Ozonation as a cost effective technology in antimicrobial resistance management of wastewater and sludge

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Abstract

Use of antibiotics in modern society is an important source of environmental contamination, especially in case of environmentally persistent molecules. Even low concentrations of antibiotics in the environment promotes the selection of antibiotic resistance genes and antibiotic resistant bacteria. This issue is getting an alarming scale, as several commonly used antibiotics are no longer effective against certain bacteria. Identifying and mitigating the risk factors and managing contamination sources becomes a key challenge for environmental engineers. The aim of our research was to develop a process that removes antibiotic activity of the selected veterinary antibiotic (Tiamulin) and make it suitable for a conventional biological treatment. Tiamulin was selected as a model substance with high environmental persistence. Ozonation was identified as one of the promising oxidation techniques, to deliver on a technology that is cost-effective and robust enough, that can be used both in wastewater and sludge management. Our study indicates that already small doses of ozone remove toxicity of the antibiotic to the activated sludge. Ozone with a selective oxidation mode is ideally suited for the technology, as it delivers desired toxicity reduction even in the complex matrix of a biological sludge.

Keywords: Antibiotic resistance, ozone, wastewater

2. Introduction

The use of antibiotics in a society of today is an important source of environmental pollution, especially in case of environmentally persistent molecules. Research shows that concentrations of some of the most commonly used antibiotics in the environment are at least an order of magnitude lower than the ones that are toxic to aquatic organisms, therefore acute toxicity of antibiotics in the environment is not likely to occur (Boxall, 2003) (Jensen, 2003) (Halling-Sorensen, 2000). In most European rivers an average concentration of antibiotics below 10 ng L^{-1} was found (Johnson, 2015). Widespread use of antibiotics and consequently the emission of these substances is not likely to cause any direct toxic effects to water organisms, but it is primarily promoting a development of bacterial antibiotic resistance genes. Research on the examples of Oxytetracycline and Ciprofloxacine indicate that the presence of these antibiotics in the aquatic environment promotes the emergence of antibiotic-resistant bacteria (Daojin, 2009). In the sludge of biological treatment plant, strains of antibiotic-resistant bacteria MRSA (methicilin-reistant Staphylococcus aureus) can be found, especially in the first stages of the biological treatment (Börjesson, 2009). In subsequent stages, number and diversity of these bacteria is decreasing, however strains with a broader spectrum of resistance and hence the higher virulence are emerging. For this reason, it is mandatory for antibiotics to be removed before entering the biological treatment plant (Songhe Zhang, 2015). Unlike chemical pollutants where concentrations in the environment gradually decreases due to the (bio)degradation or dilution, bacteria (and their antibiotic resistance genes), can be much more persistent and are able to even spread. Antibiotic resistance genes multiply within the host bacterial cells, are transferred to other bacterial populations and become part of a broader evolution. For this reason, the dissemination of antibiotic resistance in the environment is a serious risk to human health (Berendonk, 2015). As oxidation techniques are often used to abate antibiotics it is mandatory to evaluate effectiveness through the measurement of reduced toxicity to the activated sludge, as the toxicity and thus the environmental impact due to the oxidation products may change in an unexpected direction.

3. Methods

In scope of our research we have studied the removal efficiency of veterinary antibiotics Tiamulin by using ozone in a batch process mode. We were focusing mainly to low doses of ozone, as the aim was to develop a technology that will primarily reduce the environmental impact (antibiotic activity) of the molecule, but it is not necessary that the molecule is completely removed, mineralized. After the oxidative treatment, antibiotic properties of the molecule should be removed, leading to a lower toxicity to activated sludge. Biodegradability therefore increases and the molecule is suitable to be treated in a conventional aerobic biological wastewater treatment system. Toxicity of Tiamulin to activated sludge was determined before and after ozonation experiments according to the method ISO 8192, 2007 and biodegradability according to the method ISO 9408, 1999. Degradation rate of Tiamulin was monitored indirectly by measurement of COD (ISO 6060, 1989) and TOC (ISO 8245, 1999). This was an indication of its mineralization, however actual concentration was not followed, and neither were we determining any of the degradation products. As an additional insight into the degree of oxidation of the substance also an average oxidation state of carbon in the solution was followed, calculated, according to the following equation (Stumm, 1981):

$$\text{oksidation state}=\frac{4\,(\text{TOC-COD})}{\text{TOC}}$$

TOC is expressed in mol C L^{-1}, and the COD in O_2 mol L^{-1}. Oxidation state is expressed by the number of free units. The concentration of ozone in the solution was determined by using colorimetric method (118755, Merck Ozone Test).In the experimentation ozone generator by a Xylem Water Solutions GmbH Herford, type OCS Modular 8 HC was used. The operating pressure was 0.5 bar, the gas flow of 0.08 m^3 h^{-1} and the capacity of the system was 8 g h^{-1}. The nominal concentration of ozone in the gas was 100 g m^{-3}. Ozone was passed into a glass laboratory column with bubbles, a volume of 250 mL. Laboratory set-up is shown in Figure 1.

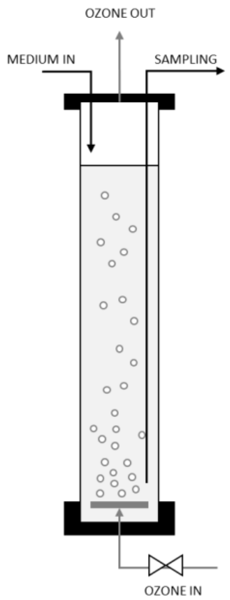

Figure 1. Laboratory set-up for batch ozonation.

Environmentally persistent, veterinary antibiotic Tiamulin was used as a model substance in the study (Figure 2, Committee for veterinary medicinal products, 1999).

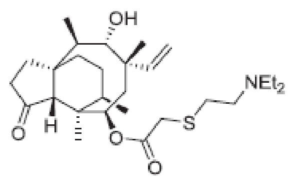

Figure 2. The structural formula of Tiamulin (Neal J. Fazakerley, 2004).

Research shows that in the period of 180 days of storage of manure, which contains antibiotics, Tiamulin is completely stable, while half-life of some other antibiotics ranges from 6 days (Salinomycin) to 41 days (Erythromycin) (Schluesener, 2006). Research of a limited scope indicates that Tiamulin stays at a concentration of 600 mg L^{-1} in distilled water at 37 °C completely stable and shows no signs of hydrolysis, while in the lake water (in the presence of various salts, natural and anthropogenic organic matter, and (micro) organisms) at the same temperature and concentration conditions, Tiamulin is losing 10% of its activity in two weeks, and half of the activity after 10 weeks (Cosgrove, 1979).Toxicity of Tiamulin to microorganisms of the activated sludge was determined by measuring the inhibition of oxygen consumption of activated sludge while oxidizing organic matter and ammonia nitrogen (ISO 8192, 2007). Biodegradability in anaerobic conditions was determined the method of determination of ultimate biodegradability of complex organic substrates by measuring biogas production (ISO 11734, 2004), with some minor modifications The increase of headspace pressure in test vessels resulting from the production of CH_4 and CO_2 (biogas) was measured by the OxiTop system at 39±1 °C (WTW Germany, 2008). Pressure gain was measured in the systems with the sample, inoculum and buffer solution.

4. Results

30minEC_{50} values determined are presented in Table 1. Tiamulin does not appear to be highly toxic to microorganism of activated sludge, however due to rather low 30minEC_{50} value for nitrifying microorganisms, it may adversely affect nitrification process.

Table 1. Toxicity of Tiamulin to microorganisms of activated sludge.

Type of microorganisms	**30minEC_{50}, mg L^{-1}**
All	700
Heterotrophic	1,660
Nitrifying	180

Biodegradation, as determined by the method of ISO 9408, 1991 is shown in Figure 3. Biodegradation does not start until the 20th day, after a very long lag phase and reaches only 40±2% in the 43 days of the test, then the biodegradation stops. Abiotic degradation has not been detected (≤ 2%).Ozonation was performed in a batch mode (250 mL reactor) with the minimum ozone dose. Tiamulin was added to the aqueous solution of ozone (10 mg L^{-1}), so that the initial concentration of was 400 mg L^{-1}. Solution was left without mixing and after 30 min ozonation was repeated with 10-minute purging with ozone (30 L h^{-1}, a nominal concentration of ozone of 100 g m^{-3}). Same procedure was repeated twice. Oxidant dose was in this case very low, in a molar ratio with respect to the COD only 1: 0.01. Effectiveness of the oxidation was measured in terms of COD and TOC, as shown in Figure 4. Although the absolute changes of TOC (8%) and COD (17%) are low, the oxidation state of the carbon in the solution reaches as high as -0.1, as shown in Figure 5. Change in the oxidation state of carbon in the solution is an indicator of the degree of oxidation of carbon atoms in the molecule, which are treated with the selected oxidation method. Complete mineralization would result in oxidation state of carbon +4 as that of the CO_2, which is the final product of mineralization. Initial oxidation state of the carbon in the solution is -0.85, therefore ozonation leads to 15% of complete mineralization.

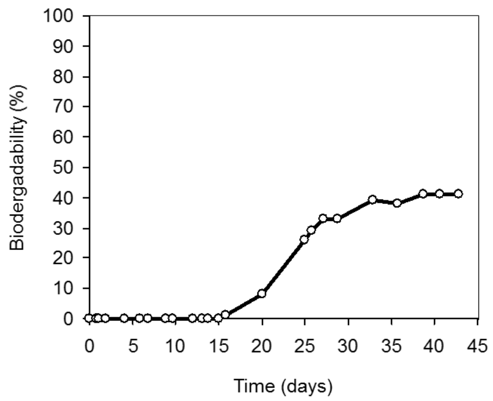

Figure 3. Biodegradation curve for Tiamulin.

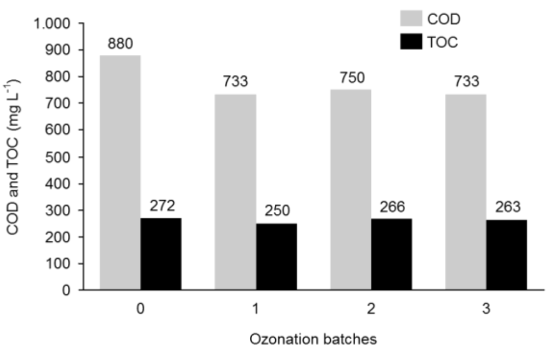

Figure 4. COD and TOC in batch ozonation of Tiamulin 400 mg L^{-1} solution

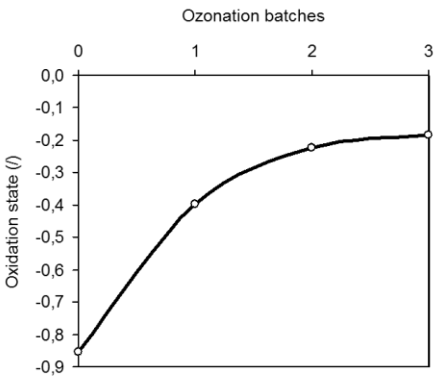

Figure 5. Change of the average oxidation state of carbon in the solution during batch ozonation.

Key development that we look for here is the change of the toxicity to microorganisms of the activated sludge. The reduction of the toxicity already after the first ozonation batch is 20% (Figure 6). Especially it is important to reduce the toxicity of the nitrifying micro-organisms which are particularly susceptible (35%, Figure 6). Considering low dose of oxidant, a significant reduction was obtained. Overall biodegradability of the model molecule is however not improving, as shown in Figure 7. Two curves in the chart are showing biodegradability of the untreated (400 mg L^{-1}) and oxidized solution by means of two consecutive batch ozonations.

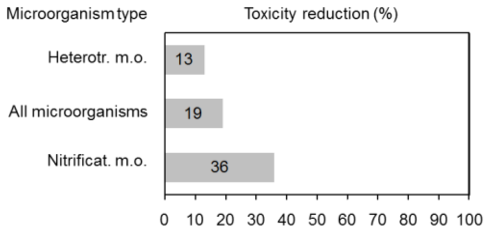

Figure 6. Reduction of toxicity in the activated sludge in a single batch oxidation with ozone.

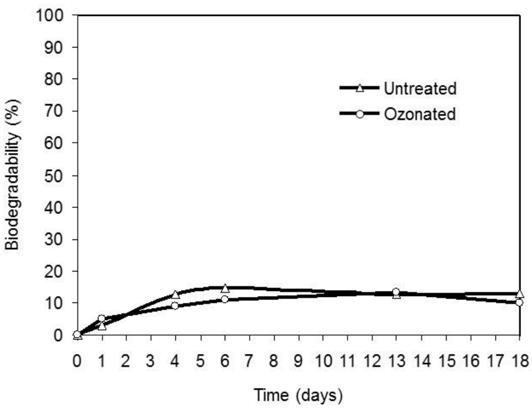

Figure 7. Biodegradability of Tiamulin 400 mg L^{-1} solution before and after ozonation.

There is no significant difference between the two curves, showing untreated and ozonated solution of 400 mg L^{-1} Tiamulin, which means that although the toxicity was reduced to all organisms by over 10% (Figure 6), this was not enough to facilitate significant change in biodegradability. Obviously, the two batch ozonations are not providing enough oxidant to deliver on the change in biodegradability. Biodegradability however improves in anaerobic conditions. Figure 8 shows degradation under anaerobic conditions, both for untreated sample of Tiamulin (400 mg L^{-1}) as well as for ozonated solution. Obvious difference between the two curves in Figure 8 indicates, that ozonation significant improves anaerobic biodegradability of Tiamulin, resulted in much higher biogas production. This is in particular important in case of anaerobic treatment of waste sludge, which could contain adsorbed Tiamulin and ozonation could improve overall treatability of both sludge and adsorbed antibiotic.

5. Conclusion

Results indicated that ozonation is a promising technology when treating wastewaters contaminated with environmentally stable antibiotic molecule, such as Tiamulin. When using ozonaton, already low doses of oxidant (in a molar ratio with respect to the COD 1: 0.01) reduces toxicity of Tiamulin to nitrification microorganisms by 36%, while toxicity to all microorganisms is reduced by only 13%. This however is not enough to improve aerobic biodegradability, while on the other hand significantly improves anaerobic biodegradation. Further work is needed in ozone doses optimization, to improve aerobic biodegradability. Furthermore, ozonation of biological sludge, contaminated with Tiamulin needs to be investigated.

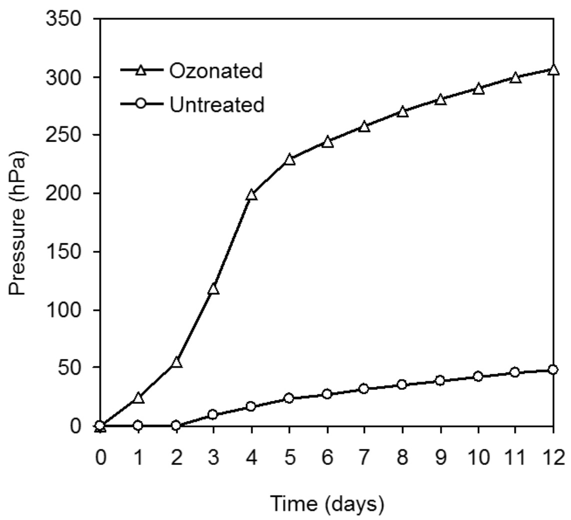

Figure 8. Biodegradability of Tiamulin 400 mg L^{-1} solution under anaerobic conditions before and after ozonation.

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References

Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., & Kreuzinger, N. (2015). Tackling antibiotic resistance: the environmental framework. Nature Reviews Microbiology, 13(5), 310-317.

Börjesson, S. (2009). Antibiotic Resistance in Wastewater: Methicillin-resistant Staphylococcus aureus (MRSA) and antibiotic resistance genes (Doctoral dissertation, Linköping University Electronic Press).

Boxall, A. B. A., Kolpin, D. W., Halling-Sorensen, B., Tolls, J. (2003). Are veterinary medicines causing environmental risks? Environmental science & technology, 37(15), 286A-294A.

Cheremisinoff P.N. (1995), Handbook of Water and Wastewater Treatment Technology, Marcel Dekker Inc., New York.

Committee for veterinary medicinal products (1999), Tiamulin, Summary report. London: The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines and Information Technology Unit.

Cosgrove, R. (1979), Tiamulin: Preliminary, small scale environmental impact studies, Report number IDL/MR/80. New York: Squibb Research and Development.

Fazakerley, N. J., & Procter, D. J. (2014). Synthesis and synthetic chemistry of pleuromulin. Tetrahedron, 70(39), 6911-6930.

Halling-Sørensen, B. (2000), Algal toxicity of antibacterial agents used in intensive farming. Chemosphere, 40(7), 731-739.

International organization for standardization (1989), International standard, ISO 6060:1989. Water quality - Determination of the chemical oxygen demand, International organization for standardization, Geneve.

International organization for standardization (1999), International standard, ISO 9408:1999. Water quality - Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer, International organization for standardization, Geneve.

International organization for standardization (1999), International standard, ISO 8245:1999. Water quality - Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC), International organization for standardization, Geneve.

International organization for standardization (2007), International standard, ISO 8192:2007. Water quality - Test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation, International organization for standardization, Geneve.

International organization for standardization (1995), International standard, ISO 11734:1995. Water quality - Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge - Method by measurement of the biogas production, International organization for standardization, Geneve.

John Jensen, P. H. (2003), Effects of the antibacterial agents tiamulin, olanquindox and metronidazole and the anthelmintic ivermectin on the soil invertebrate species Folsomia fimetaria (Collembola) and Enchytraeus crypticus (Enchytraeidae). Chemosphere, 50(3), 437–443.

Johnson, A. C., Keller, V., Dumont, E., & Sumpter, J. P. (2015). Assessing the concentrations and risks of toxicity from the antibiotics ciprofloxacin, sulfamethoxazole, trimethoprim and erythromycin in European rivers. Science of the Total Environment, 511, 747-755.

Schlüsener, M. P., Von Arb, M. A., & Bester, K. (2006). Elimination of macrolides, tiamulin, and salinomycin during manure storage. Archives of environmental contamination and toxicology, 51(1), 21-28.

Stumm, W., Morgan J.J. (1981), Aquatic Chemistry, 2ed, John Wiley & Sons, New York.

Yu D , Yi X , Ma Y , Yin B , Zhuo H , Li J , Huang Y (2009), Effects of administration mode of antibiotics on antibiotic resistance of Enterococcus faecalis in aquatic ecosystems. 76(7), Chemosphere, 915–920.

Zhang S., Han B., Gu J., Wang C., Wang P., Ma Y., Cao J., He Z. (2015). Fate of antibiotic resistant cultivable heterotrophic bacteria and antibiotic resistance genes in wastewater treatment processes. Chemosphere, 135 138–145.