cell internal components and substrate generated during ozonation (except initial $SOUR_{end}$).

To determine any correlation between the soluble COD generated during ozonation and $SOUR_{end}$, COD (COD-COD_0) was plotted against $SOUR_{end}$ ($SOUR_{end}$-$SOUR_{end0}$) (Fig. 2). Although the linearity is not good (correlation coefficient $r^2 = 0.80$), the trend clearly indicates an increase in $SOUR_{end}$ as COD due to solubilization of sludge is increased (an average increase in COD during the experiments was of 100 $mgO_2 \cdot L^{-1}$). The maximum increase in COD during ozonation was of 130 $mgO_2 \cdot L^{-1}$, whereas $SOUR_{end}$ increased by 7.1 $mgO_2 \cdot gMLVSS^{-1} \cdot h^{-1}$.

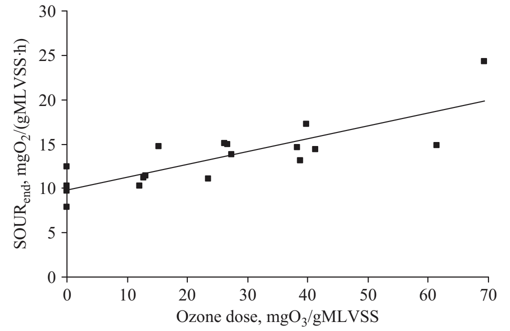

FIGURE 1. Relationship between the applied ozone dose and specific endogenous oxygen uptake rate of activated sludge samples in distilled water with added nutrients (Table 1).

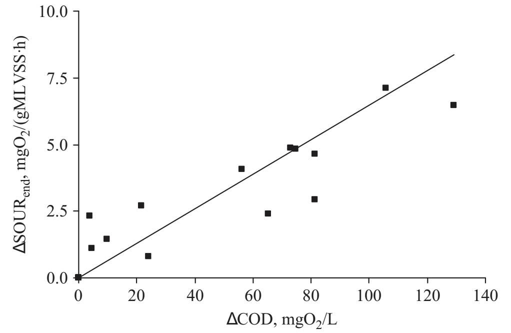

FIGURE 2. The influence of sludge-based solubilized COD on specific oxygen uptake rate (measured as $SOUR_{end}$).

COD elimination in ozone assisted activated sludge process with the aim of reducing excess sludge production is a key parameter evaluated in most of the studies (Lee et al., 2005; Deleris et al., 2002; Boehler and Siegrist, 2007; Yasui and Shibata, 1994; Yasui et al., 1997; Sakai et al., 1997; Kamiya and Hirotsuji, 1998). It is pointed out that the destruction of cells may increase the concentration of refractory organic compounds and may lead to a slight increase in the effluent COD (Yasui et al., 1997; Boehler and Siegrist, 2007; Naso et al., 2008), although others have found that whole solubilized COD can be considered as biodegradable (Paul and Debellefontaine, 2007). The results of the current study

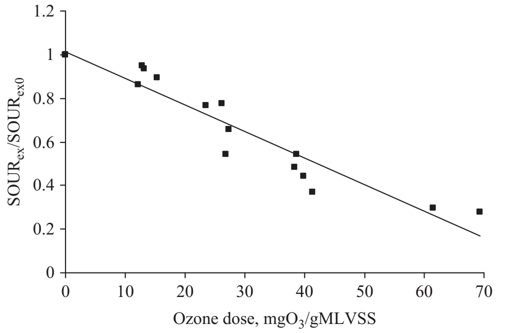

FIGURE 3. Relative $SOUR_{ex}$ of activated sludge re-suspended in distilled water as a function of transferred ozone doses.

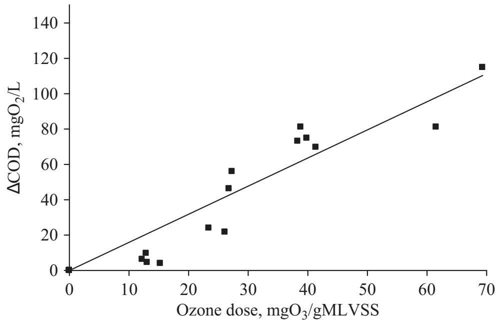

FIGURE 4. An increase in COD in activated sludge re-suspended in distilled water samples due to ozone assisted sludge solubilization.

seems to support the first finding – although COD is increased, only slight increase in OUR, detected as $SOUR_{end}$, can be seen. It has to be noted that BOD of the samples was not measured therefore it is only an estimation.

For determination of viability of activated sludge, $SOUR_{ex}$ is frequently used. Initial $SOUR_{ex}$ (ozone dose is zero) ranged from 90 to 112 $mgO_2 \cdot gMLVSS^{-1} \cdot h^{-1}$. Due to the variations of the initial $SOUR_{ex}$, relative $SOUR_{ex}$ was calculated ($SOUR_{ex}/SOUR_{ex0}$). The effect of different ozone doses on relative $SOUR_{ex}$ is presented in Figure 3. During ozonation, $SOUR_{ex}/SOUR_{ex0}$, as well as $SOUR_{ex}$, progressively decreased, i.e., inactivation of biomass starts immediately after ozone addition. An ozone dose of 70 $mgO_3 \cdot gMLVSS^{-1}$ lowered relative $SOUR_{ex}$ by 72%. By assuming that respiration activity decreases linearly as ozone dose is increased (correlation coefficient $r^2 = 0.92$), it can be estimated that 84 $mgO_3 \cdot gMLVSS^{-1}$ is needed to inactivate sludge (polynomial regression with $r^2 = 0.93$ gives a higher dose – 107 $mgO_3 \cdot gMLVSS^{-1}$).

Dziurla et al. (2005) conducted similar experiments with different activated sludges. Their results show that about 150 to 300 $mgO_3 \cdot g^{-1}COD_{sludges}$ (approximately 200 to 280, in one extreme case ~915 $mgO_3 \cdot gMLVSS^{-1}$) is needed to totally inhibit respiration. Unfortunately, no data is available for soluble COD of liquor.

The results of activated sludge solubilization, measured as COD ($COD-COD_0$), are reported in Figure 4. Solubilization is found to be proportional to transferred ozone dose. According to Paul and Debellefontaine (2007) the solubilization yield, calculated as the ratio of the solubilized COD to the quantity of ozone transferred, is close to 2.1 $gCOD \cdot gO_3^{-1}$. In current study, where sludge fed with synthetic wastewater was ozonated, it was found to be 1.6 $gCOD \cdot gO_3^{-1}$.

The Effect of Ozonation on ATP Content

A powerful tool to monitor viability or quality of microorganisms in an activated sludge system is ATP measurement. ATP is specific only for viable biomass and it is readily broken down to mono- and diphosphate nucleotides in extracellular solutions or dead cells (Holm-Hansen and Booth, 1966). Therefore, ATP concentration can be taken as the concentration of living biomass as the relationship between ATP content and viable cells remain constant.

There are many works dedicated on ATP measurement in activated sludge (Gikas and Livingstone, 1997; Archibald et al., 2001; Arretxe et al., 1997; Patterson et al., 1970; Russell and Gauthier, 1978; Levin et al., 1975; Jorgensen et al., 1992). Mainly two ATP extraction methods are used: boiling TRIS and ice-cold TCA method. Ice-cold TCA extraction is found to be least time consuming and easiest to perform, while boiling TRIS exhibits high variability and gives lower ATP yield (Arretxe et al., 1998). In current study, aimed to evaluate the influence of ozone on ATP content in activated sludge, ice-cold TCA extraction was used. Reduction of ATP content took place only during sludge ozonation i.e., ATP content measured immediately, 15 and 60 min after ozonation were equal.

Initial specific ATP concentration (SATP) of activated sludge (ATP concentration in zero-dose activated sludge) used for ozonation ranged from 2.30 to 2.80 $mgATP \cdot gMLVSS^{-1}$. These results are comparable with the results reported in earlier studies (Arretxe et al., 1998; Jorgensen et al., 1992). SATP concentration, depending on the applied ozone dose, ranged from 1.05 to 1.98 $mgATP \cdot gMLVSS^{-1}$. Reduction of SATP content in activated sludge due to the ozonation, expressed as relative SATP ($SATP/SATP_0$), is presented in Figure 5. In the range of ozone doses tested, linear relationship in relative SATP content versus ozone dose was found ($r^2 = 0.96$). The absence of the ozone threshold value, as detected by Paul and Debellefontaine (2007) and by Dziurla et al. (2005), indicates an immediate reduction in viable biomass as sludge is subjected to ozonation.

During activated sludge ozonation, ozone immediately started to influence both, SOUR and SATP – there seemed to be no compounds that could react with ozone before it

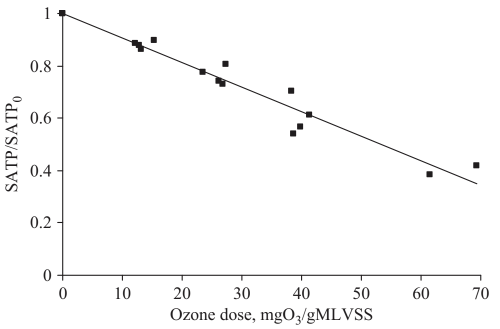

FIGURE 5. Effect of transferred ozone doses on relative SATP of activated sludge re-suspended in distilled water.

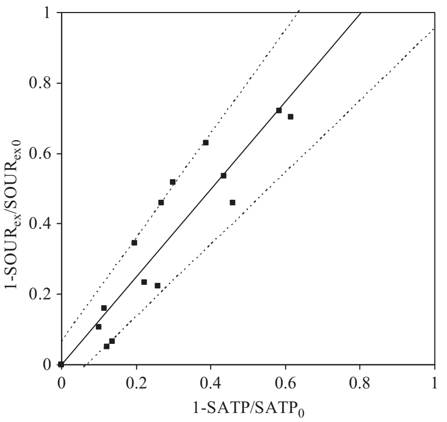

FIGURE 6. Relative decrease in ATP versus relative decrease in SOUR in ozonation of sludge in distilled water. Dotted lines represent upper and lower 95% confidence limit (obtained using regression data analysis tool in MS Excel).

comes to contact with sludge. Jorgensen et al. (1992) compared the ability of OUR and ATP to express the amount of viable biomass in activated sludge. A weak correlation between ATP and respiration measurements was found. Gikas and Livingstone (1998) on the other hand found a linear relationship between SATP and SOUR. As an objective of the current study was to compare the ability of the two methods to determine viability of activated sludge subjected to ozonation, a similar comparison was made (Figure 6).

According to our data, it can be estimated that the relationship between ATP and OUR in studied range is linear and the correlation is also rather good ($r^2 = 0.90$). Assuming the linear relationship outside experimentally studied range in Figure 6, there should still be some ATP left in the sludge (about 20% of initial) when OUR value drops to zero (1-SOUR$_{ex}$/ SOUR$_{ex0}$ is equal to 1). It could be explained by the fact that the metabolic level of the survived micro-organisms is very low (maintenance energy requirements) and because of that they do not consume oxygen.

Considering the fact that the amount of ATP in different micro-organisms differs (Ball and Atkinson, 1975), and some micro-organisms are more resistant to ozone treatment (Deleris et al., 2002; Restaino et al., 1995), further studies are planned to clarify the issue, as there is a possibility that a non-linear relationship may be more suitable to describe the situation.

It is also known that bacteria in suspended growth cultures grow in biofloc, which range in size from 0.05 to 3 mm (Grady et al., 1999; Daigger et al., 2007). A great number of micro-organisms are located inside the floc, and their contact with oxygen and/ or external substrate is limited, as oxygen and external substrate must diffuse to the interior of the floc before they can be consumed. For this reason, especially in case of OUR measurement, where contact between micro-organisms and oxygen and external substrate is essential, it may give misleading information about viability of the culture.

Figure 6 also demonstrates the sensitivity of the two techniques of viability detection – direct living biomass concentration expressed in terms of ATP concentration (SATP) and indirect methods based on determination of oxygen uptake rate (SOUR). Because of the different nature of the two methods, SATP seems to have lower sensitivity – i.e., changes in viability per milligram of ozone added, detected by relative SOUR$_{ex}$, is greater.

The Effect of Ozone on pH, NH_4^+-N and NO_3^--N

The success of biochemical wastewater treatment greatly depends on the pH in aeration basin (pH of wastewater). It is favored in pH range from 6.5 to 8.5 (Grady et al., 1999). During experiments of AS ozonation pH changed only slightly. An average initial pH was of 6.75 while at an average ozone dose of 65 $mgO_3 \cdot gMLVSS^{-1}$ it was lowered to 6.71.

Similar to Dogruel et al. (2007), an increase in NH_4^+-N and NO_3^--N was observed (Figure 7) during AS ozonation. NH_4^+-N is released as biomass is destroyed (Grady et al., 1999). Nitrate-N was detected when ozone dose was higher than 39 $mgO_3 \cdot L^{-1}$. As NH_4^+-N is not reactive toward ozone and formation of NH_3 in pH range in aeration basin is negligible, NO_3^--N formed possibly due to microbial action (nitrification).

Ozonation of Activated Sludge in Wastewater

The effect of ozonation on activated sludge in wastewater. AS+ WW ozonation was carried out identically to AS ozonation. Ozone doses ranging from 15 to 70 $mgO_3 \cdot L^{-1}$ were used. An average initial concentration of MLSS was of 2.23 (1.95 to 2.50) $g \cdot L^{-1}$ and MLVSS of 1.67 (1.48 to 1.86) $g \cdot L^{-1}$, giving MLVSS/ MLSS ratio of 0.75.

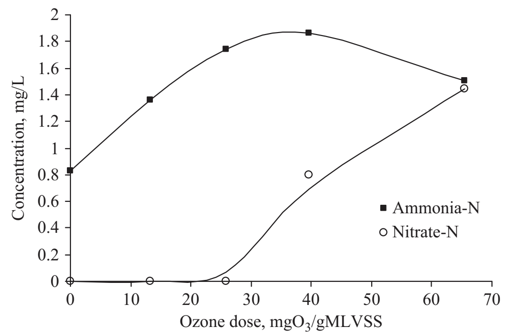

FIGURE 7. Change in average NH_4^+-N and NO_3^--N concentration after ozonation of activated sludge in wastewater.

An average initial COD due to the nature of the wastewater and short hydraulic retention time (2 days) was as high as 548 $mgO_2 \cdot L^{-1}$.

Initial $SOUR_{end}$, $SOUR_{ex}$ and SATP values in all 3 replication experiments were almost constant. $SOUR_{ex}$ ranged from 68 to 72 $mgO_2 \cdot gMLVSS^{-1} \cdot h^{-1}$, $SOUR_{end}$ from 15 to 21 $mgO_2 \cdot gMLVSS^{-1} \cdot h^{-1}$ and SATP from 2.55 to 2.70 $mgATP \cdot gMLVSS^{-1}$. Compared to experiments of AS ozonation, the behavior of the measured parameters during AS+ WW ozonation is completely different. According to OUR and ATP measurements, treating AS+ WW with ozone up to the ozone dose of 42 $mgO_3 \cdot gMLVSS^{-1}$ did not reduce the viability of sludge. In fact, during one batch, ozonation $SOUR_{ex}$ even slightly increased as ozone dose was increased. The absence of OUR inhibition and ATP reduction indicates the occurrence of fast reaction between ozone and dissolved compounds in water, i.e., ozone is depleted before it can alter the viability of sludge.

During AS ozonation an increase in COD was detected, AS+ WW ozonation on the contrary lowered an average COD value by 85 $mgO_2 \cdot L^{-1}$ (15.6%) (Figure 8a). The results are in good agreement with the results obtained when phenolic wastewater was subjected to ozone treatment: ozone dose of 83 $mgO_3 \cdot L^{-1}$ reduced COD value by 87 $mgO_2 \cdot L^{-1}$ (data not shown). Ozonation also slightly reduced pH of suspension (Figure 8b), that was also detected during phenolic wastewater ozonation. The concentration of NH_4^+-N and NO_3^--N did not change as ozone dose was increased. The findings suggest that COD reduction is mainly achieved through the action of ozone on wastewater and soluble fraction in wastewater is not increased. It also shows that in order to reduce the excess sludge production, quite a large ozone dose is needed, although the long-term effect of ozone on activated sludge is probably different and has to be studied.

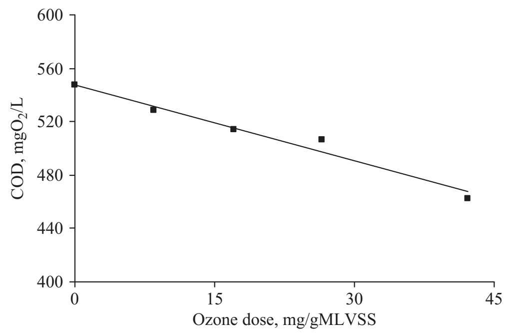

FIGURE 8a. Profiles of COD changes in batch ozonation of activated sludge in wastewater.

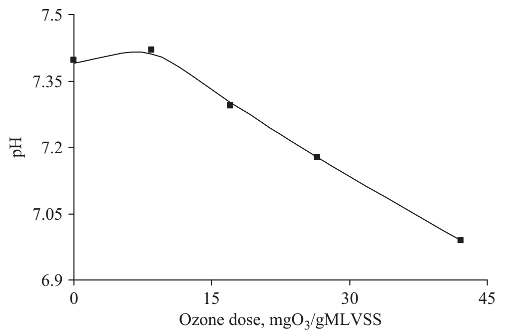

FIGURE 8b. Profiles of pH changes in batch ozonation of activated sludge in wastewater.

CONCLUSIONS

In the current paper the influence of ozone on performance of the activated sludge process, on viability (evaluated as ATP and OUR) of activated sludge and on viability of activated sludge in wastewater was studied. In experiments ozone doses ranged from 15 to 70 $mgO_3 \cdot L^{-1}$.

According to measured values, the following conclusions were drawn:

- The results of ATP measurement are valuable, giving the precise amount of living biomass. According to ATP and OUR measurements the correlation between these methods was good ($r^2 = 0.90$), and was linear in range studied.
- An immediate viability reduction was detected when only activated sludge was subjected to ozonation. Lysed cell material was estimated to be slowly biodegradable.
- The influence of ozone on activated sludge in wastewater depends on the concentration of contaminants, expressed as COD, in wastewater. It was found that during batch ozonation the viability

of sludge was not affected by ozone up to doses of ~70 $mgO_3 \cdot L^{-1}$. At the same time COD of suspension was reduced by about 16%.

Thus, based on these findings, it can be concluded that introduction of ozone to aeration tank can be effectively used to improve the effluent water quality or to reduce sludge production through viability reduction. To achieve both goals simultaneously, studies, using specific wastewater and, optimization is needed.

NOMENCLATURE

AS	Activated sludge in distilled water
AS+ WW	Activated sludge in wastewater
ATP	Adenosine-5 -triphosphate
COD	Chemical oxygen demand ($mgO_2 \cdot L^{-1}$)
CSTR	Continuous stirred tank reactor
EDTA	Ethylenediaminetetraacetic acid
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
OUR	Oxygen uptake rate ($mgO_2 \cdot L^{-1} \cdot h^{-1}$)
SATP	Specific ATP concentration ($mgATP \cdot gMLVSS^{-1}$)
SOUR	Specific oxygen uptake rate ($mgO_2 \cdot gMLVSS^{-1} \cdot h^{-1}$)
$SOUR_{end}$	Specific endogenous oxygen uptake rate ($mgO_2 \cdot gMLVSS^{-1} \cdot h^{-1}$)
$SOUR_{ex}$	Specific exogenous oxygen uptake rate ($mgO_2 \cdot gMLVSS^{-1} \cdot h^{-1}$)
$SOUR_{tot}$	Specific total oxygen uptake rate ($mgO_2 \cdot gMLVSS^{-1} \cdot h^{-1}$)
TCA	Trichloroacetic acid
TRIS	Tris(hydroxymethyl)aminomethane

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PAPER III

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Ozonation of Activated Sludge in Periodic Reactors

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Abstract: **Combining ozonation with aerobic wastewater treatment by using pre- and post-ozonation or other schemes is a known method for improving purification of industrial wastewaters. The application of mentioned methods is usually restricted due to the need of high ozone doses that significantly increases the cost of the purification process. The method of combining of ozonation and aerobic bio-oxidation into single integrated process, described in the current paper, has been found to improve wastewater purification efficiency when small ozone doses are applied. It is demonstrated how small ozone doses improve the performance of the aerobic bio-oxidation process over the conventional aerobic bio-oxidation process. Results revealed that the measurement of mixed liquor suspended solids (MLSS) is not suitable for evaluation of the activated sludge concentration in bioreactor. The measurement of ATP concentration proved to be superior over the MLSS concentration, as its response to ozone attack was immediate.**

Keywords: **Ozone, Aerobic Bio-oxidation, Activated Sludge, Integrated process, Viability of Micro-organisms, Adenosine-5'-triphosphate**

I. INTRODUCTION

Aerobic biochemical (aerobic bio-oxidation) wastewater treatment is an inexpensive and the most widely used primary treatment method used in purification of industrial wastewaters. Industrial wastewaters are often characterized by high total organic carbon content, low biodegradability, high toxicity and colour – these properties may cause great problems in biochemical treatment process.

Ozonation in combination with aerobic biochemical wastewater treatment has been considered to be an effective way to reduce several problems arising in treatment of industrial wastewaters. Earlier studies focused mainly on biochemical wastewater treatment combined with pre- and post-ozonation. Although biodegradability of the wastewater and effluent water quality were improved, those methods were found to increase remarkably the costs of the purification process. To overcome the mentioned drawback, an integrated process – introduction of ozone into activated sludge – was proposed.

There are two possible ways, sited in literature, used for introduction of ozone into activated sludge: ozonation of recycled sludge stream (1) and direct introduction of ozone into bioreactor (2). The main goal of the first method is the reduction of excess sludge production with possible improvement in sludge settling properties. The goal is usually achieved by the cost of slightly reduced effluent water quality [1]. The aim of the second method is to improve the effluent water quality (improved colour removal, reduction of chemical oxygen demand – COD) and, if possible, to reduce the excess sludge generation without adversely affecting the micro-organisms population in activated sludge (AS) responsible for the purification effect i.e. viable micro-organisms. Due to these effects of the two methods, it would be reasonable to implement the first method in municipal wastewater treatment, as the main problem there is the generation of excess sludge, while the second method (direct introduction of ozone into bioreactor) could be used in purification of industrial wastewaters.

Several works, carried out recently [2, 3, 4], have shown that combining aerobic bio-oxidation (ABO) and ozonation into a single integrated process is an efficient way to improve the purification efficiency of industrial wastewaters. Although, in a lab-scale plant, the integrated process looks promising, there are several problems that have prevented the implementation of the process in a real, large-scale plant:

- selection of ozone dose – it has to be sufficient to improve the purification process efficiency, but low enough, so that it would not inhibit the biological activity,
- micro-organisms, imposed on low ozone dose, may develop improved ozone resistance.

For example, van Leeuwen et al. [3] conducted a study where ozone doses of 45 and 30 mg·L^{-1} were used. They concluded, based on evolution of COD removal efficiency, that ozone dose of 45 mg·L^{-1}, was too high and caused inactivation of micro-organisms. The authors of the present article have studied the following processes for the purification of phenolic wastewater: ABO, ABO with activated carbon (AC) treatment and ABO with AC treatment and direct introduction of ozone into ABO process [5]. The results of the study showed that small ozone doses almost immediately increased COD and BOD removal efficiencies. A longer period of biomass ozonation led to a disturbance of the bioprocess and to the formation of soluble COD and BOD that reduced effluent water quality. Thereby it is reasonable to ask, how do find the right dose and what parameters need to be considered when choosing it?

Performance of AS system is usually evaluated by determining different values – chemical oxygen demand (COD), biochemical oxygen demand (BOD), sludge concentration, sludge yield, sludge decay rate etc. The evaluation of the impact of ozone on AS system is usually based on the same values. In most of the works dedicated to combinations of ozone and ABO, the effect of ozone on AS have been evaluated by measuring changes in sludge concentration, expressed as the concentration of mixed liquor suspended solids (MLSS) or mixed liquor volatile suspended solids (MLVSS) [4, 6, 7].

The approach to evaluate the viable micro-organisms in AS by measuring the MLSS concentration is questionable, since usually the adenosine-5'-triphosphate (ATP) concentration is considered to be the measure of micro-organisms viability. The main reason why it is not correct to use MLSS concentration for monitoring the viability of AS, is a long response time – ozone inactivates sludge and it begins to affect the sludge generation rate. An inverse relationship is valid: the greater the amount of inactivated sludge, the lower is the generation rate of new micro-organisms (sludge yield is assumed to be constant, as it depends of the type of wastewater or substrate). As solids retention time (SRT) of conventional AS system is usually at least 3 days (i.e. particles that enter the bioreactor are retained there for 3 days), a considerably long time is needed before any change in number of viable micro-organisms, detected as MLSS, can be measured. Another problem is the time needed to carry out the MLSS measurement procedure – a portion of sample has to be dried at 105 ºC as long as there is no change in mass [8]. Taking into account the shortcomings of MLSS measurement, several other applications are suggested for the measurement of changes of viable micro-organisms in AS. The most appropriate method seems to be the measurement of ATP concentration. The method of ATP measurement is a specific, precise, fast and sensitive way to detect the concentration of all viable micro-organisms in suspension.

The aim of the present study was to determine the effect of ozone on the efficiency of ABO process and on micro-organisms viability in AS.

II. MATERIALS AND METHODS

The effect of small ozone doses on efficiency of ABO process and pollutants (resorcinols) removal kinetics was evaluated using the results of the experimental study, described in [5] and additional experiments. The viability study – ozonation of AS in batch reactor – was performed to evaluate the effect of different ozone doses on viability of AS, detected as ATP, and on MLSS concentration.

A Methods

The previous study of the effect of ozone on the efficiency of ABO process was carried out in a continuous lab-scale plant consisting of two ABO reactors (volume of 10 litres). Integrated processes – ABO with AC treatment and ABO with direct introduction of ozone into ABO reactor and AC treatment – was carried out in one reactor and ABO process in another reactor (control reactor for the reference data) [5]. The results of the two integrated processes were used to evaluate the effect of ozone on ABO process.

The effect of ozone on the viability of micro-organisms in AS, as well as kinetic study was carried out in batch reactors (volume of reactors 1 and 2.6 litres, respectively).

The synthetic resorcinols containing wastewater, corresponding by its composition to the Kiviter oil-shale semicoking process effluent [2], was used in the experiments. In viability study, in order to detect the effect of ozone on pure AS (i.e. there are initially no ozone reactive compounds, other than AS, in suspension), also distilled water (with necessary nutrients – C, N, P, Mg^{2+}, Ca^{2+}, Fe^{3+}, K^+, Na^+) was used. The results of the integrated processes were compared to the results of the ABO process, carried out simultaneously. AS used in experiments was adapted to the wastewater.

For ozone introduction into continuous reactor, distilled water was saturated with ozone and pumped to the reactor – the method was used to simplify the calculation of transferred ozone dose. In kinetic study, distilled water saturated with ozone was injected via syringe. To avoid the apparent improvement of the performance of the integrated process over the ABO process, caused by the addition of distilled water saturated with ozone (effect of dilution), the same amount of distilled water was added to the ABO process at the same time. In viability study, gaseous ozone with the flow rate of 0.5 $L \cdot min^{-1}$ and inlet concentration of 24 to 26 $mgO_3 \cdot L^{-1}$, was introduced into the activated sludge in batch reactors. Ozone was produced from compressed air by Trailigaz Labo LO ozone generator (batch processes) or from air by Clear Water Tech. Inc. P-2000 ozone generator (continuous process).

B Analyses

Ozone concentration in distilled water was measured by indigo colorimetric method (4500-O_3 B) as described in [8]. In viability study, ozone concentration in the inlet gas was detected with ozone analyzer (Anseros GM-PRO), in the outlet gas by iodometry [8] (2350E).

Determination of COD, and BOD was performed according to [8] (methods 5220 D and 5210 B, respectively). MLSS and MLVSS measurements were performed via porcelain dishes as described in [8] (methods 2540 B and 2540 E).

C Determination of adenosin-5'- triphosphate (ATP)

ATP was extracted from the samples (2 ml) with an equal volume of TCA/EDTA solution (10%/4 mM) (TCA – trichloroacetic acid). The fixed samples were frozen at -18°C and analysed within four weeks. For ATP analysis samples were thawed, mixed, and MLSS was allowed to settle. Prior to assay, samples were diluted 50-fold with Tris-EDTA buffer (0.1 M/2 mM; the pH of the buffer was adjusted to 7.75 with acetic acid). ATP concentration was determined using firefly luciferin-luciferace reaction mixture (ViaLight®MDA Plus, Lonza, USA). Internal calibration with known ATP concentration was used throughout the procedure. 100 µL of diluted sample was pipetted to a cuvette, background light emission was measured; 100 µL of luciferine-luciferase enzyme mixture was added, sample was rapidly mixed and light emission was measured with 1253 luminometer (ThermoLabsystem, Finland); subsequently 10 µL of ATP internal standard ($1.65 \cdot 10^{-6}$ mol $ATP \cdot mL^{-1}$, Sigma, USA) was added, sample was mixed and light emission was measured.

D. Determination of resorcinols (kinetic study)

The concentration of resorcinols in the batch reactors was measured by gas chromatography using Thermo Electron Corporation Focus GC system with a flame ionisation detector. Resorcinols were extracted with diethyl ether (3 times), which was then exchanged with chloroform. 2 µL of sample was manually injected to split-splitless injector (300 ºC, splitless time 0.5 min). The sample was carried by carrier gas (N_2, flow rate 1 mL/min) to Restek Rtx-1 (fused silica) capillary column (30 m×0.32 mm×0.25 µm) where it was separated. Total resorcinols concentration was measured.

III. DISCUSSION

A Effect of ozone on the efficiency of ABO process

The evaluation of the effect of small ozone doses on ABO process was carried out on the basis of the results of the continuous integrated processes. An average ozone dose was 2.45 $mgO_3 \cdot L^{-1}$ (mg ozone per litre of treated water).

The concentration of viable micro-organisms was estimated by measuring the concentration of AS (as MLSS) (Figure 1a). Performance of the integrated process (direct introduction of ozone into activated sludge) was characterized by effluent water quality (as COD and BOD) (Figure 2).

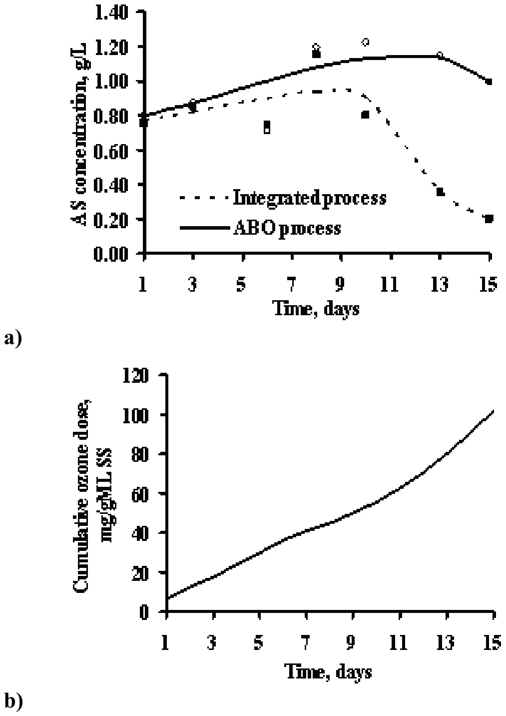


Fig. 1. Activated sludge concentration in studied processes. Lines show the trend of the concentration of AS (a). Calculated cumulative ozone doses (b)

The initial concentration of AS in both processes (integrated and ABO process), was equal (0.74 $g \cdot L^{-1}$) (Figure 1a). During the first 8 days of operation, the concentration of AS in integrated process increased by 0.39 $g \cdot L^{-1}$. In ABO process the concentration of AS at day 8 was of 1.19 $g \cdot L^{-1}$. During the whole experiment period (15 days), the concentration of AS in ABO process, compared to initial concentration, increased, reaching finally approximately to 1 $g \cdot L^{-1}$. After 8 to 10 days of operation, biomass concentration in integrated process started to drop. By this time, as part of AS was discarded from the reactors (approximately 5%) on days 1, 3, 6, 8, 10, 13, and 15, a cumulative ozone dose per 1 gram of MLSS (using an average MLSS concentration) was calculated to be from 45 to 56 $mgO_3 \cdot gMLSS^{-1}$ (Figure 1b). It was concluded that possible reason for the decrease in biomass concentration was too large ozone dose.

The changes in effluent water quality (as COD and BOD), compared to initial values (COD_{in} and BOD_{in}), are presented in Figure 2. It is shown that in the beginning, the COD removal efficiency in integrated process was calculated to be ~3% higher. On the other hand, BOD removal efficiency in integrated process was ~5% lower compared to ABO process. Increased BOD value shows that ozone can oxidise refractory organics into biodegradable oxidation intermediates and at the same time ozone may disturb the biodegradation of organic substances. Possible disturbance was caused by decreased pH (it constantly lowered in integrated process from 7.22 to 5.90 – optimal pH for ABO process is from 6.5 to 8.5) or decreased oxidation capacity (activity) of living micro-organisms. It is also possible, as stated by Kamiya and Hirotsuji [4] that the deterioration of effluent water quality was caused by increased organic loading as a side effect of the reduction of the concentration of AS.

After 8 to 10 days of operation, COD and BOD removal efficiency in integrated process started to drop. It was possibly related to the reduced biomass concentration, although, as one can see from Figure 1a, the reduction of biomass concentration was much faster compared to changes in effluent water quality. In the light of these results, it is justified to ask, what happens to the sludge when it is subjected to ozonation, i.e. how the viable (active) part of the sludge is affected?

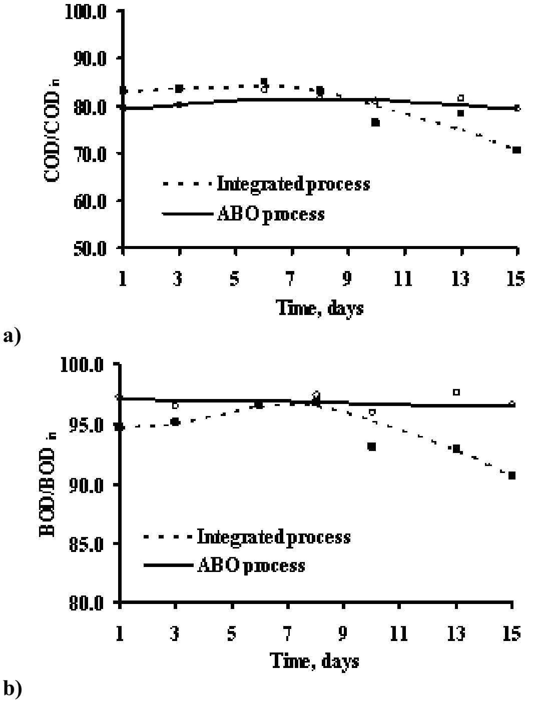


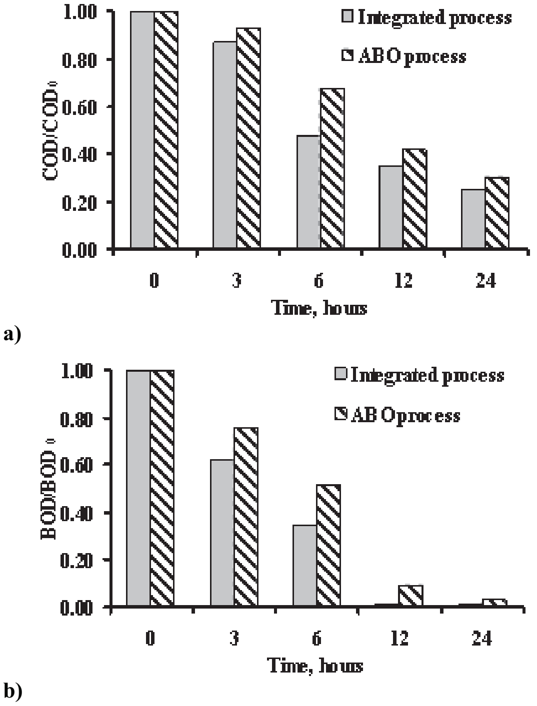
Fig. 2. Evolution of COD (a) and BOD (b) removal efficiencies in integrated and ABO processes

B Kinetics of removal of resorcinols

Study of the ABO and the integrated process in batch reactors was carried out to ascertain the kinetics of the processes. An average initial concentration of AS in both process was 1.46 g/L. Ozone dose, used in integrated process, was of 0.57 $mgO_3 \cdot L^{-1}$. At the start up of the study the wastewater was added to the bioreactor so that an average initial COD was of 1250 mg/L and BOD of 646 mg/L.

In Figure 3a and 3b changes in COD and BOD compared to initial values (COD_0 and BOD_0) are presented. The COD and BOD removal in integrated process remained higher compared to ABO process. In fact, in integrated process the same purification level was achieved approximately two times faster than in ABO process.

This finding shows that short-termed ozonation or small ozone doses actually increase biomass activity, as ozone oxidation alone (dose of 0.57 $mgO_3 \cdot L^{-1}$) can not be responsible for such a great increase in COD and BOD removal efficiency.



a)

b)

Fig. 3. Relative COD (a) and BOD (b) in batch reactors during the study of kinetics of processes

Target pollutants that were monitored during kinetic study, were resorcinols. As depicted in Figure 4, the concentration of resorcinols in integrated process decreased rapidly. Within 24 hours both processes were capable of reducing the concentration of resorcinols well below the maximum permissible concentration of 15 $mg \cdot L^{-1}$ for dibasic phenols, as stated in Estonian Water Act.

Effect of ozone on micro-organisms

According to the data, it is clear that small ozone doses have positive influence on ABO process, but it is still unclear, how to choose ozone dose that gives maximum increase in purification efficiency. In the following experiments carried out in batch reactors, an attempt to use the concentration of ATP as an indicator of concentration of viable micro-organisms in AS, is described.

In order to evaluate the effect of ozone on the ATP in AS, two different systems were subjected to batch ozonation:

1. AS in wastewater;
2. AS in distilled water (with necessary nutrients).

The results of ozonation of AS in wastewater (samples from continuous reactor) showed that transferred ozone doses up to a dose of ~30 $mgO_3 \cdot gMLSS^{-1}$ were not sufficiently large to reduce the concentration of viable micro-organisms in AS. When AS was re-suspended in distilled water, ozone started to reduce its viability almost immediately (Figure 5), whereas MLSS concentration was not changed (or the change was too small to detect). These results clearly indicate the importance of the detection of viability, as a measure of the concentration of AS, instead of MLSS concentration in ozonation of AS.

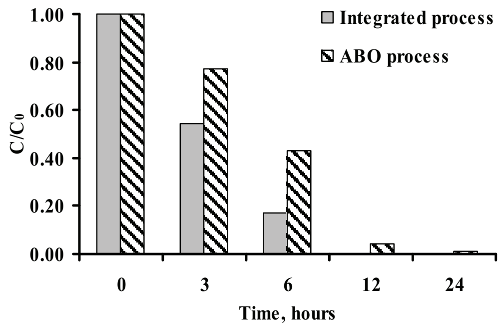


Fig. 4. Relative concentration of resorcinols in batch reactors

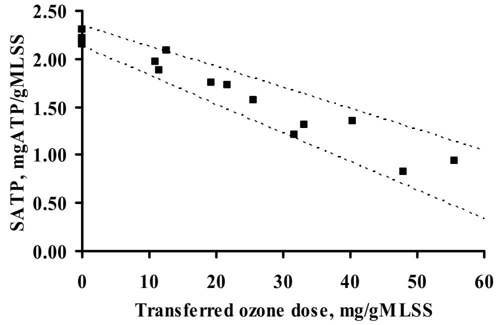


Fig. 5. Effect of transferred ozone doses on relative SATP of AS re-suspended in distilled water. Dotted lines represent upper and lower 95% confidence limit (obtained using regression data analysis tool in MS Excel)

An important factor always considered in wastewater treatment is the effluent water quality. It is usually expressed in terms of COD and BOD. In current study, during ozonation of the two systems, only changes in COD were monitored. The results of COD measurements are presented in Figure 6. An important difference between the two systems can be seen. In case of ozonation of AS only – there are no ozone reactive compounds in water that could deplete ozone before it reacts with AS – ozone attacks AS and increases the COD of effluent water (Figure 6a). When ozone reactive compounds are present, AS is not affected and ozone is used for oxidizing soluble compounds in wastewater. This decreases the COD of effluent water i.e. improves effluent water quality (Figure 6b).

The results are in good agreement with the results of the study of integrated process in continuous reactors and kinetic study. Thus the following conclusions can be made:

1. viability of AS or MLSS concentration in wastewater is not affected when ozone dose is under some specific critical value (Figure 1);
2. direct introduction of small ozone doses into ABO process may enhance the purification efficiency compared to conventional ABO process (Figures 2, 3, and 4);

when AS is attacked by ozone, it is solubilized (the concentration of ATP is decreased) and effluent water quality is deteriorated (Figure 2).

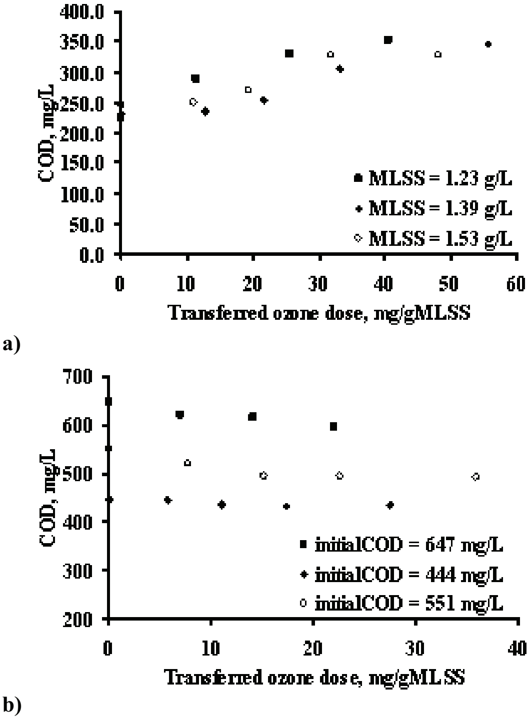


Fig. 6. Changes in effluent water quality, in terms of COD, during ozonation of AS in distilled water (a) and ozonation of AS in wastewater (b)

Thus, the direct ozonation of an activated sludge system is a complex process. Based on the results of the present study, it is clear that the optimisation of ozone dose in integrated process should be based on the experimental measurement of the ATP rather than MLSS concentration. A crucial factor to take into account is also the COD or more specifically the composition of water in bioreactor, as it may or may not contain enough solubilized compounds that react with ozone before it comes into contact with AS.

IV. CONCLUSIONS

Present study showed that small ozone doses introduced directly into bioreactor improved the COD removal efficiency of ABO of phenolic wastewater. Also, the pollutants (resorcinols) removal rate was improved in integrated process: the concentration of resorcinols was reduced approximately two times faster than in ABO process. This finding shows that short-termed ozonation or small ozone doses actually increase biomass activity and viability of micro-organisms is not affected. At higher ozone doses the performance of the process will deteriorate. This is caused by the reduced concentration of viable micro-organisms, as well as increased solubilization of micro-organisms. The optimal ozone dose depends on wastewater and activated sludge composition and should be experimentally determined.

The experiments of AS ozonation in batch reactors showed that ATP measurements are valuable, giving the precise amount of viable micro-organisms and enable to detect changes in viability.

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Olivers Jarviks, Inna Kameneva. Aktīvo dūņu ozonēšana periodiskas darbības reaktorā
Rūpniecisko un sadzīves notekūdeņu attīrīšanai plaši tiek lietota aerobā bioloģiskā oksidēšanās. Rūpnieciskie notekūdeņi bieži satur dažādas krāsvielas, toksiskas vielas, kuru biodegradēšana ir apgrūtināta.

Tādu problemātisko notekūdeņu attīrīšanai ir izstrādātas dažādas metodes, kas iekļauj sevī aerobās oksidēšanās un ozonēšanas kombināciju.

Dotās metodes ļauj samazināt notekūdeņu toksicitāti, uzlabot to biodegradāciju un kvalitāti, tomēr būtiski palielina procesa izmaksas, īpaši pie augstām ozona devām. Minēto trūkumu ir iespējams pārvarēt, izmantojot integrētu procesu, kad ozona ievadīšana notiek tieši bioreaktorā ar mērķi daļēji oksidēt bioloģiski grūti degradējošas un mikroorganismiem toksiskas vielas. Svarīga problēma ir ozona nepieciešamās dozas noteikšana, kas būtu pietiekoša attīrīšanas procesa efektivitātes palielināšanai, bet lai vienlaikus nenotiktu bioloģisko procesu inhibēšana.

Šī darba mērķis bija noteikt ozona dažādu devu ietekmi uz fenolu saturošu notekūdeņu bioloģiskās attīrīšanas efektivitāti (pēc ĶSP, BSP un rezorcīnu koncentrācijas) un uz dzīvo mikroorganismu daudzumu aktīvās dūņās. (pēc ATF daudzuma). Eksperimenti tika veikti kā nepārtrauktās darbības reaktorā (ozona deva 2,45 mg O_3/l), tā arī periodiskās darbības reaktoros (ozona deva 58 mg O_3/g aktīvo dūņu , kas aptuveni ir 80 mg O_3/l).

Eksperimentāli tika noteikts, ka nepārtrauktās darbības reaktorā nelielu ozona devu ievadīšana palielina fenolus saturošu notekūdeņu bioloģiskās attīrīšanas efektivitāti pēc ĶSP (aptuveni par 3%),taču kumulatīvi ievadītā ozona devas pieaugums no 45 līdz 56 mg O_3/g aktīvo dūņu izsauc aktīvo dūņu koncentrācijas samazināšanos, vienlaicīgi samazinājās procesa pH un tā kopējā efektivitāte.

Periodiskās darbības reaktoros veiktie eksperimenti parādīja, ka izmantojot ozona integrēšanas procesu, rezorcīnu koncentrācija samazinājusies aptuveni divas reizes ātrāk, nekā tradicionālajā bioreaktorā. Vienlaikus, pamatojoties uz dzīvo aktīvo dūņu daudzuma noteikšanu (pēc ATF) var secināt, ka ozona ietekme uz dūņu aktivitāti ir atkarīga no to vielu klātbūtnes notekūdeņos, kuras reaģē ar ozonu ātrāk par mikroorganismiem. Ja tādu vielu nav, tad ozons iedarbojas uz mikroorganismiem, kas būtiski samazina kopējo attīrīšanas efektivitāti. ATF koncentrācijas noteikšana (vai jebkāda cita analīzes metode, kas tiek izmantota dzīvu aktīvo dūņu daudzuma noteikšanai) ir ārkārtīgi svarīga ozonēšanas, kā metodes, novērtēšanai. Ozonēšanas ietekme uz notekūdeņu bioloģisko attīrīšanu ir atkarīga no notekūdeņu un aktīvo dūņu sastāva, ko ir iespējams noteikt tikai eksperimentāli.

Оливер Ярвик, Инна Каменева. Озонирование активного ила в реакторе периодического действия.

Для очистки бытовых и промышленных сточных вод широко применяется аэробное биологическое окисление. Однако известно, что некоторые промышленные сточные воды содержат плохо разлагающиеся биологически, токсичные и сильно окрашенные вещества.

Для очистки таких проблемных сточных вод разработаны различные методы, включающие в себя комбинацию аэробного окисления с пред- и постозонированием.

Данные методы позволяют снизить токсичность сточных вод, улучшить их биоразлагаемость и качество, однако существенно увеличивают стоимость процесса, особенно при высоких дозах озона. Указанный недостаток можно преодолеть, используя интегрированный процесс, при котором ввод озона осуществляется непосредственно в биореактор с целью частично окислить биологически трудно разлагаемые и токсичные для микроорганизмов вещества. Одновременно с этим, при правильном дозировании озона, можно добиться заметного снижения количества избыточного активного ила. При этом проблемой является подбор необходимой дозы озона, достаточной для увеличения эффективности процесса очистки, и одновременно с этим недостаточной для ингибирования биологических процессов.

Целью данной работы являлось определить влияние различных доз озона на эффективность биологической очистки фенольных сточных вод (по ХПК, БПК и концентрации резорцинов) и на количество живых микроорганизмов в активном иле (по количеству АТФ). Опыты проводились как в реакторе непрерывного действия (доза озона 2,45 мгO_3/л), так и в периодических реакторах (доза озона 58 мгO_3/г активного ила, что приблизительно равно 80 мгO_3/л).

Опытные данные показали, что введение в реактор непрерывного действия небольших доз озона увеличило эффективность процесса очистки фенольных сточных вод по ХПК (примерно на 3%), однако когда кумулятивно введенная доза озона возросла с 45 до 56 мгO_3/г активного ила, концентрация активного ила начала снижаться. Снизилось так же pH процесса и его общая эффективность.

Опыты, проведенные в периодических реакторах показали, что в случае использования интегрированного процесса концентрация резорцинов снижалась примерно в два раза быстрее, чем в традиционном биореакторе. Одновременно с этим, на основе определения количества живого активного ила (по АТФ), можно заключить, что влияние озона на активность ила зависит, прежде всего, от наличия в сточных водах веществ, которые реагируют с озоном быстрее, чем микроорганизмы. При отсутствии таких веществ озон атакует микроорганизмы, что ведет за собой значительное снижение результатов. На основе опытных данных можно утверждать, что определение концентрации АТФ (или любой другой метод анализа, применяемый для определения количества живого активного ила) является чрезвычайно важным для оценки озонирования на процесс биологической очистки сточных вод. Важно так же то, что влияние озонирования зависит от состава сточных вод и активного ила, что можно определить только экспериментальным путем.

PAPER IV

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Activated sludge process coupled with intermittent ozonation for sludge yield reduction and effluent water quality control

Oliver Järvik,* Andres Viiroja, Sven Kamenev and Inna Kamenev

Abstract

BACKGROUND: Ozone is applied in wastewater treatment for effluent water quality improvement (post-ozonation) as well as for excess sludge reduction (in the recirculation line). There is some evidence that ozone dosed directly to aerobic biooxidation (ABO) process enhances degradation of recalcitrant compounds into intermediates, following their biodegradation in the same reactor. However, no information regarding the influence of ozone on sludge yield in this system was found. Therefore, the current work aimed to evaluate the effect of ozone on the sludge yield when ozone is dosed directly to the ABO process. In addition, batch and continuous treatment schemes for phenolic wastewater treatment are compared.

RESULTS: The results revealed that an optimal ozone dose of ∼30 mgO$_3$ L^{-1} day^{-1} reduced the sludge yield by ∼50%, while effluent water quality in terms of total chemical oxygen demand (TCOD), compared with a conventional ABO process, was improved by 35.5 ± 3.6%. Slight improvement in soluble COD removal at the same ozone dose was also detected. The toxicity of effluent water was reduced as the ozone dose was increased.

CONCLUSIONS: In an integrated ozonation-ABO process it is possible to simultaneously reduce sludge yield and to improve effluent water quality, as COD and toxicity are reduced.

Keywords: activated sludge; bacteria; ozonation; waste minimization; wastewater; purification

INTRODUCTION

Wastewater treatment plants are facing the challenge of reducing pollutants discharge into the environment. Among the different pollutants, excess sludge is considered a possible contaminant, the production of which should be reduced. A promising sludge disintegration technique is the sludge ozonation process. Ozonation of activated sludge in a recirculation (return activated sludge) line is described in several articles. It is shown that this method allows up to 100% excess sludge reduction with an ozone dose of 150 mgO$_3$ g^{-1}TSS treated – i.e. 1 mg of ozone absorbed in suspension per gram of treated total suspended solids.[1,2] The optimal ozone dose for the highest ozone efficiency is found to be approximately 50 mgO$_3$ g^{-1}TSS treated. This dose reduces excess sludge production by 25 to 35%.[1] Ozone acts on bacterial cells by releasing intracellular compounds to the surrounding environment. Reports have pointed out that the destruction of cells slightly increases the effluent chemical oxygen demand (COD) and total organic carbon (TOC),[1−3] which would require a subsequent aerobic treatment step for the removal of the formed biodegradable cell constituents.[4,5]

It is evident that excess sludge production is reduced when the ozone dose is increased. However, ozonation may possibly result in deteriorated system performance at high ozone doses. It has also been pointed out that residual ozone may affect detrimentally the performance of the biological wastewater treatment process[6] as ozone is a strong toxicant for bacteria. Therefore, ozone introduction directly to a bioreactor is not usually considered as an option despite there being several reports of the positive effect of direct introduction of ozone into an activated sludge process. Van Leeuwen *et al.*[7,8] conducted experiments to investigate the possibility of combining ozonation and biological wastewater treatment into one integrated unit with the aim of improving effluent water quality. The target pollutants in these studies were different recalcitrant dyes. Saayman *et al.*[9] conducted full-scale experiments where ozone was introduced at doses of 0.36, 1.07 and 1.45 gO$_3$ kg^{-1}MLSS day^{-1} (gram of ozone dosed per gram of mixed liquor suspended solids per day) directly to a biological wastewater treatment unit for sludge bulking control. The results of the above mentioned studies were promising, showing that the integrated system could be beneficial for the removal of recalcitrant compounds from wastewater and for the control of sludge bulking. Thereby the effluent water quality was improved.

Kamiya and Hirotsuji[10] studied an integrated system using intermittent sludge ozonation up to a dose of 21.4 mgO$_3$ g^{-1}aeration tank MLSS day^{-1} with the aim of evaluating the effectiveness of the system. They ozonated part of the sludge (4 out of 6 L of sludge in a day in six ozonation cycles) and showed that the method is more effective for the reduction of excess sludge production than

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for continuous ozonation, mainly due to higher ozone gas concentration. Additionally, ozonation improved effluent water quality in terms of TOC, while dissolved organic carbon (DOC) was slightly increased. Ozonation also improved sludge settling properties.

Considering the results presented above, the current research aimed to evaluate the effectiveness of the activated sludge process coupled with ozonation (i.e. an integrated system) for purification of high strength oil-shale phenolic wastewater. The wastewater from the Estonian oil-shale industry is mixed before purification with domestic wastewater to increase biodegradability and to reduce the toxicity of the phenols (mono- and dibasic) in the phenolic wastewater.[11] Nevertheless, measurements show that biochemically treated effluent does not meet the standards for suspended solids, color[12] and frequently also for COD.[13] This is assumed to be caused by the phenols in wastewater, which are classified as toxic or highly toxic.[13] Ozonation is found to reduce the toxicity of oil-shale phenols efficiently;[14] among other oxidation technologies it has the highest potential for practical applications for degradation of phenols,[15] and in the recirculation line has shown the potential to reduce excess sludge production. Thus, the influence of ozone dosed directly to the aeration tank on sludge yield, viability and structure was studied. As it is usually more convenient to carry out batch experiments, the possibility of evaluating the impact of ozone on the activated sludge process using the batch ozonation procedure is discussed.

MATERIALS AND METHODS

In the study the current environmental situation and actual problems related to the treatment of oil-shale wastewaters were taken into account. In order to evaluate the effect of ozone on the activated sludge (ABO) process the following studies were carried out:

- batch ozonation of activated sludge suspension;
- intermittent ozonation of activated sludge suspension in continuous reactors.

The influence of ozone on the activity of activated sludge was evaluated using the oxygen uptake rate (OUR) and also adenosine-5′-triphosphate (ATP) measurement. The following effluent water quality parameters were measured: total COD (TCOD), soluble COD (SCOD), toxicity, polysaccharide concentration, turbidity, color, and pH. In addition, mixed liquor suspended solids (MLSS) concentration and polysaccharide concentration in MLSS were daily monitored.

Wastewater

In the experiments synthetic phenolic wastewater was used, chosen in view of the fact that purification of phenolic wastewater from the Estonian oil-shale industry is not adequate in terms of effluent water quality (mainly COD, color, and turbidity),[13] and needs to be improved. Synthetic wastewater was used because real wastewater composition may vary significantly over time depending on the process parameters, causing severe interferences with the aerobic treatment unit, making it difficult or even impossible to achieve steady-state conditions. For the preparation of synthetic wastewater, a fraction of oil-shale phenols (marketed under the name Rezol) produced in VKG Oil AS was used. Rezol was separated from the process water in the dephenolation unit (extraction) and dephenolated water was mixed with municipal wastewater before biological purification. Therefore the synthetic wastewater was assumed to closely mimic the composition of real wastewater.

The synthetic wastewater contained [mg L^{-1}] the following: Rezol [614] (supplied by the VKG Oil), $(NH_4)_2SO_4$ [205] (Lach-Ner), NH_4Cl [90] (Lach-Ner), $NaC_2H_3O_2$ [90] (Lach-Ner), $CH_3COOC_4H_9$ [200], C_2H_5OH [93] (Lach-Ner), CH_3COOH [110] (Lach-Ner), CH_3OH [90] (Merck), KH_2PO_4 [15] (Riedel-de Haen), K_2HPO_4 [39] (Lach-Ner), Na_2HPO_4 [32] (Riedel-de Haen), $FeCl_3$ [0.4] (Lach-Ner), $MgSO_4$ [27] (Lach-Ner), $CaCl_2$ [124] (Lach-Ner). The average soluble chemical oxygen demand (COD) of the wastewater was 2240 mgO_2 L^{-1}.

According to the VKG Oil AS laboratory the composition of Rezol is as follows [%]: monobasic phenols [0.06], catechol [0.05], 3-methylcatechol [0.02], 4-methylcatechol [0.07], resorcinol [2.7], 2-methylresorcinol [1.0], 4-methylresorcinol [2.4], 5-methylresorcinol [40.4], 2,5-dimethylresorcinol [7.0], 5-ethylresorcinol [10.7], 4,5-dimethylresorcinol [7.6], and unidentified compounds with higher molecular mass than 4,5-dimethylresorcinol [28.0].

Continuous activated sludge process with intermittent ozonation

The ABO process (with intermittent ozonation) was studied in four identical lab-scaled continuous stirred tank reactors (CSTRs). One served as a control reactor (conventional ABO), while ozone was intermittently added at different doses to the sludge in the other reactors. The aeration basin of each reactor had a volume of 2 L and settling basin of 0.3 L.

A magnetic stirrer (Velp® Multiposition Stirrer, Italy) was used to agitate the suspension in the aeration basins. Hydraulic retention time (HRT) during the experiments in continuous reactors was maintained at 3 days and SRT (sludge retention time (age)), depending on the applied ozone dose, ranged between 7 and 16.5 days. The reactors were kept at room temperature (21 ± 1 °C). During the ABO stage, dissolved oxygen concentration was maintained between 2 and 4 mg L^{-1} to avoid rate limitation. The sludge flocks formed under these conditions were small and effluent water was turbid as it contained filamentous organisms (possibly *Haliscomenobacter hydrossis* often found in activated sludge treating phenolic wastewater. Identification was carried out only by microscopy).

A lab-scaled unit was operated for 3 to 5 days after steady state conditions were reached (duration of one cycle from 6 to 14 days). Steady state conditions were considered to be reached when effluent water parameters were maintained nearly constant. During the period of steady state conditions, the data were recorded daily (sampling stage) and were then averaged to represent a single data point for each parameter measured. Error bars represent the standard deviation during a steady state period.

Ozonation procedure

Ozonation was carried out in a 2.6 L cylindrical glass reactor. The ozone–air gas mixture produced from compressed air by a Labo LO (Trailigaz, France) ozone generator was introduced to the reactor through the porous glass diffuser. Ozone concentration was ~20 mgO_3 L^{-1}, gas flow rate 0.4 L min^{-1}. Ozone concentration in the inlet and outlet gas was measured with an ozone analyzer (Anseros GM-PRO and PCI-Wedeco, respectively). Ozonation was carried out at room temperature (21 ± 2 °C).

For intermittent ozonation (at the conditions described above), the suspension in the continuous reactor was decanted to batch the ozonation reactor. This methodology was used to assure

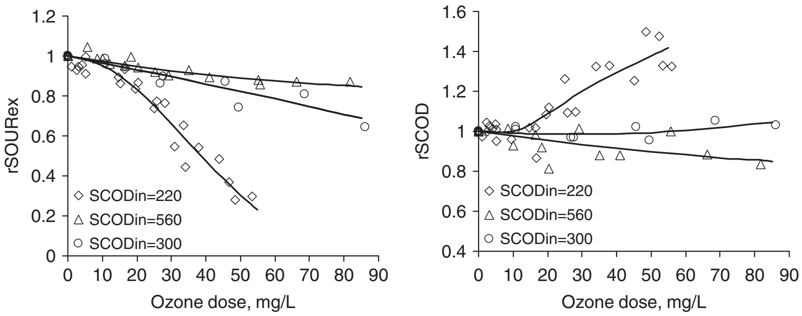

Figure 1. Influence of ozone on sludge activity (a) and SCOD (b) affected by initial SCOD during batch ozonation.

precise ozone dose detection. The ozonated suspension was then placed back in the continuous ABO reactor.

To evaluate the impact of ozone during the batch ozonation of activated sludge suspension, the suspension (2 L) was split into sludge and liquor by centrifugation (2500g, 30 min) with subsequent filtration. Sludge was then re-suspended in 2 L of BOD dilution water, where magnesium sulfate, phosphate buffer, and ferric chloride solution concentration were increased by 2.5 times and calcium chloride solution concentration by 10 times. In addition, 82 mg L^{-1} of ethanol as carbon source was added. Dilution water rather than distilled or tap water was used to ensure uniform composition throughout the studies and to avoid cytolysis. Final average initial SCOD of re-suspended suspension was measured to be approximately 220 $mgO_2\ L^{-1}$.

Oxygen uptake rate (OUR) measurement

Before OUR measurements each sample (after batch ozonation and during the sampling stage in a continuous process) was saturated with oxygen (up to 5 to 7 mg L^{-1}), pH of the samples was not adjusted. The procedure was carried out in two airtight vessels (both 100 mL), where dissolved oxygen (DO) concentration was measured by an electrochemical probe (supplied by Elke Sensor LLC, Estonia). Data were recorded using MS Excel software. The suspension in measuring vessels was stirred using a magnetic stirrer. During the first 2 min DO values in the samples were recorded to determine endogenous OUR (*OURend*), after that 1 mL of synthetic phenolic wastewater was added. DO was further recorded for another 4 min to determine the total OUR (*OURtot*). Exogenous OUR (*OURex*) for sludge activity determination was calculated as follows: ***OURex*** = ***OURtot*** − ***OURend***, where OUR is the dissolved oxygen concentration depletion in the measuring vessel over time ($mgO_2\ L^{-1}\ min^{-1}$).

Determination of adenosine triphosphate (ATP)

ATP was extracted from the samples and analyzed as described in Järvik *et al.*[16]

Analyses

To evaluate the performance of the reactors, COD,and MLSS were determined according to Standard Methods (5220 D and 2540 B, respectively).[17] pH was measured with a pH meter (Mettler Toledo SevenEasy). Turbidity and color were measured according to the Attenuated Radiation Method and the APHA Platinum-Cobalt Standard Method, respectively, using a Hach DR/2010 spectrophotometer. Polysaccharide content was measured by the phenol–sulfuric acid method,[18] using D-glucose as a standard.

Changes in the sludge structure were monitored using a Nikon microscope Microphot-fx equipped with an Olympus C-5060 digital camera. For an average flock size evaluation, micrographs of a sample and calibrated standard scale were taken (total magnification 100×, 200× and 400×) and analyzed later by measuring the length and breadth of at least 40 randomly chosen flocks in each sample.

Toxicity of the raw wastewater and effluents from the continuous activated sludge process with intermittent ozonation were estimated using *Daphnia magna*, as described by Trapido and Veressinina.[14]

RESULTS AND DISCUSSION

Influence of ozone on activated sludge during batch ozonation

In order to evaluate the possible impact of ozone on the ABO process, batch studies were carried out. The results showed that the influence of ozone on the ABO process, when ozone is dosed directly to the activated sludge, depends mainly on the soluble COD (compounds that deplete ozone) in a bioreactor. The influence of different ozone doses on the activity of activated sludge, expressed as the relative specific exogenous oxygen uptake rate (*rSOURex*) is shown in Fig. 1(a). It can be seen that the initial SCOD has a strong influence on the ability of the ozone to reduce the activity of activated sludge. When no such soluble compounds that could react with ozone before it comes into contact with activated sludge (as in the case of sludge re-suspended in dilution water with *SCODin* = 220 mg L^{-1} in Fig. 1(a)) are present in water, then the activity is reduced, as shown by the lower line in Fig. 1(a). A relatively low ozone dose is needed to deactivate sludge. The larger the quantity of compounds that will deplete most of the ozone before it reacts with sludge (expressed as SCOD), the more ozone must be dosed for activity reduction (*SCODin* = 560 mg L^{-1} – sludge in 'original' liquid and *SCODin* = 300 mg L^{-1} – sludge re-suspended in 'original' liquid and in dilution water at the ratio 1 : 1 (v/v)).

Sludge activity reduction is assumed to be caused by destruction of the active micro-organisms – ozone penetrates the cell wall and cell constituents are released to the surrounding environment. This means that SCOD, which serves as a substrate for the micro-organisms, is increased.

A comparison of the changes in *rSOURex* and relative SCOD (*rSCOD*) in Fig. 1(a) and 1(b), respectively, shows that even if *rSOURex* reduces, *rSCOD* does not change (in the cases

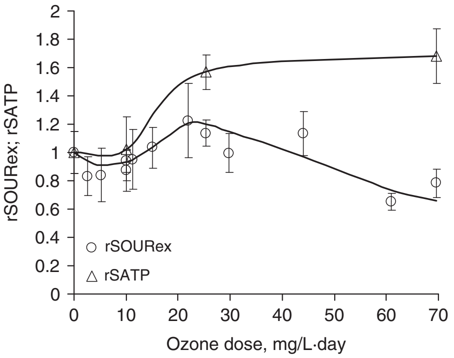

Figure 2. Activity of sludge (measured as OUR and ATP concentration) as a function of ozone dose (error bars represent standard deviation).

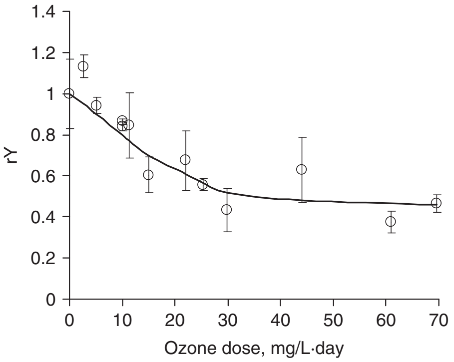

Figure 3. Relative sludge yield affected by intermittent ozonation (error bars represent standard deviation).

Table 1. Dimensions of an average sludge flock in bioreactors

Ozone dose, $mgO_3\ L^{-1}\ day^{-1}$	Dimensions, µm
0	44 ± 18 × 81 ± 31
10	52 ± 25 × 102 ± 58
25	52 ± 19 × 91 ± 42
70	69 ± 18 × 135 ± 35

$SCODin = 560\ mg\ L^{-1}$ and $SCODin = 300\ mg\ L^{-1}$), assuming that cell lysis has not taken place or lysed cell material is oxidized by ozone or/and consumed by active respiring micro-organisms. As most of the lysed cell material is considered to be readily biodegradable, the last option seems to be reasonable, although the slight decrease in pH of the suspension (an average of 0.045 units per 10 $mgO_3\ L^{-1}$) indicates that oxidation by-products are also formed.

In the case $SCODin = 220\ mg\ L^{-1}$ (no soluble compounds that could deplete ozone), the activity of sludge is reduced through cell lysis, as shown by the increased *rSCOD* value in Fig. 1(b). It is important to note that cell constituents appear not to hinder the sludge activity reduction during batch ozonation, as the trend in Fig. 1(a) is not curved upwards as ozone dose is increased (i.e. the reduction rate of *rSOURex* is constant).

In conclusion, batch ozonation studies with the measurement of activity and water quality parameters indicate a possible impact of ozone on the ABO process.

The main disadvantage of the batch studies is that they do not always describe adequately processes in continuous reactors (especially on a large scale). It is also difficult to measure changes in different parameters, e.g. in the sludge yield. To evaluate the impact of intermittent ABO process ozonation on the sludge yield and effluent water parameters, and to evaluate the usefulness of the results obtained from batch experiments, studies in continuous reactors were carried out.

Impact of ozone on ABO – study in continuous reactors

Sludge activity

Experiments in continuous reactors were carried out as described in Materials and methods. Results show an initial *rSOURex* reduction (Fig. 2) when the ozone dose is increased from 0 to about 5 $mg\ L^{-1}\ day^{-1}$ (initial average SOUR value was 39 $mgO_2\ g^{-1}MLVSS\ h^{-1}$). Further increase in the ozone dose to ∼20 $mg\ L^{-1}\ day^{-1}$ also increased sludge activity by 20% compared with the control (zero-dose) sludge. Higher ozone doses (up to 70 $mg\ L^{-1}\ day^{-1}$) gradually reduced the SOUR to 60% of its initial value, i.e. destruction of active biomass was initiated, as demonstrated by the lower sludge yield (Fig. 3).

To estimate the amount of viable activated sludge, the *Adenosine TriPhosphate* (ATP) concentration in non-ozonated and several ozonated samples (10, 25, and 70 $mgO_3\ L^{-1}\ day^{-1}$) was measured (Fig. 2). The measured ATP values were very low (ranging from 0.090 to 0.220 $mgATP\ g^{-1}MLVSS$), suggesting that the actual number of viable micro-organisms is relatively low (during batch ozonation studies SATP in non-ozonated sludge samples ranged from 2.30 to 2.80 $mgATP\ g^{-1}MLVSS$). Previous batch studies[16] have shown that the correlation between ATP and OUR is relatively good. Therefore it is not exactly clear why the ATP concentration does not correlate with the OUR at an ozone dose of 70 $mgO_3\ L^{-1}\ day^{-1}$. A possible explanation for the phenomenon is spore formation by some of the micro-organisms to survive the oxidative stress caused by sludge ozonation. Although with the concentration lower than respiring bacteria, spores still contain ATP, they do not consume oxygen.[19,20] Also, as stated by Gray,[21] the presence of large organisms, especially protozoa and rotifers, make ATP measurement less useful as a measure of bacterial viability.

Sludge yield

Average sludge yield in the ABO process ranged from 0.16 to 0.22 $gMLSS\ g^{-1}COD$, which gradually decreased for doses up to 30 $mgO_3\ L^{-1}\ day^{-1}$ and then almost leveled off (Fig. 3). It is reasonable to assume that as the portion of active biomass increases, the sludge yield also increases. Microscopic studies revealed that the suspension in the reactor contained dispersed filamentous organisms (length 20–70 µm). Compared with non-ozonated sludge, the amount of these dispersed organisms in ozonated samples was reduced, while the number of rotifers and dimensions of flocks was increased (small, pinpoint flocks) (Table 1). The abundance of rotifers was seen at an ozone dose of 10 $mgO_3\ L^{-1}\ day^{-1}$, while the destruction of dispersed non-soluble organisms was noticeable when the ozone dose exceeded 25 $mgO_3\ L^{-1}\ day^{-1}$. During ozonation dispersed organisms are broken into smaller particles with length less than 5 µm. This matter serves as a food for ciliated protozoa and rotifers who feed

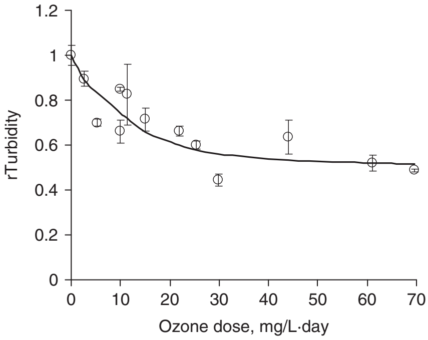

Figure 4. Effluent water turbidity during steady state conditions (error bars represent standard deviation).

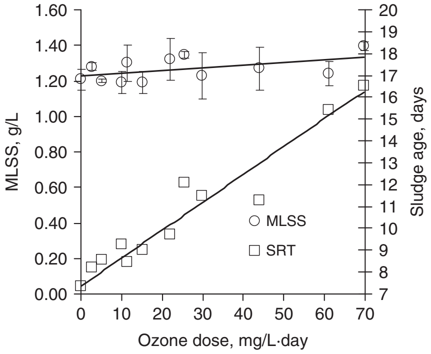

Figure 5. Average MLSS concentration and SRT during steady state conditions (error bars represent standard deviation).

on activated sludge flocks (bacteria) and non-soluble particles with the size smaller than 10 µm. Trends in the destruction of dispersed organisms and increased concentration of rotifers could be considered as the main reason for the initial increase in SOUR and SATP, while the sludge yield was reduced. In fact, investigations into bacterial predation have indicated that sludge yield is considerably lowered in the presence of large numbers of protozoa and metazoa.[22]

Kamiya and Hirotsuji[10] stated that the impact of ozone on activated sludge in the ABO process depends mainly on the applied ozone dose (at certain ozone gas concentrations) and sludge characteristics. According to their results only 11 $mgO_3\ g^{-1}MLSS\ day^{-1}$ is needed to reduce the sludge yield by 50% using intermittent ozonation (influent ozone gas concentration 32 $mgO_3\ L^{-1}$). In their work the DOC (dissolved organic carbon) concentration in a bioreactor was approximately 20 $mg\ L^{-1}$. In the current study, an average initial SCOD (although not directly comparable with DOC) in continuous ABO reactors treating phenolic wastewater was 280 $mg\ L^{-1}$. This can be considered one of the reasons why approximately 30 $mgO_3\ L^{-1}\ day^{-1}$ (influent ozone gas concentration 20 $mgO_3\ L^{-1}$) was needed to reduce the yield by 50%. Therefore, as the difference between ozone doses needed for 50% sludge reduction is remarkable, we may conclude that the concentration of soluble compounds (expressed here as SCOD) is a crucial factor when considering the impact of ozone on sludge.

Influence of ozone on effluent water quality and toxicity

Turbidity

Dispersed small particles in suspension affect detrimentally the effluent water quality as they are non-settleable. This is a problem often seen in the ABO process treating phenolic wastewaters.[24] Non-settleable particles increase effluent water turbidity and reduce other quality parameters (like COD and BOD). As microscopic studies revealed, low ozone doses used in this study seemed to act strongly on dispersed matter reducing their number greatly. This reduction was confirmed by the measurement of effluent water turbidity (Fig. 4), which was gradually improved up to a dose of 30 $mg\ L^{-1}\ day^{-1}$. This dose reduced the turbidity of the effluent water by 55.6 ± 2.8% compared with the ABO process (average turbidity in the ABO process was 325 ± 18 FAU). The turbidity remained practically constant when the ozone dose was further increased.

The reduction of dispersed particles in effluent water was the main contribution to the improvement in effluent water quality. As turbidity was reduced, the amount of sludge washed out with the effluent water decreased. Therefore, although the sludge yield was lowered (at an ozone dose ∼25 $mgO_3\ L^{-1}\ day^{-1}$ by 44.3 ± 2.8% and at a dose 70 $mgO_3\ L^{-1}\ day^{-1}$ by 53.4 ± 4.1%), the amount of sludge in the bioreactor, compared with the control reactor, was actually slightly increased (at an ozone dose ∼25 $mgO_3\ L^{-1}\ day^{-1}$ by 9.1 ± 6.6% and at a dose 70 $mgO_3\ L^{-1}\ day^{-1}$ by 14.0 ± 7.2%) (Fig. 5). Based on mass balance calculations, sludge age increased from ∼7 days for non-ozonated sludge to 16.5 days at the highest ozone dose (Fig. 5). Increased sludge age formed the basis for the development of rotifers, which unlike protozoans, are generally more abundant in processes with long sludge ages.[21]

Polysaccharides and dissolved oxygen

Although some of the turbidity removal could be attributed to the direct introduction of ozone to the bioreactor and to the increased number of rotifers, changes in polysaccharide and dissolved oxygen content may have also played an important role. Rotifers do not only clarify the effluent water by removing suspended material, they contribute also to flock formation by producing fecal pellets consisting of undigested material, which forms the 'backbone' of new flocks. It is known that most of the heterotrophic bacteria in activated sludge are negatively charged and hydrophobic. Without oxygen limitation the hydrophobic properties are retained, giving the sludge better settling properties.[26] Reactions between activated sludge/contaminant and ozone usually yield one molecule of oxygen per molecule of ozone. During intermittent sludge ozonation, depending on the applied ozone dose, oxygen concentration in the water phase temporarily increased to more than 30 $mg\ L^{-1}$, possibly increasing the oxygen concentration within the flocks.

It is estimated that in order to form a settling sludge flock, extracellular polymeric substances (EPS) (proteins, nucleic acids, humics, lipids, and polysaccharides) and multivalent cations are needed. Negatively charged bacteria can bind themselves through cationic minerals on the EPS and form flocks that are essential for settling sludge. When sludge is young, due to the excessive washout, as in the case of the conventional ABO process in the current study, weak and small sludge particles with an inadequate concentration of flocculating polysaccharides are formed.[27] As the sludge age is increased, the EPS (polysaccharide) concentration is increased.[28]

In this study polysaccharide content was measured in order to evaluate its influence on reduced turbidity. In the ABO process an average polysaccharide concentration in MLSS was found to be 23.0 ± 1.6 mgGlu g^{-1}MLSS (milligrams of D-glucose per gram of MLSS). When the ozone dose was increased, polysaccharide concentration was also increased, reaching 53.2 ± 3.0 mgGlu g^{-1}MLSS at the maximum ozone dose. These changes may be considered a reason for reduced effluent water turbidity in ozonated reactors.

According to Chu *et al.*,[28] with an ozone dose of 0 to 20 mg g^{-1}TSS polysaccharide content increased from 5 to 38 mgC L^{-1} in the supernatant. We found that indepedent of the applied ozone dose, intermittent ozonation caused practically no changes in polysaccharide concentration in the filtered effluent water which fluctuated between 1.6 ± 0.6 mgGlu L^{-1} and 3.2 ± 0.4 mgGlu L^{-1}. As stated in Gray,[21] the EPSs are not easily biodegradable, therefore the increase in their concentration would also reduce effluent water quality.

To summarize, possible reasons for increased polysaccharide concentration in activated sludge and reduced turbidity caused by intermittent ozonation are increased sludge age (as less sludge was washed out), increased loading when sludge was partly lysed,[29] and increased oxygen concentration.

COD, color, and toxicity

Ozonation of activated sludge causes cell disruption and releases intracellular compounds to the surrounding environment. Therefore sludge ozonation increases DOC concentration and COD. According to Yeom *et al.*,[23] sludge disruption by ozone at an ozone dose of 50 mg g^{-1}TSS turned 19% of sludge into SCOD and approximately 14% to dispersed particles. An ozone dose of 100 mg g^{-1}TSS increased the soluble and dispersed particles concentration to 25% and 24% of the TCOD, respectively. Further increase in the ozone dose causes sludge mineralization.

As turbidity was reduced, the TCOD (SCOD + COD of dispersed matter) of effluent water was also reduced (Fig. 6(a)). The average TCOD in ABO processes was 598 mg L^{-1}, while the lowest value was 525 mg L^{-1} (Exp. 3) and the highest value 681 mg L^{-1} (Exp. 2). The highest ozone dose introduced to the ABO process improved the effluent water quality in terms of TCOD by as much as 35.5 ± 3.6% (TCOD of effluent water 385 ± 8 mg L^{-1}), compared with the conventional ABO process.

SCOD values in the ABO process were measured to range from 239 (Exp. 1) to 323 mg L^{-1} (Exp. 2). It is important to note that up to an ozone dose of 70 mg L^{-1}, the SCOD removal efficiency was almost unaffected (Fig. 6(b)), suggesting that the soluble cell content and dispersed matter released to the bulk solution adhered to flocks or were consumed by active bacteria and rotifers. No measurable accumulation of refractory compounds could be detected. It is also interesting to note that the activity reduction expressed in terms of *rSOURex* depicted in Fig. 2 did not influence the purification efficiency. Measured changes in SCOD in intermittently ozonated CSTRs are in good agreement with the results obtained in batch ozonation experiments when initial SCOD was 300 and 560 mg L^{-1} (Fig. 1(b)). This is mainly caused by the fact that ozonolysed sludge and the intermediate products formed are biodegradable and rapidly consumed in batch and continuous processes.

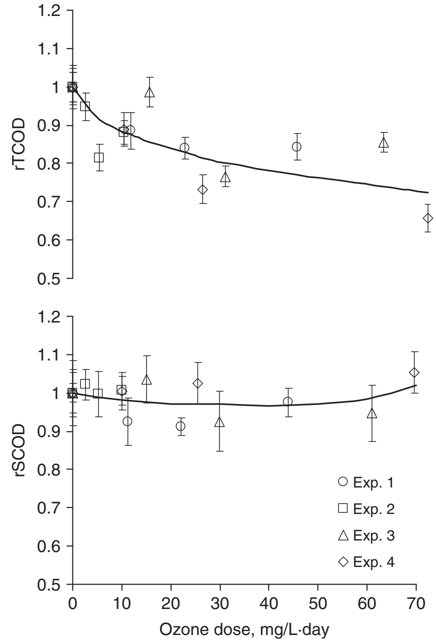

Figure 6. Influence of intermittent ozonation on the evolution of changes in rTCOD (a – upper) and rSCOD (b – lower) in effluent water (error bars represent standard deviation).

Table 2. Toxicity of oil-shale wastewater and effluents

Water	Ozone dose, mgO_3 L^{-1} day^{-1}	EC_{50} values and 95% confidence limits, %
Wastewater	0	2.0 (1.6–2.6)
Effluent	0	76.1 (70.2–83.6)
	10	83.2 (76.4–96.4)
	15	76.8 (67.2–90.3)
	25	97.8 (90.0–not toxic)
	30	not toxic
	60	not toxic
	70	not toxic

Intermittent ozonation reduced the effluent water color from an initial value of 390 ± 23 Pt-Co to 209 ± 5 Pt-Co at an ozone dose of 70 mgO_3 L^{-1} day^{-1}. Similar results were obtained when post-ozonation was used. This may be considered an expected result, as color-forming compounds contain unsaturated bonds that are prone to ozone attack. No effect of different ozone doses on pH of effluent water could be detected, showing that ozone oxidation by-products are rapidly consumed or the changes are too small to make any measurable difference.

Phenols are well known for their high toxicity, therefore, oil-shale phenolic effluents are considered to be toxic.[11,14] The toxicity of these effluents may significantly affect the quality and also the ecology of the receiving waters. The toxicity of the synthetic wastewater expressed as median effective concentration (and 95% confidence limit) was 2.0% (1.6–2.6%), while the toxicity of the effluent from the conventional activated sludge process was reduced to 76.1% (70.2–83.6%). When ozone was introduced to the activated sludge process at a dose 25 mgO_3 L^{-1} day^{-1}, the effluent was measured to be practically non-toxic. The toxicities with 95% confidence level are presented in Table 2.

Based on the changes in sludge yield and effluent water quality, it can be estimated that the optimal ozone dose for the present system is approximately 30 $mgO_3\ L^{-1}\ day^{-1}$ – further increase does not significantly improve the performance.

CONCLUSIONS

An integrated system in which ozone was intermittently dosed to the ABO process was studied. Comparison of the results obtained during batch and continuous ozonation studies showed that the former can only be used to predict changes in SCOD and color. The deviation in the values of other parameters, such as TCOD, sludge activity, turbidity, and pH, is too large. This is caused mainly by the changes in sludge composition – continuous studies last for a sufficiently long time to achieve steady state conditions and to develop its mixed liquor biota food chain. However, batch sludge ozonation experiments can be used to consider, for example, the impact of ozone on the purification process in the presence of different concentrations of soluble ozone reactive compounds.

Results showed that ozone dosing up to 30 $mgO_3\ L^{-1}\ day^{-1}$ directly to the ABO process treating phenolic wastewater, reduces an average initial sludge yield of 0.19 $gMLSS\ g^{-1}COD$ by ∼50%. Contrary to the widely studied sludge ozonation in the recirculation line, a remarkable improvement in effluent water quality in terms of turbidity and TCOD was achieved as sludge properties (dimensions) were improved and the amount of non-settleable dispersed bacteria was reduced. Deterioration in effluent water quality (in terms of soluble compounds, expressed as SCOD) as a result of ozone dosing directly to the activated sludge process could not be detected, thus the lysed cell material is biodegradable and forms a source of food for bacteria and rotifers. In contrast, effluent water quality in terms of SCOD and toxicity reduction was improved. At an ozone dose of 25 $mgO_3\ L^{-1}\ day^{-1}$ the effluent water was practically non-toxic to *Daphnia magna*. Intermittent ozonation generates conditions that favor the development of rotifers that are generally more abundant in processes with long sludge ages. Altogether, the results show that ozone, when dosed directly to the activated sludge process, can be trigger the activated sludge process and effluent water quality improvement in the case of phenolic wastewater treatment.

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PAPER V

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Influence of Activated Carbon on Ozonation of Phenolic Wastewater

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Keywords: Ozonation, activated carbon, phenolic wastewater, regression model

Abstract

The main goal of the current research was to study the ozonation of phenolic wastewater in the presence of activated carbon. In order to reveal possible interactions between ozone and activated carbon a three-level 2^{4-1} fractional factorial design was used in the experimental study. Based on the obtained experimental data, empiric models (regression equations) were developed. These models allowed the evaluation of the influence of design factors – oxygen-ozone gas volumetric flow rate, ozone concentration in the inlet gas, initial COD of phenolic wastewater, and concentration of activated carbon – on output parameters.

The adequacy of the regression equations was tested on experimental data. The results showed that the model can be used to predict the output parameters in the investigated range. However, the experimental results as well as calculations revealed that ozonation of oil-shale phenolic wastewater in the presence of activated carbon is relatively inefficient, as the highest achieved COD removal after 90 minutes of ozonation was approximately 35%. The calculations based on the empiric model indicated that by increasing the activated carbon concentration from 300 to 900 mg/L COD removal increased by 3%. An increase in gas flow rate from 0.25 to 0.75 L_{gas}/min improved COD removal by 9 % and an increase in ozone concentration in the inlet gas from 15 to 35 mgO_3/L_{gas} enhanced COD removal by 6%.

Introduction

Phenolic effluents, including oil-shale oil processing wastewater and semi-coke ash leachate, from the Estonian oil-shale industry have for many years been the major environmental issue in Estonia. Both process wastewater and leachate display high toxicity to micro-organisms [1]. Oil-shale oil process wastewater is treated together with domestic wastewater at aerobic bio-oxidation plant, where it due to its toxicity, is considered to be a potential cause of disturbances and difficulties. The phenolic wastewaters contain a wide range of mono- and dibasic phenols, carboxylic acids, ketones, basic nitrogen compounds etc. A few solutions have been tested and developed for abatement of phenols from various wastewaters [2]. Among other oxidation technologies ozonation is found to have the highest potential for practical applications for degradation of phenols [3].

Aerobic bio-oxidation with activated carbon treatment or ozonation was tested for the purification of phenolic wastewater in continuous-flow and batch reactors. The results have shown that both activated carbon addition and introduction of ozone directly to aerobic bio-oxidation (ABO) process improve the process performance. Further improvement was achieved as ABO, activated carbon treatment and ozonation were integrated into single unit [4]. In order to understand the possible interactions between activated carbon, ozone and activated sludge in aerobic bio-oxidation process, the influence of activated carbon on ozonation process must be studied.

Therefore, the main objective of the present research was to study the possibilities of ozonation of phenolic wastewater in the presence of activated carbon and to estimate the influence of activated carbon within ozonation process using a simple model consisting of the regression equations. Also the effect of activated carbon on the efficiency of ozonation of biochemically treated water (from ABO process) was evaluated.

Materials and Methods

A three-level 2^{4-1} fractional factorial design was used in the experimental study of ozonation of phenolic wastewater in the presence of activated carbon. Experiments were carried out in a 2.64 L semi-batch reactor. The independent design factors were oxygen-ozone gas volumetric flow rate G (0.25, 0.50, or 0.75 L_{gas}/min), ozone concentration in the inlet gas $C_{O3}^{initial}$ (15, 25, or 35 mgO_3/L_{gas}), initial COD of phenolic water $COD_{initial}$ (1000, 2100, 3200 mgO_2/L), and concentration of activated carbon C_{AC} (300, 600, 900 mg/L) (Table 1). Ozonation time was always 90 minutes. During the experiments, the following output parameters were measured (at 0, 10, 20, 40, 60, 90 min): pH, COD, BOD, ozone concentration in the sample C_{O3}^{liq} (mgO_3/L).

Phenolic wastewater was prepared by dissolving a fraction of oil-shale phenols (marketed under the name of Rezol, produced in VKG Oil AS) in tap water. The exact composition is described in [4]. pH of the phenolic wastewater ranged from 6 to 7. Average initial parameters of biochemically treated (HRT 5 days) effluent water were as follows: COD=354 mgO_2/L, BOD=9 mgO_2/L, pH=6.5.

COD was analysed by Hach Standard Method, and BOD was analysed according to procedure 5210 of the Standard Methods for the Examination of Water and Wastewater [5]. pH of the wastewater was measured with a pH-meter Mettler Toledo MP225. Ozone concentration in the liquid phase was analysed using the indigo method proposed by Bader and Hoigné [6] and ozone concentration in the gaseous phase was measured by ozone analyser, Anseros GM-6040.

The activated carbon used throughout the studies was Chemviron Carbon Filtrasorb 100 (F100) that was powdered, washed and dried prior to its addition to the reactor. The BET surface area was measured (Sorptometer KELVIN 1042) to be 1030 m^2/g.

Table 1. Initial values of design factors in the experiments of ozonation

Batch No	G, L_{gas}/min	$C_{O3}^{initial}$, mgO_3/L_{gas}	$COD_{initial}$, mgO_2/L	C_{AC}, mg/L
1	0.25	15	1000	300
2	0.25	35	1000	900
3	0.50	25	2100	300
4	0.50	25	2100	600
5	0.75	15	3200	300
6	0.75	35	1000	300
7	0.25	15	3200	900
8	0.75	35	3200	900
9	0.75	35	3200	300
10	0.50	25	2100	900
11	0.75	15	1000	900

The efficiency of ozonation of phenolic waters is best characterised by the removal of COD, other important output parameters of the process are BOD, changes in pH and ozone concentration in the liquid phase. The values of COD and BOD are used to calculate biodegradability expressed as the ratio of BOD/COD. The design factors that influence the efficiency are initial COD, gas volumetric flow rate, ozone concentration in inlet gas, concentration of activated carbon and treatment time. The regression model should comprise relationships between the output parameters (COD, BOD, pH and C_{O3}^{liq}) and the design factors.

In present research, the attention was mainly focused on linear and exponential regression models:

linear regression

$$Y_i = m_{i0} + \Sigma m_{ij} X_j \qquad (1)$$

and exponential regression

$$Y_i = m_{i0}(\Sigma m_{ij}^{X_j}) \qquad (2)$$

where Y_i is output parameter, X_j – design factor, m_{i0} and m_{ij} – coefficients related to design factor.

Ozonation time was considered as an independent design factor in modified regression models. Therefore coefficients m_{i0} and m_{ij} depend on the ozonation time:

$$m_{i0},\ m_{ij} = f(\tau)$$

Regression analysis was performed by using the least squares method (MS Excel functions LINEST and LOGEST). Both models have been tested for all of output parameters.

Results and Discussion

Experimental study of ozonation in the presence of activated carbon

The aim of experimental study was to supply data for modelling to reveal the interactions between activated carbon and ozone. Ozonation experiments were conducted under different conditions (different initial values of design factors) according to the fractional factorial design. Therefore the analysis of the experimental results gives only tendencies. Generally, the experimental results showed that regardless of the conditions (design factors) the removal of COD and BOD was faster during the first 10 minutes (only few cases 20 minutes) of ozonation. Although the concentrations of phenols were not measured, it may be predicted that the initial fast COD and BOD removal is a result of oxidation of oil-shale phenols due to high reaction rates between them. The decelerated decrease in COD and BOD removal is attributed to fact that ozone must react with formed oxygen-rich intermediates [3]. The overall COD removal efficiency was relatively low, ranging from 8.4 to 35%, depending on the operating conditions. In experiments the biodegradability of phenolic wastewater, expressed as BOD/COD ratio, as well as pH always decreased. The only exception was ozonation of biochemically treated effluent, where 2 to 5 fold increase in biodegradability was measured. This was explained by the fact that BOD of biochemically treated effluent was initially low (<10 mgO_2/L) and ozone oxidized refractory compounds (COD was 360 mgO_2/L) that passed ABO process.

In experiments 1, 3, 5, 6 and 9 ozonation was carried out in the presence of activated carbon at concentration of 300 mg/L. The results are presented in Table 2.

Table 2. Initial values of design factors and results of ozonation of phenolic wastewater (after 90 minutes) in the presence of activated carbon at concentration of 300 mg/L

No	$C_{O3}^{initial}$ mgO_3/L	G, L$_{gas}$/min	COD$_{initial}$ mgO_2/L	Δn, mgO_3/L	ΔCOD		ΔBOD,		$\Delta\left(\frac{COD}{BOD}\right)$ %	C_{O3}^{liq} mgO_3/L	$\frac{\Delta n}{\Delta COD}$ mgO_3/mgΔCOD
					mgO_2/L	%	mgO_2/L	%			
1	14.8	0.25	1100	221	195	18	139	27	11.3	0.10	1.14
3	25.4	0.50	2043	507	395	19	435	41	27.4	0.53	1.28
5	14.7	0.75	3178	632	555	17	453	30	30.2	0.08	1.14
6	35.1	0.75	1024	525	321	31	223	40	40.2	2.42	1.64
9	34.7	0.25	3209	570	477	15	466	27	14.7	0.00	1.20

The highest relative purification efficiency in terms of COD removal (%) was achieved in experiment 6. As also BOD removal (%) was high, the biodegradability was remarkably reduced indicating that subsequent biochemical purification is not feasible. The highest purification efficiency was achieved at the cost of the high amount of ozone consumed per one unit of COD removed (specific ozone consumption), namely 1.64 mgO_3/mgΔCOD. The high specific ozone consumption is possibly caused by the self-decomposition of ozone since rate of reactions between ozone and contaminants were low (as indicated by the highest concentration of soluble ozone).

In absolute values the best results in terms of COD removal were achieved in experiment 5 as a result of high gas flow rate. BOD removal was also high, while specific ozone consumption was lower than in experiments 3 and 6. The results of experiment 9 are almost comparable to those of 5. The difference between the experimental conditions in the two experiments was in volumetric gas flow rate and initial ozone concentration in the gas phase. In experiments 1 and 5 the flow rate is equal but ozone concentration in the inlet gas differs. Relative COD removals as well as relative BOD removals and specific ozone consumptions in those experiments were almost equal. The comparison of experiments 6 and 9 shows that increased gas flow rate improved mass transfer and ozone concentration in liquid phase was also increased (as high as 2.42 mgO$_3$/L in experiment 6). Therefore it can be concluded that ozonation is mainly limited by the rate of chemical reactions. On the other hand, in experiment 9 the low volumetric gas flow rate and high ozone concentration in the inlet gas was used. Under these conditions dissolved ozone was consumed in the reactions (ozone concentration in liquid phase was zero), indicating that ozonation process is limited by mass transfer. Thus, beside reaction kinetics, mass transfer plays an important role in ozonation.

In experiments 2, 7, 8, 10, and 11 activated carbon was added to the ozonation reactor with the concentration of 900 mg/L (Table 3).

Table 3. Initial values of design factors and results of ozonation of phenolic wastewater (after 90 minutes) in the presence of activated carbon at concentration of 900 mg/L

No	$C_{O3}^{initial}$	G,	$COD_{initial}$	Δn,	ΔCOD		ΔBOD,		$\Delta\left(\frac{COD}{BOD}\right)$	C_{O3}^{liq}	$\frac{\Delta n}{\Delta COD}$
	mgO$_3$/L	L$_{gas}$/min	mgO$_2$/L	mgO$_3$/L	mgO$_2$/L	%	mgO$_2$/L	%	%	mgO$_3$/L	mgO$_3$/mgΔCOD
2	34.5	0.25	967	336	247	26	160	35	12.4	0.37	1.36
7	14.9	0.25	3065	247	258	8	292	17	9.7	0.14	0.96
8	34.5	0.75	3027	1723	728	24	688	44	26.3	1.00	2.37
10	25.1	0.50	1954	492	480	25	382	38	17.7	0.55	1.03
11	15.9	0.75	997	375	353	35	265	50	23.4	0.28	1.06

Ozonation in the presence of activated carbon at concentration of 900 mg/L was in most cases more efficient in terms of relative COD and relative BOD removal compared to conditions when only 300 mg/L of activated carbon was added (Table 2 and 3). Also specific ozone consumption was lower ($COD_{initial}$=2100 mgO$_2$/L, G=0.5 L$_{gas}$/min, $C_{O3}^{initial}$ =25 mgO$_3$/L$_{gas}$).

The influence of activated carbon on specific ozone consumption is presented in Fig. 1. Under the light of these results, it may be concluded that activated carbon addition increases the efficiency of ozonation process. However, the results are not that straightforward – the lowest purification efficiency (COD and BOD removal 8% and 17%, respectively) was achieved in experiment 7, where activated carbon concentration and initial COD were fixed at highest values, but gas flow rate, and ozone concentration in the inlet gas were set to lowest values. Highest efficiency was achieved in experiment 11 (COD and BOD removal 35% and 50%, respectively), where activated carbon concentration and gas flow rate were set to highest values, initial COD and ozone concentration in the inlet gas were set to lowest values.

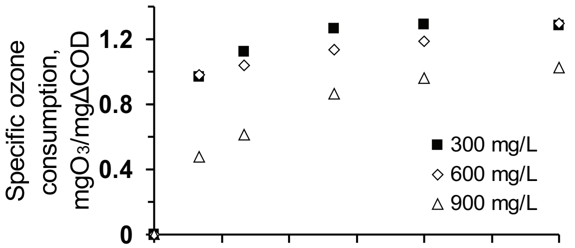


Figure 1. The dependence of specific ozone consumption of ozonation time and activated carbon concentration (the results of experiments 3, 4 and 10)

Additional experiments were carried out to evaluate the efficiency of only ozone (C_{AC}=0 mg/L), the impact of air stripping and air stripping with activated carbon addition ($C_{O3}^{initial}$=0 mgO_3/L_{gas}) on phenolic wastewater. The conditions were chosen according to average initial design factors – gas flow rate 0.5 L_{gas}/min, ozone concentration in the inlet gas 25 mgO_3/L_{gas}, $COD_{initial}$=2100 mg/L and C_{AC}=600 mg/L. The comparison of the results with those of experiment 4 revealed that COD removal efficiency in integrated process was 1.1% higher compared to the sum of the efficiencies of ozonation and air stripping processes in the presence of activated carbon adsorption carried out separately (Fig. 2). These results are in agreement with the findings of Lei *et al.* [7] who claimed that in case of concentrated industrial wastewaters and under acidic conditions there is no synergy between ozone and activated carbon. The reason for this is explained by Alvárez et al. [8]. Namely, under acidic conditions the hydrogen peroxide formed in the reactions between ozone and surface of activated carbon, does not dissociate into hydroperoxyl ion which is known as an initiator of the radical type ozone decomposition chain reactions that eventually leads to formation of hydroxyl radicals.

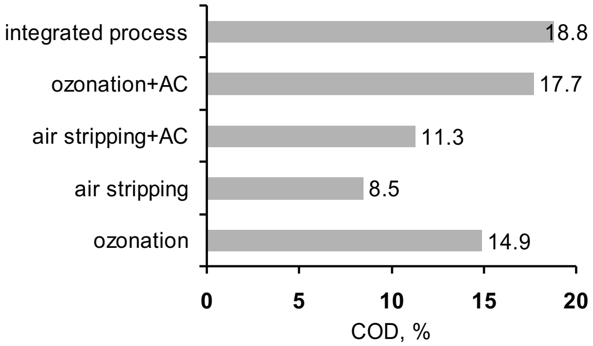


Figure 2. The comparison of integrated process with individual processes

In case of biochemically treated effluent specific ozone consumption was approximately 3 times higher compared to wastewater ozonation. The results showed that activated carbon addition (at dose of 300 mg/L) decreased ozone consumption by 8.9% compared to ozonation alone. This was achieved because purification efficiency in terms of COD removal was increased by 2%. Further increase in activated carbon concentration from 300 to 600 mg/L improved COD removal by 4%. Thereby also specific ozone consumption was further reduced by 7.2%.

Regression models

Based on the experimental data, the regression equations for estimation of COD, BOD, pH, and dissolved ozone concentration in water were found.

Comparison of the results of different regression models, the most accurate equation for evaluation of COD during ozonation was modified linear regression model:

$$COD_{\tau} = m_0 + m_1 C_{O3}^{initial} + m_2 G + m_3 COD_{initial} + m_4 C_{AC}\,, \qquad (3)$$

where m_0 to m_4 are time dependent coefficients:

$$m_0 = 1.14 \cdot 10^{-6}\tau^4 + 1.67 \cdot 10^{-4}\tau^3 - 8.07 \cdot 10^{-2}\tau^2 + 7.29\tau - 2.26\,,\ r^2=0.99$$

$$m_1 = -1.62 \cdot 10^{-5}\tau^3 + 3.34 \cdot 10^{-3}\tau^2 - 0.234\tau + 0.188\,,\ r^2=1.0$$

$$m_2 = \frac{527}{\left[1 + \left(\frac{\tau}{17.1}\right)^{0.653}\right] - 527}\,,\ r^2=1.0$$

$$m_3 = -9.07 \cdot 10^{-9}\tau^4 + 1.78 \cdot 10^{-6}\tau^3 - 1.03 \cdot 10^{-4}\tau^2 + 2.30 \cdot 10^{-4}\tau + 1.0\,,\ r^2=1.0$$

$$m_4 = -3.37 \cdot 10^{-10}\tau^5 + 9.32 \cdot 10^{-8}\tau^4 - 1.00 \cdot 10^{-5}\tau^3 + 5.26 \cdot 10^{-4}\tau^2 - 1.27 \cdot 10^{-2}\tau - 2.53 \cdot 10^{-11},\ r^2=0.99$$

The analysis of the calculated results with the results measured in experiments revealed that modified linear regression model describes adequately changes in COD when gas flow rate and ozone concentration in the inlet gas are not set simultaneously to highest or lowest values (except the case when all independent design parameters are set to highest values). The deviation was usually below 5%.

For evaluation of changes in BOD during ozonation, modified exponential regression model gave the best fit with experimental data:

$$BOD_\tau = m_0\left(m_1^{C_{O3}^{initial}} \cdot m_2^{G} \cdot m_3^{COD_{initial}} \cdot m_4^{C_{AC}}\right), \qquad (4)$$

where m_0 to m_4 are time dependent coefficients that are calculated using the following equations:

$$m_0 = 1.01 \cdot 10^{-6}\tau^4 - 3.44 \cdot 10^{-4}\tau^3 + 4.39 \cdot 10^{-2}\tau^2 + 2.49\tau + 292,\ r^2=0.94$$

$$m_1 = -1.14 \cdot 10^{-8}\tau^3 + 2.1 \cdot 10^{-6}\tau^2 - 1.36 \cdot 10^{-4}\tau + 1.0,\ r^2=1.0$$

$$m_2 = -7.2 \cdot 10^{-10}\tau^5 + 2.16 \cdot 10^{-7}\tau^4 - 2.46 \cdot 10^{-5}\tau^3 + 1.32 \cdot 10^{-3}\tau^2 - 3.4 \cdot 10^{-2}\tau + 1.03,\ r^2=1.0$$

$$m_3 = -1.16 \cdot 10^{-11}\tau^4 + 2.86 \cdot 10^{-9}\tau^3 - 2.45 \cdot 10^{-7}\tau^2 + 7.98 \cdot 10^{-6}\tau + 1.0,\ r^2=1.0$$

$$m_4 = 8.58 \cdot 10^{-12}\tau^4 - 2.05 \cdot 10^{-9}\tau^3 + 1.72 \cdot 10^{-7}\tau^2 - 6.26 \cdot 10^{-6}\tau + 1.0,\ r^2=0.99.$$

The comparison of calculated and measured parameters showed that the deviation between these values may be relatively low (4.4% and 5.7% in experiments 1 and 9, respectively). The highest deviation of calculated values from measured values was in experiments 2 and 10 (14.5% and 12.0%, respectively).

For pH and dissolved ozone concentration determination, modified exponential regression (5) and exponential regression (6), respectively gave the best correlation with experimental data:

$$pH_\tau = m_0\left(m_1^{C_{O3}^{initial}} \cdot m_2^{G} \cdot m_3^{COD_{initial}} \cdot m_4^{C_{AC}}\right) \qquad (5)$$

where m_0 to m_4 are time dependent coefficients:

$$m_0 = 2.62 \cdot 10^{-5}\tau^3 - 4.41 \cdot 10^{-3}\tau^2 + 0.172\tau + 7.9,\ r^2=1.0$$

$$m_1 = 6.23 \cdot 10^{-11}\tau^4 - 6.75 \cdot 10^{-8}\tau^3 + 1.07 \cdot 10^{-5}\tau^2 - 5.57 \cdot 10^{-4}\tau + 1.0,\ r^2=1.0$$

$$m_2 = 1.39 \cdot 10^{-8}\tau^4 - 4.61 \cdot 10^{-6}\tau^3 + 5.27 \cdot 10^{-4}\tau^2 - 2.36 \cdot 10^{-2}\tau + 1.02,\ r^2=0.87$$

$$m_3 = 6.39 \cdot 10^{-12}\tau^4 - 1.35 \cdot 10^{-9}\tau^3 + 9.47 \cdot 10^{-8}\tau^2 - 2.77 \cdot 10^{-6}\tau + 10.0,\ r^2=1.0$$

$$m_4 = -4.14 \cdot 10^{-10}\tau^3 + 8.27 \cdot 10^{-8}\tau^2 - 1.92 \cdot 10^{-6}\tau + 1.0,\ r^2=1.0$$

$$C_{O3,\tau} = 5.61 \cdot 10^{-5}\left(1.1^{\tau} \cdot 1.09^{C_{O3}^{initial}} \cdot 27.4^{G} \cdot 0.999^{COD_{initial}} \cdot 1.0^{C_{AC}}\right),\ r^2=0.79 \qquad (6)$$

Exponential regression was relatively accurate for calculation of low dissolved ozone concentrations. It has to be mentioned that the usability of the model is limited, as the relationship between ozone concentration in liquid phase and ozonation time is not exponential.

The adequacy of the regression equations was tested on experimental data (0.55 L_{gas}/min, 20.55 mgO_3/L_{gas}, 2538 mgO_2/L, 450 mg/L). The comparison of the experimental and calculated data shows that the regression equation predicts adequately changes in COD during ozonation (Fig. 3). BOD is not predicted very adequately, although final values (after 90 minutes of ozonation) are very close. Changes in pH follow the same trend. Therefore it may be concluded that the model can be used to predict the output parameters of ozonation of phenolic wastewater in the presence of activated carbon in the investigated range.

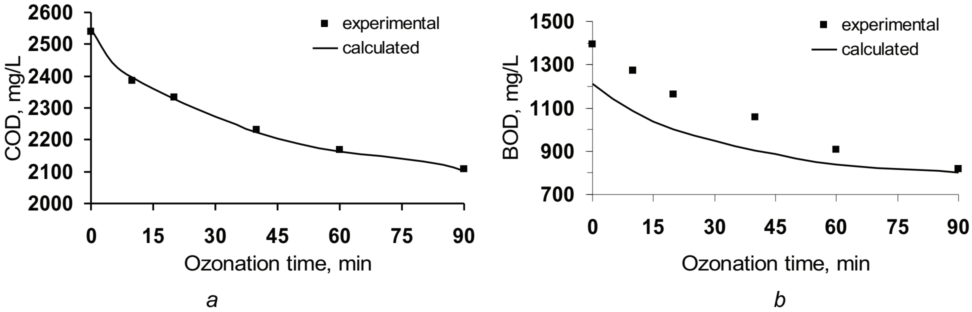


Figure 3. Experimental and calculated COD (a) and BOD (b) versus time at ozonation of phenolic wastewater

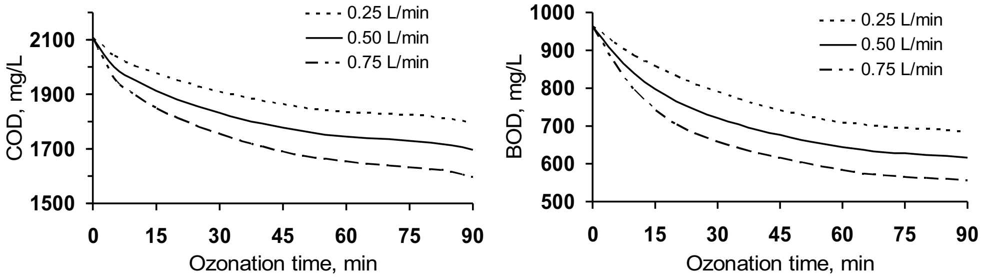
Impact of different factors on ozonation efficiency

The impact of independent design factors was assessed using the obtained regression equations. The equations allowed to predict the evolution of ozonation process efficiency in time (in terms of COD and BOD removal) influenced by initial COD, gas volumetric flow rate, ozone concentration in inlet gas and concentration of activated carbon.

For the consistency, the following results were calculated at an average design factors values (0.50 L_{gas}/min, 25 mgO_3/L_{gas}, 2100 mgO_2/L, 600 mg/L) after 90 minutes, where only the value of the design factor under study is varied. The efficiencies of the processes are obtained by dividing the calculated results with initial values.

Initial COD has a remarkable impact on the purification efficiency in terms of COD and BOD removal. The highest COD and BOD removal efficiency is achieved at the lowest initial COD value (28.8% and 40.0%, respectively). At the same time reduction in the biodegradability at these conditions was the lowest (16.6%). As the concentration of activated carbon was increased, COD and BOD removal efficiencies were reduced, while reduction in biodegradability was highest at $COD_{initial}$=2100 mgO_2/L.

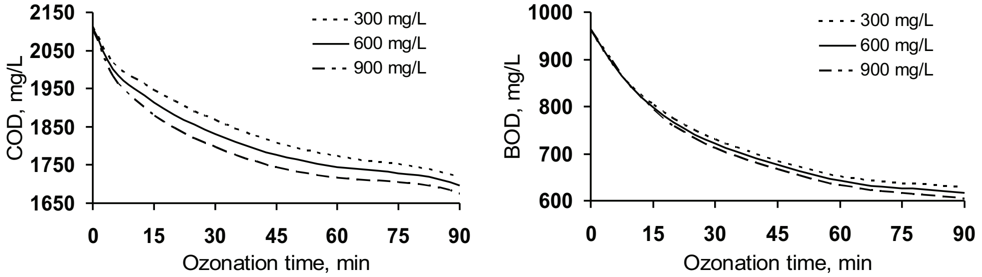
To evaluate the influence of ozone concentration in the inlet gas for ozonation process efficiency, ozone concentration was varied between the values of 15, 25 and 35 mgO_3/L_{gas}. The analysis of the calculated data shows that an increase in $C_{O3}^{initial}$ from 15 to 35 mgO_3/L_{gas} improves COD removal only by 5.7% (efficiency increased from 16.4 to 22.1%), BOD removal is improved by 2.3%. On the other hand, gas flow rate has slightly stronger influence on both COD and BOD removal (Fig. 4). At 0.25 L_{gas}/min COD and BOD removal efficiencies were 14.6 and 33.7% respectively. Consequently also biodegradability was reduced. An increase in gas flow rate from 0.25 to 0.75 L_{gas}/min improved COD removal by 9% and BOD removal by as much as 14.4%. Improvement in purification efficiency caused by the increased $C_{O3}^{initial}$ and *G* suggest that ozonation in the presence of activated carbon is mass transfer limited.
Although the exact mechanism of the studied process is unclear, the obtained results can only be explained by the theory that COD and BOD removal takes preferably place on the surface of activated carbon. As this process involves diffusion, both ozone concentration in the inlet gas and gas flow rate must be increased to reduce the resistance to mass transfer.



a *b*

Figure 4. Influence of oxygen-ozone gas volumetric flow rate on the COD (a) and BOD (b) in ozonation of phenolic wastewater

The calculations showed that an increase in activated carbon concentration from 300 to 900 mg/L had only minor effect on COD removal (Fig. 5) – it was increased by 3%. Increase in BOD removal efficiency was approximately 4%.



a *b*

Figure 5. Influence of activated carbon concentration on the COD (a) and BOD (b) in ozonation of phenolic wastewater

Based on the results it may be concluded that volumetric gas flow rate had the strongest influence on the efficiency of ozonation of phenolic wastewater, closely followed by the ozone concentration in the inlet gas. The calculations showed that the purification efficiency can be improved by increasing the values of design factors. However the results indicate that from the practical point of view, ozonation in the presence of activated carbon can not be considered as a potential application for treatment of oil-shale phenolic wastewater, especially when the ozonated effluent is subjected to subsequent biochemical treatment.

Conclusions

The present research focused on ozonation of oil-shale phenolic wastewater in the presence of activated carbon. The impact of various factors was assessed using the fractional factorial design. Experimental results showed that ozonation in the presence of activated carbon is not very efficient – COD decreased at most by 35%. In all experiments also the biodegradability reduced.

The calculations indicated that by increasing the activated carbon concentration from 300 to 900 mg/L COD removal increased by 3%. It means that expected catalytic effect of the carbon on the ozonation process was not discovered in this case. An increase in gas flow rate from 0.25 to 0.75 L_{gas}/min improved COD removal by 9 % and an increase in ozone concentration in the inlet gas from 15 to 35 mgO_3/L_{gas} enhanced COD removal by 6%.

Additional experimental studies showed that in the case of ozonation of biochemically treated phenolic wastewater the addition of activated carbon at concentration of 300 mg/L improved purification efficiency in terms of COD by 2%. Further increase in activated carbon concentration from 300 to 600 mg/L improved COD removal by 4%. Also the addition of activated carbon reduces the consumed ozone dose per one gram of removed COD.

Acknowledgements

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APPENDIX II
CURRICULUM VITAE

ELULOOKIRJELDUS

1. Isikuandmed

Ees- ja perekonnanimi: Oliver Järvik
Sünniaeg ja koht: 28. veebruar 1982, Tallinn
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2. Kontaktandmed

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3. Hariduskäik

Õppeasutus (nimetus lõpetamise ajal)	Lõpetamise aeg	Haridus (eriala/kraad)
Tallinna Tehnikaülikool	2007	Tehnikateaduste magister
Tallinna Tehnikaülikool	2005	Tehnikateaduste bakalaureusekraad

4. Keelteoskus

Keel	Tase
Eesti keel	Kõrgtase
Inglise keel	Kesktase
Vene keel	Algtase
Soome keel	Algtase

5. Teenistuskäik

Töötamise aeg	Organisatsiooni nimetus	Ametikoht
2007–k.a.	TTÜ	Assistent
2005–2007	TTÜ	Insener
2009–2009	UTTP doktorikool	Erakorraline teadur
2004–2004	ES Sadolin AS	Operaator-õpilane

6. Kaitstud lõputööd

Tehnikateaduste magistrikraad (2007): Kombineeritud protsessid põlevkivitööstuse reovee puhastamisel. Juhendaja Keemiatehnika instituudi dotsent Inna Kamenev.

Tehnikateaduste bakalaureusekraad (2005): Reovee puhastamine aeroobse biooksüdatsiooniga kombineeritult aktiivsöetöötluse ja osoonimisega. Juhendaja Keemiatehnika instituudi dotsent Inna Kamenev.

7. Teadustöö põhisuunad

Aeroobse biooksüdatsioon uurimine ja parendamine, osoonimise uurimine, jääkaktiivmuda tekke vähendamine.

8. Teised uurimisprojektid

Projekt SF0142719s06 (2006–k.a.): Tehnoloogiliste protsesside intensiivistamine aktuaalsete keskkonnaprobleemide lahendamiseks.

Projekt Lep9026 (2009): Osoonimise kasutusvõimaluste uurimine pärmitööstuse reovee puhastusprotsessis.

Projekt G5607 (2003–2005): Jääkaktiivmuda käitlemine – aktiivsöe valmistamine ja selle kasutamine reovee puhastuses.

CURRICULUM VITAE

1. Personal data

Name: Oliver Järvik
Date and place of birth: 28th February 1982, Tallinn
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2. Contact information

Address: TUT, Department of Chemical Engineering, Ehitajate tee 5,
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Phone: +372 620 2850
E-mail: oliver.jarvik@ttu.ee

3. Education

Educational institution	Graduation year	Education (field of study/degree)
Tallinn University of Technology	2007	Master of Science
Tallinn University of Technology	2005	Bachelor of Science

4. Language competence/skills

Language	Level
Estonian	Fluent
English	Average
Russian	Basic skills
Finnish	Basic skills

5. Professional Employment

Period	Organisation	Ametikoht
2007–onwards	TUT	Teaching Assistant
2005–2007	TUT	Engineer
2009–2009	UTTP Doctoral School	Extraordinary Researcher
2004–2004	ES Sadolin AS	Learner operator

6. Defended theses

Master of Science (2007): Purification of Oil Shale Industry Wastewater Using Combined Processes. Supervisor – Associate Professor Inna Kamenev, Department of Chemical Engineering.

Bachelor of Science (2005): Wastewater purification using aerobic bio-oxidation combined with activated carbon treatment and ozonation. Supervisor – Associate Professor Inna Kamenev, Department of Chemical Engineering.

7. Main areas of scientific work

The research and improvement of aerobic biooxidation, the research of ozonation, reduction of excess sludge generation.

8. Other research projects

Project SF0142719s06 (2006–onwards): Intensification of technological processes for the solution of actual environmental problems.

Projekt Lep9026 (2009): Possibilities of application of ozone for treatment of wastewater from the yeast industry.

Projekt G5607 (2003–2005): Handling of excess activated sludge – production of activated carbon for wastewater treatment.

DISSERTATIONS DEFENDED AT TALLINN UNIVERSITY OF TECHNOLOGY ON *CHEMISTRY AND CHEMICAL ENGINEERING*

1. **Endel Piiroja**. Oxidation and destruction of polyethylene. 1993.

2. **Meili Rei**. Lihatehnoloogia teaduslikud alused. Fundamentals of Food Technology. 1995.

3. **Meeme Põldme**. Phase transformations in hydrothermal sintering processing of phosphate rock. 1995.

4. **Kaia Tõnsuaadu**. Thermophosphates from Kovdor and Siilinjärvi apatites. 1995.

5. **Anu Hamburg**. The influence of food processing and storage on the N-nitrosamines formation and content in some Estonian foodstuffs. 1995.

6. **Ruth Kuldvee**. Computerized sampling in ion chromatography and in capillary electrophoresis. 1999.

7. **Külliki Varvas**. Enzymatic oxidation of arachidonic acid in the coral *Gersemia fruticosa*. 1999.

8. **Marina Kudrjašova**. Application of factor analysis to thermochromatography and promotion studies. 2000.

9. **Viia Lepane**. Characterization of aquatic humic substances by size exclusion chromatography and capillary electrophoresis. 2001.

10. **Andres Trikkel**. Estonian calcareous rocks and oil shale ash as sorbents for SO_2. 2001.

11. **Marina Kritševskaja**. Photocatalytic oxidation of organic pollutants in aqueous and gaseous phases. 2003.

12. **Inna Kamenev**. Aerobic bio-oxidation with ozonation in recalcitrant wastewater treatment. 2003.

13. **Janek Reinik**. Methods for purification of xylidine-polluted water. 2003.

14. **Andres Krumme**. Crystallisation behaviour of high density polyethylene blends with bimodal molar mass distribution. 2003.

15. **Anna Goi**. Advanced oxidation processes for water purification and soil remediation. 2005.

16. **Pille Meier**. Influence of aqueous solutions of organic substances on structure and properties of pinewood (*Pinus sylvestris*). 2007.

17. **Kristjan Kruusement**. Water conversion of oil shales and biomass. 2007.

18. **Niina Kulik**. The application of Fenton-based processes for wastewater and soil treatment. 2008.

19. **Raul Järviste**. The study of the changes of diesel fuel properties at its long term storage. 2008.

20. **Mai Uibu**. Abatement of CO_2 emissions in Estonian oil shale-based power production. 2008.

21. **Valeri Gorkunov**. Calcium-aluminothermal production of niobium and utilization of wastes. 2008.

22. **Elina Portjanskaja**. Photocatalytic oxidation of natural polymers in aqueous solutions. 2009.

23. **Karin Reinhold**. Workplace assessment: determination of hazards profile using a flexible risk assessment method. 2009.

24. **Natalja Savest**. Solvent swelling of Estonian oil shales: low temperature thermochemical conversion caused changes in swelling. 2010.

25. **Triin Märtson**. Methodology and equipment for optical studies of fast crystallizing polymers. 2010.

26. **Deniss Klauson**. Aqueous Photocatalytic Oxidation of Non-Biodegradable Pollutants. 2010