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Bacterial inactivation and regrowth in ozonated activated sludges

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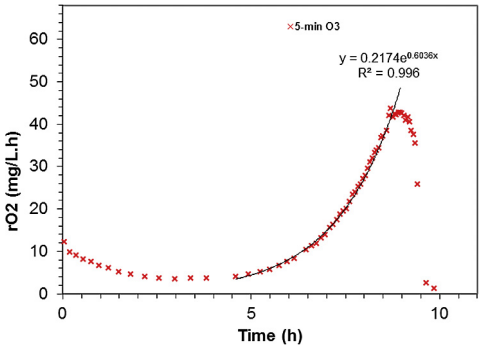
HIGHLIGHTS

- Microbial diversity changes due to ozone may have quantitative ASMs implications.
- Heterotrophic growth rate (μ_{Hmax}) rose from 3.5 d^{-1} to 10 d^{-1} in ozonated sludges.
- A lag period of up to 12 h preceded the growth phase after the O_3 treatments.
- Microbes that survived to digestion and ozone were fast-growing species.
- Study is of interest when applying the ASM models to the ozone-AS process.

GRAPHICAL ABSTRACT



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ABSTRACT

Ozonation of the return activated sludge (AS) flow is an emerging option for excess-sludge reduction. This study aimed to evaluate the potential changes suffered by some kinetic parameters of the activated sludge models (ASMs) in the combined ozone-AS process. The heterotrophic maximum specific growth rate (μ_{Hmax}) was determined by respirometry in three model-sludges (S1 to S3) treated in batch with different O_3 doses. S1 was a fresh synthetic biosolid composed by only two particulate fractions. S2 was a digestate of S1 almost made by the endogenous residues. S3 was from a municipal wastewater treatment plant. μ_{Hmax} increased significantly from 3.5 d^{-1} originally, to more than 10 d^{-1} in the ozonated sludges. Ozonation promoted the selection of fast-growing bacteria in the activated sludges, after transitory inactivation and long lag times. Some microorganisms survived to 3 months of digestion and subsequent ozonation, and then regrow faster than before, once fed again with acetate. The research is of interest from the point of view of the application of the ASM models to the ozone-AS process, but also for wastewater disinfection in general.

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1. Introduction

Conventional activated sludge (AS) combined with ozonation of the return activated sludge flow (RAS) is being used for waste biosolids minimization (Chiavola et al., 2013; Semblante et al., 2017). Ultimately, many efforts were done by researchers to find ways for extending the dynamic activated sludge models (ASMs, Henze et al., 2000) to the case of the ozone-AS process (Frigon and Isazadeh, 2011; Isazadeh et al., 2015; Semblante et al., 2017). The main interests in past studies were about the sludge reduction performances and the physico-chemical changes suffered by the biosolids, as function of the O_3 doses (Zhang et al., 2016; Ren et al., 2017). However, a question that remained was the extent to which

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the ozonation process can alter the biokinetic parameters of the ASMs.

For modeling the excess-sludge reduction processes, heterotrophic biomass inactivation is one of the most important phenomena that need be followed during sludge ozonation. This is often well done, by respirometry mostly, cell counting, flow cytometry and adenosine tri-phosphate analysis (Dziurla et al., 2005; Paul and Debellefontaine, 2007; Järvik et al., 2010; Lee et al., 2016). However, aspects that were fairly studied are the microbial diversity changes in the aeration tanks of the ozone-AS process and their ASMs modeling consequences.

Isazadeh et al. (2016) did not observe a significant shift on biomass species unlike many others studies that claimed that activated sludge bacterial communities are modified by ozone and other disinfectants (Yan et al., 2009; Sun et al., 2013; Lee et al., 2016). Bacterial shifts were also reported in water and wastewater disinfection (Becerra-Castro et al., 2016; Sousa et al., 2017). Generally, structural changes on the species were revealed by 16 S rRNA gene sequencing, or by flow cytometry techniques. Certain microorganisms in sludge were highly resistant to ozone (Semblante et al., 2017). In the present research, it was hypothesized that qualitative affectations of microbial diversity may have modeling implications. The biokinetic parameters of the ASM models can be affected, more especially the heterotrophic growth constant, and consequently the effluents qualities and sludge production rates (Qiang et al., 2013; Zhang et al., 2016; Semblante et al., 2017). Friedrich et al. (2017) underlined the potential benefits of incorporating physiological adaptation of heterotrophic populations (metabolic survival optimization) in future ASMs.

With the introduction of the dynamic mathematical models for the AS process (ASM1, 2 and 3, Henze et al., 2000), many respirometric methods were developed as an easier way for assessing the kinetic constants (Kappeler and Gujer, 1992; Spanjers and Vanrolleghem, 1995; Vital-Jacome et al., 2016). Some of the most important parameters of the ASM1 model, such as the heterotrophic specific maximum growth rate (μ_{Hmax}) and the decay coefficient (b_H), are currently determined by respirometry. In all cases, the acquired data are the oxygen uptake rates (OUR or r_{O2}), as a function of time in a batch reactor. Under high substrate/biomass (S_0/X_0) conditions, the exogenous OUR increases exponentially, which allows estimating the μ_{Hmax} constant. Under low S_0/X_0 ratio, the respirogram behaves as a plateau (constant maximum r_{O2} value); at the depletion of the substrate, the O_2 consumption rates suddenly decrease until the endogenous respiration level. Independent of the shape of the exogenous OUR curves (exponential vs plateau), the initial r_{O2} values were assumed to be proportional to the active biomass fraction present in the inoculum of the tests; this allows determining the relative activity of the biomass in sludges that have different X_H contents (e.g., a biosolid ozonated at different O_3 doses). The low and high S_0/X_0 levels correspond generally to less than 0.5, versus greater than 4 g COD/g COD (Kappeler and Gujer, 1992). Furthermore, measuring the endogenous respiration rates (sludge without substrate) over several days provides the heterotrophic decay rate coefficient (b_H).

Despite that qualitative changes in microbial diversity of the ozone-AS process were revealed, as far, their effects on the ASMs parameters were not quantified in the literature. In addition, OUR measurement was widely used to monitor the level of biomass inactivation in biosolids, but not far enough to detect changes in the microbial diversity and kinetics (μ_{Hmax}) of ozonated sludges, and even less to reveal regrowth occurrences after ozonation. In this research, respirometry was used to study the biomass inactivation process due to ozone, give insights into the regrowth process and estimate the heterotrophic maximum specific growth rates in activated sludges treated by ozonation. Three model-sludges having only one, only two, or all the three main organic fractions (X_P, X_H and X_I) were produced and subjected to 5 levels of ozonation in semi- batch reactors. The respirograms of the O_3-treated biosolids were obtained in batch respirometric tests (sludge spiked with acetate in excess) designed so as to be able to estimate the activity of the remaining biomass and their maximum heterotrophic growth rate. The data from the exponential phase of the growth curves were analyzed according to Kappeler and Gujer (1992, μ_{Hmax} estimation), in addition to discussing the particular behaviors observed (latencies, regrowth and selection processes) at the different O_3 doses and sludge types.

2. Material and methods

2.1. Biosolids used in the experiments

Based on ASM1 (Henze et al., 2000), the main components of secondary biological sludge are the heterotrophic biomass (X_H), the endogenous residues from decay (X_P), and two others inert fractions from the influent. The latter are the particulate non-biodegradable organic matter, X_I, and the inorganic suspended solids or ISS. Three model-sludges identified as S1, S2 and S3 were used in the experiments. The biosolids were also referred to as S1-X_H, S2-X_P and S3-wwtp with the intention of remembering its main components. They were projected to have respectively only one, only two, or all of the three main organic fractions (X_P, X_H and X_I). S1-X_H originated from a culture in the laboratory (30 L SBR at 15 d SRT) fed with acetate-based synthetic wastewater (WW, 500 mg/L chemical oxygen demand) and contained two fractions: X_H predominantly and X_P. The synthetic WW was prepared with the following chemicals (mg/L): $NaCH_3COO$ (641), NH_4Cl (107), KH_2PO_4 (12), KCl (36), $FeCl_3 \cdot 6H_2O$ (0.5), $MgSO_4 \cdot 7H_2O$ (90), $CaCl_2 \cdot 2H_2O$ (14), yeast extract (1), EDTA (1). The minor salts components were (mg/L): H_3BO_3 (0.045), $ZnSO_4 \cdot 7H_2O$ (0.036), $MnCl_2 \cdot 4H_2O$ (0.036), $CuSO_4 \cdot 5H_2O$ (0.009), KI (0.054), $Na_2MoO_4 \cdot 2H_2O$ (0.018) and $CoCl_2 \cdot 6H_2O$ (0.045). The second sludge S2-X_P resulted from three months of aerobic digestion of an aliquot from S1. So, it was expected that S2 was made by one fraction (X_P) (Labelle et al., 2011). The third material (S3-wwtp) was from a municipal wastewater treatment plant (WWTP, conventional AS at 7 d SRT) and was assumed to contain all the three particulate organic fractions (X_I, X_H and X_P).

The initial characteristics of the biosolids were obtained according to standards methods (APHA, 2005). The parameters that were measured were the total, the particulate and the soluble chemical oxygen demands (COD_{tot}, COD_{part} and COD_{sol}), the total and the volatile suspended solids (TSS and VSS) and the pH. During the respirometric tests, the dissolved oxygen (DO) concentrations, the oxygen uptake rates and the initial activities of the biomass were determined.

2.2. Ozone reaction system

Fig. 1 represents the experimental set-up used for the ozonation of the sludges. The O_3 treatments took place at around 20 °C in semi-batch reactors (batch addition of sludge and continuous bubbling of the O_3 mixture gas). An ozone generator with a maximum capacity of 10 g O_3 per hour was used; it was fed with pure oxygen bought in tank cylinders. For each run, 900 mL of mixed liquor were transferred to a 2-L glass reactor equipped with a magnetic stirrer. The reactor was closed with a silicone stopper through which 2 holes were made for the incoming and the outlet gases. The inlet gas flow was brought into contact with the biosolids through a porous stone arranged at the bottom of the reactor. A 3-ways by-pass valve in stainless steel and teflon was installed

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