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DISINFECTION BY PERACETIC ACID (PAA): EVALUATION OF THE EFFECT OF WASTEWATER COMPOSITION ON PAA DECAY AND BACTERIAL INACTIVATION KINETICS AND DEFINITION OF PREDICTIVE MODELS

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CONTENTS

ABSTRACT

Disinfection of municipal wastewater plays a very important role in public health protection by controlling the release of pathogenic microorganisms into receiving water bodies. Chlorination is the most common method for disinfection of municipal wastewaters, largely due to its low cost and efficiency. However, it is well documented that chlorine-based disinfectants are highly reactive towards several compounds present in the waters, inducing the formation of a wide range of harmful disinfection by-products (DBPs) of concern to human health and the environment. Peracetic acid (PAA) has gained increasing attention over the last decade as a safe and suitable alternative to chlorine-based products for wastewater disinfection. PAA decomposes rapidly in water solution; thus in order to ensure a sufficient residual concentration of disinfectant while avoiding over dosages that entail environmental risks and over costs, it is fundamental foreseeing the disinfectant decay. Previous studies have suggested that the water matrix composition has a significant effect on PAA decomposition. However, there is a high uncertainty on the specific physical-chemical characteristics of the water that affect PAA decay and therefore the available concentration for bacterial inactivation during wastewater disinfection. A further key aspect is related to the disinfectant dose (mg/L min) as the main parameter determining disinfection efficiency, whose estimation is based on the actual concentration of disinfectant at which bacteria are exposed, defined by the area under the curve that describes PAA decay over time.

The present doctoral dissertation is focused on the optimization of PAA dosage in order to reach bacterial inactivation targets avoiding high residual concentrations that can entail environmental risks. The integration of both PAA decay and bacterial inactivation kinetics is fundamental to achieve this goal, and requires the definition of predictive models for both aspects.

First, a critical literature review has been performed regarding the environmental fate of PAA residuals and their effects on aquatic ecosystems, presenting a comprehensive overview covering aspects such as formation of DBPs, toxicity and potential genotoxic and mutagenic effects. This analysis highlights that PAA is an environmentally-friendly disinfection option, producing less environmental impacts than chlorine-based disinfectants, given that limited DBPs are formed while the toxic, mutagenic and genotoxic effects on different aquatic organisms are very modest. Then, considering the monitoring of PAA concentration over time to define its decay kinetics, which requires the implementation of an accurate and rapid measurement, a method to achieve this purpose has been validated and an additional measurement method for the H_2O_2 fraction in equilibrium with PAA has been proposed. For both methods, kinetic aspects and potential interferences were investigated. This constituted a valuable and reliable tool to be implemented in the subsequent aspects to be studied.

As regards to PAA decay, the effect of wastewater composition was evaluated firstly assessing the contribution of dissolved matter, with focus on the identification of the specific inorganic and organic compounds that affect PAA decay. Five inorganic compounds and six organic compounds were selected to perform decay tests, based on the typical composition of secondary effluents and one experimental plan based on the statistical method of Design of Experiments (DoE) was carried out for each set of compounds, in order to assess their effect in multicomponent test solutions. The inorganic compounds, namely the presence of reduced iron and phosphate, displayed an important effect on the exponential decay rate whereas the organic compounds, mainly proteins, had a significant instantaneous consumption of PAA. Stepwise regressions were carried out for each set of compounds in order to select the most relevant ones to describe both aspects of PAA decay kinetics, the exponential decay rate and the initial consumption for the inorganics and the organic respectively. Subsequently, two models were interpolated by means of linear least-square regressions based on the most relevant compounds. A unique predictive model for PAA decay was then formulated by merging the two aforementioned models and it was validated by means of a set of experiments assessing PAA decay in the simultaneous presence of inorganics and organics, which were compared with the outcomes of Monte Carlo simulations, used to propagate the uncertainty through the model.

The contribution of suspended matter on PAA decay was evaluated by preparing two different stock suspended solids (TSS) solutions from the activated sludge of two wastewater treatment plants. Various TSS concentrations were studied during PAA decay tests, which are representative of secondary effluents of good (well settled) and medium (not well-settled) quality, and combined sewer overflows. In addition, the contribution of the soluble matter associated to the suspended solids on PAA decay was also evaluated by performing the decay experiments in the presence of the equivalent volume of stock TSS solution, prior removal of the solids through filtration. The contributions of the suspended and the soluble fractions were found to be independent; therefore, a predictive model formed by two additive sub-models was proposed to describe the overall decay kinetics of PAA.

Lastly, the disinfectant dose was evaluated as the main parameter determining the inactivation performance of PAA by means of inactivation tests with a pure strain of *E.coli*. This was performed in two phases that aimed at (i) define the dose-response curve of the organism and (ii) test the invariance of the dose in determining the disinfection performance by comparing *E.coli* inactivation level by changing the combination of C_0 (initial disinfectant concentration) and t (contact time) at a fixed dose. The same levels of inactivation were observed for different combinations of C_0 and t and a model for bacterial inactivation kinetics has been proposed based on the response of *E.coli* to different disinfectant doses. Finally, the effect of TSS on the inactivation performance of PAA was investigated with a focus on “free-swimming” *E. coli*. Solids demonstrated to have an effect beyond PAA decay, interfering with the disinfection efficiency leading to a reduction in the log-inactivation obtained. It was hypothesized that this might be due to probable formation of bacteria aggregates, as defense mechanism, enhanced by the presence of the suspended solids.

CHAPTER 1. STATE OF THE ART

Peracetic acid (PAA) is an organic peroxide and as such, it is a strong oxidizing agent. PAA properties have been known since 1902 [1], however its application was not well extended until the mid-1960s [2]. Since then, PAA has been widely used as disinfectant in various industries [3], such as food processing, beverage, brewery, pharmaceutical, pulp and paper, as well as medical applications, water in cooling systems and water process among others [2,4]. PAA is typically commercially available at 5-40 w/w % in an equilibrium mixture with acetic acid (CH_3COOH), hydrogen peroxide (H_2O_2), and water [5]. Since peroxides are highly unstable, to prevent a reverse reaction, PAA commercial solutions are enriched with CH_3COOH and H_2O_2. In the last decades, PAA has gained attention for wastewater disinfection and it is considered a suitable alternative to chlorine-based compounds claiming that only harmless disinfection by-products (DBPs) are formed, allowing low costs of implementation and operation, as well as easy retrofitting of the already existent equipment for chlorination.

The present chapter is dedicated to present an overview of the literature related to some of the key aspects of wastewater disinfection by PAA, such as the influence of wastewater composition on PAA stability and disinfection performance, defining a framework of the research topic. In detail, the current relevant aspects regarding disinfection mechanisms and PAA reactions in water with different compounds are discussed.

1.1. Disinfection and oxidation mechanisms

It is known that the mechanism of action of oxidative disinfectants (such as chlorine and peroxides) consist in the removal of electrons from specific chemical groups, oxidizing them. Microorganisms have defense mechanisms to counteract oxidation processes, however at high biocide concentrations these mechanisms can be overwhelmed [6], causing massive cellular damage at a macromolecular level and irreversible damage in the functionality and structure of cell wall/membranes, leading to cell lysis and releasing intra-cellular components, which are also oxidized [6,7]. It has been suggested [6,8] that oxidizing agents have different mechanisms of action and in consequence, several targets and types of specific damage within a cell wall/membrane and biomolecules, depending on the chemical nature of the biocide.

Regarding the oxidation mechanism of PAA, previous studies have been addressed to elucidate this aspect [6,8–12]. It has been proposed that this mechanism comprises the PAA homolytic cleavage and the presence of hydroxyl radical ($OH^{\bullet}$) as a primary radical product of PAA cleavage [9–11]. The formed hydroxyl radical is able to attack not only the organic pollutant but also the PAA molecule itself, entailing subsequent chain reactions with different pathways in which different free radicals are produced, such as the superoxide anion (O_2^{-}), the hydroperoxyl radical (HO_2^{-}) and peroxyl radical [9,10]. These highly oxidative radical species are pointed out as responsible for many of the possible damaging reactions in microorganisms [6,8,13]. However,

the exact mechanism by which PAA oxidizes and inactivates microorganisms is still controversial because of the complexity of the reaction pathway [10]. Despite of this fact it is thought that PAA denatures proteins, increasing cell wall permeability by free-radical oxidation and disruption of enzymes, sulfhydryl (-SH) and sulfur (-S) bonds, damaging the chemosmotic function and causing metabolic inhibition [2,5,6,8,13]. It has also been suggested that the organic part of the PAA molecule might help it to penetrate into the microbial cells [14].

In a similar fashion as for bacteria, virus inactivation by PAA may occur by damaging the virus surface, such as the protein coat of the sites needed for the infection of the host cells. The rate of virus inactivation by PAA decreased when viruses were present as aggregates. Mattle et al. [15] hypothesized the possible blockage of viral pores, a reduction of void spaces among viruses, adsorption of PAA on the viral proteins, and consumption of PAA during diffusion through the aggregate [16].

Regarding the role of the H_2O_2 fraction in equilibrium with PAA, although H_2O_2 is also a disinfectant contributing to the disinfection power of the PAA mixture, the last one is a more potent antimicrobial agent than H_2O_2 [5,17–19]. Therefore, H_2O_2 as a single compound requires much larger doses than PAA for the same level of disinfection [5,20]. This can be explained because it is known that some microorganisms may be protected against H_2O_2 by their catalase enzymatic activity [11,13,20]. This enzyme does not act against PAA; indeed, this last one can inactivate or inhibit catalase activity [2,11]. Nevertheless, even if PAA has a significantly higher biocidal activity than H_2O_2, the synergistic effects cannot be ruled out [4,11]. H_2O_2 might have an important role in the effectiveness of PAA solutions, since products with higher concentration of H_2O_2 than PAA, and therefore high H_2O_2:PAA ratios, have demonstrated higher effectiveness against pathogens [21–23].

1.2. PAA reactions in water solution: PAA decay and water matrix composition

Some of the factors affecting the decomposition rate of PAA are the initial concentration dosed, pH, the presence of organic material, suspended solids, transition metal ions, salinity, and water hardness [24–30]. As reported by Yuan et al. [30,31], the decomposition of PAA is driven by three mechanisms: spontaneous decomposition, hydrolysis, and decomposition catalyzed by transition metal, according to reactions 1, 2 and 3, respectively.

$$CH_3CO_3H \rightarrow 2\,CH_3CO_2H + O_2 \quad \text{(e.1)}$$

$$CH_3CO_3H + H_2O \rightarrow CH_3CO_2H + H_2O_2 \quad \text{(e.2)}$$

$$CH_3CO_3H \xrightarrow{M^{n+}} CH_3CO_2H + O_2 + other\ products \quad \text{(e.3)}$$

Figure 1 summarizes the reactions of PAA with different chemical constituents present in aqueous environments. In the following paragraphs the main physical-chemical characteristics of the wastewater affecting PAA decomposition and/or disinfection performance, are briefly described.

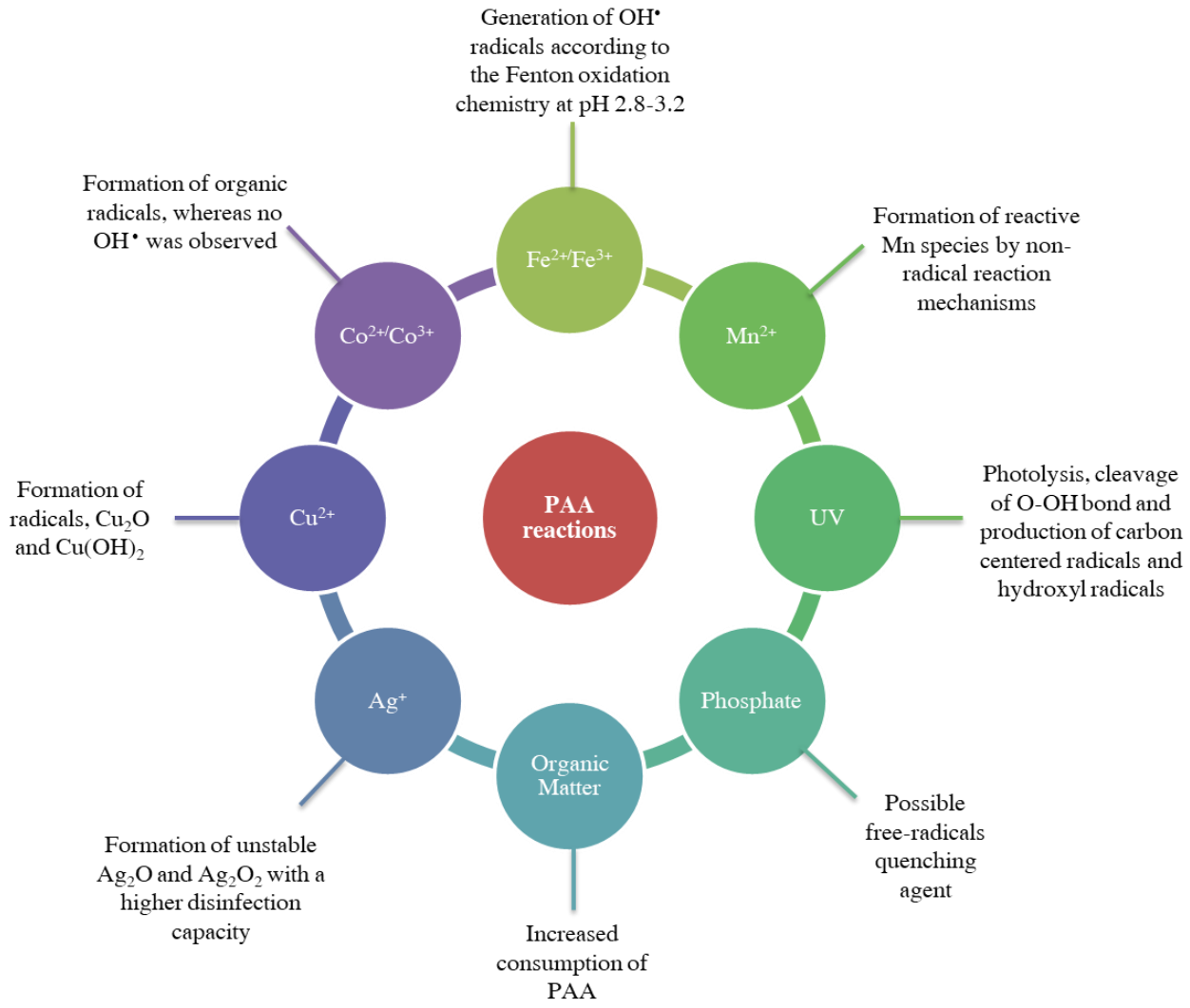

Figure 1. Reactions of PAA with various physical and chemical agents in aqueous solution [16].

1.2.1. pH

The effect of pH on both PAA stability and disinfection performance has been documented by various authors [5,16,31,32], who have observed that alkaline pH values lead to higher PAA decomposition rates and lower bacterial inactivation rates. As for the first aspect, various authors have reported that pH values above 9 lead to higher PAA decomposition rates. Given that the pK_a of PAA is 8.2, it is expected that at pH values higher than this value the equilibrium will move toward the dissociated form ($CH_3CO_3^-$). According to Yuan et al. [31], who studied the kinetics of alkaline hydrolysis of PAA, in a pH range of 5.5-8.2 the hydrolysis (reaction 2) is negligible and PAA consumption is mainly due to the spontaneous decomposition (reaction 1), whereas at pH between 8.2 to 9.0, PAA consumption is due to the spontaneous decomposition and hydrolysis. The dissociation of PAA as a function of the pH is presented in Figure 2. In agreement with this findings, Pedersen et al. [33] found that PAA decay was comparable at pH adjusted between values of 6.4 and 8.6.

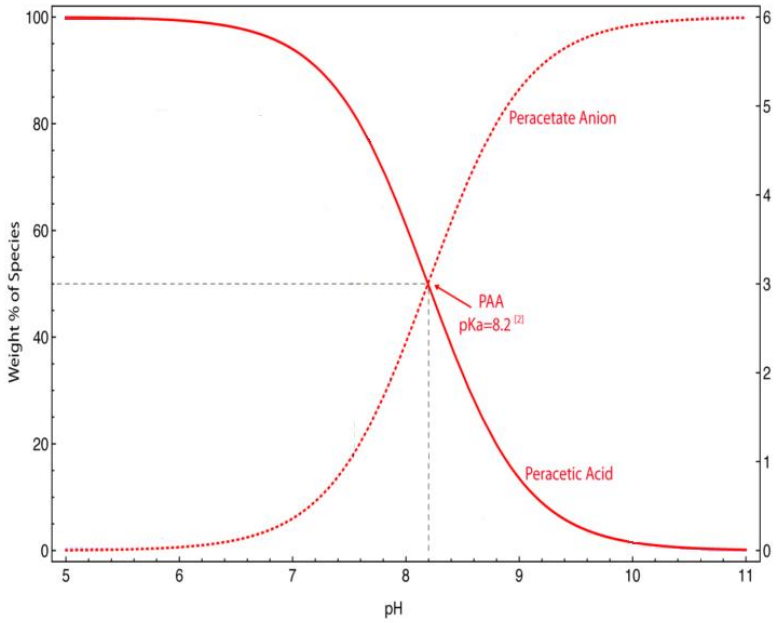

Figure 2. Dissociation of PAA as a function of the pH [31].

As for the effect of pH on the disinfection performance, it is has been reported that PAA is more effective in acidic conditions and above pH 9 the efficiency starts to decrease [5,32]. This might be related to the afore-discussed dissociation of PAA at alkaline pH, since the non-dissociated form (CH_3CO_3H) is thought to be a more active disinfectant than the dissociated form ($CH_3CO_3^-$). Therefore, at typical pH values (6.5-8.5) for secondary effluents it is not expected to observe an effect of pH on PAA stability or disinfection performance.

1.2.2. Inorganic compounds

The following paragraphs describe the roles of different inorganic compounds present in water on PAA decay and disinfection kinetics. One of the aspects to highlight regarding the currently available information about PAA decay and its reactions in water solution, is that most of the studies have been addressed to study one parameter at the time and the potential interactions among the different parameters have not been investigated to date.

It should be stressed as well that no information is presented in literature regarding the role of inorganic nitrogen-containing compounds, which are always present in the biological treated effluents and vary in type and concentration according to the treatment scheme.

1.2.2.1. Transition metals

Several authors have reported that transition metals catalyzed PAA decomposition [5,16,20,30–34]. In detail, Yuan et al. [30,31] reported that Fe^{2+}, Mn^{2+}, Cu^{2+} and Co^{2+} can catalyze the decomposition of PAA according to reaction 3. The same authors observed that chelants, such as diethylenetriaminepentaacetic acid (DTPA) and diethylene-triaminepentamethylenephosphonic acid (DTMPA), stop the catalytic effect of these transition metal ions, which however, cannot be entirely eliminated. The decomposition of PAA by Mn^{2+} proceeds via

complex redox reactions, where a number of different oxidation states of Mn are involved. Moreover, the kinetics display oscillatory features that are characteristic of autocatalytic processes [35]. Similar behavior was observed when Mn^{2+} was used in the catalytic oxidative degradation of dyes by PAA [36]. As for other transition metal ions commonly present in bleaching systems such as Co^{2+} and Co^{+3}, they cause PAA to decompose mainly to acetoxy ($CH_3CO_2^{\cdot}$) and peracetoxy ($CH_3CO_3^{\cdot}$) radicals, whereas no hydroxyl ($OH^{\cdot}$) radical formation was observed [37].

Besides catalyzing the decomposition of PAA, the presence of transition metals, such as copper (Cu) and silver (Ag), can also enhance the formation of radicals that improve the disinfection efficiency of PAA. For instance, Orta de Velasquez et al. [38] observed the synergy between Ag^{+} and PAA in the removal of *V. cholerae, Salmonella* sp., *Shigella, P. aeruginosa*, helminth eggs, and fecal coliforms. In agreement with these observations, Luna-Pabello et al. [39] also reported a synergistic effect between Ag^{+}/PAA and Ag^{+}/Cu^{2+}/PAA in the removal of helminth eggs. The authors highlighted that one major advantage of the addition of Cu and/or Ag ions is a decrease in the required contact time [38,39]. The authors proposed that the improvement of the disinfection efficiency is due to the reaction between Ag and PAA, which produces Ag^{+}, Ag_2O and Ag_2O_2, while the products of the reaction between Cu and PAA correspond to Cu^{+}, Cu^{2+}, Cu_2O and $Cu(OH)_2$, being the oxidized states are very unstable due to their high reactivity [39]. It is known that the oxidized forms, such as trivalent silver, are significantly faster and more effective disinfectants than monovalent silver [16,40].

1.2.2.2. Phosphate

In a recent study, Mattle et al. [15] observed that high phosphate concentrations (400 mM, ~746 mg/L) decreased the inactivation rate of bacteriophage MS2 by a factor of 5.1 compared to the inactivation rate at lower phosphate concentrations (15 mM, ~28 mg/L). The authors hypothesized that the protective effect of phosphate may arise from its capacity to remove free radicals [15,41].

1.2.2.3. Hardness and Salinity

Liu et al. [28] studied the effect of salinity and water hardness on different PAA formulations. In detail, the authors studied the effect of salinity with NaCl and sea salt dissolved in distilled water to reach the concentrations of 1% (10 g/L) and 3% (30 g/L). The results of this study demonstrated that salinity affects PAA degradation in all the formulations that were tested. However, the sea salt solution caused higher degradation of PAA than pure NaCl solutions. Indeed, the main components of sea salt used were NaCl and KCl, which represent about 85 % w/w of the sea salt. The rest of the 15% w/w is composed of SO_4^{2-}, Mg^{2+} and Ca^{2+}. This outcome indicates that the ion composition and complexity also have a role on PAA decay. The authors hypothesized that this might be due to Mg^{2+}/Ca^{2+} and Na^{+}/K^{+} ratios. Higher Mg^{2+}/Ca^{2+}ratios (85% in standard dilution water) lead to slower PAA decay, while higher Na^{+}/K^{+} ratios (85% in sea salt) resulted in faster PAA decay. In contrast, the same authors found that water hardness had only a slight impact on PAA degradation.

1.2.3. Organic matter

Numerous studies on PAA have reported that the presence of organic matter in solution leads to a significant PAA consumption [28,29,33,42]. To date, all the works that have documented this effect have adopted macroparameters of organic content such as chemical oxygen demand (COD) or dissolved organic carbon (DOC). The impact of different levels of organic matter content (up to 70.8 mg/L of COD) on PAA decay (from 0.29 to 2.0 mg/L) in water samples from a recirculating aquaculture system was studied by Pedersen et al. [33]. The authors found that PAA decay is significantly and positively correlated to the COD levels and a significant PAA consumption was observed at high COD concentrations. The instantaneous PAA consumption was significantly higher in water with high COD content (70.8 and 61.2 mg/L of COD) compared to water with low COD (10.3 mg/L) at the same PAA concentration. Moreover, the authors found that both organic matter content and nominal PAA concentration significantly affected PAA decay since lower decay rates were observed as PAA concentrations increased.

In another study [29] using water samples from two different recirculating aquaculture systems, a minor difference on PAA decay was observed between water samples with COD concentrations of 28.6 and 48.8 mg/L. In agreement with the results in aquaculture systems, Liu et al. [28] observed that PAA decay increases with DOC content in synthetic media in which two levels of dissolved organic carbon (high: 24 mg/L, low: 8 mg/L) were obtained from a commercial product composed mainly of humic substances.

Other authors [27] have observed the effect of organic matter on PAA decay on real sewage effluents. For instance, Gehr et al. [18] reported a high negative influence of the organic matter content on the PAA consumption and disinfection performance on municipal wastewater after enhanced primary treatment, which presented high COD concentrations (124-240 mg/L), total suspended solids (TSS) (16-45 mg/L) and turbidity (16-31 NTU). In agreement with this observation, Koivunen et al. [42] reported that PAA residues are typically very low or absent after the disinfection of primary effluents with high COD, TSS and turbidity levels. In contrast, PAA residual concentrations were typically present after the disinfection of secondary and tertiary effluents due to a low reactivity of PAA with high-quality effluents, characterized by low COD, TSS and turbidity levels, which improves PAA stability and therefore the efficiency for disinfection action.

It should be highlighted that an important limitation of adopting macro-parameters such as COD and DOC to evaluate the effect of organic matter content on PAA decay is that they do not allow discriminating the effect of the different organic macromolecules (carbohydrates, lipids, protein and nucleic acids). Due to the different nature of these macromolecules, they can potentially display different effect on PAA decay. For instance, Finnegan et al. [6] investigated the biochemical mechanisms of action of oxidizing biocides such as chlorine dioxide (ClO_2), PAA and H_2O_2 at a macromolecular level using amino acids, protein and an enzyme as model substrates. In particular, PAA oxidized amino acids efficiently, degraded bovine serum albumin and reduced the efficiency of the enzyme alkaline phosphatase.

The oxidative capacity of PAA towards proteins was observed again by Kerkaert et al. [43] on dairy proteins.

In detail, the authors studied the oxidation of whey proteins and caseins and different markers for protein oxidation were monitored. Cysteine, tryptophan, and methionine were found to be the most vulnerable amino acids for degradation upon PAA treatment, whereas tyrosine and histidine were fairly stable in the presence of PAA. These results suggest that PAA has a specific oxidative action on proteins, which can entail PAA consumption. As for the other organic macromolecules, no specific effect on PAA decay has been documented to date.

Regarding the evaluation of the effect of organic load on PAA consumption in complex and heterogeneous mixtures, such as real effluents in which the observed effect can be due to other water quality parameters (e.g. TSS or turbidity); current available literature does not permit to determine which factors drive PAA consumption. Moreover, the organic matter is distributed in different proportions in the suspended and soluble fractions in real effluents and it changes in the effluent throughout the stages of the treatment. This is a critical aspect to consider when different types of effluent are used to investigate the influence of organic load on PAA decay. For instance, according to Dignac et al. [44] most of the organic matter in the treated effluent is found in the soluble fraction (86% of the COD) whereas in the raw wastewater it represents less than a third part (31%). In addition, the authors found that not all the macromolecules are removed in the same proportion after the biological treatment, therefore the composition of the organic matter changes after the treatment [45]. Proteins and lipids are removed in a higher proportion (95 and 99%, respectively) than carbohydrates (91%) from the soluble fraction, suggesting that lipids are the most easily biodegradable organic macromolecule, followed by proteins, and carbohydrates.

1.2.4. Particulate matter

The same as soluble organic matter, particulate matter has a heterogeneous composition, which varies with the characteristics of the wastewater, depending mainly on the source (domestic, industrial, agricultural, storm) and on the type of the sewer (combined or separated); therefore, a detailed characterization of their composition is very difficult. The effects of TSS on PAA decay have been scarcely studied, although it has been observed in different works [25,42,46–52]. Several authors have indicated that combined sewer overflows (CSOs) and primary effluents require higher PAA concentrations for disinfection than secondary and tertiary effluents [32,42,52,53]. Furthermore, Chhetri et al. [52] observed that the pretreatment of CSOs and the removal of suspended solids decreased the required disinfectant dose. However, according to several authors the most relevant effect of suspended solids on PAA disinfection is related to the inactivation of bacteria. Indeed, suspended solids are usually blamed for impairing water disinfection with chemical (chlorine-based compounds, ozone, PAA) or physical methods (ultraviolet radiation). In detail, solids can afford protection to embedded microorganisms within their porosity, allowing them to resist even to high disinfectant dosages [49,54]. Bayoudh et al. [55] proposed that bacteria can be retained by suspended solids through non-specific Lif-shitzevan der Waals forces and hydrogen and chemical bonding. In the last decade, the research on this aspect has been addressed mainly to the effect of particle size. Falsanisi et al. [49] observed that TSS can afford

a protection of 0.6 and 1.3 logs for particles between 10 -120 μm and greater than 120 μm, respectively, whereas McFadden et al. [50] observed that solid size (10-100 μm) had a minor effect on PAA disinfection.

As for the effect of TSS concentrations on the disinfection efficiency of PAA, a detrimental effect of high concentrations has been reported in various studies [5,42,46,48,50]. Lefevre et al. [46] found that TSS concentrations high ranging between 10 to 50 mg/L had no effect on bacterial inactivation, but TSS concentrations over 100 mg/L would reduce the extent of inactivation by 1 log in secondary effluents. On the other hand, Lazarova et al. [25] and Stampi et al. [48] found that the effect on PAA disinfection performance is moderate and constant for TSS concentrations between 10 and 40 mg/L.

1.3. References

[1] P.C. Freer, F.G. Novy, On the formation, decomposition and germicidal action of benzoylacetyl and diacetyl peroxides, J. Am. Chem. Soc. (1902) 161–93.

[2] S.S. Block, Disinfection, sterilization, and preservation, 5th ed., Lippincott Williams & Wilkins, 2001.

[3] P.S. Malchesky, Peracetic Acid and Its Application to Medical Instrument Sterilization, Artif. Organs. 17 (2008) 147–152.

[4] European Chemicals Agency, Regulation (EU) No 528 / 2012 concerning the making available on the market and use of biocidal products. Assessment Report for peracetic acid, 2015.

[5] M. Kitis, Disinfection of wastewater with peracetic acid: A review, Environ. Int. 30 (2004) 47–55.

[6] M. Finnegan, E. Linley, S.P. Denyer, G. McDonnell, C. Simons, J.Y. Maillard, Mode of action of hydrogen peroxide and other oxidizing agents: Differences between liquid and gas forms, J. Antimicrob. Chemother. 65 (2010) 2108–2115.

[7] US EPA, Alternative Disinfection Methods Fact Sheet : Peracetic Acid, (2012).

[8] S.P. Denyer, G.S.A.B. Stewart, Mechanisms of Action of Disinfectants, Int. Biodeterior. Biodegrad. 41 (1998) 261–268.

[9] R. Bach, P. Ayala, H.B. Schlegel, A Reassessment of the Bond Dissociation Energies of Peroxides. An ab Initio Study. 118 (1996) 12758-12765.

[10] E. V. Rokhina, K. Makarova, E.A. Golovina, H. Van As, J. Virkutyte, Free Radical Reaction Pathway, Thermochemistry of Peracetic Acid Homolysis, and Its Application for Phenol Degradation: Spectroscopic Study and Quantum Chemistry Calculations, Environ. Sci. Technol. 44 (2010) 6815–6821.

[11] M.J. Flores, M.R. Lescano, R.J. Brandi, A.E. Cassano, M.D. Labas, A novel approach to explain the inactivation mechanism of *Escherichia coli* employing a commercially available peracetic acid, Water Sci. Technol. 69 (2014) 358.

[12] S. Wessels, H. Ingmer, Modes of action of three disinfectant active substances: A review, Regul. Toxicol. Pharmacol. 67 (2013) 456–467.

[13] G. McDonnell, A.D. Russell, Antiseptics and disinfectants: activity, action, and resistance., Clin. Microbiol. Rev. 12 (1999) 147–79.

[14] J. Koivunen, H. Heinonen-Tanski, Inactivation of enteric microorganisms with chemical disinfectants, UV irradiation and combined chemical/UV treatments, Water Res. 39 (2005) 1519–1526.

[15] M.J. Mattle, B. Crouzy, M. Brennecke, K. R. Wigginton, P. Perona, T. Kohn, Impact of virus aggregation on inactivation by peracetic acid and implications for other disinfectants, Environ. Sci. Technol. 45 (2011) 7710–7717.

[16] T. Luukkonen, S.O. Pehkonen, Peracids in water treatment: A critical review, Crit. Rev. Environ. Sci. Technol. 0 (2016) 1–39.

[17] M.G.C. Baldry, The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid, J. Appl. Bacteriol. 54 (1983) 417–423.

[18] M.G.C. Baldry, M.S. French, Disinfection of sewage effluent with peracetic acid, Wat. Sci. Tech. 21 (1989) 203–206.

[19] J.A.L. Fraser, A.F. Godfree, F. Jones, Use of peracetic acid in operational sewage sludge diposal to pasture, Wat. Sci. Tech. 17 (n.d.) 451–466.

[20] M. Wagner, D. Brumelis, R. Gehr, Disinfection of wastewater by hydrogen peroxide or peracetic acid: development of procedures for measurement of residual disinfectant and application to a physicochemically treated municipal effluent., Water Environ. Res. 74 (2002) 33–50.

[21] P.A. Marchand, T.M. Phan, D.L. Straus, B.D. Farmer, A. Stüber, T. Meinelt, Reduction of in vitro growth in Flavobacterium columnare and Saprolegnia parasitica by products containing peracetic acid, Aquac. Res. 43 (2012) 1861–1866.

[22] A.D. Shah, Z.Q. Liu, E. Salhi, T. Höfer, U. Von Gunten, Peracetic acid oxidation of saline waters in the absence and presence of H2O2: Secondary oxidant and disinfection byproduct formation, Environ. Sci. Technol. 49 (2015) 1698–1705.

[23] D. Liu, D.L. Straus, L.-F. Pedersen, T. Meinelt, Comparison of the Toxicity of Wofasteril Peracetic Acid Formulations E400, E250, and Lspez to *Daphnia magna*, with Emphasis on the Effect of Hydrogen Peroxide, N. Am. J. Aquac. 77 (2015) 128–135.

[24] D. Falsanisi, R. Gehr, D. Santoro, A. Dell'Erba, M. Notarnicola, L. Liberti, Kinetics of PAA demand and its implications on disinfection of wastewaters, Water Qual. Res. J. Canada. 41 (2006) 398–409.

[25] V. Lazarova, M. Janex, L. Fiksdal, C. Oberg, I. Barcina, M. Pommepuy, Advanced wastewater disinfection technologies: Short and long term efficiency, Water Sci. Technol. 38 (1998) 109–117.

[26] J. Howarth, M. Harvey, Method of analyzing low levels of peroxyacetic acid in water (Patent No: US 7,651,860 B2), 2010.

[27] C. Sanchez-Ruiz, S. Martinez-Royano, I. Tejero-Monzon, An evaluation of the effciency and impact of raw WW disinfection with PAA prior to Ocean discharge, Water Sci. Technol. 32 (1995) 159–166.

[28] D. Liu, C. Steinberg, D.L. Straus, L. Pedersen, T. Meinelt, Salinity, dissolved organic carbon and water hardness affect peracetic acid (PAA) degradation in aqueous solutions, Aquac. Eng. 60 (2014) 35–40.

[29] L.F. Pedersen, P.B. Pedersen, J.L. Nielsen, P.H. Nielsen, Peracetic acid degradation and effects on

nitrification in recirculating aquaculture systems, Aquaculture. 296 (2009) 246–254.

[30] Z. Yuan, Y. Ni, A.R. Van Heiningen, Kinetics of the peracetic acid decomposition: Part II: pH effect and alkaline hydrolysis, Can. J. Chem. Eng. 75 (1997) 42–47.

[31] Z. Yuan, Y. Ni, A.R.P. Van Heiningen, Kinetics of peracetic acid decomposition: Part I: Spontaneous decomposition at typical pulp bleaching conditions, Can. J. Chem. Eng. 75 (1997) 37–41.

[32] T. Luukkonen, J. Teeriniemi, H. Prokkola, J. Rämö, U. Lassi, Chemical aspects of peracetic acid based wastewater disinfection, Water SA. 40 (2014) 73–80.

[33] L.F. Pedersen, T. Meinelt, D.L. Straus, Peracetic acid degradation in freshwater aquaculture systems and possible practical implications, Aquac. Eng. 53 (2013) 65–71.

[34] X. Zhao, K. Cheng, J. Hao, D. Liu, Preparation of peracetic acid from hydrogen peroxide, part II: Kinetics for spontaneous decomposition of peracetic acid in the liquid phase, J. Mol. Catal. A Chem. 284 (2008) 58–68.

[35] E. Popov, J. Eloranta, V. Hietapelto, V.-M. Vuorenpalo, R. Aksela, J. Jäkärä, Mechanism of decomposition of peracetic acid by manganese ions and diethylenetriaminepentaacetic acid (DTPA), Holzforschung. 59 (2005) 507–513.

[36] S. Rothbart, E.E. Ember, R. van Eldik, Mechanistic studies on the oxidative degradation of Orange II by peracetic acid catalyzed by simple manganese (ii) salts. Tuning the lifetime of the catalyst, New J. Chem. 36 (2012) 732–748.

[37] X.Z. Zhang, R.C. Francis, D.B. Dutton, R.T. Hill, Decomposition of peracetic acid catalyzed by Cobalt(II) and Vanadium(V), Can. J. Chem. 76 (1998) 1064–1069.

[38] M. Orta de Velásquez, I. Yáñez-Noguez, B. Jiménez-Cisneros, V.M. Luna Pabello, Adding silver and copper to hydrogen peroxide and peracetic acid in the disinfection of an advanced primary treatment effluent, Environ. Technol. 29 (2017) 1209–1217.

[39] V. Luna-Pabello, M. Miranda Ríos, B. Jiménez, M. Orta de Velasquez, Environmental Technology Effectiveness of the use of Ag, Cu and PAA to disinfect municipal wastewater Effectiveness of the use of Ag, Cu and PAA to disinfect municipal wastewater, Environ. Technol. 30 (2009) 129–139.

[40] R.L. Davies, S.F. Etris, The development and functions of silver in water purification and disease control, Catal. Today. 36 (1997) 107–114.

[41] R.A. Booth, J.N. Lester, The potential formation of halogenated by-products during peracetic acid treatment of final sewage effluent, Water Res. 29 (1995) 1793–1801.

[42] J. Koivunen, H. Heinonen-Tanski, Peracetic acid (PAA) disinfection of primary, secondary and tertiary treated municipal wastewaters, Water Res. 39 (2005) 4445–4453.

[43] B. Kerkaert, F. Mestdagh, T. Cucu, P.R. Aedo, S.Y. Ling, B. De Meulenaer, Hypochlorous and peracetic acid induced oxidation of dairy proteins, J. Agric. Food Chem. 59 (2011) 907–914.

[44] M.F. Dignac, P. Ginestet, D. Rybacki, A. Bruchet, V. Urbain, P. Scribe, Fate of wastewater organic pollution during activated sludge treatment: Nature of residual organic matter, Water Res. 34 (2000)

4185–4194.

[45] H.K. Shon, S. Vigneswaran, S.A. Snyder, Effluent organic matter (EfOM) in wastewater: Constituents, effects, and treatment, Crit. Rev. Environ. Sci. Technol. 36 (2006) 327–374.

[46] F. Lefevre, J.M. Audic, F. Ferrand, Peracetic acid disinfection of secondary effluents discharged off coastal seawater, Water Sci. Technol. 25 (1992) 155–164.

[47] C. Sánchez-Ruiz, S. Martínez-Royano, I. Tejero-Monzón, An evaluation of the efficiency and impact of raw wastewater disinfection with peracetic acid prior to ocean discharge, Water Sci. Technol. 32 (1995) 159-166.

[48] S. Stampi, G. De Luca, F. Zanetti, Evaluation of the efficiency of peracetic acid in the disinfection of sewage effluents, J. Appl. Microbiol. 91 (2001) 833–838.

[49] D. Falsanisi, R. Gehr, L. Liberti, M. Notarnicola, Effect of suspended particles on disinfection of a physicochemical municipal wastewater with peracetic acid, Water Qual. Res. J. Canada. 43 (2008) 47–54.

[50] M. McFadden, J. Loconsole, A.J. Schockling, R. Nerenberg, J.P. Pavissich, Comparing peracetic acid and hypochlorite for disinfection of combined sewer overflows: Effects of suspended-solids and pH, Sci. Total Environ. 599–600 (2017) 533–539.

[51] R.K. Chhetri, D. Thornberg, J. Berner, R. Gramstad, U. Öjstedt, A.K. Sharma, H.R. Andersen, Chemical disinfection of combined sewer overflow waters using performic acid or peracetic acids, Sci. Total Environ. 490 (2014) 1065–1072.

[52] R.K. Chhetri, A. Bonnerup, H.R. Andersen, Combined Sewer Overflow pretreatment with chemical coagulation and a particle settler for improved peracetic acid disinfection, J. Ind. Eng. Chem. 37 (2016) 372–379.

[53] R. Gehr, D. Cochrane, Peracetic acid (PAA) as a disinfectant for municipal wastewaters: encouraging performance results from physicochemical as well as biological effluents, Proc. Water Environ. Fed. 2002, 182–198.

[54] J.P. Dietrich, F.J. Loge, T.R. Ginn, H. Başağaoğlu, Inactivation of particle-associated microorganisms in wastewater disinfection: Modeling of ozone and chlorine reactive diffusive transport in polydispersed suspensions, Water Res. 41 (2007) 2189–2201.

[55] S. Bayoudh, A. Othmane, L. Mora, H. Ben Ouada, Assessing bacterial adhesion using DLVO and XDLVO theories and the jet impingement technique, Coll. Surfaces B Biointerfaces. 73 (2009) 1–9.

CHAPTER 2. DESIGN OF THE RESEARCH

2.1. Rationale

As outlined in the State of the Art, peracetic Acid (AA) decomposition in water solution is highly affected by the composition of the water matrix. Moreover, the presence of suspended matter might have an effect beyond PAA decomposition, interfering with bacterial inactivation performance, potentially affording a mechanism of defense to microorganisms against disinfection. Guarantying a sufficient amount of the disinfectant to reach bacterial inactivation targets, avoiding excessive residuals is fundamental for the design of a disinfection process, in particular when disinfected effluents are intended to be discharged in sensitive water bodies or re-used in agriculture and large residuals can pose detrimental environmental effects. Consequently, a compromise should be met between an adequate dosage of disinfectant to achieve the bacterial inactivation targets and the prevention of high residual concentrations. Considering this, the integration of both aspects, PAA decay and bacterial inactivation kinetics is of the uppermost importance in order to establish a comprehensive approach for PAA disinfection design and management.

The present work is focused on the definition of predictive models for PAA decay and bacterial inactivation kinetics, in order to integrate them into a dosage optimization strategy. To achieve this, different elements of both aspects were studied as summarized in the figure 1.

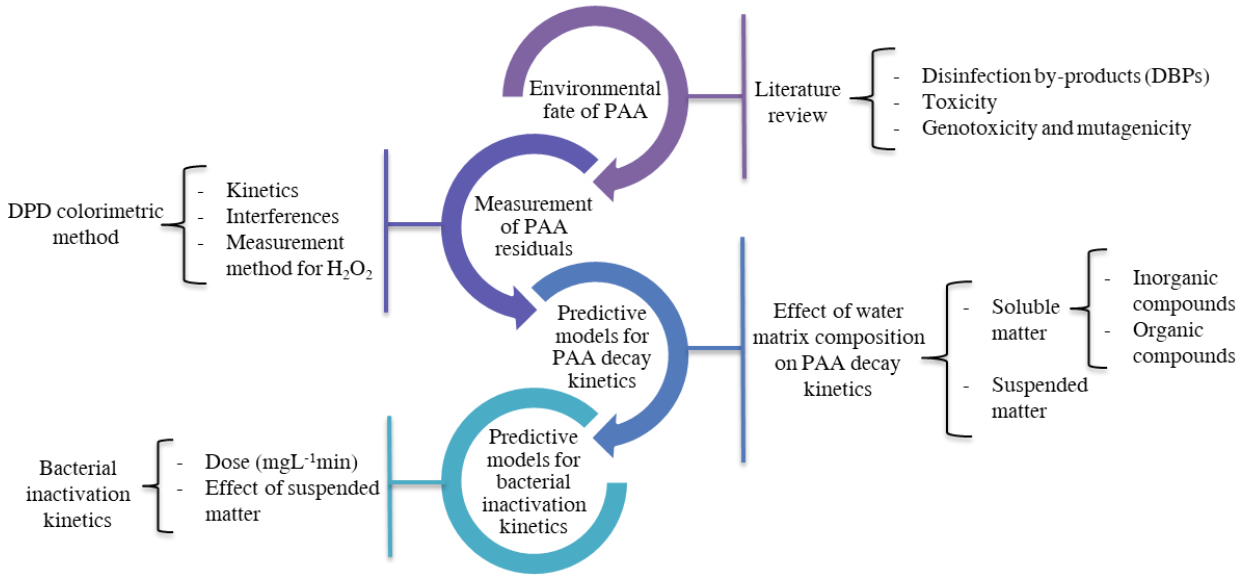

Figure 1. Schematic representation of the experimental research plan.

Firstly, a thorough literature review of the environmental fate of PAA residuals and the ecological impact of effluents is presented, exploring different aspects on the topic. Then, a reliable measurement method for PAA residual concentrations to be implemented in the subsequent stages of this study was defined, studied and

validated. Regarding the definition of predictive models for the decay kinetics of PAA, a thorough experimental plan was carried out in order determine the physical-chemical characteristics of the effluent that affect the decay of PAA, investigating separately the contribution of the soluble and suspended matter. As for the soluble matter, the investigation was addressed to identify the specific inorganic and organic compounds that affect PAA decomposition, whereas for the suspended matter the focus was on the extent of the effect of different total suspended solids (TSS) concentrations. In addition, the contribution of the uncharacterized soluble fraction associated to the TSS to PAA decay, was also studied. Concerning the development of a predictive model for bacterial inactivation, the parameter of dose, defined as the area under the PAA decay curve, was evaluated as the main factor determining disinfection efficiency of PAA on *E.coli*. Firstly, the dose-response curve of the organism was determined and subsequently the invariance of the effect of the dose was assessed by selecting different operating conditions to define the PAA dose. Subsequently, the effect and a potential interference of TSS with PAA disinfection performance were tested.

2.2. Research Design

The present doctoral dissertation is structured in four chapters; each one intended to be published on an international peer-reviewed scientific journal. In consequence, each chapter follows an article-based structure that includes an introduction that presents the specific state-of-the-art on the topic and the objectives, experimental methodologies, presentation and discussion of the results and conclusions. The chapters are related among them, based on the design briefly described in Figure 1. Their content is briefly described in the following paragraphs.

- Chapter 3: A critical review of the literature on the environmental fate of the residual PAA is presented, exploring the ecotoxicological effects on aquatic environment associated to effluents treated with PAA and its residual concentration. A comprehensive and updated overview on the topic, covering aspects of PAA disinfection such as DBPs formation, toxic, genotoxic and mutagenic effects on aquatic ecosystems is introduced in this chapter.
- Chapter 4: An experimental method to measure the residual concentration of PAA in solution is proposed based on the one implemented by various authors in previous studies on PAA. This method was adapted from the DPD colorimetric method for the measurement of total chlorine, proposed by the US EPA (method # 330.5). A method to measure the associated fraction of H_2O_2 in equilibrium with PAA, is also described. In addition, some relevant aspects related to the operating conditions, kinetics and the possible interference of H_2O_2 on PAA measurement are elucidated.
- Chapter 5: The contribution of soluble matter on the decay kinetics of PAA is evaluated, with a focus on the identification of the specific inorganic and organic compounds, typically present in secondary effluents. The statistical method of the Design of Experiments (DoE) was used to define experimental plans for both set of compounds. Considering that the inorganic and organic compounds displayed different effects on PAA decay, a predictive model for each set of compounds was interpolated based on

the most relevant compounds to describe the decay kinetics. Finally, a unique predictive model to describe the decay kinetics of PAA is proposed based on the combined effect of the inorganic and the organic compounds. A validation of this final model is presented as a comparison between set of experiments assessing PAA decay in the simultaneous presence of inorganics and organics and the outcomes of Monte Carlo simulations, which were used to propagate the uncertainty through the model.

- Chapter 6: The contribution of suspended solids on the decay kinetics of PAA is evaluated by testing their effect at different concentrations of TSS, representative of secondary effluents of good (well settled) and medium (not well settled) quality, and combined sewer overflows. The contribution of the soluble matter associated to the suspended solids on PAA decay is also evaluated and a predictive model for the decay kinetics of PAA is presented based on the contribution of both suspended and soluble matter. This chapter also presents an assessment of the dose (actual concentration of PAA at which bacteria are exposed) as the main parameter determining the inactivation performance of PAA and a model to describe bacterial inactivation kinetics based on this parameter is proposed. Furthermore, an evaluation of the invariance of the dose, namely different combinations of initial concentrations of PAA and contact time for a fixed dose, to reach the same levels of bacterial inactivation is also presented. Finally, a potential defense mechanism for bacteria against disinfection, afforded by suspended solids is studied.

CHAPTER 3. ECOTOXICOLOGICAL EFFECTS OF EFFLUENTS TREATED WITH PAA: A REVIEW

Abstract

Peracetic acid (PAA) has gained increasing attention over the last decades as a suitable and environmental-friendly alternative to chlorine-based compounds for wastewater disinfection, claiming limited disinfection by-products formed and no persistent residues in the environment. The present work aims at presenting a comprehensive up-to-date review of the ecotoxicological effects of effluents treated with PAA and the environmental fate of the residual concentrations. Modest concentrations of disinfection by-products (DBPs) have been observed after PAA treatment, mainly carboxylic acids, which are not recognized as mutagenic. Moreover, there is no evidence of any endocrine disruption potential of PAA in human health or in the ecotoxicological studies. Considering that PAA is available commercially as an equilibrium solution of PAA and hydrogen peroxide (H_2O_2), the contribution of the associated H_2O_2 fraction to the ecotoxicological effects of PAA has been reviewed as well. Effluents disinfected with PAA at concentrations typical of the wastewater treatment field have displayed limited toxic, mutagenic and genotoxic effects on different aquatic organisms, particularly low respect to chlorine-based disinfectants.

Keywords: Peracetic acid, hydrogen peroxide, disinfection by-products, toxicity, mutagenicity, genotoxicity.

The section of the present chapter dedicated to the review of the DBPs formed during PAA disinfection, has been properly adapted to contribute to a chapter of a handbook on PAA disinfection, currently in preparation and edited by the Water Environment Federation (WEF).

The present chapter has been submitted for publication to the journal “Critical Reviews in Environmental Science and Technology”.

3.1. Introduction

Chlorination has played for many decades a leading role for drinking and wastewater applications; however, concerns over the formation of toxic, carcinogenic, mutagenic and teratogenic DBPs began in the 1970s and since then it has been extensively reported that drinking and wastewater chlorination promotes the formation of such DBPs. The concerns about the adverse effects on human health and aquatic ecosystem of these DBPs [1–5] have motivated the investigation on alternative disinfection processes that do not produce harmful DBPs. These alternatives include ultraviolet (UV) radiation, ozone and organic peracids, such as PAA. Nevertheless, disinfection processes such as UV radiation and ozonation require expensive equipment, long time of implementation and they are very sensitive to wastewater physical-chemical characteristics [6–10].

Peroxides are high-energy-state compounds and as such, they are thermodynamically unstable and can be easily split into reactive radicals via homolitic cleavage, making them strong oxidizers [11,12]. The disinfectant properties of PAA are derived from its high oxidation potential (1.81 V) [13], which is greater than the one of H_2O_2 (1.76 V) and ClO_2 (1.27 V) [14]. For this reason, PAA displays a broad spectrum of antimicrobial activity, exerting virucidal, sporicidal, bactericidal and fungicidal effects on microorganisms [12,15,16]. PAA has been used widely as a disinfectant in various industries [17] and has gained increasing attention over the last decade for wastewater disinfection, establishing itself as a suitable alternative for chlorine-based compounds [18]. The choice of a suitable disinfectant for wastewater depends on the following criteria: safe and easy handling, storage, capital, operational and infrastructure costs, alongside the lack of neither toxic nor mutagenic disinfection by-products (DBPs), which are key aspects to take into account. Regarding this last aspect during PAA disinfection, the contribution of the H_2O_2 fraction in equilibrium with PAA to the potential ecotoxic effects of disinfected effluents cannot be ruled out [19,20].

PAA decomposes rapidly in water solution and its decay rate is highly affected by the water composition [21-27], as outlined in the State of the Art in Chapter 1. The half-life time (t50) of PAA (1-15 mg/L) in tap water estimated by Rossi et al. [22] was 100 minutes, whereas Luukkonen et al. [23] (15 mg/L) estimated a t50 of 469 and 710 minutes. For secondary effluents t50 of PAA (1-15 mg/L) ranged from 77 to 248 minutes [24,22,25,26] and for tertiary effluents (15 mg/L of PAA) it ranged from 168 to 189 minutes [23]. The t50 of PAA (1-5 mg/L). in aquaculture systems reported by Pedersen et al. [27] ranged from 36 to 318 minutes depending on the organic matter content and PAA concentration.

On the other hand, H_2O_2 naturally degrades to water and oxygen by various mechanisms, including chemical reduction and enzymatic (catalase and peroxidase) decomposition by algae, zooplankton, and heterotrophic bacteria. The t50 of H_2O_2 in natural water can vary from several hours to several days or more, depending on the characteristics of receiving water (e.g. chemical, biological, and physical factors) [28]. Longer t50 (in the order of several days) occur in very clear and pristine waters, nearly devoid of microorganisms, algae, and organic matter, whereas much shorter t50 (120-480 minutes) occur in nutrient-rich eutrophic water containing a larger biomass of microorganisms [29]. Cooper et al. [30] reported the t50 of H_2O_2 in surface water as 264,

282, 384, 1146 and 3522 minutes in unfiltered, 64 mm filtered (zooplankton removed), 12 mm filtered (large algae removed), 1 mm filtered (small algae removed), and 0.2 mm filtered (bacteria removed) samples, respectively [31].

As for their environmental fate, being both PAA and H_2O_2 strong oxidizing agents that are highly reactive and can readily oxidize several compounds present in water, such as dissolved organic matter, the possibility that they might potentially lead to the formation of DBPs cannot be completely neglected [32,33]. Furthermore, given that disinfectants are intended to inactivate microorganisms, causing massive cellular damage due to their oxidizing properties [34], it is reasonable to expect that their residues (which will remain in the receiving water according to their t50) might pose as well a significant risk to ecosystems and organisms other than the targeted ones for disinfection [21,35–37]. It should be stressed that there is no evidence of any endocrine disruption potential of PAA in human health or in any of the ecotoxicological studies performed to date. Furthermore, PAA is not included in the list of endocrine disruptors in the Commission Staff Working Document [38] that establishes the scientific criteria for their determination in the context of the EU legislation on plant protection products and biocidal products.

The present work presents a critical review on the environmental fate and ecotoxicological effects of water treated with PAA. The purpose is to present a comprehensive and updated overview on the topic, covering aspects of PAA disinfection such as DBPs formation, toxic, genotoxic and mutagenic effects on aquatic environment. In addition, the documented effects of H_2O_2 in equilibrium with PAA are discussed.

3.2. Disinfection by-products (DBPs) formation

Disinfection by-products are formed when disinfectants react with naturally occurring organic matter (e.g. humic and fulvic acids), anthropogenic contaminants, bromide, and iodide during the production of drinking water [39,40] and disinfection of wastewater [4,41,42]. The most studied DBPs derive from chlorination, being trihalomethanes (THMs) and haloacetic acids (HAAs), which were also the first chlorine DBPs reported [2]. They are currently regulated in the EU (THMs) and the USA (THMs, HAAs) [39]. So far, about 600 DBPs are identified to be formed during chlorination and chloramination, among them there are highly toxic compounds such as iodo- and bromo-compounds [3,43–45], halofuranones [45,46], halonitromethanes [45], and N-nitroso-dimethylamine [47].

Regarding PAA, even though it decomposes to oxygen, water and acetic acid, the possibility that DBPs can be formed, cannot be ruled out. It should be pointed out that the general criterion used to identify a compound as a DBP is its presence in the treated water at levels at least two to three times greater than in the untreated raw water (as judged by comparing chromatographic peak areas) [39,48]. Indeed, raw water (e.g. surface water for drinking water applications) can already contain several contaminants which act as DBPs precursors or contribute as such to the toxicity of the effluents, as aldehydes, aromatic compounds such as naphthalene, tert-butylbenzoic acid and pentafluorobenzaldehyde, phthalates (dimethyl phthalate), and alkanes, such as 2,2,4,6,6-pentamethylheptane and 2,2,4,4-tetramethyloctane [4,48]. DBPs formed during PAA disinfection

reported in literature by different authors are summarized in Table 1.

Most of the DBPs identified during PAA disinfection are carboxylic acids, which are not recognized as mutagenic [3,48] and have been also reported as commonly formed during drinking water disinfection with other agents, such as ClO_2, O_3 and chloramines, due to the oxidation of organic matter. Carboxylic acid concentration increases mainly due to the acetic acid present in the equilibrium solution and the decomposition of PAA [18]. Furthermore, the amount of long chain carboxylic acids, such as octanoic, nonanoic, decanoic, lauric, myristic, and hexanedioic acids, may increase due to the oxidation of aqueous organic compounds in surface water after PAA treatment (1.5-3 mg/L) [18]. However, no exact formation mechanism of these long chain carboxylic acids has been presented so far [18].

Modest concentrations of several low-molecular-weight aldehydes and ketones (μg/L levels) have been reported [4,48–50] to form during drinking and wastewater disinfection by PAA, being many of them already present in raw water in the same order of magnitude. These aldehydes and ketones, such as propanal, butanal, hexanal, glyoxal and acetone, are the products of the oxidation of amino acids, phenols and other aromatic substances present in the raw effluent [16,41,49,51,52]. For instance, Liberti and Notarnicola [42] found that about 120 ppb of aldehydes were already present in the wastewater before PAA disinfection; hence, their eventual further formation was ignored. Formaldehyde has been reported to be formed as well during the disinfection of secondary effluents, although below the limit of detection (1 μg/L) [41]. Moreover, the formation of aldehydes is proportional to PAA dosage and varies with PAA/COD ratio, reaching a maximum around 0.3 mg $_{PAA}$/mg $_{COD}$ and then tending to vanish [4]. Once formed, aldehydes can be further oxidized by PAA excess to the corresponding carboxylic acids, or ultimately to carbon dioxide and disappear according to reactions 1 and 2, as proposed by Dell'Erba et al. [4].

$$2\,CH_3COOOH + CH_3OH \rightarrow 2\,CH_3COOH + HCHO + H_2O \quad \text{(e.1)}$$

$$CH_3COOOH + HCHO \rightarrow 2\,CH_3COOH + CO_2 + H_2O \quad \text{(e.2)}$$

Aldehydes, in particular, are recognized as hepatotoxins at mg/L concentration, although much lower levels are formed after PAA disinfection of municipal sewage (<30 μg/L) [24,49–51]. However other authors [24], have not observed any by-products such as aldehydes, phenols and benzene derivatives during the disinfection of secondary effluents with PAA (10 mg/L).

The potential formation of halogenated DBPs during PAA disinfection of drinking and wastewater treatment has been investigated under conventional treatment conditions (1-10 mg_{PAA}/L, contact time 30-90 minutes), finding limited or undetectable amounts of halogenated DBPs (e.g. THMs, HAAs, bromate, chlorinated and brominated organics, adsorbable organic halides (AOXs)) [4,24,41,42,48–50,52]. Monarca et al. [53] observed a very limited formation of halogenated organic DBPs (as AOXs) after PAA treatment of surface water; however, no specific compounds were identified. Similarly, Pedersen et al. [50] and Dell'Erba et al. [4] found

bromophenols, chlorophenols and formaldehyde below the detection limit (0.1 μg/L) after PAA disinfection at conventional operating conditions (PAA concentration ≤ 4.8 mg/L contact time ≤ 37 min). However, in the presence of high concentrations of PAA or halides can promote the formation of halogenated DBPs. Under these conditions halide ions are oxidized forming hypohalous acids, which subsequently react with dissolved organic matter to form halogenated DBPs [4,18,52,54]. Shah et al. [54] found that PAA can lead to the formation of brominated by-products during the disinfection of sea or brackish water with high concentrations of PAA (150 mg/L) and extended contact time (CT of 5 days) was able to form bromoform (up to 920 μg/L) and dibromochloromethane (up to 110 μg/L), whereas no formation of chloroform or bromodichloromethane was observed. In agreement with these findings, Booth and Lester [52] observed that high concentrations of PAA (30 mg/L) can oxidize bromide to hypobromous acid (HOBr) in solutions enriched with bromide (400 μg/L) and phenol (400 ng/L) and extended contact time (14 h), forming 2- and 4-bromophenol and increasing AOXs. This suggests that PAA can lead to the formation of brominated by-products, although under unrealistic conditions in conventional drinking or wastewater treatment. Conversely, it has been reported that the addition of PAA to an aqueous solution of humic acids enriched with chloride does not produce an increase of AOXs, suggesting that chlorides are not oxidized to HClO [41,49]. Indeed, the electrochemistry of PAA enables the oxidation of bromide to hypobromous acid but not the one of chloride to hypochlorous acid, thus it does not lead to the formation of chlorine-based by-products [52]. Bromine is usually more reactive than chlorine, especially towards phenolic compounds [5,55], due to the much lower rate constant for the PAA reaction with chloride compared to the reaction with bromide [18,56]. Moreover, ammonia content of secondary effluents (20-25 mg/L) can minimize the formation of brominated organic compounds since it competes with the halogenation reaction. Indeed, ammonia reaction rate with HOBr is much greater than the one between HOBr and organic matter [42].

Even though PAA is commonly used as epoxidation agent in the chemical industry [57], no formation of epoxides has been observed during PAA disinfection of wastewater [18,42]. Indeed, epoxides are very unstable and in the case they are formed, it is expected that they behave as reaction-intermediates that immediately decompose to H_2O_2 and carbonyl-containing products [42].

Since the discovery of the occurrence of DBPs during chlorination, research has been focused on carbonaceous DBPs, particularly THMs and HAAs, rather than nitrogenous disinfection by-products (N-DBPs), which include families of compounds such as halonitroalkanes, halonitriles, haloamides and N-nitrosamines [58]. Unlike the other groups, N-nitrosamines are not halogenated, therefore they are not included in the total organic halogen (TOX) analysis. Although N-nitrosamines are generally formed at lower concentrations than THMs or HAAs, they have shown more carcinogenic and mutagenic effects than THMs and HAAs. For instance, the most prominent one, N-nitroso-dimethylamine (NDMA) has an associated cancer risk approximately 600 times greater than any of the regulated THMs and its concentration as low as 0.7 ng/L in drinking water is associated with a 10^6 lifetime cancer risk [58,59]. No formation of NDMA or other N-nitrosamines was detected taking place during PAA disinfection (5 and 10 mg/L) of drinking water, except for N-nitroso-di-n-

propylamine (NDPA) (<10 ng/L) when amine-based precursors were added to the water samples [60].

Table 1. DBPs formed during PAA disinfection. Contact time (t) in brackets.

DBP	PAA concentration (mg/L)	Type of effluent	Test Scale	References
Non-halogenated DBPs				
Nonanal	1-2, 3, 15, 25	Lake water and secondary Effluent	PS, FS	[41,48,53]
Decanal	1-2, 15, 25	Lake water and secondary Effluent	PS	[41,53]
1-Methoxy-4-methylbenzene	1-2	Lake water (t: 60 min)	PS	[53]
Aldehydes	1.5	River water	PS, FS	[48]
CHOH (sum parameter of aldehydes)	1.5, 2, 4, 8, 15, 25	Secondary effluent (t: 6,12, 18, 36, 40 min)	LS, PS, FS	[4,41,48]
Formaldehyde	1,3,5	River water (t: 30, 45 min)	PS	[41]
Acetaldehyde	1,3,5	River water (t: 30, 45 min)	PS	[41]
Benzoic acid*	1.5-3	River water (t: 90 min)	PS, FS	[48]
Octanoic acid	1.5-3	River water (t: 90 min)	PS, FS	[48]
Nonanoic acid	1.5-3	River water (t: 90 min)	PS, FS	[48]
Decanoic acid	1.5-3	River water (t: 90 min)	PS, FS	[48]
Lauric acid	1.5-3	River water (t: 90 min)	PS, FS	[48]
Myristic acid	1.5-3	River water (t: 90 min)	PS, FS	[48]
Hexanedioic acid	3	River water (t: 90 min)	PS, FS	[48]
2-Ethyl-1-hexanol *	1.5	River water (t: 90 min)	PS, FS	[48]
Palmitic acid[a]	1.5-3	River water (t :90 min)	PS, FS	[48]
Stearic acid[a]	1.5-3	River water (t: 90 min)	PS, FS	[48]
Acetone[a]	1.5	River water (t: 90 min)	PS, FS	[48]
Propanal[a]	1.5	River water (t: 90 min)	PS, FS	[48]
Butanal[a]	1.5	River water (t: 90 min)	PS, FS	[48]
Hexanal[a]	1.5	River water (t: 90 min)	PS, FS	[48]
3-Hexanone[a]	1.5	River water (t: 90 min)	PS, FS	[48]
2-Methylvaleraldehyde[a]	1.5	River water (t: 90 min)	PS, FS	[48]
Glyoxal[a]	1.5	River water (t: 90 min)	PS, FS	[48]
4-Hydroxy-4-methyl-2-pentanone[a]	1.5-3	River water (t :90 min)	PS, FS	[48]
Naphthalene[a]	3	River water (t: 90 min)	PS, FS	[48]
Tetrahydronaphthalene[a]	3	River water (t: 90 min)	PS, FS	[48]
Benzothiazole[a]	3	River water (t: 90 min)	PS, FS	[48]
6-Methyl-5-hepten-2-one[a]	3	River water (t: 90 min)	PS, FS	[48]
o-Hydroxybiphenyl[a]	3	River water (t: 90 min)	PS, FS	[48]
4-Hydroxy-3-methylbenzoate[a]	3	River water (t: 90 min)	PS, FS	[48]
Cyclodecanol[a]	3	River water (t: 90 min)	PS, FS	[48]
Tributyl phosphate[a]	1.5-3	River water (t: 90 min)	PS, FS	[48]

Diethyltoluamide[a]	3	River water (t: 90 min)	PS, FS	[48]
Halogenated DBPs				
Bromophenol	30	Filtered secondary effluent. Phenol spiked effluent (400 ng/L) enriched with NaCl (400 mg/L) and KBr (400 µg/L). Enhanced reaction conditions to promote the formation of halogenated DBPs	LS	[52]

LS (Lab scale), PS (pilot scale) and FS (full scale)

* Present only in the treated water and not in the raw water sample

[a] Compounds present in the raw water in the same quantities as in the treated samples

So far, the influence of H_2O_2 in the formation DBPs during PAA disinfection is poorly documented. However, it has been observed that H_2O_2 fraction in equilibrium with PAA in the mixture has an influence on the formation of DBPs during disinfection of brackish waters. In detail, in PAA disinfection (150 mg/L, contact time 18-24 h) of ballast waters with PAA formulations characterized by higher molar ratio H_2O_2:PAA (2:1), H_2O_2 was able to reduce HOCl, HOBr, or HOI to chloride, bromide or iodide respectively, leading to a limited formation of halogenated DBPs. In contrast, when PAA formulations with low molar ratio H_2O_2:PAA (0.3:1) were used, significant concentrations of brominated DBPs, such as bromoform (260 µg/L), bromoacetic acid (106 µg/L), dibromoacetic acid (230 µg/L), and tribromoacetic acid (89 µg/L) were formed [56]. Therefore, H_2O_2 can potentially serve as an important quencher for secondary oxidants, such as hypohalous acids, and minimize their further reaction with organic matter to form THMs and HAAs.

3.3. Toxicity on aquatic organisms

The conventional approach to evaluate the effect of discharged effluents on aquatic ecosystems is based on a set of physical-chemical parameters rather than ecotoxicological aspects. Nevertheless, water pollution includes a large number of compounds of different nature, whose effects and interactions are not always well understood. Thus, determination of single compounds do not always provide enough information to assess the potential harmful effects of the discharged effluents on aquatic environment, as they cannot detect synergistic or antagonistic effects of pollutants [61]. Therefore, beyond the formation of DBPs and their analytical determination, the assessment of the global effect of disinfected effluents discharged in aquatic ecosystems and the extent of the possible ecotoxicological repercussions, allow ensuring their environmental safety. The most common used disinfectants (chlorine, ozone, PAA and UV radiation) agents display some degree of toxic potential, being capable to cause harmful effects on aquatic species [62]. However, no universal statement can be made regarding the effect of disinfectants on effluent toxicity [63]. The response of aquatic ecosystems to the potential acute toxic effect of PAA disinfected effluents has been evaluated by several authors on living indicator organisms from all trophic levels of aquatic ecosystems (producers, consumers, saprophytes) by

means of bioassays for acute or chronic toxicity with the following endpoints:

- EC50 (half-maximal effective concentration): the dose causing a response (i.e. growth inhibition, immobility) to 50% of the population tested, determined by running several doses and estimating the EC50 statistically.
- LC50 (lethal concentration, 50%): the dose causing lethality to 50% of the population tested, determined by running several doses and estimating the LC50 statistically.
- NOEC (no-observed-effect concentration): the highest dose tested that does not cause any toxic effects to the organisms tested.

The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) reviewed thoroughly back in 2001 the environmental fate and ecotoxicity of PAA, as part of the Joint Assessment of Commodity Chemicals (JACC) program [21]. Table 2 presents an updated and extended summary of the literature results of the acute toxicity tests of effluents disinfected by PAA on aquatic organisms, which includes the ones reported by the ECETOC. Giving the limited DBPs reported to be formed during drinking and wastewater disinfection by PAA, the toxic effects observed on different indicator organisms have been mainly attributed to the residual disinfectant, rather than to the DBPs formed in the water matrix [61]. A toxic effect on bacteria was expected, as PAA is used for disinfection (Figure 1). The 0.25-h EC50 for *Vibrio fischeri* reported for secondary effluent [61] and sewer overflow [64] are similar, 0.102 and 0.109 mg/L, respectively. Furthermore, it has been reported that for *Vibrio fischeri* the inhibition effect was negative in the undisinfected effluent, meaning that the bacteria were more active in the biological effluent than in the culture media (higher bioluminescence emission) [61]. This was probably due to the presence in the effluent of different potentially toxic substances, including mostly long-chain-aldehydes, which are likely to enhance the light emission, modifying the organisms response [61,65,66]. An increase of the EC50 values with time has been observed [61], which was linked to a defense mechanism of an apparent detoxification of reactive oxygen species [66]. This effect depends upon both PAA concentration and exposure period, being the last parameter relevant at low PAA concentrations (0.1-0.25 mg/L). The extent of apparent detoxification decreases with increasing PAA concentration, suggesting a shift of PAA action from bacteriostatic to bactericidal [61]. Similar results were obtained by Rossi et al. [22] for *E. coli* inactivation where PAA concentration is more relevant than exposure time in determining the inactivation extent.

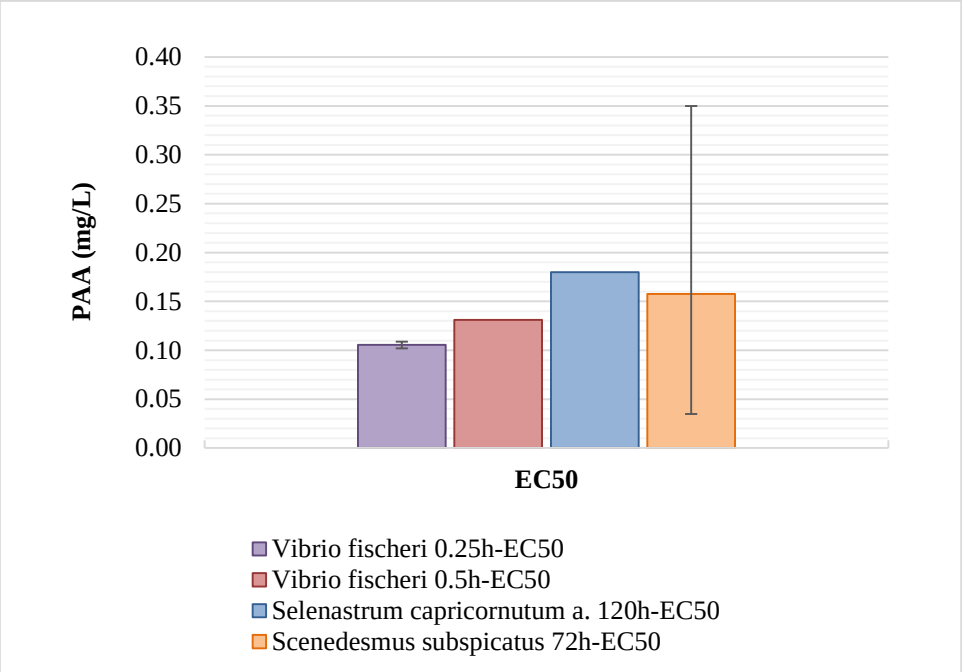

Figure 1. EC50 for bacteria and algae.

As for algae, PAA was toxic for *Scenedesmus subspicatus* with a 72h- EC50 of 0.035-0.35 mg/L [67] and *Selenastrum capricornutum* with 72h- EC50 of 1.38 mg/L [68] and 120h- EC50 of 0.18 mg/L [69], as shown in Figure 1. Contrarily, low toxicity was observed in the study of Antonelli et al. [61] on *Selenastrum capricornutum*, with 72h- EC50 of 8.89 mg/L, being this value confirmed by the presence of green algae in the disinfection basin of the WWTP [61,65].

PAA is also toxic to *Daphnia magna* as shown in Figure 2, with 48-h EC50 values ranging from 0.152 to 1.1 mg/L with the exception of the value reported by Licata-Mesana [70] who observed much higher toxicity (48-h EC50 0.035-0.35 mg/L PAA). This value might not be representative due to the high H_2O_2 fraction in the commercial product used in the study (PAA:H_2O_2 molar ratio of 0.02). Liu et al. 2015 [71] studied the toxic effect of different PAA:H_2O_2 molar ratios on *Daphnia magna* in different water matrixes. The 24h-LC values for *Daphnia magna* ranged between 0.176 and 2.6 mg/L. The authors observed a positive correlation between the PAA: total peroxides (PAA+H_2O_2) molar ratios and the 24-h LC50, indicating that the toxicity of PAA formulations to *Daphnia magna* is due to the combined effect of both PAA and H_2O_2. However, no significant differences were observed among the 24h- LC values for PAA formulations with higher PAA:H_2O_2 molar ratios (0.37 and 1.39), such as the products conventionally used for wastewater treatment. In that case, toxicity on *Daphnia magna* was attributed to PAA, whereas for an unconventionally lower PAA:H_2O_2 molar ratio of 0.034, mortality was more likely to be caused by the total peroxide concentration than by PAA alone [71]. The variations on the 24h- LC values among the different water matrix compositions were attributed to the different PAA decay rates, which resulted in different PAA residues and therefore toxic effects. The sensitivity to chemicals varies even within different groups of cladocerans [63,72–74], as found for daphnids [63]. For instance, the EC50 is much higher for *Daphnia magna* than for *Thamnocephalus platyurus*, even if both

organisms belong to the same trophic level [61]. Indeed, the freshwater crustacean (*Thamnocephalus platyurus*) proved to be a very sensitive organism, which according to Antonelli et al. [61], is not adequate to perform a reliable toxicity assessment of effluents for monitoring purposes.

PAA has a very low toxicity in brown shrimp (*Crangon crangon*) with a 96-h LC50 value of 15 mg PAA/L [75]. Indeed, these saltwater crustaceans are much larger than daphnids [75], which may explain the lower sensitivity of the shrimp [21]. The high LC50 value of the brown shrimp could also be related to the rapid degradation of PAA in brackish water. The tests to assess the effect of PAA on marine oysters (*Mytilus edulis* [76] and *Crassostrea gigas* [77]) during the first 48 hours of their development from embryo to larva (period where a protective D-shaped shell is formed) showed that without a shell, the embryos are very sensitive, as shown in Figure 2.

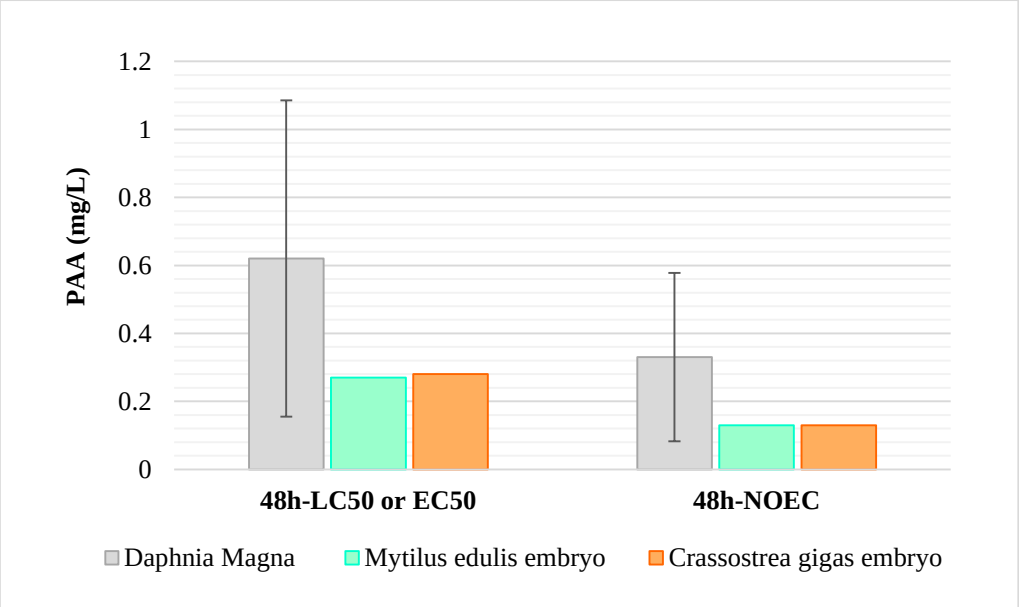

Figure 2. 48h-EC50 or LC50 and 48h-NOEC for planktonic crustaceans (*Daphnids*) and mollusks.

Toxicity to fish was lower than for the other organisms and 96-h LC50 values ranged from 0.9 to 3.3 mg/L in freshwater species such as rainbow trout (*Oncorhynchus mykiss*), blue gill (*Lepomis macrochirus*), zebrafish (*Danio rerio*), as presented in Figure 3. The 96h- EC50 value for zebrafish (*Danio rerio*) reported by Licata-Mesana [78] shows a particularly high toxicity (0.35 mg/L), which might not be representative. Indeed, as in the case of the EC50 value for *Daphnia magna* reported by the same author, this probably might be due to the high H_2O_2 fraction in the commercial product used in both studies.

Toxicity was lower in shorter tests and 24-h LC50 ranged from 2.6 to 5.97 mg/L for rainbow trout, blue gill, channel catfish (*Ictalurus punctatus*) and other freshwater species used in aquaculture, as shown in Figure 4. Toxicity displayed by PAA was lower for shorter tests and 24-h LC50 ranged from 2.6 to 5.97 mg/l for rainbow trout, blue gill, channel catfish (*Ictalurus punctatus*) and other freshwater species used in aquaculture, as presented in Figure 4. The reduction in fish survival caused by exposure to PAA can be attributed to severe

lesions of the gills and to degeneration of the renal tubules, as demonstrated by Straus et al. [79] in histopathology studies on channel catfish exposed to 2 mg PAA/L. For a saltwater fish, such as the European plaice (*Pleuronectes platessa*), PAA was less toxic with a 96-h LC50 of 11 mg/L. According to this, PAA tends to be less toxic to marine and estuarine organisms, compared to fresh water species, probably due to the shorter half-life of PAA in brackish and hard water matrixes [80–82].

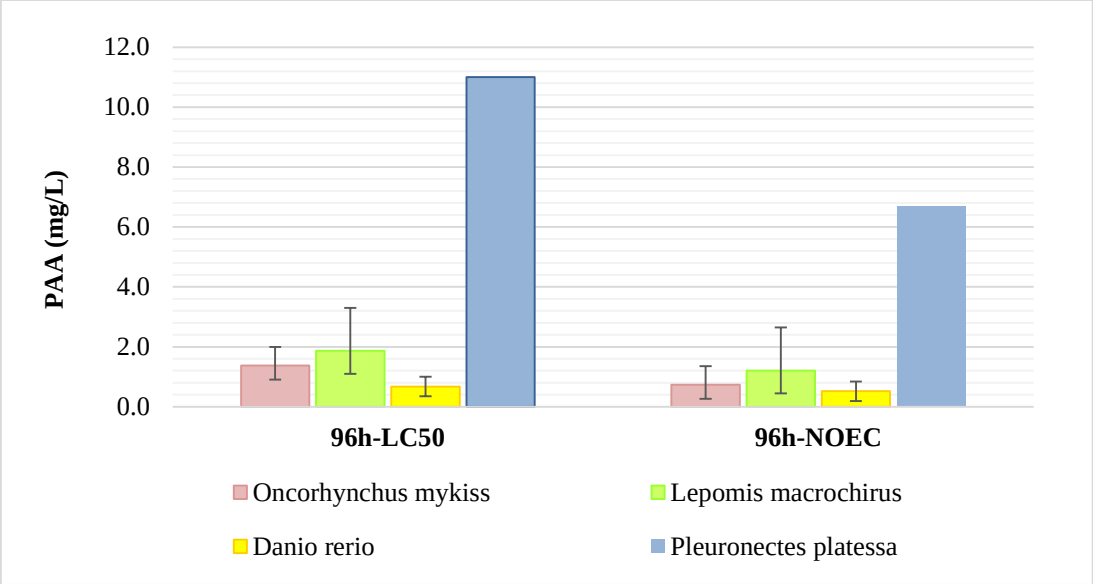

Figure 3. 96h-EC50 and 96h-NOEC for fish**.**

Toxicity on *Danio rerio* embryos was assessed by Marchand et al. [83] in different water matrixes with six different formulations of the PAA solution [83]. As shown in Table 2, toxic effects significantly different were observed among the PAA products; however, no apparent toxicity trend was observed among the responses and the PAA formulations. Contrarily, it was observed that the water matrix characteristics play a relevant role, reflected in the significantly lower 24h- LC50 values observed in low-hardness water than values observed in high-hardness water [83]. A negative correlation between water hardness and toxicity to *Danio rerio* embryos was found, probably because a given PAA concentration caused a lower pH in low-hardness water than in high hardness water, due to a potential greater buffering capacity of the high-hardness water [83], in additional acidosis to the embryos. For instance, an increase of 1 mg/L PAA caused an increment in the mortality rate by 5.3-fold, 3.3-fold and 1.6-fold in low, medium and high-hardness waters, respectively.

As for the role of H_2O_2, its effect might not be limited to the formation of DBPs. In general, the responses during aquatic toxicity tests are similar for a determined organism among authors if concentrations were expressed as PAA independently of the concentration of H_2O_2 [21], since PAA concentration alone may explain the toxicity of PAA formulations. However, PAA solutions with low PAA:H_2O_2 ratios are more toxic, indicating that H_2O_2 has an additive toxic effect; therefore the toxicity of PAA formulations is due to the combined effect of both PAA and H_2O_2 [71].

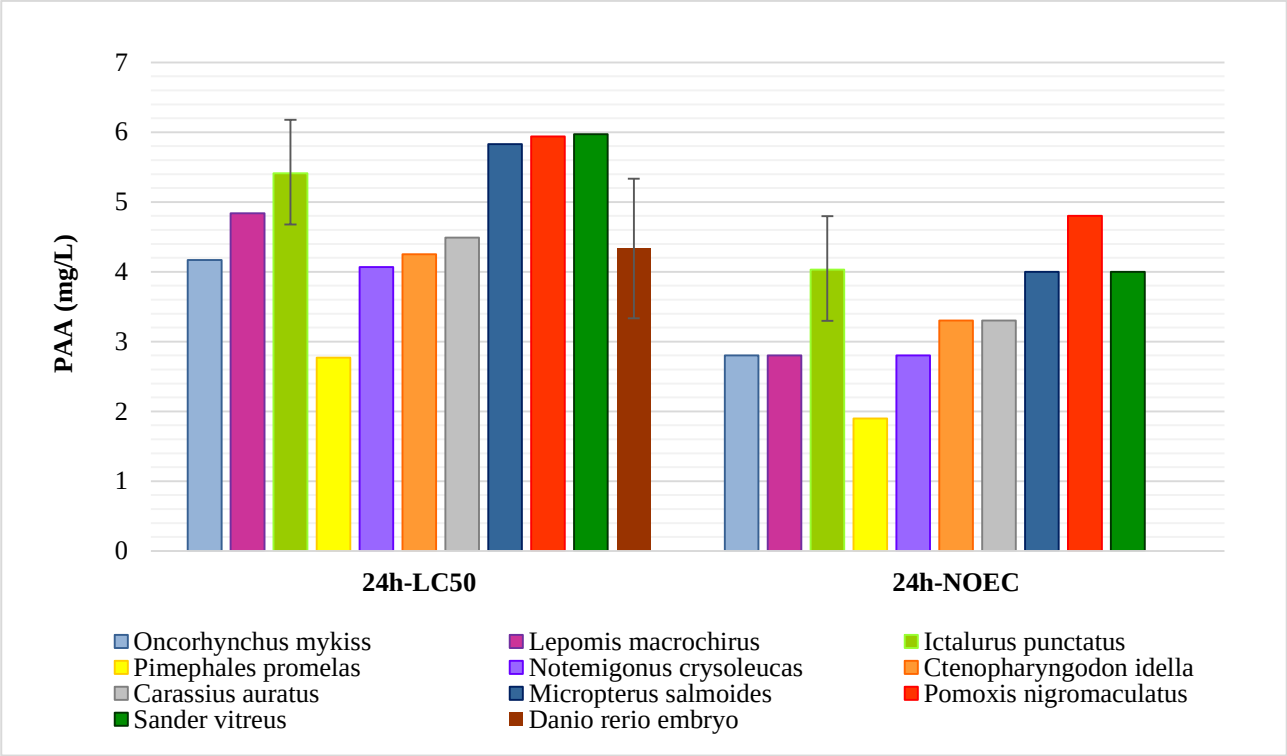

Figure 4. 24h-EC50 and 24h-NOEC for freshwater fish**.**

Moreover, PAA residues degrade rapidly at a rate that is highly dependent on the water matrix composition such as hardness, salinity, presence of transition metals, organic and particulate matter content [24,80,81,83–88]. For instance, the low toxicity displayed by PAA on saltwater species, such as brown shrimp and plaice, can be related to the rapid degradation of PAA in seawater. The rapid decay of PAA is beneficial from an environmental point of view, since it reduces the exposure risk.

In addition, the results obtained in ecotoxicological studies can depend on the sensitivity of the test organisms to environmental modifications [63]. Indeed, smaller indicator organisms tend to be more sensitive than larger ones.

The low octanol-water partition coefficients (K_{ow}) of PAA, H_2O_2 and acetic acid, 0.3 0.4 and 0.68 respectively, indicate that their bioaccumulation in aquatic organisms is unlikely [21,89], therefore it is expected that the environmental impact of PAA disinfection will be minimal and short-lived.

Table 2. Results of toxicity with various aquatic organisms for PAA disinfected effluents.

Species	Duration (h)	End point result (mg_{PAA}/L)	NOEC (mg_{PAA}/L)	Water matrix characteristics	Composition (%)				Reference
					PAA	H_2O_2	CH_3CO_3H	PAA:H_2O_2	
Bacteria		**Growth inhibition (EC50)**							
Vibrio fischeri	0.25	0.102	-	Secondary effluent	15	23	15	0.29	[66]
Vibrio fischeri	0.25	0.109	-	Sewer overflow	-	-	-	-	[90]
Vibrio fischeri	0.5	0.131	-	Secondary effluent	15	23	15	0.29	[66]
Algae		**Growth inhibition (EC50)**							
*Selenastrum capricornutum a.**	72	<1.0	<1.0	-	18	0.3	-	26.8	[91]
*Selenastrum capricornutum a.**	72	8.89	-	Secondary effluent	15	23	-	0.29	[66]
*Selenastrum capricornutum a.**	72	1.38	-	-	-	-	-	-	[73]
*Selenastrum capricornutum a.**	120	0.18		-	5.2	20	-	0.12	[74]
Scenedesmus subspicatus	72	0.035-0.35	0.035	-	0.35	7	-	0.02	[72]
**Crustaceans*		**Immobility (EC50)**							
Daphnia magna	48	0.5	0.15	-	15	14	28	0.48	[92]
Daphnia magna	48	1.1	0.45	-	4.5	27.5	-	0.07	[93]
Daphnia magna	48	0.69	0.16	-	15.5	22	15	0.32	[94]
Daphnia magna	48	0.73	0.56	-	5.2	20	-	0.12	[95]
Daphnia magna	48	<1.0	<1.0	-	18	0.3	-	26.8	[96]
Daphnia magna	48	0.035-0.35	>0.035	-	0.35	7	-	0.02	[75]

**Selenastrum capricornutum a.* also known as *Pseudokirchneriella subcapitata*

Daphnia magna	24 (=48)	0.152	-	Secondary effluent	15	23	15	0.29	[66]
Thamnocephalus platyurus	1.5	0.049	-	Secondary effluent	15	23	15	0.29	[66]
		Lethal concentration (LC50)							
Daphnia magna	24	0.181, 0.547, 0.774	-	Standard dilution water	3-40	12-40		0.034, 0.37, 1.49	[76]
Daphnia magna	24	0.202, 0.739, 0.731	-	Extra hardness	3-40	12-40		0.034, 0.37, 1.49	[76]
Daphnia magna	24	0.176, 0.487, 0.81	-	Extra NaCl	3-40	12-40		0.034, 0.37, 1.49	[76]
Daphnia magna	24	0.393, 1.695, 1323	-	Extra sea salt	3-40	12-40		0.034, 0.37, 1.49	[76]
Daphnia magna	24	0.196, 0.79, 2.617	-	Extra DOC (20 mg/L HuminFeed)	3-40	12-40		0.034, 0.37, 1.49	[76]
Mytilus edulis embryo	48	0.27	0.13	-	12.5	19	18	0.29	[81]
Crassostrea gigas embryo	48	0.28	0.13	-	12.5	19	18	0.29	[82]
Crangon crangon	96	15	6.7	-	12	20	8	0.27	[80]
Fish		**Lethal concentration (LC50)**							
Oncorhynchus mykiss	24	4.17	2.8	Well water	-	-	-	-	[97]
Oncorhynchus mykiss	96	2	1.5	-	15	14	28	0.48	[98]
Oncorhynchus mykiss	96	0.91	0.16	-	15.5	22	15	0.32	[99]
Oncorhynchus mykiss	96	1	0.45	-	4.5	27.5	-	0.07	[100]
Oncorhynchus mykiss	96	1.6	0.82	-	5.2	20	-	0.12	[101]
Lepomis macrochirus	24	4.84	2.8	Well water	-	-	-		[97]
Lepomis macrochirus	96	1.2	0.45	-	4.5	27.5	-	0.07	[102]
Lepomis macrochirus	96	3.3	2.7	-	15.5	22	15	0.32	[99]

Lepomis macrochirus	96	1.1	0.47	-	5.2	20	-	0.12	[103]
Danio rerio embryo	24	2.24-4.49	-	Low hardness water	3-40	6-40	-	0.034, 0.156, 0.28, 0.37,1.49, 2.91	[87]
Danio rerio embryo	24	3.01-6.14	-	Medium hardness water	3-40	6-40	-	0.034, 0.156, 0.28, 0.37,1.49, 2.91	[87]
Danio rerio embryo	24	4.16-7.14	-	High hardness	3-40	6-40	-	0.034, 0.156, 0.28, 0.37,1.49, 2.91	[87]
Danio rerio	96	1	<1.0	-	18	0.3	-	26.8	[104]
Danio rerio	96	0.35	>0.035	-	0.35	7	-	0.02	[86].
Pleuronectes platessa	96	11	6.7	-	12	20	8	0.27	[105]
Ictalurus punctatus (yolk-sac fry)	24	2.6	2.2	Filtered well water (75 μm)	4.5	22	9	0.09	[83]
Ictalurus punctatus (swim-up fry)	48	1.6	2.3	Filtered well water (75 μm)	4.5	22	9	0.09	[83]
Ictalurus punctatus	24	5.64	4	Well water	-	-	-	-	[97]
Ictalurus punctatus	24	4.8	3.3	Well water	-	-	-	-	[97]
Ictalurus punctatus	24	5.8	4.8	Well water	-	-	-	-	[97]
Pimephales promelas	24	2.77	1.9	Well water	-	-	-	-	[97]
Notemigonus crysoleucas	24	4.07	2.8	Well water	-	-	-	-	[97]
Ctenopharyngodon idella	24	4.25	3.3	Well water	-	-	-	-	[97]
Carassius auratus	24	4.49	3.3	Well water	-	-	-	-	[97]
Micropterus salmoides	24	5.83	4	Well water	-	-	-	-	[97]
Pomoxis nigromaculatus	24	5.94	4.8	Well water	-	-	-	-	[97]
Sander vitreus	24	5.97	4	Well water	-	-	-	-	[97]

3.1. Mutagenicity, genotoxicity and cytotoxicity

The limitations to perform chemical analyses for DBPs identification and long-term epidemiological studies for carcinogenicity have encouraged the use of short-term mutagenicity and genotoxicity tests, which are rapid, cheap and allow predicting carcinogenic activity, evaluating the combined action of DBPs present in drinking water as complex mixtures [53,106]. Several authors have studied the potential mutagenicity, genotoxicity and cytotoxicity in drinking water and secondary effluent samples before and after disinfection by PAA in the last two decades [53,106]. Most of the authors who have studied this aspect of PAA disinfection have focused on samples collected at different months of the year in order to assess seasonal variability.

Mutagenic activity of the water samples disinfected by PAA and other disinfectants (such as NaOCl and ClO_2) has been assessed by means of *in vitro* tests on bacteria (Ames test with *Salmonella typhimurium*; mutagenicity test (Mutatox) with *Vibrio fischeri*; SOS Chromotest with *E. coli*) [48,53,62,107,108] and yeast (*Saccharomyces cerevisiae* test) [109]. Enzymatic activity and cytotoxicity (neutral red) have also been studied *in vitro* on human hepatic cells and trout hepatocytes [110,111].

Genotoxicity has been widely studied with *in vivo* tests performed by directly exposing bio-indicators to water samples disinfected by PAA. The clastogenic effects derived from chromosome breakages has been studied with the micronucleus test in plants (*Tradescantia* pollen and root cells of *Vicia faba*) [48,53,62,108,112,113], fish (*Cyprius carpio*) [53,109,111,114–116] and molluscs (*Dreissena polimorpha*) [53,117] and DNA damage, as strand breakages, has been studied with the Comet assay (alkaline single-cell gel electrophoresis - SCGE) on the same fish and molluscs species as the micronucleus test. Genotoxicity of water samples disinfected by PAA has been studied as well with *in vitro* assays on human lymphocytes and leukocytes (micronucleus test and comet test) [53,118]. Furthermore, given that a single short-term test cannot predict mutagenicity, genotoxicity or cytotoxicity of a compound or a mixture, several authors [53,108,113,116,117,119,120] have performed batteries of tests with different genetic end-points to determine the effects of disinfected samples on indicator organisms.

Table 3 summarizes the *in vivo* and *in vitro* tests reported by different authors that have evaluated the mutagenicity and genotoxicity of water samples disinfected with PAA on different indicator organisms. PAA disinfection of wastewater (2 and 4 mg/L) [107] and drinking water (0.6-3 mg/L) did not induce genotoxicity in the *Allium cepa* root based on anaphase aberration test, and *Tradescantia* and *Vicia faba* based on micronucleus test [48,53,62,112,113]. Indeed, it has been reported that the formation of *Allium cepa* chromosomal aberrations was reduced after disinfection by PAA, decreasing the genotoxic load of surface waters [48,108]. However, genotoxic effects were observed during wastewater disinfection with PAA (1 mg/L) in fall and winter in the *Allium cepa* chromosomal aberration tests [62,112] and the *Tradescantia*/micronucleus test [112]. No clastogenic effects derived from chromosome breakages in

the micronucleus test or DNA damage in the comet test, were detected in common carp (*Cyprinus carpio*) [53,109,111,114,115] or freshwater mussel (*Dreissena polymorpha*) [53,117] as a result of PAA disinfection of lake water and of well water with addition of humic acids. Nevertheless, genotoxic effects were observed in the bile of *Cyprinus carpio* after PAA (0.6 mg/L) disinfection of surface water, indicating potential co-carcinogic effects of PAA disinfection [116].

DNA damage in human leukocytes was also observed after PAA (0.6-5 mg/L) disinfection of lake water, as determined by the comet test [53,109,118]. However, no chromosomal breakage was observed in the micronucleus test in human leukocytes [53,118]. PAA disinfection (2-4 mg/L) of wastewater did not induce mutagenicity in wastewater according to the Ames test (*Salmonella typhimurium*) [107]. However, slight bacterial mutagenicity was observed in surface waters treated with PAA (1, 1.5-3 mg/L) during the Ames test [48,62]. In addition, *Saccharomyces cerevisiae* D7 test showed a mutagenic response only at 5- to 10-fold doses of PAA typically used for water disinfection [109]. Slight mutagenic effects during summer and winter were observed in *Vibrio fischeri* and *E. coli* respectively, while in the other seasons these effects were not observed [53,108].

Cytochrome P450 (CYP) enzyme modulations were induced in common carp (*Cyprinus carpio*) biomarkers and freshwater mussel (*Dreissena polymorpha*) after PAA (0.6-2 mg/L) disinfection of lake water [110,111], therefore PAA is capable to perturb fish microsomal metabolism, posing a potential hazard through a non-genotoxic mechanism [111]. In addition, glutathione (GHS) content variation increased reactive oxygen species (ROS) according to Ferraris et al. [37]. However, Elia et al. [121] did not observed oxidative stress or antioxidant defense variations of carp liver, whereas NaOCl and ClO_2 displayed significant antioxidant responses. No cytotoxic effects were observed in rainbow trout (*Oncorhynchus mykiss*) hepatocytes after exposure to surface water treated with PAA [42,60].

Most of the authors who have studied mutagenicity and genotoxicity of PAA disinfected effluents have performed comparative analysis with other agents such as NaOCl and ClO_2 and they have consistently reported that these two compounds increase in a larger extent than PAA, the mutagenic and genotoxic effects of effluents, in agreement with the DBPs formed during chlorination. For instance, the thorough study carried out by Monarca et al. [53] on water lake disinfected with different agents (PAA, NaOCl and ClO_2) in different seasons and using a battery of *in vivo* and *in vitro* short-term tests, including DBPs analysis, indicated consistently lower DBP levels formed by PAA with respect to NaOCl and ClO_2 disinfection. Furthermore, PAA reduces raw water genotoxic activity, in agreement with other authors. For instance in the *Allium cepa* analysis performed by Monarca et al. [48], PAA disinfected samples exhibit lower frequency of aberrations than the raw water samples, possibly due to the oxidation of unknown genotoxins present in the raw water.

Additionally, the toxic and genotoxic effects of the disinfectant appeared to be modulated by the season of exposure [122]. It has been observed that the sampling period presumably has an effect on the

concentrations of potential DBPs precursors and physical-chemical parameters of the raw water, such as TOC and UV254 absorbance [53,62,113,118,119]. In addition, temperature is an important variable in the exposure of organisms for their testing. Some organisms, particularly plants and bacteria, are highly sensitive to temperature changes and clearly show specific temperature ranges suitable for their growth and survival. Thus, test results might be influenced by temperature beyond the variation of other parameters, e.g. physical conditions and chemical composition of the lake water [122].

Table 3. Qualitative results of in vivo and in vitro tests reported by different authors to evaluate toxicity, genotoxicity and mutagenicity of water samples disinfected by PAA.

Test	Genetic end-point	Type of Effluent	PAA concentration	Main outcome	Author
***In vivo* tests with plants**					
Tradescantia/ micronucleous test (Clone #4430, hybrid of T. hirsutiflora and T. subacaulis)	Chromosomal breakage as micronucleus in pollen cells	Secondary effluent	2 and 4 mg/L.	-	[107]
		Secondary effluent	1 mg/L of PAA in winter and summer.*	-	[62]
		Drinking water (river water)	1.5 and 3 mg/L.	-	[48]
		Drinking water and raw water (lake water)	1 mg/L in Oct., 0.6 mg/L in Feb. and 0.9 mg/L of PAA in June. *	- (June), + (Feb. and Oct.)	[112]
		Drinking water (lake water)	1 mg/L in Oct. *	-	[53]
		Organic-free redistilled water (pH 5–6)	0.2, 0.5, 1, 2, and 10 mg/l.	- + (acid pH and neutral pH at 10 mg/L)	[113]
Allium cepa test (Allium cepa)	Chromosomal aberration in anaphase root cells	Secondary effluent	2 and 4 mg/L	-	[107]
		Secondary effluent	1 mg/L of PAA in winter and summer.*	- + (winter)	[62]
		Drinking water (river water)	1.5 and 3 mg/L.	-	[48]
		Drinking water (lake water)	1 mg/L in Oct., 0.6 mg/L in Feb. and 0.9 mg/L of PAA in June. *	- + (Oct.)	[112]
		Drinking water (lake water)	1 mg/L in Oct. *	-	[53]
		Organic-free redistilled water (pH 5–6)	0.1, 0.2 and 0.5.	-	[113]

Vicia faba/MCN test (Vicia faba)	**Chromosomal breakage as micronucleus in root cells**	**Drinking water (lake water)**	**1 mg/L in Oct., 0.6 mg/L in Feb. and 0.9 mg/L of PAA in June. ***	-	**[112]**
		Drinking water (lake water)	1 mg/L in Oct. *	for 6 hr + for 72 hr -	[53]
		Organic-free redistilled water (pH 5–6) and	0.1, 0.2 and 0.5 (unbuffered solutions) and 0.1, 0.2, 0.5, 1, and 2 mg/l (buffered solutions)	- + (acid pH and neutral pH at 2 mg/L)	[113]
***In vitro* tests with bacteria**					
Ames test (Salmonella typhimurium (TA98/TA100) (-/+S9))	Point mutation	Secondary effluent	2 and 4 mg/L	-	[107]
		Drinking water (lake water))	1 mg/L in July, 1 mg/L in Oct., 0.6 mg/L in Feb. and 0.9 mg/L of PAA in June. *	-	[108]
		Secondary effluent	1 mg/L of PAA in winter and summer.*	+	[62]
		Drinking water (lake and river water)	0.2, 0.5, 1 mg/L for lake water 1.5 and 3 mg/L for rive water	- (lake water) + (river water)	[48]
		Drinking water (lake water)	1 mg/L in Oct. *	- (+S9) - (- S9)	[53]
Mutatox (Vibrio fischeri (M169))	Point mutation	Drinking water (lake water)	1 mg/L in July, 1 mg/L in Oct., 0.6 mg/L in Feb. and 0.9 mg/L of PAA in June. *	- - (+S9, summer)	[108]
		Drinking water (lake water)	1 mg/L in Oct. *	- (+S9) - (- S9)	[53]
SOS Chromotest (Escherichia coli (PQ37))	Genetic mutation	Drinking water (lake water)	1 mg/L in July, 1 mg/L in Oct., 0.6 mg/L in Feb. and 0.9 mg/L of PAA in June. *	- + (Feb.)	[108]
		Drinking water (lake water)	1 mg/L in Oct. *	- (+S9) - (- S9)	[53]
Toxicity (Vibrio fischeri (NRRL-B-11177))	EC50	Drinking water (lake water)	1 mg/L in July, 1 mg/L in Oct., 0.6 mg/L in Feb. and 0.9 mg/L of PAA in June. *	July, Oct. and Feb. EC50=20 June EC50=60	[108]
		Secondary effluent	1 mg/L of PAA in winter and summer.*	EC50 = 20	[62]
		Drinking water (lake water)	1 mg/L in Oct. *	EC50 = 19	[53]
***In vitro* test with yeast**					
Saccharomyces cerevisiae test (Saccharomyces cerevisiae (D7) (-/+P450) (-/+S9))	Point mutation, mitotic gene conversion and mitochondrial DNA mutation	Drinking water (lake water)	1 mg/L in July, 1 mg/L in Oct., 0.6 mg/L in Feb. and 0.9 mg/L of PAA in June. *	- (Oct.) + (Feb, June and July)	[108]
		Drinking water	0-15 ppm.	- + (10-15 mg/L)	[123]
		Drinking water (lake water)	1 mg/L in Oct. *	-	[53]

***In vivo* tests with fish**					
Comet test in erythrocytes (Cyprinus carpio)	DNA damage as single and double- strand breakages	Drinking water (lake water)	0.19 mg/L in Oct., 0.04 mg/L in Feb., 0.05 mg/L in June. *	-	[109]
		Drinking water (lake water)	1 mg/L in Oct. *	-	[53]
		Well water (+ humic acid)	0.02-0.5 ppm.	-	[115]
Comet test in bile (Cyprinus carpio)	DNA damage	Drinking water (lake water)	0.6 mg/L.*	+	[116]
Micronucleus test in erythrocytes (Cyprinus carpio)	Chromosomal breakage as micronucleus in erythrocytes	Drinking water (lake water)	0.19 mg/L in Oct., 0.04 mg/L in Feb., 0.05 mg/L in June. *	-	[109]
		Drinking water (lake water)	Winter and Summer, 1 mg/L.	-	[111]
		Drinking water (lake water)	1 mg/L in Oct. *	-	[53]
		Well water (+ humic acid)	0.02-0.5 ppm.	-	[115]
***In vivo* tests with mollusk**					
Comet test in hemocytes (Dreissena polymorpha)	DNA damage as single and double- strand breakages	Drinking water (lake water)	1 mg/L in Oct., 0.6 mg/L in Feb. and 0.9 mg/L of PAA in June. *	-	[117]
		Drinking water (lake water)	1 mg/L in Oct. *	-	[53]
Micronucleus test in hemocytes (Dreissena polymorpha)	Chromosomal breakage as micronucleus in hemocytes	Drinking water (lake water)	1 mg/L in Oct., 0.6 mg/L in Feb. and 0.9 mg/L of PAA in June. *	-	[117]
		Drinking water (lake water)	1 mg/L in Oct. *	-	[53]
***In vitro* test with human cells**					
Micronucleus test (Human leukocytes)	Chromosomal breakage as micronucleus in and morphological analyses as necrosis or apoptosis	Drinking water (lake water)	1 mg/l of PAA in July, 1 mg/L in Oct., 0.6 mg/L in Feb. and 0.9 mg/L of PAA in June. *	- + (Oct., Feb.)	[118]
		Drinking water (lake water)	1 mg/L in Oct. *	-	[53]
Comet test (Human leukocytes)	DNA damage as double-strand breakage	Drinking water (lake water))	1 mg/l of PAA in July, 1 mg/L in Oct., 0.6 mg/L in Feb. and 0.9 mg/L of PAA in June. *	+	[118]
		Drinking water	0-5 ppm.	+	[123]
		Drinking water (lake water)	1 mg/L in Oct. *	+	[53]

Enzymatic activity (Human hepatic cells (HepG2))	**Glutathione and free radicals contents**	**Drinking water (lake water)**	**1 mg/L in Oct. ***	+	[53]
Cytotoxicity test (neutral red) (Human hepatic cells (HepG2))	Cell viability	Drinking water (lake water)	Different seasons (Oct., Feb. and June). *	- (winter and summer) + (autumn)	[119]
		Drinking water (lake water)	1 mg/L in Oct. *	+	[53]
***In vitro* test with fish cells**					
Enzymatic activity test in hepatocytes (Oncorhynchus mykiss)	Glutathione and free radicals contents	Drinking water (lake water)	1 mg/L Autumn, 0.6 mg/L in winter, 0.9 -1 mg/L in summer.	+	[37]
		Drinking water (lake water)	0.6 mg/L	-	[121]
		Drinking water (lake water)	1 mg/L in Oct. *	+	[53]
Cytotoxicity test (neutral red) in hepatocytes (Oncorhynchus mykiss)	Cellular viability	Drinking water (lake water)	1 mg/L Autumn, 0.6 mg/L in winter, 0.9 -1 mg/L in summer.	-	[37]
		Drinking water (lake water)	1 mg/L in Oct. *	-	[53]

3.2. Conclusions

PAA has increasingly gained acceptance as an alternative disinfectant agent, particularly for wastewater disinfection, mainly due to environmental concerns. Current literature consistently shows that PAA produces less environmental impacts than conventional disinfectants, such as NaOCl, ClO_2, in terms of formation of disinfection by-products and toxic, carcinogenic, mutagenic or genotoxic effects on different indicator organisms.

Most of the formed DBPs are carboxylic acids and the concentrations of other DBPs such as aldehydes, epoxides, halogenated compounds, and N-nitrosamines are either non-existing or limited. Limited toxicity, mutagenicity and genotoxicity of disinfected effluents have been observed in different organisms, particularly in comparison with NaOCl and ClO_2. However, possible co-carcinogenic effects of PAA and formation of emerging DBPs should be further studied. A relevant aspect to take into account regarding PAA ecotoxicity are the different equilibrium compositions, namely PAA:H_2O_2 ratio, present in different commercial PAA products, which may entail different environmental effects.

3.3. References

[1] T. Sirivedhin, K.A. Gray, Comparison of the disinfection by-product formation potentials between a wastewater effluent and surface waters, Water Res. 39 (2005) 1025–1036.

[2] J.J. Rook, Formation of haloforms during chlorination of natural waters, 23 (1974) 234–243.

[3] S.D. Richardson, M.J. Plewa, E.D. Wagner, R. Schoeny, D.M. DeMarini, Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking

water: A review and roadmap for research, Mutat. Res. 636 (2007) 178–242.

[4] A. Dell'Erba, D. Falsanisi, L. Liberti, M. Notarnicola, D. Santoro, Disinfection by-products formation during wastewater disinfection with peracetic acid, Desalination. 215 (2007) 177–186.

[5] H. Gallard, U. Von Gunten, Chlorination of natural organic matter: Kinetics of chlorination and of THM formation, Water Res. 36 (2002) 65–74.

[6] V. Mezzanotte, M. Antonelli, S. Citterio, C. Nurizzo, Wastewater Disinfection Alternatives : Chlorine, Ozone, Peracetic Acid, and UV Light, Water Environ. Res. 79 (2007) 2373–9.

[7] J. Koivunen, H. Heinonen-Tanski, Inactivation of enteric microorganisms with chemical disinfectants, UV irradiation and combined chemical/UV treatments, Water Res. 39 (2005) 1519–1526.

[8] R. Gehr, M. Wagner, P. Veerasubramanian, P. Payment, Disinfection efficiency of peracetic acid, UV and ozone after enhanced primary treatment of municipal wastewater, Water Res. 37 (2003) 4573–4586.

[9] I. Wojtenko, M.K. Stinson, R. Field, Performance of ozone as a disinectant for combined sewer overflow, Crit. Rev. Environ. Sci. Technol. 31 (2001) 295–309.

[10] I. Wojtenko, M.K. Stinson, R. Field, Challenges of combined sewer overflow disinfection by ultraviolet light irradiation, Crit. Rev. Environ. Sci. Technol. 31 (2001) 223–239.

[11] D.E. Clark, Peroxides and peroxide- forming compounds, Chem. Health Saf. 9098 (2001).

[12] S.S. Block, Disinfection, sterilization, and preservation, 5th ed., Lippincott Williams & Wilkins, 2001.

[13] S.M. Rahman, T. Durance, Handbook of Food Preservation, 2002.

[14] C.W. Jones, Applications of Hydrogen Peroxide and Derivatives, Royal Society of Chemistry, Cambridge, 1999.

[15] M.G.C. Baldry, The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid, J. Appl. Bacteriol. 54 (1983) 417–423.

[16] M. Kitis, Disinfection of wastewater with peracetic acid: A review, Environ. Int. 30 (2004) 47–55.

[17] P.S. Malchesky, Peracetic Acid and Its Application to Medical Instrument Sterilization, Artif. Organs. 17 (2008) 147–152.

[18] T. Luukkonen, S.O. Pehkonen, Peracids in water treatment: A critical review, Crit. Rev. Environ. Sci. Technol. 0 (2016) 1–39.

[19] European Chemicals Agency, Regulation (EU) No 528 / 2012 concerning the making available on the market and use of biocidal products. Assessment Report for peracetic acid, 2015.

[20] M.J. Flores, M.R. Lescano, R.J. Brandi, A.E. Cassano, M.D. Labas, A novel approach to explain the inactivation mechanism of *Escherichia coli* employing a commercially available peracetic acid, Water Sci. Technol. 69 (2014) 358.

[21] European Centre for Ecotoxicology and Toxicology of Chemicals, Joint Assessment of Commodity Chemicals (JACC) programme. JACC No. 40 - Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions, Brussels, Belgium, 2001.

[22] S. Rossi, M. Antonelli, V. Mezzanotte, C. Nurizzo, Peracetic acid disinfection: a feasible alternative to wastewater chlorination., Water Environ. Res. 79 (2007) 341–350. doi:10.2175/106143006X101953.

[23] T. Luukkonen, T. Heyninck, J. Rämö, U. Lassi, Comparison of organic peracids in wastewater treatment: Disinfection, oxidation and corrosion, Water Res. 85 (2015) 275–285.

[24] G.S. Cavallini, S.X. De Campos, J.B. De Souza, C.M. De Sousa Vidal, Evaluation of the physical-chemical characteristics of wastewater after disinfection with peracetic acid, Water. Air. Soil Pollut. 224 (2013).

[25] D. Falsanisi, R. Gehr, D. Santoro, A. Dell'Erba, M. Notarnicola, L. Liberti, Kinetics of PAA demand and its implications on disinfection of wastewaters, Water Qual. Res. J. Canada. 41 (2006) 398–409.

[26] A. Dell'Erba, D. Falsanisi, L. Liberti, M. Notarnicola, D. Santoro, Disinfecting behaviour of peracetic acid for municipal wastewater reuse, Desalination. 168 (2004) 435–442.

[27] L.F. Pedersen, P.B. Pedersen, J.L. Nielsen, P.H. Nielsen, Peracetic acid degradation and effects on nitrification in recirculating aquaculture systems, Aquaculture. 296 (2009) 246–254.

[28] B. Herut, E. Shoham-Frider, N. Kress, U. Fiedler, D.L. Angel, Hydrogen Peroxide Production Rates in Clean and Polluted Coastal Marine Waters of the Mediterranean, Red and Baltic Seas, Mar. Pollut. Bull. 36 (1998) 994–1003.

[29] L.J. Schmidt, M.P. Gaikowski, W.H. Gingerich, Environmental Assessment for the Use of Hydrogen Peroxide in Aquaculture for Treating External Fungal and Bacterial Diseases of Cultured Fish and Fish Eggs, United States Geological Survey, Biological Resources Division Upper, Upper Midwest Environmental , La Crosse, Wisconsin, 2006.

[30] W.J. Cooper, C. Shao, D.R.S. Lean, A.S. Gordon, F.E. Scully, Factors Affecting the Distribution of H2O2 in Surface Waters., in: Ed. L.A. Baker. American Chemical Society (Ed.), Adv. Chem. Ser. 237, Environ. Chem. Lakes Reserv., Washington, D.C., 1994: pp. 391–422.

[31] V. Randhawa, M. Thakkar, L. Wei, Applicability of Hydrogen Peroxide in Brown Tide Control – Culture and Microcosm Studies, PLoS One. 7 (2012) e47844.

[32] S.M. Díaz-Cruz, M. Llorca, D. Barceló, Organic UV filters and their photodegradates, metabolites and disinfection by-products in the aquatic environment, TrAC Trends Anal. Chem. 27 (2008) 873–887.

[33] E. Choe, D.B. Min, Chemistry and Reactions of Reactive Oxygen Species in Food, J. Food Sci. 70 (2005) 142–159.

[34] M. Finnegan, E. Linley, S.P. Denyer, G. McDonnell, C. Simons, J.Y. Maillard, Mode of action

of hydrogen peroxide and other oxidizing agents: Differences between liquid and gas forms, J. Antimicrob. Chemother. 65 (2010) 2108–2115.

[35] G. Frenzilli, M. Nigro, B.P. Lyons, The Comet assay for the evaluation of genotoxic impact in aquatic environments, Mutat. Res. - Rev. Mutat. Res. 681 (2009) 80–92.

[36] E. Cabiscol, J. Tamarit, J. Ros, Oxidative stress in bacteria and protein damage by reactive oxygen species, Int. Microbiol. 3 (2000) 3–8.

[37] M. Ferraris, E. Chiesara, S. Radice, A. Giovara, S. Frigerio, R. Fumagalli, L. Marabini, Study of potential toxic effects on rainbow trout hepatocytes of surface water treated with chlorine or alternative disinfectants, Chemosphere. 60 (2005) 65–73.

[38] Commission to the European Parliament and the Council, Commission Staff Working Document. Impact Assessment. Defining criteria for identifying endocrine disruptors in the context of the implementation of the plant protection products regulation and biocidal products regulation, Brussels, 2016.

[39] S.D. Richardson, Disinfection by-products and other emerging contaminants in drinking water, TrAC - Trends Anal. Chem. 22 (2003) 666–684.

[40] L. Guzzella, F. Di Caterino, S. Monarca, C. Zani, D. Feretti, I. Zerbini, G. Nardi, A. Buschini, P. Poli, C. Rossi, Detection of mutagens in water-distribution systems after disinfection, Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 608 (2006) 72–81.

[41] C. Nurizzo, M. Antonelli, M. Profaizer, L. Romele, By-products in surface and reclaimed water disinfected with various agents, Desalination. 176 (2005) 241–253.

[42] L. Liberti, M. Notarnicola, Advanced treatment and disinfection for municipal wastewater reuse in agriculture, Water Sci. Technol. 40 (1999) 235–245.

[43] Y. Bichsel, U. von Gunten, Formation of Iodo-Trihalomethanes during Disinfection and Oxidation of Iodide-Containing Waters, Environ. Sci. Technol. 34 (2000) 2784–2791.

[44] M.J. Plewa, E.D. Wagner, S.D. Richardson, A.D. Thruston, Y.-T. Woo, A.B. McKague, Chemical and Biological Characterization of Newly Discovered Iodoacid Drinking Water Disinfection Byproducts, Environ. Sci. Technol. 38 (2004) 4713–22.

[45] S.W. Krasner, H.S. Weinberg, S.D. Richardson, S.J. Pastor, R. Chinn, M.J. Sclimenti, G.D. Onstad, A.D. Thruston, Occurrence of a New Generation of Disinfection Byproducts, Environ. Sci. Technol. 40 (2006) 7175–85.

[46] G.D. Onstad, H.S. Weinberg, Evaluation of the stability and analysis of halogenated furanones in disinfected drinking waters, Anal. Chim. Acta. 534 (2005) 281–292.

[47] W.A. Mitch, J.O. Sharp, R.R. Trussell, R.L. Valentine, L. Alvarez-Cohen, D.L. Sedlak, N-Nitrosodimethylamine (NDMA) as a Drinking Water Contaminant: A Review, Environ. Eng. Sci. 20 (2003).

[48] S. Monarca, S.D. Richardson, D. Feretti, M. Grottolo, A.D. Thruston, C. Zani, G. Navazio, P.

Ragazzo, I. Zerbini, A. Alberti, Mutagenicity and disinfection by-products in surface drinking water disinfected with peracetic acid., Environ. Toxicol. Chem. 21 (2002) 309–18.

[49] B. Crathorne, J. Fawell, T. Irving, N. Harris, S. Denny, T. Whitmore, H. Horth, H. James, B. Roddie, D.J. Smith, L. Taylor, Sewage disinfection: by-product formation, ecotoxicology and microbiological efficacy, Report NR 2727, National Rivers Authority, Medmenham, UK., 1991.

[50] P.O. Pedersen, E. Brodersen, D. Cecil, Disinfection of tertiary wastewater effluent prior to river discharge using peracetic acid; treatment efficiency and results on by-products formed in full scale tests, Water Sci. Technol. 68 (2013) 1852–1856.

[51] D. Santoro, R. Gehr, T.A. Bartrand, L. Liberti, M. Notarnicola, A. Dell'Erba, D. Falsanisi, C.N. Haas, Wastewater disinfection by peracetic acid: assessment of models for tracking residual measurements and inactivation., Water Environ. Res. 79 (2007) 775–87.

[52] R.A. Booth, J.N. Lester, The potential formation of halogenated by-products during peracetic acid treatment of final sewage effluent, Water Res. 29 (1995) 1793–1801.

[53] S. Monarca, C. Zani, S.D. Richardson, A.D. Thruston, M. Moretti, D. Feretti, M. Villarini, A new approach to evaluating the toxicity and genotoxicity of disinfected drinking water, Water Res. 38 (2004) 3809–3819.

[54] A.D. Shah, Z.-Q. Liu, E. Salhi, T. Höfer, B. Werschkun, U. Von Gunten, Formation of disinfection by-products during ballast water treatment with ozone, chlorine, and peracetic acid: influence of water quality parameters, Environ. Sci. Water Res. Technol. 1 (2015) 465–465.

[55] J.L. Acero, P. Piriou, U. Von Gunten, Kinetics and mechanisms of formation of bromophenols during drinking water chlorination: Assessment of taste and odor development, Water Res. 39 (2005) 2979–2993.

[56] A.D. Shah, Z.Q. Liu, E. Salhi, T. Höfer, U. Von Gunten, Peracetic acid oxidation of saline waters in the absence and presence of H2O2: Secondary oxidant and disinfection byproduct formation, Environ. Sci. Technol. 49 (2015) 1698–1705.

[57] N. Kaur, D. Kishore, Peroxy Acids: Role in Organic Synthesis, Synth. Commun. 44 (2014) 721–747.

[58] A.D. Shah, W.A. Mitch, Halonitroalkanes, halonitriles, haloamides, and N-nitrosamines: A critical review of nitrogenous disinfection byproduct formation pathways, Environ. Sci. Technol. 46 (2012) 119–131.

[59] W. Lee, P. Westerhoff, J.-P. Croué, Dissolved organic nitrogen as a precursor for chloroform, dichloroacetonitrile, N-nitrosodimethylamine, and trichloronitromethane., Environ. Sci. Technol. 41 (2007) 5485–90.

[60] D.M. West, Q. Wu, A. Donovan, H. Shi, Y. Ma, H. Jiang, J. Wang, N-nitrosamine formation by monochloramine, free chlorine, and peracetic acid disinfection with presence of amine precursors in drinking water system, Chemosphere. 153 (2016) 521–527.

[61] M. Antonelli, V. Mezzanotte, M. Panouillères, Assessment of peracetic acid disinfected effluents by microbiotests, Environ. Sci. Technol. 43 (2009) 6579–6584.

[62] S. Monarca, D. Feretti, C. Collivignarelli, L. Guzzella, I. Zerbini, G. Bertanza, R. Pedrazzani, The influence of different disinfectants on mutagenicity and toxicity of urban wastewater, Water Res. 34 (2000) 4261–4269.

[63] J.B. da Costa, S. Rodgher, L.A. Daniel, E.L.G. Espíndola, Toxicity on aquatic organisms exposed to secondary effluent disinfected with chlorine, peracetic acid, ozone and UV radiation, Ecotoxicology. 23 (2014) 1803–1813.

[64] R.K. Chhetri, D. Thornberg, J. Berner, R. Gramstad, U. Öjstedt, A.K. Sharma, H.R. Andersen, Chemical disinfection of combined sewer overflow waters using performic acid or peracetic acids, Sci. Total Environ. 490 (2014) 1065–1072.

[65] M. Antonelli, A. Turolla, V. Mezzanotte, C. Nurizzo, Peracetic acid for secondary effluent disinfection: A comprehensive performance assessment, Water Sci. Technol. 68 (2013) 2638–2644.

[66] R. Łyżeń, G. Węgrzyn, Sensitivity of dark mutants of various strains of luminescent bacteria to reactive oxygen species, Arch. Microbiol. 183 (2005) 203–208.

[67] L. Licata-Messana, Inhibition test (72 hours) in freshwater unicellular algae, semi-static system (1, 10 and 100 mg/L), test substance Dialox. Unpublished report F227 SEPC, Sarcey, France., Paris, France, 1995.

[68] R.K. Chhetri, A. Baun, H.R. Andersen, Algal toxicity of the alternative disinfectants performic acid (PFA), peracetic acid (PAA), chlorine dioxide (ClO2) and their by-products hydrogen peroxide (H2O2) and chlorite (ClO2-), Int. J. Hyg. Environ. Health. (2016).

[69] L. Hicks, T. Ziegler, J. Bucksath, Acute toxicity of 5% peracetic acid (Vigor Ox) to Selenastrum capricornutum Printz. Unpublished report 42866., Princeton NJ, USA., 1996.

[70] L. Licata-Messana, Test to evaluate acute toxicity (48 hours) in Daphnia, semi-static system (1, 10 and 100 mg/l), test substance Dialox. Unpublished report F237, SEPC, Sarcey, France. SEPPIC., Paris, France., 1995.

[71] D. Liu, D.L. Straus, L.-F. Pedersen, T. Meinelt, Comparison of the Toxicity of Wofasteril Peracetic Acid Formulations E400, E250, and Lspez to *Daphnia magna*, with Emphasis on the Effect of Hydrogen Peroxide, N. Am. J. Aquac. 77 (2015) 128–135.

[72] D.J. Versteeg, M. Stalmanst, S.D. Dyer, C. Janssen, Ceriodaphnia and Daphnia: a comparison of their sensitivity to xenobiotics and utility as a test species, Chemosphere. 34 (1997) 869–892.

[73] S.S.S. Sarma, V.M. Peredo-Alvarez, S. Nandini, Comparative study of the sensitivities of neonates and adults of selected cladoceran (Cladocera: Crustacea) species to acute toxicity stress, J. Environ. Sci. Heal. J. J. Environ. Sci. Heal. Part A. 42 (2007) 1093–4529.

[74] H. Mano, M. Sakamoto, Y. Tanaka, A comparative study of insecticide toxicity among seven

cladoceran species, Ecotoxicology. 19 (2010) 1620–1625.

[75] D. Tinsley, I. Sims, The acute toxicity of Oxymaster to brown shrimp (Crangon crangon) under semi-static conditions. Unpublished report CO1649-M/EV8687, WRc Environment, Medmenham, UK. ., Widnes, Cheshire, UK., 1987.

[76] F. Fairhurst, Determination of the 48 hour median effect concentration (EC50) of Oxymaster to the common mussel (Mytilus edulis), in terms of larval survival and development. Unpublished report CO1644-M/EV8687., Cheshire, UK., 1987.

[77] R. Butler, Determination of the 48 hour median effect concentration (EC50) of Oxymaster to the pacific oyster (Crassostrea gigas) in terms of larval survival and development. Unpublished report CO1643-M/EV8687., Cheshire, UK., 1987.

[78] L. Licata-Messana, Test to evaluate acute toxicity (96 hours) in freshwater fish (Brachydanio rerio), semi-static system (1, 10 and 100 mg/l), test substance Dialox. Unpublished report F236, SEPC. Sarcey, France., Paris, France, 1995.

[79] D.L. Straus, T. Meinelt, B.D. Farmer, B.H. Beck, Acute toxicity and histopathology of channel catfish fry exposed to peracetic acid, Aquaculture. 342–343 (2012) 134–138.

[80] J. Koivunen, H. Heinonen-Tanski, Peracetic acid (PAA) disinfection of primary, secondary and tertiary treated municipal wastewaters, Water Res. 39 (2005) 4445–4453.

[81] D. Liu, C. Steinberg, D.L. Straus, L. Pedersen, T. Meinelt, Salinity, dissolved organic carbon and water hardness affect peracetic acid (PAA) degradation in aqueous solutions, Aquac. Eng. 60 (2014) 35–40.

[82] L.F. Pedersen, T. Meinelt, D.L. Straus, Peracetic acid degradation in freshwater aquaculture systems and possible practical implications, Aquac. Eng. 53 (2013) 65–71.

[83] P.-A. Marchand, D.L. Straus, A. Wienke, L.-F. Pedersen, T. Meinelt, Effect of water hardness on peracetic acid toxicity to zebrafish, Danio rerio, embryos, Aquac. Int. 21 (2013) 679–686.

[84] C. Sanchez-Ruiz, S. Martinez-Royano, I. Tejero-Monzon, An evaluation of the effciency and impact of raw WW disinfection with PAA prior to Ocean discharge, Water Sci. Technol. 32 (1995) 159–166.

[85] V. Lazarova, M. Janex, L. Fiksdal, C. Oberg, I. Barcina, M. Pommepuy, Advanced wastewater disinfection technologies: Short and long term efficiency, Water Sci. Technol. 38 (1998) 109–117.

[86] D. Falsanisi, R. Gehr, L. Liberti, M. Notarnicola, Effect of suspended particles on disinfection of a physicochemical municipal wastewater with peracetic acid, Water Qual. Res. J. Canada. 43 (2008) 47–54.

[87] Z. Yuan, Y. Ni, A.R. van Heiningen, Kinetics of the peracetic acid decomposition: Part II: pH effect and alkaline hydrolysis, Can. J. Chem. Eng. 75 (1997) 42–47.

[88] S. Stampi, G. De Luca, M. Onorato, E. Ambrogiani, F. Zanetti, Peracetic acid as an alternative

wastewater disinfectant to chlorine dioxide, J. Appl. Microbiol. 93 (2002) 725–731.

[89] US Environmental Protection Agency, Summary Review of Available Literature for Hydrogen Peroxide and Peroxyacetic Acid for New Use to Treat Wastewater, Washington D.C., 2007.

[90] R.K. Chhetri, D. Thornberg, J. Berner, R. Gramstad, U. Öjstedt, A.K. Sharma, H.R. Andersen, Chemical disinfection of combined sewer overflow waters using performic acid or peracetic acids, Sci. Total Environ. 490 (2014) 1065–1072.

[91] V. Petit-Poulsen, M. Lamy, L. Chauvire, F. Gondelle, M. Bazzon, J. Giacalone, S. Bailleul, M. Rose, Essai d'inhibition de la croissance de l'algue d'eau douce Pseudokirchneriella subcapitata. Substance d'essai: solution aqueuse d'acide peracétique. Unpublished report BA567c, INERIS (Institut National de l'Environnement Industriel et des Risques), Verneu, Jouy en Josas, France., 1997.

[92] M. Douglas, I. Pell, The acute toxicity of Proxitane 1507 to Daphnia magna. Unpublished report LPT44/851641. Huntingdon Research Centre, Huntingdon, Cambridgeshire, UK. Laporte Industries, Widnes, Cheshire, UK., 1986.

[93] D. Burgess, A. Forbis, Acute toxicity of Oxonia Active to Daphnia magna. Unpublished report report 30724., Iowa, USA., 1983.

[94] Y. Terrell, Acute toxicity bioassay of Divosan Forte on Daphnia magna. Unpublished report, project 86-718, American Standards Biosciences Corporation, Reading, PA, USA., Wyandotte, Michigan, USA ., 1987.

[95] C. Gardner, Bucksath, JD., Static acute toxicity of 5 % peracetic acid (Vigor Ox) to Daphnia magna. Amended final report 42349, ABC Unpublished report, study I95-2021. Laboratories, Columbia, MO, USA., Princeton, N.J, USA., 1996.

[96] M. Lamy, L. Chauvire, F. Gondelle, M. Bazzon, G. J., S. Bailleul, M. Rose, Toxicité aiguë vis-à-vis des daphnies, substance d'essai: solution aqueuse d'acide péracétique. Unpublished report, study Ba567b., Jouy en Josas, France., 1997.

[97] D.L. Straus, C.K. Ledbetter, B.D. Farmer, T. Meinelt, L.-F. Pedersen, Acute toxicity of peracetic acid to various fish species, in: 17th Int. Conf. Dis. Fish Shellfish, European Association of Fish Pathologists, Las Palmas, 2015: pp. 188–188.

[98] M. Douglas, I. Pell, The acute toxicity of Proxitane 1507 to rainbow trout (Salmo gairdneri). Unpublished report LPT43/851643. Huntingdon Research Centre Huntingdon, Cambridgeshire, UK., Widnes, Cheshire, UK., 1986.

[99] Y. Terrell, Acute toxicity bioassay of Divosan Forte on rainbow trout and bluegill sunfish. Unpublished report, project 86-719/720 American Standards Biosciences Corporation, Reading, PA, USA., Wyandotte, Michigan, USA., 1987.

[100] P. Cohle, W. McAllister, Acute toxicity of Oxonia Active to rainbow trout (Salmo gairdneri). Unpublished report 30723, Analytical Bio-Chemistry Laboratories, Columbia MO, USA.

Bonewitz Chemical Services., Burlington, Iowa, USA., 1983.

[101] C. Gardner, J. Bucksath, Static acute toxicity of 5 % peracetic acid (Vigor Ox) to rainbow trout (Oncorhynchus mykiss). Final report 42348, ABC Laboratories, Columbia, MO, USA. Unpublished report, study I95-2023., Princeton NJ, USA., 1996.

[102] W. McAllister, P. Cohle, Acute toxicity of Oxonia Active to bluegill sunfish (Lepomis macrochirus). Unpublished report 30722, Analytical Bio-Chemistry Laboratories, Columbia, MO, USA. Bonewitz Chemical Services., 1983.

[103] C. Gardner, J. Bucksath, Static renewal toxicity of 5 % peracetic acid (Vigor Ox) to bleugill (Lepomis macrochirus). Final report 42347, ABC Laboratories, Columbia, MO, USA. Unpublished report, study I95-2029., Princeton, New Jersey, USA., 1996.

[104] M. Bazzon, L. Chauvire, J. Giacalone, F. Gondelle, M. Lamy, V. Petit-Poulsen, S. Bailleul, M. Rose, Toxicité aiguë vis-à-vis des poissons Brachydanio rerio. Substance d'essai: solution aqueuse d'acide peracétique. Unpublished report, study Ba567a, INERIS (Institut National de l'Environnement Industriel et des Risques), Verneuil-en-Halatte, France., Jouy en Josas, France., 1997.

[105] D. Tinsley, I. Sims, The acute toxicity of Oxymaster to plaice (Pleuronectes platessa) under semi-static conditions. Unpublished report CO1650-M/EV8687. WRc Environment, Medmenham, UK., Widnes, Cheshire, UK., 1987.

[106] E. Ceretti, M. Moretti, I. Zerbini, M. Villarini, C. Zani, S. Monarca, D. Feretti, Occurrence and control of genotoxins in drinking water: a monitoring proposal, J. Public Health Res. 116 (2016).

[107] R. Crebelli, L. Conti, S. Monarca, D. Feretti, I. Zerbini, C. Zani, E. Veschetti, D. Cutilli, M. Ottaviani, Genotoxicity of the disinfection by-products resulting from peracetic acid- or hypochlorite-disinfected sewage wastewater, Water Res. 39 (2005) 1105–1113.

[108] L. Guzzella, S. Monarca, C. Zani, D. Feretti, I. Zerbini, A. Buschini, P. Poli, C. Rossi, S.D. Richardson, In vitro potential genotoxic effects of surface drinking water treated with chlorine and alternative disinfectants, Mutat. Res. 564 (2004) 179–193.

[109] A. Buschini, A. Martino, B. Gustavino, M. Monfrinotti, P. Poli, C. Rossi, M. Santoro, A.J.M. Dörr, M. Rizzoni, Comet assay and micronucleus test in circulating erythrocytes of Cyprinus carpio specimens exposed in situ to lake waters treated with disinfectants for potabilization, Mutat. Res. 557 (2004) 119–129.

[110] A. Sapone, D. Canistro, F. Vivarelli, M. Paolini, Perturbation of xenobiotic metabolism in Dreissena polymorpha model exposed in situ to surface water (Lake Trasimene) purified with various disinfectants, Chemosphere. 144 (2016) 548–554.

[111] D. Canistro, S. Melega, D. Ranieri, A. Sapone, B. Gustavino, M. Monfrinotti, M. Rizzoni, M. Paolini, Modulation of cytochrome P450 and induction of DNA damage in Cyprinus carpio exposed in situ to surface water treated with chlorine or alternative disinfectants in different

seasons, Mutat. Res. 729 (2012) 81–89.

[112] S. Monarca, M. Rizzoni, B. Gustavino, C. Zani, A. Alberti, D. Feretti, I. Zerbini, Genotoxicity of surface water treated with different disinfectants using in situ plant tests, Environ. Mol. Mutagen. 41 (2003) 353–359.

[113] S. Monarca, D. Feretti, C. Zani, M. Rizzoni, S. Casarella, B. Gustavino, Genotoxicity of drinking water disinfectants in plant bioassays, Environ. Mol. Mutagen. 46 (2005) 96–103.

[114] D. Canistro, S. Melega, D. Ranieri, A. Sapone, B. Gustavino, M. Monfrinotti, M. Rizzoni, M. Paolini, Modulation of cytochrome P450 and induction of DNA damage in Cyprinus carpio exposed in situ to surface water treated with chlorine or alternative disinfectants in different seasons, Mutat. Res. 729 (2011) 81–89.

[115] B. Gustavino, A. Buschini, M. Monfrinotti, M. Rizzoni, L. Tancioni, P. Poli, C. Rossi, Modulating effects of humic acids on genotoxicity induced by water disinfectants in Cyprinus carpio, Mutat. Res. 587 (2005) 103–113.

[116] M. Villarini, M. Moretti, L. Dominici, C. Fatigoni, A. Josef, M. Dörr, A. Concetta Elia, S. Monarca, A protocol for the evaluation of genotoxicity in bile of carp (Cyprinus carpio) exposed to lake water treated with different disinfectants, Chemosphere. 84 (2011) 1521–1526.

[117] C. Bolognesi, A. Buschini, E. Branchi, P. Carboni, M. Furlini, A. Martino, M. Monteverde, P. Poli, C. Rossi, Comet and micronucleus assays in zebra mussel cells for genotoxicity assessment of surface drinking water treated with three different disinfectants, Sci. Total Environ. 333 (2004) 127–136.

[118] F. Maffei, A. Buschini, C. Rossi, P. Poli, G.C. Forti, P. Hrelia, Use of the comet test and micronucleus assay on human white blood cells for in vitro assessment of genotoxicity induced by different drinking water disinfection protocols, Environ. Mol. Mutagen. 46 (2005) 116–125.

[119] L. Marabini, S. Frigerio, E. Chiesara, S. Radice, Toxicity evaluation of surface water treated with different disinfectants in HepG2 cells, Water Res. 40 (2006) 267–272.

[120] C. Pellacani, A. Buschini, M. Furlini, P. Poli, C. Rossi, A battery of in vivo and in vitro tests useful for genotoxic pollutant detection in surface waters, Aquat. Toxicol. 77 (2006) 1–10.

[121] A.C. Elia, V. Anastasi, A.J.M. Dörr, Hepatic antioxidant enzymes and total glutathione of Cyprinus carpio exposed to three disinfectants, chlorine dioxide, sodium hypochlorite and peracetic acid, for superficial water potabilization, Chemosphere. 64 (2006) 1633–1641.

[122] B. Gustavino, Influence of Temperature on Mutagenicity in Plants Exposed to Surface Disinfected Drinking Water, J. Water Resour. Prot. 4 (2012) 638–647.

[123] A. Buschini, P. Carboni, M. Furlini, P. Poli, C. Rossi, Sodium hypochlorite-, chlorine dioxide- and peracetic acid-induced genotoxicity detected by the Comet assay and Saccharomyces cerevisiae D7 tests, Mutagenesis. 19 (2004) 157–162.

CHAPTER 4. EVALUATION OF A COLORIMETRIC METHOD FOR THE MEASUREMENT OF LOW CONCENTRATIONS OF PAA AND H_2O_2 IN WATER

Abstract

The recent growing interest in peracetic acid (PAA) as disinfectant for wastewater treatment demands reliable and readily-available methods for its measurement. In detail, the monitoring of PAA in wastewater treatment plants requires a simple, accurate, rapid and inexpensive measurement procedure. In the present work, a method for analyzing low concentrations of PAA, adapted from the US EPA colorimetric method for total chlorine, is assessed. This method employs the N,N-diethyl-p-phenylelnediamine (DPD) in the presence of an excess of iodide in a phosphate buffer system. Pink colored species are produced proportionally to the concentration of PAA in the sample. Considering that PAA is available commercially as an equilibrium solution of PAA and hydrogen peroxide (H_2O_2), a measurement method for H_2O_2 is also investigated. This method is based on the same reactions as the one for the PAA measurement, with the addition of ammonium molybdate to catalyze the oxidation reaction between H_2O_2 and iodide. The two methods are suitable for concentration ranges from 0.1 to 2 mg/L and from 0.3 to 3.5 mg/L for PAA and total peroxides (Cl_2), respectively. Moreover, the work elucidates some relevant aspects related to the operational conditions, kinetics and the possible interference of H_2O_2 on PAA measurement.

Keywords: Peracetic acid, hydrogen peroxide, spectrophotometry, residual concentration measurement, wastewater disinfection.

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4.1. Introduction

For its application as wastewater disinfectant, as recently reviewed [1], the determination of residual PAA concentration at low values, is essential to assess decay kinetics, disinfection performance, and toxicity of effluents. In particular, regarding the design of the disinfection process, a reliable and accurate method for PAA measurement is needed for estimating the actual PAA dose, due to the rapid decay of PAA in wastewater [2]. Moreover, since PAA coexists with H_2O_2, such analytical method must be highly selective in distinguishing PAA and H_2O_2, in view of the different behavior of PAA and H_2O_2 as disinfectants [3,4] and lifetime of the residues in water, despite the important chemical similarities of the species.

While a wide literature was published on the determination of each compound alone, several analytical methods were reported to measure PAA and H_2O_2 simultaneously [1]. These methods are based on the difference in the oxidizing power of both species, and include chromatographic, potentiometric, titrimetric and colorimetric techniques. For some of these methods important operating disadvantages were evidenced, as low sensitivity, complex procedures, expensive instruments and reagents. In addition, in case of wastewater disinfection, the time required to perform the measurement is an important feature, due to the instability of PAA diluted solutions. In the following, a brief discussion of the most relevant methods in literature is reported.

Concerning chromatographic methods, Di Furia et al. [5] proposed the determination of PAA and H_2O_2 based on the selective oxidation of methyl-p-tolyl sulfide (MTS) to the corresponding sulfoxide species (MTSO) which is then separated by gas chromatography. Alternatively, a method based on the same reaction has been proposed for liquid chromatography by Pinkernell et al. [6]. Although gas and liquid chromatographic techniques offer selectivity and low limits of detection (0.1-10 mg/L, approximately), expensive instrumentation and reagents are required; in addition, they are not suitable for field applications. As for potentiometric methods, Awad et al. [7] proposed a technique based on the detection of the transient change of the electrode potential due to the oxidation of iodide by PAA and H_2O_2. The electrical response is obtained within few seconds while maintaining high sensitivity and selectivity down to the micromolar range. Although this method presented good recovery of PAA and H_2O_2 concentrations, it is labor intensive, cumbersome and more suitable for on-line analysis.

Titration methods, such as permanganometry, cerimetry and iodometry titrations [8–11], are among the oldest and most widely used techniques due to the good selectivity between PAA and H_2O_2, but their application is inappropriate for the determination of low concentrations in the order of few mg/L, but rather in as % w/w [12].

Finally, colorimetric methods allow the measurement of the compounds at low concentrations (0.1-10 mg/L approximately) with good selectivity. A method, adapted from Pütter and Becker [13], was proposed by Wagner et al. [4], based on the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline)-6-

sulfonate (ABTS) and horseradish peroxidase by PAA and H_2O_2. An intensely green radical cation is produced and its spectrophotometric detection is performed at 405 nm. This method is accurate and reliable, although it is complex to perform, involving several steps. Nevertheless, its main limitation is the impossibility to distinguish between the two species, providing a total quantification of peroxides. The availability and cost of the reagents as well as the time required to perform the analysis are minor drawbacks of this method. Pinkernell et al. [14] proposed another procedure based on the oxidation of ABTS by peroxides, reporting good results. However, in this case, the time required for the development of the color is in the order of minutes and it is highly dependent on the pH. Therefore, limitations in existing analytical methods suggest the need for further investigations.

A promising colorimetric method allowing the determination of low concentrations of PAA and H_2O_2 was adapted from the US EPA DPD (N,N-diethyl-p-phenylenediamine) colorimetric method (method #330.5) [15] for the determination of total chlorine; it has been used in numerous previous works related to PAA disinfection [10,16–23]. In detail, the samples containing PAA are treated with an excess of potassium iodide (KI). The PAA oxidizes iodide to iodine, which subsequently oxidizes the DPD to a pink colored species. The PAA concentration can be estimated measuring the absorbance at 530 nm by means of a linear calibration curve. A major potential drawback of this method is low selectivity, since both PAA and H_2O_2 can oxidize iodide. Indeed, when PAA concentration is to be selectively determined by the DPD method, catalase is usually added to decompose H_2O_2 in solution [10,19–21,24–26]. However, in many other cases the occurrence of interference related to H_2O_2 is neglected [17,22,23,27–29]. Moreover, the literature is also ambiguous about the quenching procedure by catalase: two papers reported that PAA is unaffected by catalase [23,30], whereas the partial decomposition of PAA is highlighted in other two papers [4,31], resulting in an under-estimation of PAA concentration. On the other hand, the DPD method can be effectively used for the selective determination of PAA and H_2O_2, in case that the effect of the two species on the oxidation of iodide can be accurately distinguished.

The purpose of this work is to assess the DPD method as a technique to simultaneously determine PAA and H_2O_2 in diluted solutions at low concentrations, and to discuss the aspects that are still unclear in literature, specifically addressing to its application for wastewater disinfection. Furthermore, the effect of pH on the measurement, kinetic aspects of the reaction as well as possible interferences are addressed.

4.2. Material and methods

4.2.1. Reagents

All solutions were prepared using deionized water and all chemicals were of analytical grade, purchased from Sigma Aldrich (USA), except for DPD (N,N-diethyl-p-phenylenediaminesulfate) salt for total chlorine that was provided by Hach Lange (USA). Commercial PAA technical-grade solution (15% w/w of PAA, 23% w/w of H_2O_2 and 16% w/w of acetic acid) was supplied by PeroxyChem (USA).

4.2.2. Analytical methods

4.2.2.1. Iodometric titration for PAA and H_2O_2

The concentration of PAA and H_2O_2 in the commercial PAA solution was determined by iodometric titration [9]. PAA and H_2O_2 oxidize iodide to iodine in presence of sulfuric acid (H_2SO_4) according to Equations 1 and 2, respectively. A starch indicator is added to develop an intense blue starch-iodine complex. Then, iodine is titrated with sodium thiosulfate ($Na_2S_2O_3$) until the disappearance of the color, according to Equation 3. This iodometric titration is performed in two separate steps to measure PAA and H_2O_2, as detailed in the following.

$$CH_3CO_3H + 2\,KI + H_2SO_4 \rightarrow CH_3CO_2H + I_2 + K_2SO_4 + H_2O \quad \text{(e.1)}$$

$$H_2O_2 + 2\,KI + H_2SO_4 \rightarrow I_2 + K_2SO_4 + H_2O \quad \text{(e.2)}$$

$$I_2 + Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI \quad \text{(e.3)}$$

First, in order to determine PAA concentration, an aliquot of commercial PAA solution is placed into a 250-mL volumetric flask and diluted up to the mark with deionized water. A 25-mL aliquot of the aforementioned solution is placed into a 250-mL Erlenmeyer flask containing 50 mL of deionized water and 10 mL of phosphate buffer solution (0.14 mol/L of $NaHPO_4 \cdot 12H_2O$, 0.34 mol/L of KH_2PO_4, 0.003 mol/L of EDTA). The pH of the buffer solution is adjusted to 5.5 with H_2SO_4. H_2O_2 is quenched with 500 µg of bovine catalase (2900 units/mg). After 5 minutes, in which the bovine catalase reacts with H_2O_2, 15 mL of H_2SO_4 (12 N) and 2.5 g of KI are added. The mixture is covered and maintained for 20 minutes in dark conditions. 50 mL of deionized water are added and the mixture is titrated with standardized $Na_2S_2O_3$ (0.1 N) until the solution becomes pale yellow. Then, 2 mL of the starch solution are added and the titration is carried out under constant stirring until the disappearance of the color. According to the stoichiometry of the reactions presented in Equations 1 and 3, the following relationship can be established to determine the mass of PAA present in the sample titrated:

$$mg\ of\ PAA = A\ (ml) * \left[\frac{0.1\ m_{Equiv}\ Na_2S_2O_3}{1\ ml\ Na_2S_2O_3}\ \frac{1\ m\ mol\ Na_2S_2O_3}{2\ m_{Equiv}\ Na_2S_2O_3}\ \frac{1\ mmol\ PAA}{1\ m\ mol\ Na_2S_2O_3}\ \frac{76\ mg\ PAA}{1\ mmol\ PAA}\right] \quad \text{(e.4)}$$

Simplifying Equation 4, the concentration of PAA (mg/g) can be obtained by means of Equation 5:

$$[PAA] = \frac{A * [3.803]}{m} \quad \text{(e.5)}$$

where A is the volume of $Na_2S_2O_3$ solution consumed in the titration (in mL), m is the weight of the titrated sample (in g). In this case and according to the procedure, m corresponds to the mass of the sample of commercial solution contained in the 25 mL aliquot placed in the Erlenmeyer flask, which is estimated with the density of the pure commercial solution. Then, the H_2O_2 fraction in equilibrium with PAA can be determined following the above-described procedure without quenching H_2O_2 with bovine

catalase. The reaction rate of H_2O_2 with iodide is slower than PAA [32], therefore ammonium molybdate (Mo(VI)) is added as catalyst. The Mo(VI) solution is prepared dissolving 9 g of ammonium heptamolybdate ($(NH_4)_6Mo_7O_{24}$) in 10 mL of 6 N ammonium hydroxide. Then, 24 g of ammonium nitrate are added and the mixture is diluted up to 100 mL. 1 mL of Mo(VI) solution is added subsequently to the KI when performing the titration.

Given that this method quantifies all the oxidizing agents in the sample, the aforementioned procedure quantifies both H_2O_2 and PAA. In order to determine the concentration of H_2O_2, the contribution of PAA can be subtracted as follows:

$$Equivalents = \frac{0.1\ m_{Equiv}\ Na_2S_2O_3}{1\ ml\ Na_2S_2O_3} * (B - A) \quad \text{(e.6)}$$

where A and B are the volumes (in mL) of $Na_2S_2O_3$ solution consumed in the titration procedure in presence and absence of both the Mo(VI) catalyst bovine catalase, respectively. According to the stoichiometry of the reactions presented in Equations 1 and 2, the following relationship can be established to determine the milligrams of H_2O_2 present in the sample titrated:

$$mg\ of\ H_2O_2 = Equivalents * \left[\frac{1\ m\ mol\ Na_2S_2O_3}{2\ m_{Equiv}\ Na_2S_2O_3} \frac{1\ mmol\ H_2O_2}{1\ m\ mol\ Na_2S_2O_3} \frac{34\ mg\ H_2O_2}{1\ mmol\ H_2O_2} \right] \quad \text{(e.7)}$$

Simplifying Equation 7 the concentration of H_2O_2 (mg/g) can be obtained by means of Equation 8:

$$[H_2O_2] = \frac{Equivalents \cdot 17}{m} \quad \text{(e.8)}$$

where m is the weight of the titrated sample (in g). In an analogous way as the titration for PAA, m corresponds to the mass of the sample of commercial solution contained in the 25 mL aliquot placed in the Erlenmeyer flask, which is estimated with the density of the pure commercial solution.

4.2.2.2. Determination of low concentrations of PAA

To determine PAA concentration in diluted solution, 50-mL sample is placed in a 250-mL Erlenmeyer flask continuously mixed by a magnetic stirrer. Subsequently, 2 mL of phosphate buffer solution at pH 5.5 and 2 mL of KI (1 M) are added followed by one dose of DPD salt (0.01 g). After 10 seconds, 10 mL of the sample are transferred to a quartz cuvette (optical path 40 mm) and the absorbance at 530 nm is measured by means of a spectrophotometer Hach Lange CADAS 200. The calibration curve is constructed by repeating the measurement procedure for three series of standards (each value replicated three times) covering the PAA concentration range from about 0.1 to 2 mg/L, as shown in Figure 1, being its Equation the following:

$$ABS\ (\lambda = 530\ nm) = (0.552 \pm 0.005) \cdot [PAA] + (0.024 \pm 0.005) \quad \text{(e.9)}$$

In case of samples with PAA concentration above 2 mg/L, the sample must be diluted before measurement. While the 95% confidence interval of parameters is reported in Equation 4, the R^2 and the predicted R^2 values of the calibration curve were 0.9972 and 0.9967, respectively.

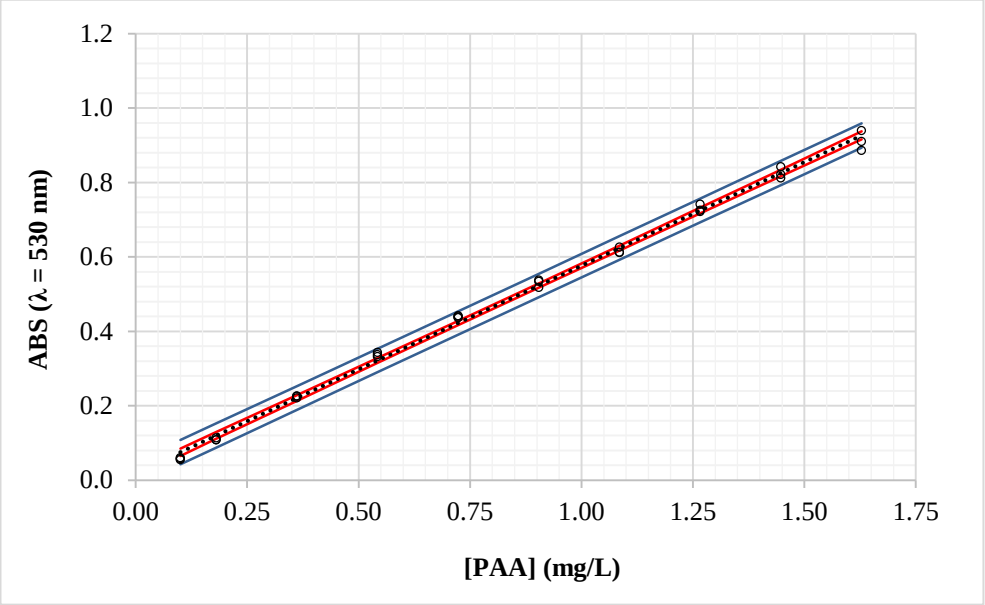

Figure 1. Calibration curve for PAA measurement by the DPD method.
The 95% confidence and prediction intervals are reported in red and blue respectively.

4.2.2.3. Determination of low concentrations of H_2O_2

For the measurement of H_2O_2 concentration in diluted solutions, 94.7 mL of the sample are placed in a 100-mL volumetric flask, and the volume is filled up to 100 mL by adding 5 mL of KI (1 M), 0.2 mL of Mo(VI) solution and 0.1 mL of phosphoric acid (H_3PO_4) (20% w/w). The volumetric flask is capped and shaken gently. After 6 minutes of reaction time, the sample is transferred to an Erlenmeyer flask of 250 mL and two doses of DPD are added, subsequently 10 mL of the sample are transferred to a quartz cuvette (optical path 40 mm) and the absorbance is measured by means of a spectrophotometer Hach Lange CADAS 200. As for titration to determine PAA and H_2O_2 concentration, the procedure quantifies all the oxidizing compounds present in the sample, namely both peroxides. PAA and H_2O_2 are accounted in terms of total Cl_2 by means of their molecular weight ratios to Cl_2, according to Equations 10 and 11.

$$[Cl_2]_{as\ PAA} = \frac{[PAA]}{1.072} \quad \text{(e.10)}$$

$$[Cl_2]_{as\ H_2O_2} = \frac{[H_2O_2]}{0.480} \quad \text{(e.11)}$$

The absorbance at 530 nm is converted to total Cl_2 concentration using the calibration curve shown in Figure 2, being its Equation reported:

$$ABS\ (\lambda = 530\ nm) = (0.6706 \pm 0.0091) \cdot [Cl_2]_{as\ PAA+H2O2} + (0.1125 \pm 0.0183) \qquad \text{(e.12)}$$

The R^2 and the predicted R^2 values of the calibration curve were 0.997 and 0.9961, respectively.

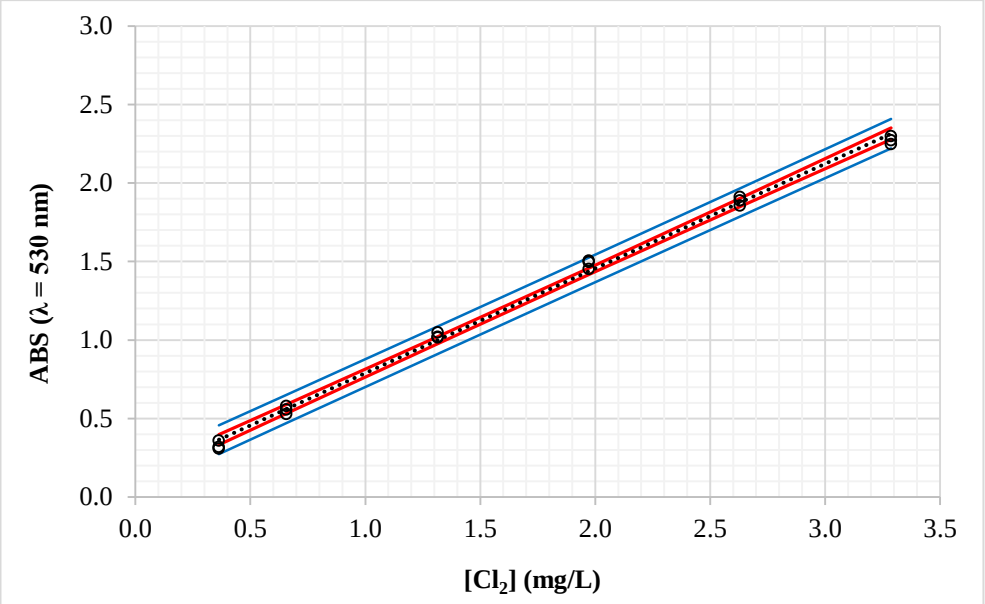

Figure 2. Calibration curve for PAA and H_2O_2 measurement by the DPD method.

The 95% confidence and prediction intervals are reported in red and blue respectively.

In order to determine the concentration of H_2O_2, the contribution of PAA determined by the procedure described in section 4.2.2.2, in terms of Cl_2, can be subtracted according to Equation 13.

$$[H_2O_2] = 0.480 \cdot \left([Cl_2]_{as\ PAA+H_2O_2} - [Cl_2]_{as\ PAA}\right) \qquad \text{(e.13)}$$

4.2.3. Effect of pH on the measurement of PAA and H_2O_2 concentrations

The effect of solution pH on the measurement of PAA concentration was assessed by measuring PAA concentration by the procedure described in section 4.2.2.2 on a sample containing 0.36 mg/L of PAA and 0.47 mg/L of H_2O_2, using phosphate buffer solutions at different pH (2.5, 4, 5.5, 6.5, 7.5, 9 and 10.5). The phosphate buffer solutions were prepared according to the procedure described in section 3.2.2.1 and the pH was adjusted with H_2SO_4 (pH 2.5, 4 and 5.5) or NaOH (pH 7.5, 9 and 10.5). Each measurement was replicated three times. The effect of pH on the measurement of H_2O_2 concentration was evaluated by analyzing the same sample using the procedure described in section 4.2.2.3, repeated in presence and absence of H_3PO_4.

4.2.4. Effect of H_2O_2 on the measurement of PAA concentration

The interference of H_2O_2 on the determination of PAA concentration was assessed by means of two tests to determine the kinetics of the reaction in presence and absence of H_2O_2 plus a third experiment to

assess solely H_2O_2 kinetics. In the first case, (a) the method to measure PAA concentration was performed on a sample containing 0.36 mg/L of PAA in equilibrium with 0.47 mg/L of H_2O_2, and the absorbance was monitored for 120 minutes at 5-minute intervals. In addition, the experiment was repeated monitoring the absorbance at 10-second intervals for 6 minutes. In the second case, (b) the test was performed and monitored as in the first case but an additional step to quench H_2O_2 in the sample was carried out by adding 200 μL of a solution containing 10 mg of bovine catalase (2900 units/mg) dissolved in 25 mL of deionized water. In the procedure, the bovine catalase solution was added after the phosphate buffer solution at pH 5.5 and before the KI. Finally, a third test (c) was carried out to assess the reaction for H_2O_2 alone in a sample containing 0.47 mg/L of H_2O_2 using the same procedure and it was monitored for 120 minutes at 5-minute intervals. In addition, the experiment was repeated monitoring the absorbance at 20-second intervals for 6 minutes. In addition, blank tests were performed for the measurement methods described in section (d) 4.2.2.2 and (e) 4.2.2.3, and performing the same monitoring as for case (a). All the experiments were performed in the dark.

4.2.5. Effect of Mo(VI) on the oxidation of iodide by H_2O_2

The catalyzed and uncatalyzed oxidation rates of iodide by H_2O_2 were compared in two tests for samples containing 0.47 mg/L of H_2O_2. For the assessment of the reaction rate of the catalyzed reaction, the procedure to measure H_2O_2 concentration described in section 4.2.2.3 was used, and the absorbance was monitored for 180 minutes at 5-minute intervals. The reaction rate of the uncatalyzed reaction was evaluated using the procedure to measure PAA concentration described in section 4.2.2.2, monitoring the absorbance at same time intervals. All the experiments were performed in the dark.

4.2.6. Method validation

The composition of the equilibrium mixture was determined by the standard iodometric titration describe in section 4.2.2.1 (15.6% PAA and 22.3% H_2O_2). This was used to prepare a stock solution containing 0.905 mg/L of PAA and 1.309 mg/L of H_2O_2 by placing 0.5 mL of the titrated equilibrium mixture in a volumetric flask of 100 mL and making up to volume with deionized water. Subsequently, this solution was used to dose tap water (prior dechlorination) in order to prepare solutions with six different nominal concentrations of PAA (0.1, 0.25, 0.5, 1, 1.25 and 1.5 mg/L), which were immediately analyzed for PAA concentration following the procedure described in section 4.2.2.2. Thereafter, the same procedure was carried out to measure the associated fractions of H_2O_2 (0.13, 0.324, 0.65, 0.970, 1.23, 1.94 mg/L) in equilibrium with the aforementioned concentrations of PAA, according to the procedure described in section 4.2.2.3. Each experiment was replicated four times.

4.3. Results and discussion

4.3.1. Effect of pH on the measurement of PAA and H_2O_2 concentrations

The parent method to measure total chlorine, hypochlorite ion, hypochlorous acid and chloramines

stoichiometrically generates iodine from iodide at pH 4 or lower [15]. The DPD method can be adapted to PAA measurement on the basis of the capacity of PAA to act like chlorine on iodide [27].

In the case of PAA measurement, the maximum peak of absorbance at the same operating conditions is reached at pH comprised between 4 and 6.5 for a PAA concentration of 0.36 mg/L, as shown in Figure 3. In this range, the difference in the absorbance values at different pH is not relevant, although the variability of the absorbance values is slightly higher at pH 6.5. For pH values below 4 and above 6.5, an important decrease of the absorbance values is observed, thus it can be inferred that pH values outside the aforementioned range are not optimal for the reaction kinetics, and the reaction is not completed. This pH range agrees with that reported by other authors who implemented the DPD method employing a pH between 5.5 and 6.5 [10,15,17,33]. Moreover, it is in agreement as well with the kinetic study carried out by Awad et al. [7] for the potentiometric determination of PAA, in which the first order rate constants for the reaction between iodide and PAA were found to be independent of solution pH in the range 3.5-5.4. Therefore, pH 5.5 was selected as the optimum for the determination of PAA concentration in agreement with other authors and in the view of reducing the variability of the response.

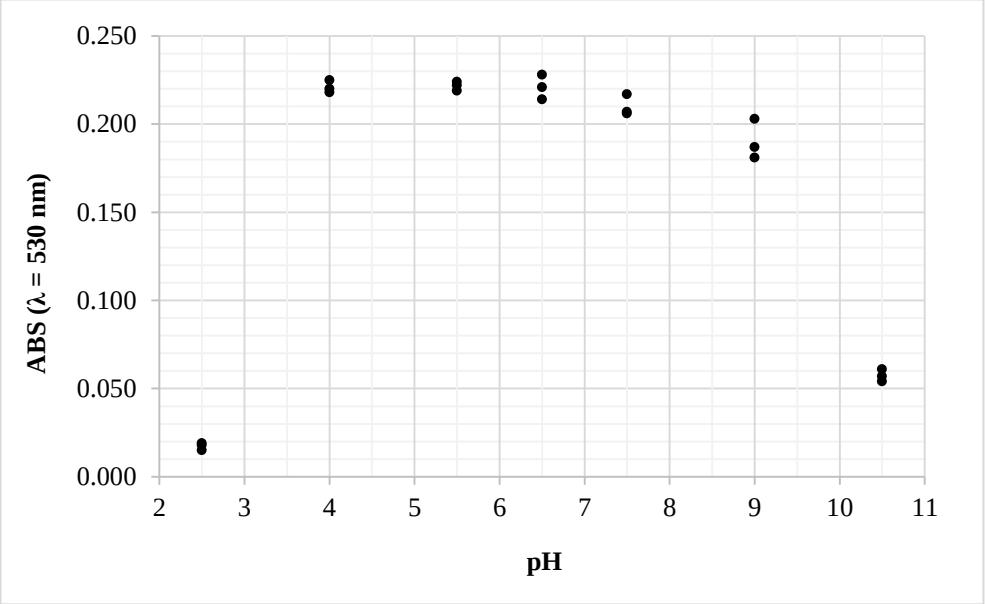

Figure 3. Effect of pH on the measurement of PAA concentration by the DPD method.

On the other hand, the rate of the reaction of iodide with H_2O_2 is known to be largely dependent on pH, even when the reaction is catalyzed with Mo(VI) [7,34]. The absorbance at 530 nm of a sample containing 0.36 mg/L of PAA and 0.47 mg/L of H_2O_2 treated with H_3PO_4 prior the oxidation of iodide by H_2O_2 catalyzed by Mo(VI) was 1.016, whereas the absorbance was 0.697 without the addition of H_3PO_4. For the sample treated with H_3PO_4, the reaction occurred in acidic media (pH 3.5), while in the other case at more alkaline conditions (pH 6). In agreement with the kinetic study reported by Awad et al. [32], the reaction of iodide and H_2O_2 becomes slower as the pH increases, being the optimal conditions reported for pH 4 for the Mo(VI) catalyzed oxidation of KI by H_2O_2 [35,36].

The procedure for the determination of PAA and H_2O_2 cannot be simplified avoiding the addition of a pH buffer (PAA) or an acid (H_2O_2) and the pH values reported in the literature should be strictly observed.

4.3.2. Effect of H_2O_2 on the measurement of PAA concentration

It is well-documented that the rate of oxidation of iodide by PAA is much higher than that by H_2O_2 [7,34,37]. The uncatalyzed reaction rate of iodide and PAA is reported to be five orders of magnitude higher than that of iodide and H_2O_2 [7]. For instance, the pseudo-first order kinetic rate constant for the uncatalyzed reaction between PAA and iodide calculated by Awad et al. [32] was $4.22 \cdot 10^2$ $M^{-1} s^{-1}$, being the rate expressed as $r_{uncatalyzed} = 4.22 \cdot 10^2$ $[PAA][I^-]$, whereas the rate constant for the uncatalyzed reaction of H_2O_2 and iodide calculated by Cooper and Koubek [36] was $9.5 \cdot 10^{-3}$ $M^{-1} s^{-1}$, with a rate expressed as $r_{uncatalyzed} = 9.5x10^{-3}$ $[H_2O_2][I^-]$. In order to confirm the absence of a detrimental effect by H_2O_2 on the measurement of PAA, the absorbance at 530 nm was monitored over 100 minutes on a sample containing 0.36 mg/L of PAA and 0.47 mg/L of H_2O_2 with and without removing the H_2O_2 fraction with bovine catalase, as shown in Figure 4.

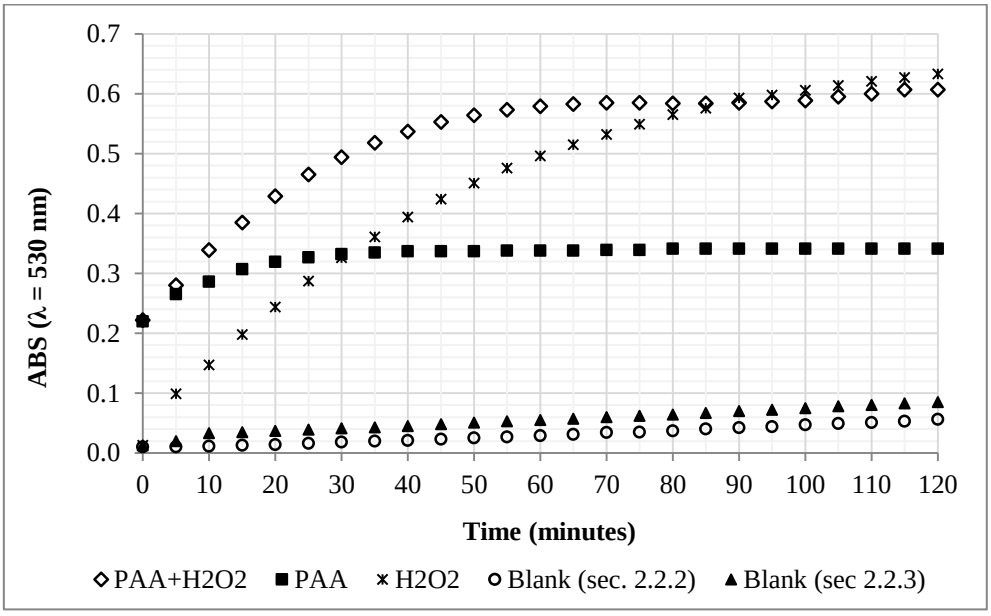

Figure 4. Absorbance at λ = 530 nm over 100 minutes (5-minute intervals) for experiments with: (a) 0.36 mg/L of PAA in equilibrium with 0.47 mg/L of H_2O_2, (b) 0.36 mg/L of PAA after the removal of H_2O_2 with bovine catalase, (c) 0.47 mg/L of H_2O_2, and the blanks described in sections (d) 4.2.2.2 and (e) 4.2.2.3.

Bovine catalase is an oxidoreductase enzyme that selectively catalyzes the decomposition of H_2O_2 in equilibrium with PAA. When H_2O_2 was not quenched (PAA+H_2O_2), absorbance values were noticeably higher than the ones obtained when H_2O_2 was quenched (PAA), particularly after 5 minutes. For the curve PAA+H_2O_2, a plateau of the absorbance values, indicating the completeness of the reaction, was

reached after approximately 70 minutes, whereas for the PAA curve, the levelling of the absorbance was reached after approximately 20 minutes. In both cases, the absorbance reached a value (~0.220) at t = 0, that remained briefly steady for approximately 30 seconds, approximately 60 seconds after the addition of DPD, considering that the spectrophotometrical measurement was carried out approximately 30 seconds after the addition of the DPD.

A close-up to the first six minutes, is shown in Figure 5. Indeed, during the first 20 seconds, the absorbance values are the same for both curves (PAA and PAA+H_2O_2), indicating that the absorbance values can be attributed solely to PAA if they are measured within this time frame. Therefore, no interference of H_2O_2 is expected in the measurement of PAA within about 60 seconds after adding DPD, in agreement with the memorandum of the US EPA and the patent of Howarth et al. [27,29]. This evidence is confirmed by the curve for the reaction of H_2O_2 alone, in which the absorbance values at the beginning of the experiment are negligible, thus no significant oxidation of iodide by H_2O_2 occurs in the first minute. Furthermore, the trend is very similar to the curve for PAA and H_2O_2, although it is shifted upwards, starting at the corresponding value for the PAA concentration. Regarding the slow-paced growth of the absorbance for the oxidation of iodide by PAA, it can be attributed to the slow decomposition of H_2O_2 by the bovine catalase.

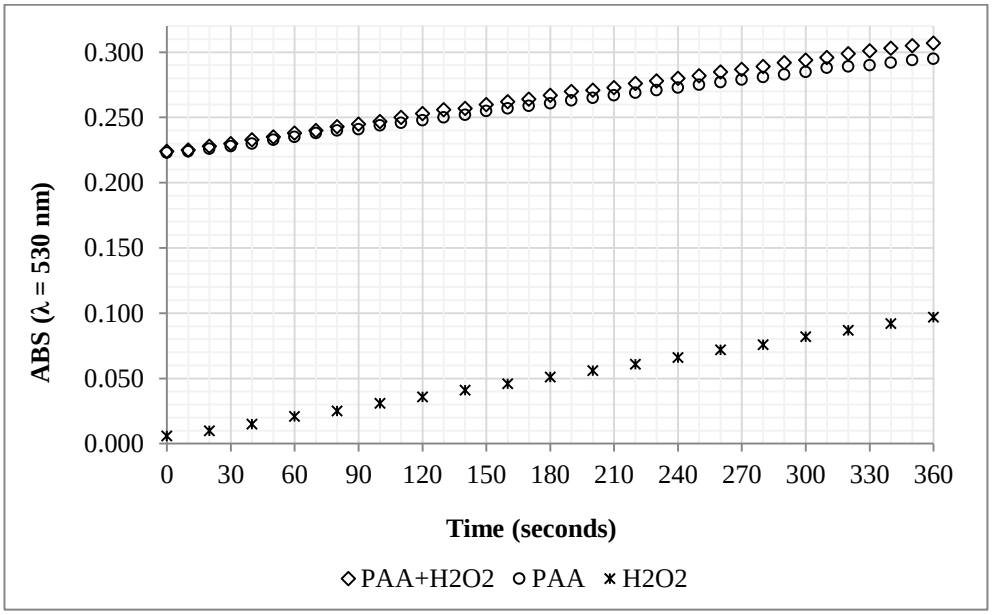

Figure 5. Absorbance at λ = 530 nm over 360 seconds (10 and 20-second intervals) for experiments with: (a) 0.36 mg/L of PAA in equilibrium with 0.47 mg/L of H_2O_2, (b) 0.36 mg/L of PAA after the removal of H_2O_2 with bovine catalase, and (c) 0.47 mg/L of H_2O_2.

Therefore, the reaction of iodide with H_2O_2 can be considered negligible in the time scale used to carry out the reaction of iodide with PAA, confirming the faster rate of the oxidation of iodide with PAA respect to H_2O_2 [7]. This large difference allows determining PAA concentration without the addition of catalase to quench H_2O_2 as long as the absorbance is read promptly, within about 60 seconds after the

addition of DPD, making time a key parameter to measure accurately PAA concentration.

4.3.3. Effect of Mo(VI) on the oxidation of iodide by H_2O_2

The reaction rate of iodide and H_2O_2 can be increased either by the addition of Mo(VI) solution as catalyst or decreasing the solution pH [7], in agreement with the method described for the measurement of H_2O_2 concentration, implementing the dosage of catalyst and the acidification by H_3PO_4 jointly. When the reaction is catalyzed with Mo(VI), the reaction rate of iodide and H_2O_2 is reduced by two orders of magnitude [7,32,34].

The effect of the Mo(VI) solution on the measurement of H_2O_2 concentration was evaluated following the absorbance values over 180 minutes for a sample containing 0.47 mg/L of H_2O_2 in presence and absence of Mo(VI). Experimental results are reported in Figure 6.

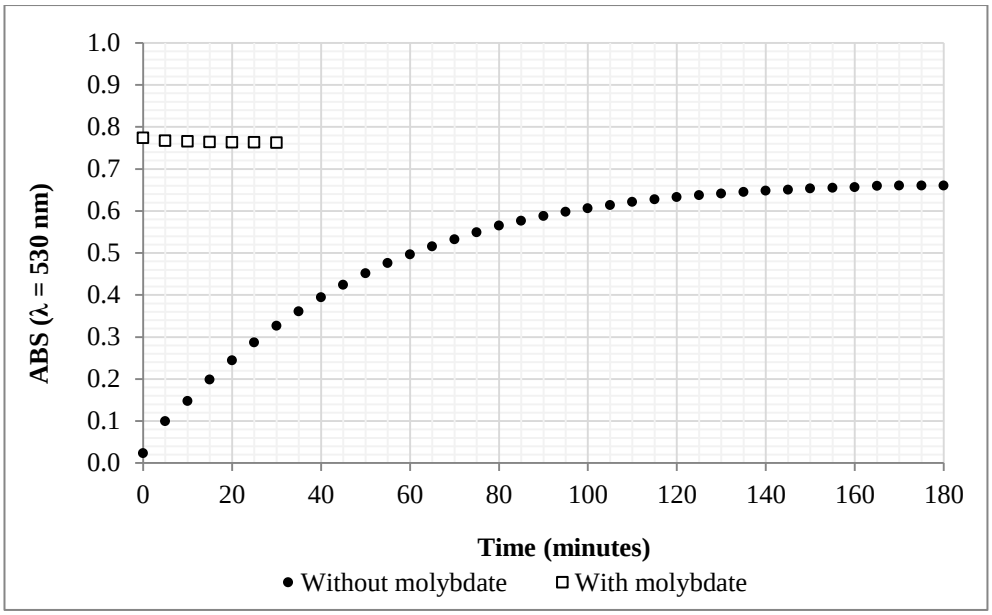

Figure 6. Absorbance at λ = 530 nm over 180 minutes (5-minute intervals) for the oxidation of iodide by 0.47 mg/L of H_2O_2 with and without the dosage of Mo(VI) solution as catalyst.

In agreement with the previous results (Figure 5), no significant increase in the absorbance occurs in the first minutes for the curve of the uncatalyzed reaction. The levelling-off of the absorbance values seems to occur after 180 minutes, without reaching the same value as the Mo(VI) catalyzed reaction, suggesting that even if the absorbance is stable, the reaction is not completed. For the reaction catalyzed with Mo(VI), the maximum absorbance and, therefore, the completion of the reaction, are reached immediately and maintained for 30 minutes without significant changes.

Consequently, the oxidation of iodide by H_2O_2 is highly catalyzed by Mo(VI). As already introduced and reported by other authors [7,32,34,36], the rate constant of the Mo(VI) catalyzed reaction is about three orders of magnitude larger than that of the uncatalyzed one. However, the catalytic effect of Mo(VI) on the oxidation of iodide by different peroxides is different depending on the type of peroxide.

It has been also reported that the rate of the oxidation of iodide by organic peroxide decreases with increasing the complexity of the organic molecules [38,39].

4.3.4. Method validation

The results of the measurement of different concentrations of PAA are presented in Table 1. The recovery of PAA ranged between 95.5 and 97.8%, whereas for H_2O_2 the recovery ranged between 96.6 and 97.8%. At lower concentrations, the recoveries tend to be higher and the standard deviations lower.

Several authors [10,17,19–29] have applied the DPD method to the determination of PAA, whereas only few of them [10,29] have described the performance and accuracy of the method. As for the measurement of H_2O_2, to the best knowledge of the authors, the DPD method has not been applied in disinfection studies to date. Summarizing, both methods display optimal recoveries and high precision, with relative percentage standard deviation below 2% even for the lowest investigated concentration of 0.1 mg/L.

Table 1. Recovery of PAA, total peroxides and H_2O_2 concentrations in tap water. Recovered concentrations reported as mean ± standard deviation.

PAA dose applied (mg/L)	PAA recovered (mg/L)	% recovery PAA	Total peroxides applied (mg/L as Cl_2 eq.)	Total peroxides recovered (mg/L as Cl_2 eq.)	% recovery total peroxides	H_2O_2 dose applied (mg/L)	H_2O_2 recovered	% recovery H_2O_2
0.1	0.098±0.0017	97.8%	0.363	0.355±0.0021	97.8%	0.130	0.127±0.0027	97.8%
0.25	0.242±0.0031	97.0%	0.908	0.886±0.0059	97.6%	0.324	0.317±0.0067	97.8%
0.5	0.486±0.0044	97.3%	1.816	1.782±0.0099	97.7%	0.648	0.63±0.011	97.8%
0.75	0.730±0.0040	97.3%	2.725	2.65±0.014	97.4%	0.971	0.95±0.015	97.5%
1	0.97±0.014	96.7%	3.633	3.53±0.036	97.1%	1.295	1.26±0.039	97.2%
1.5	1.43±0.032	95.5%	5.449	5.25±0.061	96.3%	1.943	1.88±0.069	96.5%

Comparison of the present Figures of merit with literature data may be performed for PAA determination and the study of Cavallini et al. [10]. Lower accuracy and precision is reported in the latter, which may partially be accounted for by the different optical path used in the present work (40 mm) and in the cited paper [10] (10 mm; addition of catalase).

4.4. Conclusions

A colorimetric method for the measurement of low concentrations of PAA and H_2O_2 in solution was assessed. The method is based on the oxidation of iodide by PAA and H_2O_2, and on the subsequent generation of colored by-products that can be selectively measured by spectrophotometry. Experimental results evidenced that the reaction is independent of pH in the range 4-6.5 for the measurement of PAA, whereas the reaction for the measurement of H_2O_2 catalyzed by Mo(VI) is dependent on the pH, and the optimal pH value for the application of the method is 3.5. Due to the differences in the oxidation rate of both compounds, interferences due to the presence of H_2O_2 when measuring PAA are negligible if the

absorbance reading is taken rapidly, within 60 seconds after the addition of the DPD: the quenching of H_2O_2 with bovine catalase is accordingly not necessary. Therefore, time is a key parameter for getting a reliable measurement. Given the slow oxidation rate of iodide by H_2O_2, the reaction for the measurement of H_2O_2 must be catalyzed by Mo(VI). The optical path should be selected according to the needs of the research; in general, longer optical paths provide higher accuracy and precision whenever low concentrations of the analytes are to be determined.

4.5. References

[1] T. Luukkonen, S.O. Pehkonen, Peracids in water treatment: A critical review, Crit. Rev. Environ. Sci. Technol. 0 (2016) 1–39.

[2] D. Santoro, F. Crapulli, M. Raisee, G. Raspa, C.N. Haas, Nondeterministic Computational Fluid Dynamics Modeling of *Escherichia coli* Inactivation by Peracetic Acid in Municipal Wastewater Contact Tanks, Environ. Sci. Technol. 49 (2015) 7265–7275.

[3] M.J. Flores, M.R. Lescano, R.J. Brandi, A.E. Cassano, M.D. Labas, A novel approach to explain the inactivation mechanism of *Escherichia coli* employing a commercially available peracetic acid, Water Sci. Technol. 69 (2014) 358.

[4] M. Wagner, D. Brumelis, R. Gehr, Disinfection of wastewater by hydrogen peroxide or peracetic acid: development of procedures for measurement of residual disinfectant and application to a physicochemically treated municipal effluent, Water Environ. Res. 74 (2002) 33–50.

[5] F. Di Furia, M. Prato, U. Quintily, S. Salvagno, G. Scorrano, Gas-liquid chromatographic method for the determination of peracids in the presence of a large excess of hydrogen peroxide, Analyst. 109 (1984) 985.

[6] U. Pinkernell, S. Effkemann, U. Karst, Simultaneous HPLC Determination of Peroxyacetic Acid and Hydrogen Peroxide, Anal. Chem. 69 (1997) 3623–3627.

[7] M.I. Awad, T. Oritani, T. Ohsaka, Simultaneous potentiometric determination of peracetic acid and hydrogen peroxide, Anal. Chem. 75 (2003) 2688–2693.

[8] F.P. Greenspan, D.G. Mackellar, Analysis of Aliphatic Peracids, Anal. Chem. 20 (1948) 1061–1063.

[9] B.D. Sully, P.L. Williams, The analysis of solutions of per-acids and hydrogen peroxide, Analyst. 87 (1962) 653.

[10] G.S. Cavallini, S.X. De Campos, J.B. De Souza, C.M.D.S. Vidal, Comparison of methodologies for determination of residual peracetic acid in wastewater disinfection, Int. J. Environ. Anal. Chem. 93 (2013) 906–918.

[11] X. Zhao, K. Cheng, J. Hao, D. Liu, Preparation of peracetic acid from hydrogen peroxide, part II: Kinetics for spontaneous decomposition of peracetic acid in the liquid phase, J. Mol. Catal. A Chem. 284 (2008) 58–68.

[12] S. Effkemann, S. Brødsgaard, P. Mortensen, S.A. Linde, U. Karst, Determination of gas phase

peroxyacetic acid using pre-column derivatization with organic sulfide reagents and liquid chromatography, J. Chromatogr. A. 855 (1999) 551–561.

[13] Pütter and Becker, Peroxidases. In Methods of Enzymatic Analysis. J. Bergmeyer and M. Grassl (Eds.), Verl. Chemie, Weinheim, Germany, 1983.

[14] U. Pinkernell, H.-J. Lüke, U. Karst, Selective Photometric Determination of Peroxycarboxylic Acids in the Presence of Hydrogen Peroxide, Analyst. 122 (1997) 567–571.

[15] US Environmental Protection Agency, Chlorine, Total Residual (Spectrophotometric, DPD), 1978.

[16] M. Profaizer, Peracetic acid as an alternative technology for drinking water disinfection: a new modelling approach (in Italian), PhD. Dissertation, Politecnico di Milano, 1998.

[17] D. Falsanisi, R. Gehr, D. Santoro, A. Dell'Erba, M. Notarnicola, L. Liberti, Kinetics of PAA demand and its implications on disinfection of wastewaters, Water Qual. Res. J. Canada. 41 (2006) 398–409.

[18] S. Rossi, M. Antonelli, V. Mezzanotte, C. Nurizzo, Peracetic acid disinfection: a feasible alternative to wastewater chlorination., Water Environ. Res. 79 (2007) 341–350.

[19] M. Antonelli, S. Rossi, V. Mezzanotte, C. Nurizzo, Secondary effluent disinfection: PAA long term efficiency, Environ. Sci. Technol. 40 (2006) 4771–4775.

[20] S. Monarca, D. Feretti, C. Zani, M. Rizzoni, S. Casarella, B. Gustavino, Genotoxicity of drinking water disinfectants in plant bioassays, Environ. Mol. Mutagen. 46 (2005) 96–103.

[21] S. Monarca, C. Zani, S.D. Richardson, A.D. Thruston, M. Moretti, D. Feretti, M. Villarini, A new approach to evaluating the toxicity and genotoxicity of disinfected drinking water, Water Res. 38 (2004) 3809–3819.

[22] A. Dell'Erba, D. Falsanisi, L. Liberti, M. Notarnicola, D. Santoro, Disinfecting behaviour of peracetic acid for municipal wastewater reuse, Desalination. 168 (2004) 435–442.

[23] D. Santoro, R. Gehr, T.A. Bartrand, L. Liberti, M. Notarnicola, A. Dell'Erba, D. Falsanisi, C.N. Haas, Wastewater disinfection by peracetic acid: assessment of models for tracking residual measurements and inactivation., Water Environ. Res. 79 (2007) 775–87.

[24] R. Crebelli, L. Conti, S. Monarca, D. Feretti, I. Zerbini, C. Zani, E. Veschetti, D. Cutilli, M. Ottaviani, Genotoxicity of the disinfection by-products resulting from peracetic acid- or hypochlorite-disinfected sewage wastewater, Water Res. 39 (2005) 1105–1113.

[25] L. Guzzella, S. Monarca, C. Zani, D. Feretti, I. Zerbini, A. Buschini, P. Poli, C. Rossi, S.D. Richardson, In vitro potential genotoxic effects of surface drinking water treated with chlorine and alternative disinfectants, Mutat. Res. 564 (2004) 179–193.

[26] A. Buschini, A. Martino, B. Gustavino, M. Monfrinotti, P. Poli, C. Rossi, M. Santoro, A.J.M. Dörr, M. Rizzoni, Comet assay and micronucleus test in circulating erythrocytes of Cyprinus carpio specimens exposed in situ to lake waters treated with disinfectants for potabilization,

Mutat. Res. 557 (2004) 119–129.

[27] US Environmental Protection Agency, BioSide HS15%-1. Evaluation of the Enviro Tech Chemical Services, Inc. Submission for Peroxyacetic Acid Wastewater EUP, Washington D.C., 2006.

[28] L.F. Pedersen, P.B. Pedersen, J.L. Nielsen, P.H. Nielsen, Peracetic acid degradation and effects on nitrification in recirculating aquaculture systems, Aquaculture. 296 (2009) 246–254.

[29] J. Howarth, M. Harvey, Method of analyzing low levels of peroxyacetic acid in water, 2010.

[30] S.S. Block, Disinfection, sterilization, and preservation, 5th ed., Lippincott Williams & Wilkins, 2001.

[31] European Centre for Ecotoxicology and Toxicology of Chemicals, Joint Assessment of Commodity Chemicals (JACC) programme. JACC No. 40 - Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions, Brussels, Belgium, 2001.

[32] M.I. Awad, T. Oritani, T. Ohsaka, Kinetic studies on the oxidation of iodide by peroxyacetic acid, Inorganica Chim. Acta. 344 (2003) 253–256.

[33] M. Antonelli, S. Rossi, V. Mezzanotte, C. Nurizzo, Secondary effluent disinfection: PAA long term efficiency, Environ. Sci. Technol. 40 (2006) 4771–4775.

[34] U. Pinkernell, U. Karst, K. Cammann, Determination of Peroxyacetic Acid Using High-Performance Liquid Chromatography with External Calibration, Anal. Chem. 66 (1994) 2599–2602.

[35] E. Koubek, M.L. Haggett, C.J. Battaglia, K.M. Ibne-Rasa, H.Y. Pyun, J.O. Edwards, Kinetics and Mechanism of the Spontaneous Decompositions of Some Peroxoacids, Hydrogen Peroxide and t-Butyl Hydroperoxide, J. Am. Chem. Soc. 85 (1963) 2263–2268.

[36] C.L. Copper, E. Koubek, Kinetics of the molybdate and tungstate catalyzed oxidation of iodide by hydrogen peroxide, Inorganica Chim. Acta. 288 (1999) 229–232.

[37] D.M. Davies, M.E. Deary, Determination of peracids in the presence of a large excess of hydrogen peroxide using a rapid and convenient spectrophotometric method, Analyst. 113 (1988) 1477–1479.

[38] B.E. Saltzman, N. Gilbert, lodometric Microdetermination of Organic Oxidants and Ozone. Resolution of Mixtures by Kinetic Colorimetry, Anal. Chem. 31 (1959) 1914–1920.

[39] R.D. Cadle, H. Huff, The The Oxidation of Iodide to Iodine by Dilute Solutions of Organic Peroxides., J. Phys. Colloid Chem. 54 (1950) 1191–1195.

CHAPTER 5. INFLUENCE OF INORGANIC AND ORGANIC COMPOUNDS ON PAA DECAY DURING WASTEWATER DISINFECTION

Abstract

The aim of this study was to evaluate the influence of the physical-chemical characteristics of wastewater on peracetic acid (PAA) decay, in multi-component solutions of inorganic and organic compounds (11 compounds in total) representative of a secondary effluent of a wastewater treatment plant, disinfected at various PAA concentrations (2 to 5 mg/L). Batch experiments were defined using the statistical method of the Design of Experiments (DoE) in order to evaluate the effect of each compound and/or their interaction on PAA decay. Results showed that the organics consumed immediately a considerable amount of PAA, independently from the initial PAA concentration, and consumption dropped rapidly to almost nil after 5 minutes, whereas PAA consumption due to the inorganics was slow, depending from the initial PAA concentration and persisted until the end of the experiments (60 minutes). In detail, inorganics (such as reduced iron and orthophosphate) have shown to be the main drivers of the exponential decay: iron, particularly, has proved to directly consume PAA due to its catalyzing capacity, whereas orthophosphate has shown to mainly interact with iron, acting as chelating compound toward iron and consequently reducing the iron effect in consuming PAA. As for organics, proteins such as, casein and peptone, have been highlighted as the main cause of the initial PAA demand, probably due to the homolytic fission of PAA to generate peroxyl and hydroxyl radicals, which are known to have a high reactivity towards proteins. Finally, a model for predicting the residual PAA concentration was obtained and validated; uncertainty analysis was also performed by a series of Monte Carlo simulations to propagate input uncertainties to the model output.

Keywords: Wastewater disinfection, peracetic acid, decay rate, oxidative demand, uncertainty analysis.

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5.1. Introduction

The ongoing population growth has often challenged the water supply systems in many areas of the world [1], leading to severe shortage of water. Consequently, the reuse of treated wastewater to reduce the gap between water demand and available water resources is drawing increasing attention in the recent years [2]. In particular, reclaimed water from treated municipal effluents is increasingly used as an alternative source for a wide range of applications, such as irrigation of public areas and crops, industrial use and groundwater recharge [3]. Regardless of the type of wastewater reuse, a disinfection stage is required in wastewater treatment plants (WWTPs) to ensure a good microbiological quality of the discharged effluent [4]. Chlorine-based compounds have been the most widely used disinfectants for over a century even though many studies [5–7] have reported that reactions between chlorine and organic matter lead to the formation of carcinogenic disinfection by-products in the treated effluents. For this reason, alternative disinfecting agents, such as PAA, have been investigated for wastewater treatment over the past years [8]. PAA has proved to be effective against many microorganisms [9–12], guaranteeing no appreciable re-growth after disinfection [13]. In addition, no harmful disinfection by-products (DBPs) are formed during PAA treatment [14–17] as presented in Chapter 3. Concerning the application of PAA disinfection in WWTPs, initial PAA concentration and contact time have been widely studied as the most relevant operating parameters for determining PAA disinfection efficacy against microorganisms. In particular, several works [11,18–20] showed that initial PAA concentrations from 1 to 15 mg/L and contact times from 15 to 60 minutes can result in proper disinfection of primary, secondary and tertiary effluents. However, PAA decomposes rapidly in water solution and the rate of this process is highly affected by the water matrix composition [8,11,12,21–23], as outlined in the State of the Art in Chapter 1. A key step for guarantying a sufficient disinfectant residual concentration to reach bacterial inactivation targets, avoiding excessive residuals consists in the assessment of the influence of wastewater characteristics on PAA decay, in terms of easy-to-measure parameters, and thus, on the actual PAA dose.

Currently, experimental works on this topic are either missing or were carried out as preliminary assessments. Moreover, most of past research works focused on investigating the effect of one factor at a time, without considering the occurrence of interaction effects between two or more factors. The purpose of this study is firstly to identify which inorganic and organic compounds commonly present in the soluble fraction of wastewater can individually and/or jointly affect PAA decay, and secondly the development of a reliable mathematical model to predict residual PAA concentration over time as a function of the constituents of the WWTP effluent. In detail, eleven compounds were evidenced as potentially relevant for PAA decay based on a preliminary literature review as well as on the expertise of experimenters. Inorganic compounds were ammonia nitrogen (NH^{4+}), nitrate (NO^{3-}), nitrite (NO^{2-}), reduced iron (Fe^{2+}) and orthophosphate (PO_4^{3-}), whereas organic compounds were surrogates of carbohydrates (glucose and cellulose), lipids (butyric and oleic acids) and proteins (peptone and casein).

Experiments were divided in two experimental plans, one for inorganics and one for organics, which were defined using the statistical method of the Design of Experiments (DoE). This methodology allowed to evaluate the effect exerted by each compound and to evidence the occurrence of interaction effects. After the development of the model and its validation by a set of experiments in which inorganics and organics displaying a significant effect were simultaneously present, an uncertainty analysis was performed by Monte Carlo simulations to propagate the input uncertainties to model output.

5.2. Material and methods

5.2.1. Reagents and experimental setup

PAA technical grade solution (VigorOx® WWTII) was supplied by PeroxyChem (USA), whose composition as weight percentage is 15% of PAA, 23% of H_2O_2 and 16% of acetic acid. The PAA concentration in the commercial solution was checked monthly by iodometric titration [24]. All chemicals were reagent grade purchased from Sigma Aldrich (USA), except for DPD (N,N-diethyl-p-phenylenediaminesulfate) salt that was purchased from Hach Lange (USA). All the stock solutions for the inorganic compounds were prepared according to Standard Methods [25]. The stock solutions containing the organic compounds or their surrogates, were prepared to have a concentration 1000 mg/L in terms of COD for each compound. The calculations required to prepare the stock solutions were based on the Theoretical Oxygen Demand (ThOD) values of each organic compound [26].

1-hour PAA decay tests were performed in completely mixed batch reactors (1 L glass beakers) mixed by magnetic stirrer in dark conditions at room temperature (20±1°C). In each beaker, a given aliquot of the stock solutions, according to the experimental plan described in sections 5.2.3 and 5.2.4, was added to deionized water and the pH of the solutions was adjusted to 7.5 at the beginning of the tests with sodium hydroxide (NaOH) or sulfuric acid (H_2SO_4) and monitored during the tests. After pH adjustment, a selected aliquot of PAA stock solution was added to obtain the given PAA initial concentration. Samples were collected at five contact times (2, 5, 10, 30 and 60 minutes). Preliminary tests were performed in deionized water to estimate PAA decay in absence of inorganic and organic compounds (blank tests). Experiments were performed in triplicate for three initial PAA concentrations (2, 5 and 10 mg/L).

5.2.2. Experimental plan for inorganics and organics

Two fractional factorial designs, one for inorganics and one for organics, were defined to identify the most important factors affecting PAA decay within a large number of compounds and to assess the occurrence of interaction effects. The choice of having two separate experimental plans was based on two main reasons: (i) a unique experimental plan would have required too many experiments and, (ii) the chemical properties of molecules, as size, polarity, type of molecular bonds, are such that interaction effects between inorganic and organic compounds are neither expected nor, in case of occurrence,

comparable with PAA decay generated by the interaction between two inorganics or two organics [27]. Moreover, the fractional factorial design was selected due to sequential assembly feature allowing experimenters to modulate and develop the design to increase the degree of detail when needed [28].

5.2.3. DoE for inorganics

As a first step, the decay of two initial PAA concentrations (2 and 10 mg/L) over time was tested in 16 batch experiments where 5 inorganic compounds (factors) were combined at two pre-defined concentrations (levels) according to a fractional factorial design of resolution V (Table 1): (A) ammonia nitrogen, (B) nitrate, (C) nitrite, (D) reduced iron and (E) orthophosphate. The levels were set as follows: A and B (0.5 and 25 mg/L as N), C (0.1 and 1 mg/L as N), D (0.1 and 5 mg/L) and E (1 and 5 mg/L as P). Each experiment was repeated three times for a total of 96 experiments. As a second step, PAA decay at 2 and 10 mg/L was also evaluated in presence of either D or E at the following concentrations: 0.1 and 5 mg/L for D, 1 and 5 mg/L for E. Each experiment was performed in triplicate for a total of 24 experiments. The stability of nitrite over 1 hour was verified in preliminary tests by dosing the maximum concentration of nitrite with and without all other inorganic compounds at the highest concentration used in the experimental plan.

5.2.4. DoE for organics

Firstly, the decay of two initial PAA concentrations (2 and 10 mg/L) over time was tested in 16 batch experiments where 6 compounds (factors), namely (A) glucose, (B) cellulose, (C) butyric acid, (D) oleic acid, (E) casein and (F) peptone, were combined at two pre-defined levels (10 and 35 mg/L in terms of COD) according to a fractional factorial design of resolution IV (Table 1). Surrogates of compounds B, C and D were used for the tests, namely carboxymethylcellulose, sodium butyrate and sodium oleate, respectively. Each experiment was repeated three times for a total of 96 experiments.

Secondly, the following steps have been accomplished: (i) the performance of a full factorial design to evaluate the combined effect of factors E and F on PAA decay at 5 mg/L, and (ii) the evaluation of PAA decay at 2 and 5 mg/L in presence of either E or F at the concentrations of 10 and 35 mg/L as COD for both factors. Twelve experiments (4 for the full factorial and 8 for the single trials) were performed and replicated three times for a total of 36 experiments. Finally, (iii) the effect of urea as a single compound on PAA decay was tested by *ad hoc* experiments at two different concentrations of PAA (2 and 5 mg/L). In detail, the urea concentrations were set to have the same concentration of the organic nitrogen present in both casein and peptone at their lowest and highest concentrations used in the decay experiments, namely 2 and 8 mg/L as N.

Table 1. Experimental plan (fractional factorial design) for inorganic and organic compounds in coded units. The two levels of concentration for each compound are identified by '-' for low concentration value and '+' for high concentration value.

Inorganics					Experiment	**Organics**					
A	**B**	**C**	**D**	**E**		**A**	**B**	**C**	**D**	**E**	**F**
-	-	-	-	+	1	-	-	-	-	-	-
+	-	-	-	-	2	+	-	-	-	+	-
-	+	-	-	-	3	-	+	-	-	+	+
+	+	-	-	+	4	+	+	-	-	-	+
-	-	+	-	-	5	-	-	+	-	+	+
+	-	+	-	+	6	+	-	+	-	-	+
-	+	+	-	+	7	-	+	+	-	-	-
+	+	+	-	-	8	+	+	+	-	+	-
-	-	-	+	-	9	-	-	-	+	-	+
+	-	-	+	+	10	+	-	-	+	+	+
-	+	-	+	+	11	-	+	-	+	+	-
+	+	-	+	-	12	+	+	-	+	-	-
-	-	+	+	+	13	-	-	+	+	+	-
+	-	+	+	-	14	+	-	+	+	-	-
-	+	+	+	-	15	-	+	+	+	-	+
+	+	+	+	+	16	+	+	+	+	+	+

5.2.5. Validation of the model

The validation of the final model was performed by testing the consumption of two PAA concentrations (2 and 5 mg/L) in four *ad hoc* batch experiments where the inorganic and organic compounds that displayed a significant effect on PAA decay were added simultaneously, as shown in Table 2. In detail, four compounds namely (A) casein, (B) peptone, (C) reduced iron and (D) orthophosphate were combined at two pre-defined concentrations, which were set at the corresponding ones for each compound in the DoEs of inorganics and organics. Each experiment was performed in triplicate for a total of 24 experiments.

Table 2. Experimental plan for the validation of the model. The two levels of concentration for each compound are identified by '-' for low concentration value and '+' for high concentration value.

Experiment	**Factor**			
	A	**B**	**C**	**D**
1	+	+	-	-
2	+	+	+	+
3	-	-	-	-
4	-	-	+	+

5.2.6. Analytical procedures

The residual PAA concentration was measured by means of the DPD colorimetric method described in Chapter 4 - Evaluation of a colorimetric method for the measurement of low concentrations of PAA and H_2O_2 in water. Preliminary tests were performed to assess the interference of inorganic and organic compounds on the DPD method for the measurement of residual PAA concentration. In detail, two experiments (one with all inorganics and another one with all organics) were performed in absence of PAA. In each test the compounds were added at the highest concentrations used in the experimental plans and four repetitions were carried out.

The stability over 60 minutes of the nitrite ion (NO_2^-) in solution at the experimental conditions adopted was verified using a Hach Lange kit LCK 341. The COD of the stock solutions of organic compounds was measured by Hach Lange kit LCK 514, whereas the total nitrogen concentrations of the stock solutions of casein and peptone were measured by Hach Lange kit LCK 338. These analyses were performed using a spectrophotometer Dr. Lange XION 500. The pH was measured during the tests with a Eutech 6+ pH meter.

5.2.7. Data processing

The standard curve for PAA measurement was obtained through a linear least-square regression (R^2 = 0.9995). The equation is: ABS = $\alpha + \beta \cdot$[PAA], where ABS is the absorbance value at 530 nm and [PAA] is the PAA concentration (mg/L). Estimated coefficients were: α = 0.0624, β = 0.5563.

Values of residual PAA concentration measured during the tests were plotted over time for each experiment (5 PAA residual concentration in triplicate, for a total of 15 data points) and interpolated with a non-linear least-square regression using as models both the first-order kinetic model (Equation 1) and the Haas and Finch kinetic model (Equation 2) [29].

$$[PAA] = [PAA]_o \cdot e^{-kt} \quad \text{(e.1)}$$

$$[PAA] = ([PAA]_o - D) \cdot e^{-kt} \quad \text{(e.2)}$$

where $[PAA]_0$ is the initial PAA concentration (mg/L), k is the decay kinetic rate constant (min^{-1}) and D is the initial oxidative consumption (mg/L). Mathworks Matlab R2015a software was used for the estimation of coefficients. Furthermore, the estimated coefficients were used as inputs in Minitab 17 software for the statistical analysis of data. As for the uncertainty analysis, a Monte Carlo simulation framework has been coded in Mathworks Matlab R2015a.

5.3. Results and discussion

5.3.1. Preliminary tests

The preliminary tests demonstrated the stability of nitrite in solution under different conditions over 1-

hour contact time, making nitrite adequate for the experimentation. As for the interference tests on PAA measurements due to inorganic and organic compounds, a negligible effect was observed in all the performed experiments: absorbance values were always below the lowest extreme of the standard curve, corresponding to PAA concentration of 0.176 mg/L, suggesting that inorganic and organic compounds do not bias PAA measurement.

Concerning the blank tests, the regression of experimental data by the Haas and Finch kinetic model [29] evidenced the negligible role of oxidative demand on PAA decay (p-values > 0.05) with values lower than 0.03 mg/L for all tested concentrations, meaning that first-order kinetic model is adequate to describe PAA decay in deionized water. Estimated values for the decay kinetic rate constant k_{blank} differed depending on the initial PAA concentration as shown in Table 3. Finally, k_{blank} was modelled as reported by Equation 3, resulting from linear interpolation among initial PAA concentrations and estimated k_{blank} values ($R^2 = 0.9439$, N = 3):

$$k_{blank} = 0.00128 - 6.25e^{-5} \cdot [PAA]_0 \quad \text{(e.3)}$$

Table 3. Main statistics and regression estimates of k_{blank} for the first-order kinetic model (CI: confidence interval at 95% of significance; N: number of data used for the fitting).

$[PAA]_0$ (mg/L)	**k_{blank} (min^{-1})**					
	Estimate	**SE (Standard Error)**	**CI (95%)**	**R^2**	**p-value**	**N**
2	0.0012	0.0001	0.0010, 0.0015	0.9013	< 0.000	15
5	0.0009	0.0001	0.0006, 0.0012	0.8000	< 0.000	15
10	0.0007	0.0001	0.0005, 0.0009	0.8400	< 0.000	15

5.3.2. Effect of inorganics on PAA decay

The Haas and Finch kinetic model demonstrated to fit better all experimental data for inorganics than first-order kinetic model, as reported in Supplementary Material (Figure S1 and S2). In particular, PAA decay exhibited a scarce oxidative demand (ranging intervals 1.8% - 12.4% and 1.5% - 11.5% for an initial PAA concentration of 2 and 10 mg/L, respectively) followed by a pronounced exponential decay (up to 14 and 18 times higher than k_{blank} for 2 and 10 mg/L, respectively). In Figure 1 the model coefficients for the 16 experiments with corresponding confidence intervals at 95% of significance are reported. The decay rate constants have been normalized with respect to the decay rate constant of the blank tests (k_{blank}).

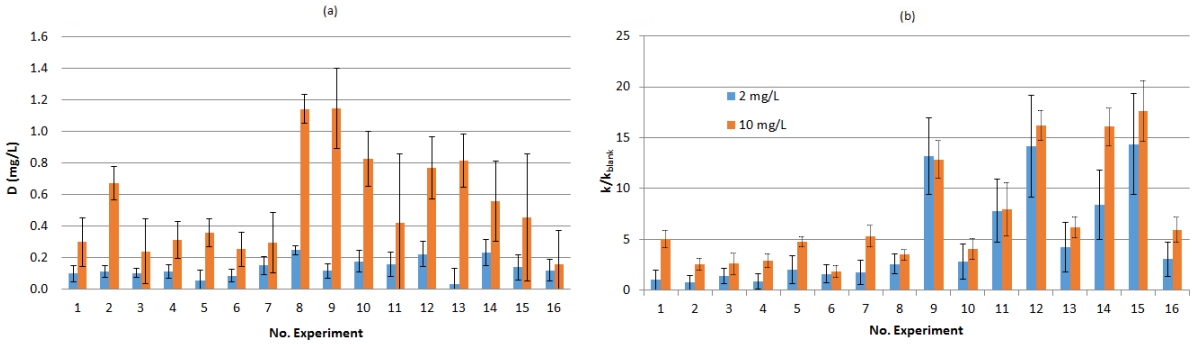

Figure 1. Oxidative demand D (a) and normalized decay rate constant k/k_{blank} (b) of Haas and Finch kinetic model for experiments at 2 and 10 mg/L on inorganics. Confidence intervals at 95% of significance are reported.

5.3.3. Effect of inorganics on oxidative demand

With regard to the oxidative demand, a rigorous analysis of data did not highlight any statistically significant correlation between the D values and the presence of inorganics. Therefore, a graphical inspection (often efficient for data obtained using DoE methods) was adopted. D values in Figure 1a can be sub-grouped as follow: from experiment 1 to 7, where the D values are low (with exception of experiment 2 for 10 mg/L) and the confidence intervals are narrow, and from experiment 8 to 16, where high D values and wide confidence intervals are observed (with the exception of experiment 8 where a very narrow confidence interval is observed for both initial PAA concentrations). Specifically, these two modes for confidence intervals are not fortuitous, being the same trend also noticeable for k/k_{blank} values of Figure 1b. Indeed, experiments from 9 to 16 were performed with high iron concentration (see Table 1). A variability analysis subsequently determined a strong relation between the variability of D values and the presence of iron (Supplementary Material, Figure S3). As for the means of D values, the highest values were observed for experiment 8 (0.25±0.03 and 1.15±0.25 mg/L for 2 and 10 mg/L, respectively), where iron and orthophosphate are present at low concentration, whereas ammonia nitrogen, nitrate and nitrite are present at high concentrations. In detail, the contribution of each of these nitrogen compounds on oxidative demand can be observed by looking at D values of experiments 2, 3 and 5, where only ammonia nitrogen, or nitrate, or nitrite were present at high concentration (see Table 1). It is important to highlight that the addition of these values (0.27±0.13 and 1.27±0.40 mg/L for 2 and 10 mg/L, respectively) is statistically comparable (confidence interval at 95% of significance) with the D value of experiment 8, meaning that the effect of these compounds is additive, and no interaction between these inorganics occurs. As a consequence of these considerations, *ad hoc* experiments were performed in absence of iron to precisely investigate the effect that ammonia nitrogen, nitrate and nitrite have on oxidative demand. In particular, a full factorial design was carried out (Supplementary Material, Table S1). Experimental results evidenced negligible D values ranging from no consumption to maximum values of 0.04±0.02 and 0.12±0.06 mg/L respectively for 2 and 10 mg/L, confirming the additive effect. However, estimated D values are low with respect to those in Figure 1a, meaning that oxidative demand

is mainly generated by iron or orthophosphate or an interaction between them.

5.3.4. Effect of inorganics on decay rate

As for the decay rate constants, reported in Figure 1b, the statistical analysis was accomplished through a step-wise regression that sorted each compound by the relevance in affecting the decay rate. Data analysis, as shown in Figure 2, highlighted that iron, orthophosphate and their interaction are the only significant factors (confidence level at 95%). Subsequently, *ad hoc* experiments were performed to investigate the experimental sub-space of iron and orthophosphate towards null values of one or both compounds, as shown in Figure S4 of Supplementary Material, and to incorporate the experimental data from blank tests. Finally, a least-square regression was used to interpolate a linear model as reported by Equation 4:

$$k = k_{blank}(b_0 + b_1 \cdot Fe^{2+} + b_2 \cdot PO_4^{3-} + b_{12} \cdot Fe^{2+} \cdot PO_4^{3-}) \qquad \text{(e.4)}$$

Table 4 shows the main statistics and regression estimates for the models at 2 and 10 mg/L, while a graphical representation of the model is given in Figure S5 in Supplementary Material. The choice to interpolate data with a linear model is reasonable (p-value for the lack-of-fit test higher than 0.05). Both models describe experimental data satisfactorily (R^2 equals to 0.8870 and 0.9420), with high accuracy on predictions (R^2_{pred} equals to 0.8247 and 0.9119). Residual analysis (not reported) assessed the appropriateness of linear regression hypothesis (normality and homoscedasticity).

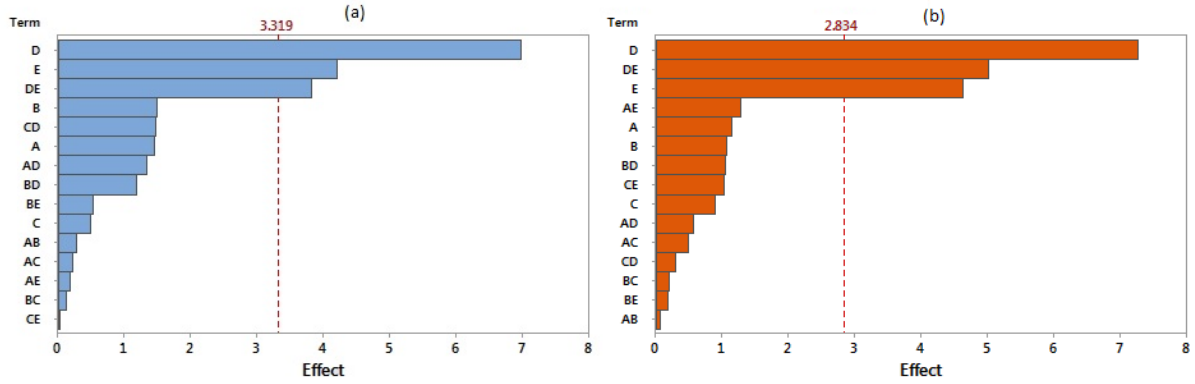

Figure 2. Pareto chart showing factors affecting PAA decay rate at 2 (a) and 10 (b) mg/L: the red dashed line represents the significance threshold at 0.05.

Table 4. Main statistics and regression estimates of the model for the effect of inorganics on PAA decay rate.

$[PAA]_0$ (mg/L)	Symbol	Estimate	SE (Standard Error)	CI (95%)	T-Value	p - value
2	b_0	1.312	0.761	-0.286, 2.910	1.72	0.102
	b_1	2.459	0.238	1.958, 2.960	10.31	< 0.000
	b_2	0.033	0.231	-0.453, 0.519	0.14	0.887
	b_{12}	-0.367	0.069	-0.512, -0.222	-5.31	< 0.000
	$R^2 = 0.8870$; $R^2_{adj} = 0.8682$; $R^2_{pred} = 0.8247$; N = 21; Lack-of-fit (p-value) = 0.706					
10	b_0	2.788	0.661	1.399, 4.177	4.22	0.001
	b_1	3.149	0.207	2.714, 3.585	15.19	< 0.000
	b_2	0.144	0.201	-0.278, 0.566	0.72	0.500
	b_{12}	-0.531	0.060	-0.657, -0.405	-8.85	< 0.000
	$R^2 = 0.9420$; $R^2_{adj} = 0.9323$; $R^2_{pred} = 0.9119$; N = 21; Lack-of-fit (p-value) = 0.610					

Summarizing, reduced iron proved to be the most important factor affecting PAA decay (p-value for b_1 < 0.0001 for both initial PAA concentrations), probably due to its capacity to catalyze PAA decomposition, as discussed by Yuan et al. [30]. On the other hand, orthophosphate does not affect PAA decay (p-value for b_2 equals to 0.887 and 0.500 for 2 and 10 mg/L, respectively). However, the term is kept in the model for hierarchical reasons, whereas significantly interacts with iron (p-value for b_{12} < 0.0001 for both PAA concentrations). The chemical effect of this interaction can be attributed to the role of orthophosphate in acting as chelating compound towards iron, inhibiting its catalyzing effect towards PAA. In accordance with the literature [31–36], phosphate molecules can surround heavy metals avoiding their precipitation (known as sequestration), as it occurred in present case, in which no precipitate was observed during experiments.

Based on experimental data, when both compounds are at high concentration, the orthophosphate is enough to sequester iron, leading to k/k_{blank} values that are comparable with those observed when low concentration of iron is present. On the other hand, when iron and orthophosphate are at high and low concentration respectively, orthophosphate do not sequester iron completely, resulting in the highest k/k_{blank} values observed. As for the role of initial PAA concentration, the two models can be statistically considered different (see Figure S5b in Supplementary Material which shows that the confidence intervals do not overlap), meaning that the initial PAA concentration affects PAA decay. In particular, modeling–results demonstrate that the higher the initial PAA concentration, the stronger the PAA interaction with iron and orthophosphate leading to a fast PAA decay.

Finally, supposing a linear behavior between the coefficients of models and the initial PAA concentration, a unique formulation for decay rate constants is proposed in the equations:

$$b_0 = 0.943 + 0.185 \cdot [PAA]_0 \quad \text{(e.5)}$$

$$b_1 = 2.286 + 0.086 \cdot [PAA]_0 \quad \text{(e.6)}$$

$$b_2 = 0.005 + 0.138 \cdot [PAA]_0 \quad \text{(e.7)}$$

$$b_{12} = -0.326 - 0.020 \cdot [PAA]_0 \quad \text{(e.8)}$$

5.3.5. Effect of organics on PAA decay

The regression on experimental data at 2 mg/L highlighted a sizeable oxidative demand within the first five minutes (D values ranging between 9.1% and 27.2% of the initial PAA concentration) followed by a smooth exponential decay (k values from 1 to 8 times higher than k_{blank}) (see also Figure S6 of Supplementary Material for model fitting). Figure 3 shows the model coefficients for the 16 experiments with the corresponding confidence interval at 95% of significance. Contrarily to the results for inorganics, only oxidative demand values were processed by statistical analysis: as for decay rate constants, the high variability results in mean values not statistically different (ANOVA test with p-value > 0.05). Moreover, the small values of normalized decay rate constants lead to conclude that organics slightly affect PAA decay after the first five minutes. Consequently, it can be inferred that all organics are oxidized by PAA in a short time and their oxidized products, which cannot further affect significantly PAA decay, remain in solution; however, the influence of these reaction by-products on PAA decay could explain the observed k values slightly higher than the k_{blank}. Nevertheless, further investigations are needed.

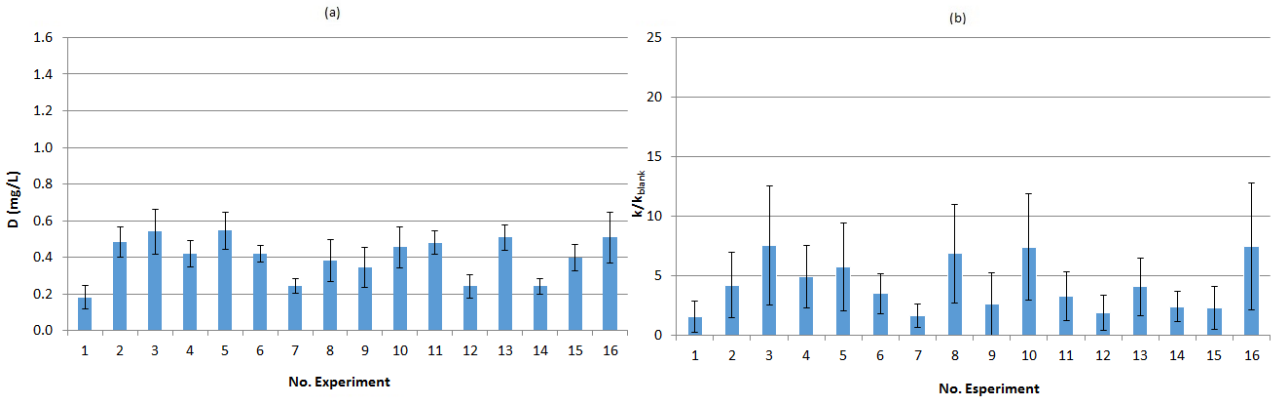

Figure 3. Oxidative demand D (a) and normalized rate constant k/k_{blank} (b) of Haas and Finch kinetic model for experiments at 2 mg/L when organics are present. The confidence intervals are at 95% of significance.

As for 10 mg/L initial PAA concentration, the high variability of the experimental data lead to poor quality of raw data (Supplementary Material, Figure S8). The reason for this outcome is not clear and

further investigations are needed. Furthermore, based on the assumption that the high initial PAA concentration could have been the cause of the poor quality of the raw data, an extra DoE has been performed at the intermediate concentration of 5 mg/L. In this case, a full factorial design in two factors has been run. The choice of these factors comes from the results of the step-wise regression for the D values at 2 mg/L, later detailed in paragraph 3.3.1, where only proteins (casein and peptone) were highlighted as influencing factors for PAA decay. At 5 mg/L a strong oxidative demand within the first five minutes was observed (D values ranging between 4.0% and 12.8% of the initial PAA concentration) followed by a negligible exponential decay (k values ranging from 1 to 3 times higher than the k_{blank}).

5.3.6. Effect of organics on oxidative demand

In Figure 4, D values at 2 mg/L (obtained from the fractional factorial design) and 5 mg/L (obtained from the full factorial design) are reported. It is important to point out that D values at 2 mg/L can be used for the comparison, thanks to the projection property of the fractional factorial design. Indeed, the planned fractional factorial design has resolution IV and projectivity III, meaning that if two of the 6 factors are inert, the design results in a complete 2^4 full factorial design with the remaining factors. Consequently, the fractional factorial design at 2 mg/L can be projected from a six-dimensional space to a two-dimensional space considering only casein and peptone, that were evidenced as statistically significant.

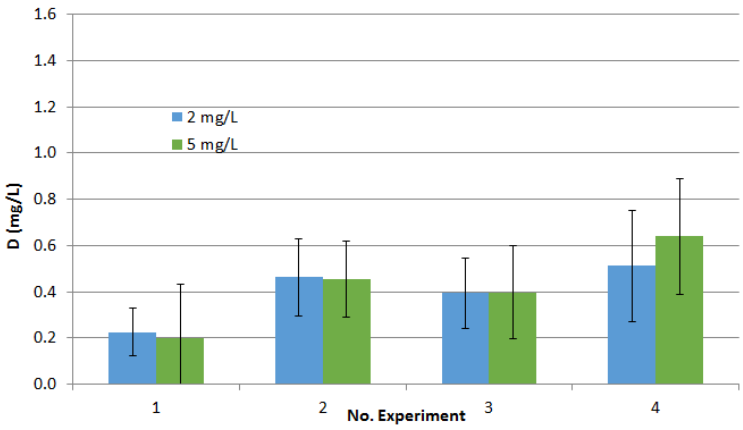

Figure 4. Oxidative demand D of Haas and Finch kinetic model for experiments at 2 and 5 mg/L when organics are present. The confidence intervals are at 95% of significance.

As shown in Figure 4, mean values at 2 and 5 mg/L almost coincide, suggesting that the organic compounds are limiting the reaction with PAA for both initial PAA concentrations. Finally, *ad hoc* experiments were performed to investigate the experimental sub-space of casein and peptone towards null values of one or both compounds, and to incorporate the results from the blank test. A linear least-square regression was used to interpolate a linear model as reported by Equation 9:

$$D = b_3 + b_4 \cdot caseine + b_5 \cdot peptone \qquad \text{(e.9)}$$

In Table 5 the main statistics and regression estimates for the model are shown. The choice to interpolate data by a unique model for both initial PAA concentrations is justified by the complete overlap between the confidence intervals of D values at 2 and 5 mg/L, indicating a non-dependence from this parameter. Instead, a linear model is not the most reasonable for data interpolation (p-value for the lack-of-fit test lower than 0.05). However, the moderately high values of R-square (R^2 = 0.9205) and predicted R-square (R^2_{pred} = 0.9032) indicate that the model is enough accurate and predictive.

Table 5. Main statistics and regression estimates of the model for the effect of organics on PAA oxidative demand.

Symbol	Estimate	SE (Standard Error)	CI (95%)	T-Value	p -value
b_3	0.046	0.018	0.010, 0.083	2.61	0.014
b_4	0.009	0.006	0.008, 0.010	9.98	< 0.000
b_5	0.006	0.006	0.005, 0.007	15.37	< 0.000
R^2 = 0.9205; R^2_{adj} = 0.9153; R^2_{pred} = 0.9032; N = 34; Lack-of-fit (p-value) < 0.044					

In conclusion, both casein and peptone individually affect oxidative demand (p-value < 0.001 for both b_3 and b_4) while no interaction seems to occur, as also supported by a forward step-wise regression that has excluded the interaction term.

Moreover, casein seems to consume more PAA than peptone ($b_3 > b_4$). The oxidative demand determined by these compounds can be attributed to the role of PAA in acting as a protein denaturant [37,38]. Indeed, the peroxyl and hydroxyl radicals that PAA forms through homolytic fission might oxidize sulfhydryl and sulfur bonds present in proteins. It can be inferred that PAA acts towards protein compounds as it does with microorganisms, mainly attacking the lipoprotein cytoplasmic membrane and disrupting their chemiosmotic function [39–42]. However, this hypothesis requires specific investigations to be confirmed.

As a final step, an easy-to-measure indicator of organic compounds that displayed a significant effect on PAA decay has been assessed. Casein and peptone are a protein and a peptide respectively, both consisting of chains of amino acids, which are mainly formed by an amine and a carboxyl group. Particularly, amines are nitrogen-containing groups that allow to differentiate proteins from other organic compounds such as carbohydrates and lipids. Therefore, experimental results stress the previous statements about the ineffectiveness of conventional macro-indicators of organic content. Accordingly, in the view of referring the presented model to a unique indicator, the organic nitrogen could represent a valuable alternative. In order to verify such hypothesis, the effect of urea on PAA decay was evaluated by *ad hoc* experiments. However, as reported in Figure S7 in Supplementary Material no significant effect was observed with respect to blank tests, indicating that not all organic nitrogen but protein nitrogen affects PAA decay.

In conclusion, the experimental evidence suggests that the model can be expressed in terms of protein nitrogen if the values for the coefficients are re-calculated and merged by considering the composition

of the two organic compounds (0.138 $mg_{protein\ N}/mg_{caseine}$, 0.130 $mg_{protein\ N}/mg_{peptone}$). The result is $b_{4\text{-}5}$ = 0.123, that is a unique coefficient for describing the influence of protein nitrogen on PAA decay. For the application of this model to WWTP effluents, given the issues related to analytical determination, the protein nitrogen can be roughly estimated as a percentage of the total organic nitrogen, according to data in literature for various types of effluents and sources [43,44].

5.3.7. Uncertainty analysis and validation of the model

Equations 3, 4 and 9 were integrated in the Haas and Finch kinetic model (e.2) in order to obtain a unique model for predicting the residual PAA concentration over time as a function of inorganics, organics and initial PAA concentration. Subsequently, the uncertainty analysis was performed in three steps: (i) identification and quantification of uncertainty in model inputs, (ii) propagation of the uncertainties through a Monte Carlo simulation, and (iii) statistical analysis of the model results. As for the first step, model inputs were divided in two groups: a first group formed by inorganics (Fe^{2+},PO_4^{3-}), organics (casein and peptone) and initial PAA concentration ($[PAA]_0$), which can be either measured or spiked into a water matrix with a certain precision and accuracy depending on instruments and labware, and a second group formed by model coefficients (b_1, b_2, b_3, b_4, and b_5), which are the results of the linear least-square regressions previously described. Uncertainties associated with the first group can be quantified only by a precise evaluation of the errors due to measurement and laboratory procedures (e.g., dilution error, sampling error, etc.). These uncertainties were not considered in the present work. Only uncertainties associated to the second group were quantified and propagated through the model, being normal probability density functions (normal pdf) assumed for all coefficients. In particular, at each function a mean and a standard deviation, respectively equal to the estimate value and the standard error of the coefficient, were assigned. Moreover, correlation between coefficients was taken into account by the correlation matrixes obtained from the regression. As for the second step, a classical Monte Carlo simulation was used to propagate coefficient uncertainties to the system output. Specifically, the code simulates 10^4 lives of the system: for each life, all system coefficients are randomly sampled from correlated normal distributions and a value of k and D is calculated. Then, the residual PAA concentration is simulated over time. Figure 6 shows the output of the Monte Carlo simulation carried out for the input values and the correlation matrix reported in Table 6. As for the last step, the outputs of simulations were processed from a frequentist point of view. As an example, frequency histograms for exemplary Monte Carlo simulation results at three different times (2, 30 and 60 min) and the related interpolations with normal pdf (blue, green and red line) are shown in Figure 5. The normal pdf fits the data with good accuracy and a confidence interval for residual PAA concentration can be easily obtained. For example, it can be concluded with a 95% of confidence that the predicted residual PAA concentration after 60 minutes ranges within 0.716 and 0.856 mg/L (estimated mean and standard deviation of the normal pdf equals to 0.786 and 0.035 mg/L, respectively).

Finally, a series of *ad hoc* experiments were planned with a dual objective: (i) to validate the final model,

and (ii) to detect the occurrence of interaction effects between inorganics and organics. In particular, PAA decay at two initial PAA concentrations, namely 2 and 5 mg/L, was evaluated in case of simultaneous presence of inorganics and organics. In Figure 6 the experimental data with 95% confidence intervals were compared with simulated data under various operating conditions. Overall, the model effectively predicts residual PAA concentrations for most of the tested cases. Additionally, these results highlight the absence of significant interaction effects between inorganic and organic compounds, confirming what was expected initially by the experimenters.

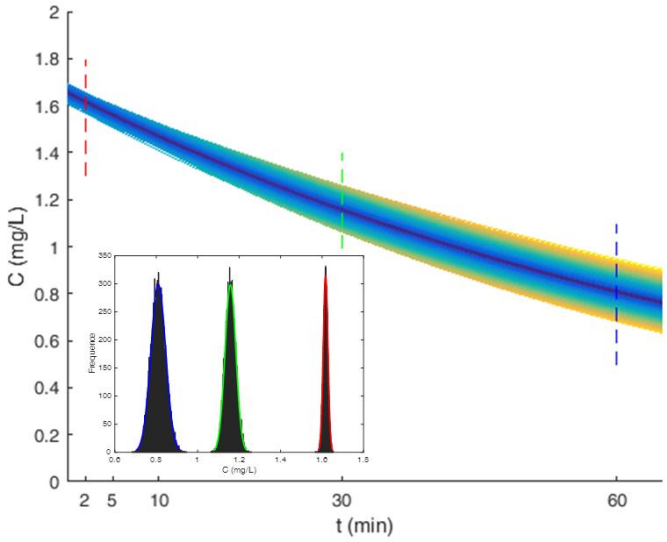

Figure 5. Results of the exemplary Monte Carlo simulation for 10^4 simulations. In the detail the output distribution (approximated by a normal pdf) of the model at three different sections (2, 30 and 60 minutes).

Table 6. Input values and correlation matrix used for the exemplary Monte Carlo simulation.

Input values	**Coefficient**	**Correlation matrix**						
		b_0	b_1	b_2	b_{12}	b_3	b_4	b_5
$[PAA]_0$ = 2 mg/L	b_0	1	-0.65	-0.76	0.51			
	b_1	-0.65	1	0.49	-0.78			
Fe^{2+} = 4 mg/L	b_2	-0.76	0.49	1	-0.68			
PO_4^{3-} = 1 mg/L	b_{12}	0.51	-0.78	-0.68	1			
	b_3					1	-0.59	-0.59
casein = 25 mg/L	b_4					-0.59	1	-0.07
peptone = 10 mg/L	b_5					-0.59	-0.07	1

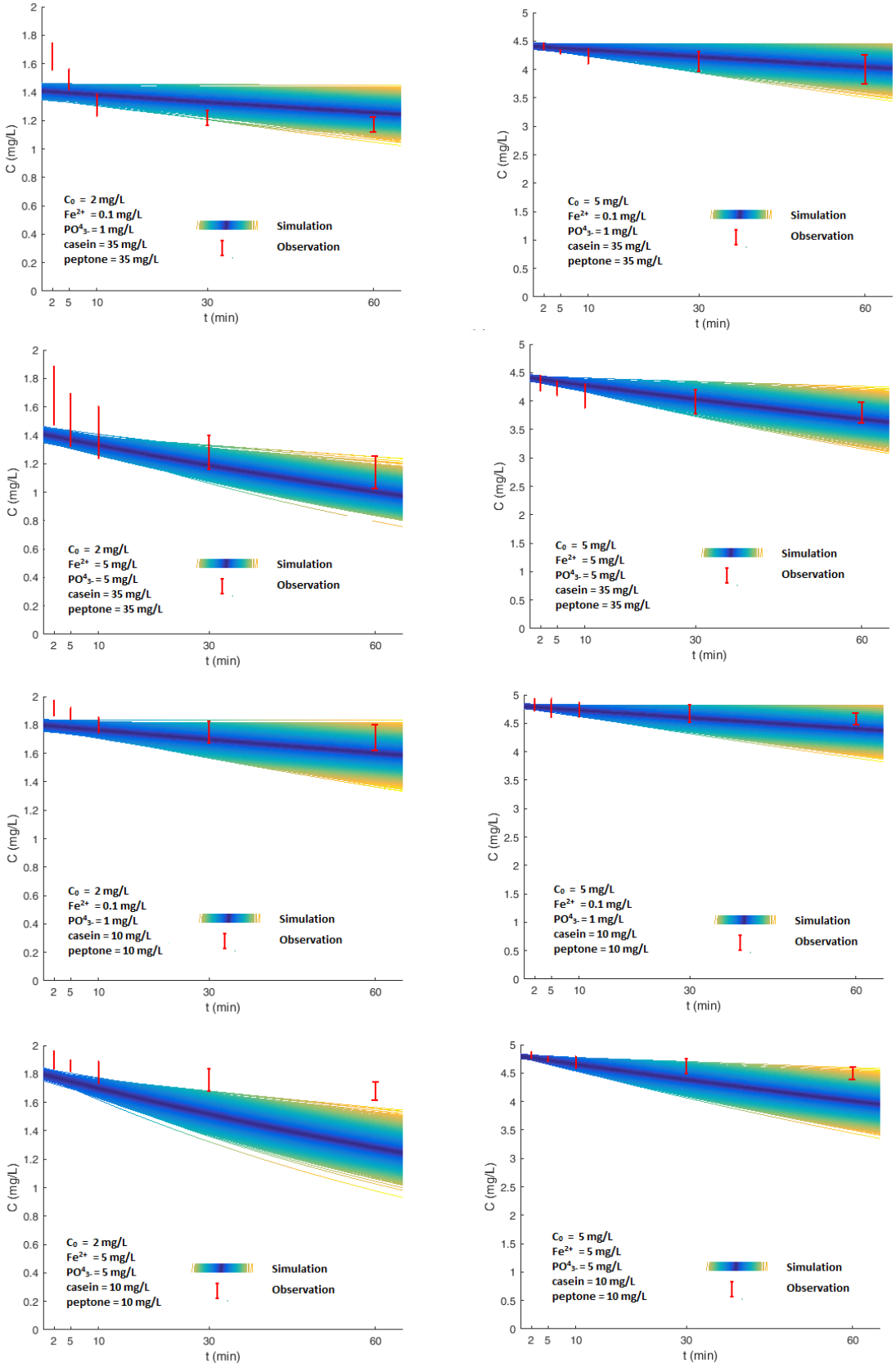

Figure 6. Experimental (mean±CI) and simulated data for the validation of PAA decay model.

5.4. Conclusions

Inorganics and organics compounds displayed different roles in PAA decay, specifically inorganics affected the rate constant, being responsible of PAA residual concentration at a given contact time, while organics defined the instantaneous PAA demand, indicating the required minimum initial PAA concentration. In particular, PAA decay rate was driven mainly by the presence of transition metals (reduced iron) and was slightly depending on initial PAA concentration. On the other hand, PO_4^{3-} acts as a chelating agent, counteracting the effect of transition metals and stabilizing residual PAA concentration. Inorganic nitrogen compounds at concentrations typical of secondary effluents do not display any significant effect in determining PAA decay rate. As for the organics, among the compounds used as surrogates of the main components of secondary effluents, only proteins affected PAA initial demand, while carbohydrates and lipids did not display any effect. In detail, proteins consumed instantaneously a significant amount of PAA, independently from the initial PAA concentration, and PAA consumption dropped rapidly after 5 minutes to almost nil.

Based on experimental evidences, a unique predictive model has been formulated to define the proper PAA dosage based on effluent chemical composition to obtain the actual concentration available for disinfection. The usefulness of this predictive model for PAA decay is in achieving specific bacteria inactivation targets avoiding disinfectant overdosage. This has not only economic implications, but also allows to prevent high residual concentrations of the disinfectant in the effluents, which might lead to toxic effects in aquatic ecosystems and the potential formation of genotoxic and mutagenic disinfection by-products.

5.5. References

[1] D. Pimentel, M. Pimentel, World Population, Food, Natural Resources, and Survival, World Futur. J. Gen. Evol. 59 (2003) 145–167.

[2] US Environmental Protection Agency, Guidelines for Water Reuse. Report EPA/625/R-04/108, Washington D.C., 2012.

[3] D. Bixio, C. Thoeye, J. De Koning, D. Joksimovic, D. Savic, T. Wintgens, T. Melin, Wastewater reuse in Europe, Desalination. 187 (2006) 89–101.

[4] World Health Organization, Guidelines for Drinking-water Quality. Recommendations, Geneva, 2008.

[5] J.J. Rook, Formation of haloforms during chlorination of natural waters, 23 (1974) 234–243.

[6] S.D. Richardson, M.J. Plewa, E.D. Wagner, R. Schoeny, D.M. DeMarini, Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research, Mutat. Res. 636 (2007) 178–242.

[7] S. Monarca, S.D.S.D. Richardson, D. Feretti, M. Grottolo, A.D.A.D. Thruston, C. Zani, G. Navazio, P. Ragazzo, I. Zerbini, A. Alberti, Mutagenicity and disinfection by-products in surface

drinking water disinfected with peracetic acid., Environ. Toxicol. Chem. 21 (2002) 309–18.

[8] T. Luukkonen, S.O. Pehkonen, Peracids in water treatment: A critical review, Crit. Rev. Environ. Sci. Technol. 0 (2016) 1–39.

[9] M.G. C Baldry, M.S. French, D. Slater, The activity of peracetic acid on sewage indicator bacteria and viruses, Wat. Sci. Tech. 24 (1991) 353–357.

[10] J. Koivunen, H. Heinonen-Tanski, Inactivation of enteric microorganisms with chemical disinfectants, UV irradiation and combined chemical/UV treatments, Water Res. 39 (2005) 1519–1526.

[11] J. Koivunen, H. Heinonen-Tanski, Peracetic acid (PAA) disinfection of primary, secondary and tertiary treated municipal wastewaters, Water Res. 39 (2005) 4445–4453.

[12] T. Luukkonen, J. Teeriniemi, H. Prokkola, J. Rämö, U. Lassi, Chemical aspects of peracetic acid based wastewater disinfection, Water SA. 40 (2014) 73–80.

[13] M. Antonelli, S. Rossi, V. Mezzanotte, C. Nurizzo, Secondary effluent disinfection: PAA long term efficiency, Environ. Sci. Technol. 40 (2006) 4771–4775.

[14] C. Nurizzo, M. Antonelli, M. Profaizer, L. Romele, By-products in surface and reclaimed water disinfected with various agents, Desalination. 176 (2005) 241–253.

[15] A. Dell'Erba, D. Falsanisi, L. Liberti, M. Notarnicola, D. Santoro, Disinfection by-products formation during wastewater disinfection with peracetic acid, Desalination. 215 (2007) 177–186.

[16] S.D. Richardson, Disinfection by-products and other emerging contaminants in drinking water, TrAC - Trends Anal. Chem. 22 (2003) 666–684.

[17] S. Monarca, C. Zani, S.D. Richardson, A.D. Thruston, M. Moretti, D. Feretti, M. Villarini, A new approach to evaluating the toxicity and genotoxicity of disinfected drinking water, Water Res. 38 (2004) 3809–3819.

[18] F. Lefevre, J.M. Audic, F. Ferrand, Peracetic acid disinfection of secondary effluents discharged off coastal seawater, Water Sci. Technol. 25 (1992) 155–164.

[19] V. Lazarova, M. Janex, L. Fiksdal, C. Oberg, I. Barcina, M. Pommepuy, Advanced wastewater disinfection technologies: Short and long term efficiency, Water Sci. Technol. 38 (1998) 109–117.

[20] M. Wagner, D. Brumelis, R. Gehr, Disinfection of wastewater by hydrogen peroxide or peracetic acid: development of procedures for measurement of residual disinfectant and application to a physicochemically treated municipal effluent., Water Environ. Res. 74 (2002) 33–50.

[21] M. Kitis, Disinfection of wastewater with peracetic acid: A review, Environ. Int. 30 (2004) 47–55.

[22] D. Liu, C. Steinberg, D.L. Straus, L. Pedersen, T. Meinelt, Salinity, dissolved organic carbon and water hardness affect peracetic acid (PAA) degradation in aqueous solutions, Aquac. Eng. 60 (2014) 35–40.

[23] L.F. Pedersen, T. Meinelt, D.L. Straus, Peracetic acid degradation in freshwater aquaculture systems and possible practical implications, Aquac. Eng. 53 (2013) 65–71.

[24] D. Falsanisi, R. Gehr, D. Santoro, A. Dell'Erba, M. Notarnicola, L. Liberti, Kinetics of PAA demand and its implications on disinfection of wastewaters, Water Qual. Res. J. Canada. 41 (2006) 398–409.

[25] APHA/AWA/WEF, Standard Methods for the Examination of Water and Wastewater, 22nd ed., Washington, DC., 2012.

[26] J.R. Baker, M.W. Milke, J.R. Mihelcic, Relationship between chemical and theoretical oxygen demand for specific classes of organic chemicals, Water Res. 33 (1999) 327–334.

[27] M. Deborde, U. von Gunten, Reactions of chlorine with inorganic and organic compounds during water treatment-Kinetics and mechanisms: A critical review, Water Res. 42 (2008) 13–51.

[28] G.E.P. Box, J.S. Hunter, W.G. Hunter, Statistics for experimenters : design, innovation, and discovery, Wiley-Interscience, 2005.

[29] C.N. Haas, G.R. Finch, Methodologies for the determination of disinfection effectiveness, AWWA Research Foundation and American Water Works Association, 2001.

[30] Z. Yuan, Y. Ni, A.R. van Heiningen, Kinetics of the peracetic acid decomposition: Part II: pH effect and alkaline hydrolysis, Can. J. Chem. Eng. 75 (1997) 42–47.

[31] R.R. Irani, W.W. Morgenthaler, Iron sequestration by polyphosphates, J. Am. Oil Chem. Soc. 40 (1963) 283–285.

[32] K.G. Klueh, R.B. Robinson, Sequestration of Iron in Groundwater by Polyphosphates, J. Environ. Eng. 114 (1988) 1192–1199.

[33] A. Aklil, M. Mouflih, S. Sebti, Removal of heavy metal ions from water by using calcined phosphate as a new adsorbent, J. Hazard. Mater. 112 (2004) 183–190.

[34] D.L. Varner, S. Skipton, D. Hay, P.J. Jasa, G96-1280 Drinking Water: Iron and Manganese Drinking Water: Iron and Manganese Sources of Iron and Manganese in Drinking Water, 1996.

[35] Z. Elouear, J. Bouzid, N. Boujelben, M. Feki, F. Jamoussi, A. Montiel, Heavy metal removal from aqueous solutions by activated phosphate rock, J. Hazard. Mater. 156 (2008) 412–420.

[36] T.E. Larson, Evaluation of use of polyphosphates in water industry, J. Am. Water Work. Assoc. 49 (1957).

[37] B. Kerkaert, F. Mestdagh, T. Cucu, P.R. Aedo, S.Y. Ling, B. De Meulenaer, Hypochlorous and peracetic acid induced oxidation of dairy proteins, J. Agric. Food Chem. 59 (2011) 907–914.

[38] C.C. Winterbourn, M.B. Hampton, Thiol chemistry and specificity in redox signaling, Free Radic. Biol. Med. 45 (2008) 549–561.

[39] E. Cabiscol, J. Tamarit, J. Ros, Oxidative stress in bacteria and protein damage by reactive oxygen species, Int. Microbiol. 3 (2000) 3–8.

[40] S.P. Denyer, G.S.A.B. Stewart, Mechanisms of Action of Disinfectants, Int. Biodeterior.

Biodegrad. 41 (1998) 261–268.

[41] G. Storz, J.A. Imlayt, Oxidative stress, Curr. Opin. Microbiol. 2 (1999) 188–194.

[42] M. Finnegan, E. Linley, S.P. Denyer, G. McDonnell, C. Simons, J.Y. Maillard, Mode of action of hydrogen peroxide and other oxidizing agents: Differences between liquid and gas forms, J. Antimicrob. Chemother. 65 (2010) 2108–2115.

[43] P.J. Westgate, C. Park, Evaluation of proteins and organic nitrogen in wastewater treatment effluents, Environ. Sci. Technol. 44 (2010) 5352–5357.

[44] E. Pehlivanoglu-Mantas, D.L. Sedlak, Measurement of dissolved organic nitrogen forms in wastewater effluents: Concentrations, size distribution and NDMA formation potential, Water Res. 42 (2008) 3890–3898.

5.6. Supporting Information

Effect of inorganics on PAA decay: An example representative of experimental data and the interpolation functions for both models are shown in Figure S1, referred to the first replicate of experiment #15 of the experimental plan for inorganics (see DoE in Table 1) at 2 mg/L. As it can be observed in Figure S1, the R^2 value for the Haas and Finch model is higher than the value for first-order kinetic model. Same results have been obtained for all the experiments of the experimental plan for inorganics. The all set of raw data is shown in Figure S2.

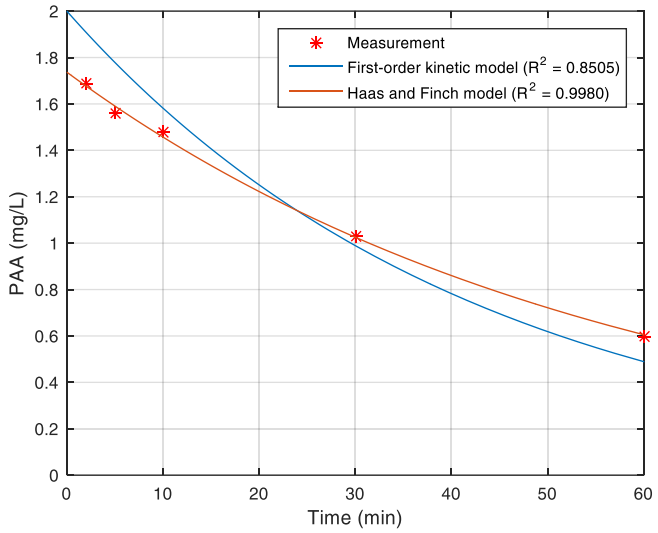

Figure S1. Residual PAA concentration vs. contact time: experimental data (dots) and interpolation functions for the first-order kinetic (blue line) and Haas and Finch (red line) models (2 mg/L, first replicate of experiment #15 of the DoE for inorganics).

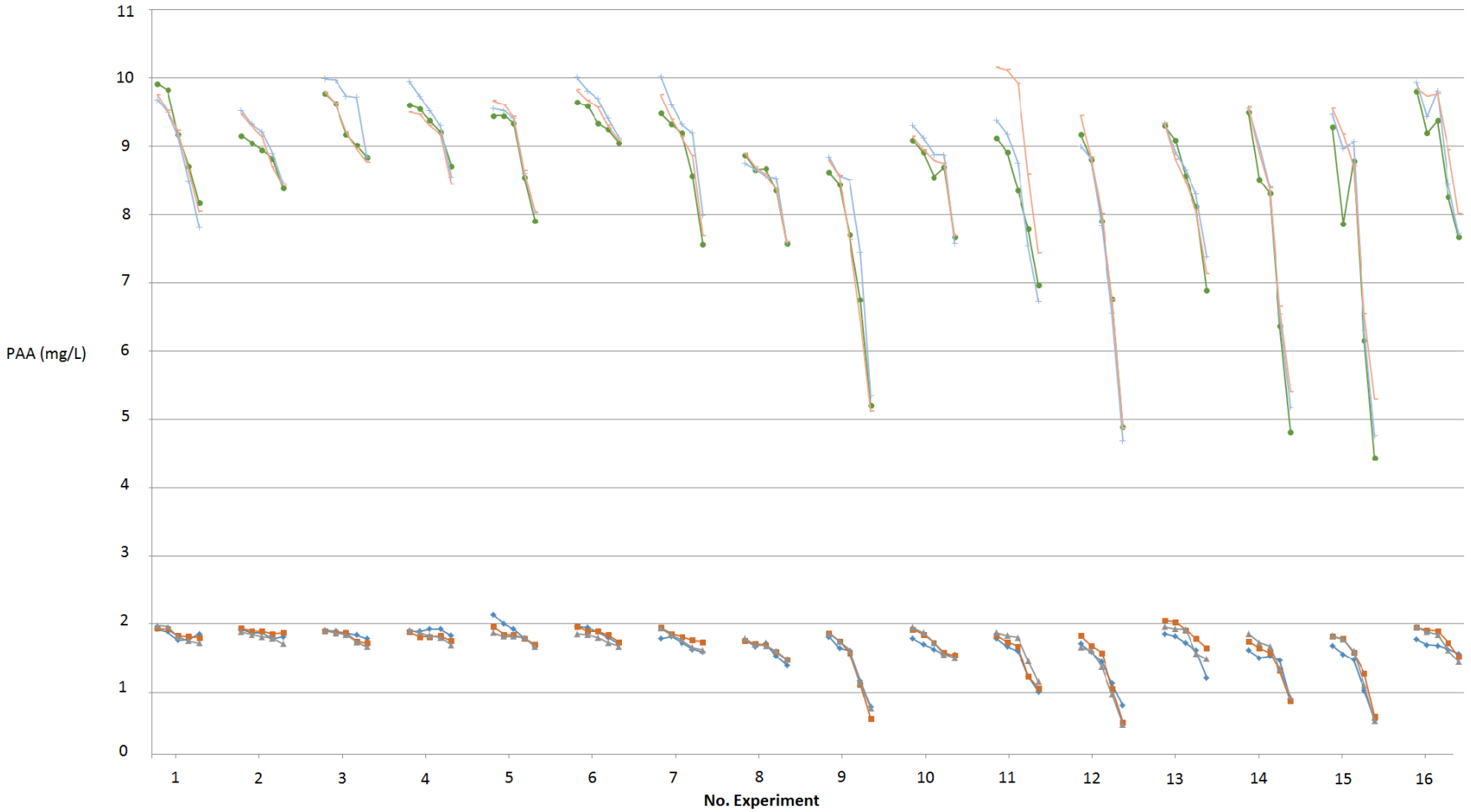

Figure S2. Raw data for the experimental plans for inorganics.

Effect of inorganics on oxidative demand: The factors that mainly contribute to the variability of the oxidative demand are identified by examining the standard deviation of the replicate values obtained for each experiments. As can be observed in Figure S3, reduced iron (D) is the factor that contribute the most at both PAA concentrations, explaining the trend of the confidence intervals observed in Figure 1b. Table S1 shows a full factorial design for the following compounds: ammonia nitrogen (A), nitrate (B) and nitrite (C). The levels were set as follows: A and B (0 and 25 mg/L as N) and C (0 and 1 mg/L as N). Each experiment was repeated three times for a total of 24 experiments. In Table S1 the mean values of oxidative demand coefficient (D_2 and D_{10} for 2 and 10 mg/L, respectively) and corresponding confidence intervals at 95% of significance are also reported.

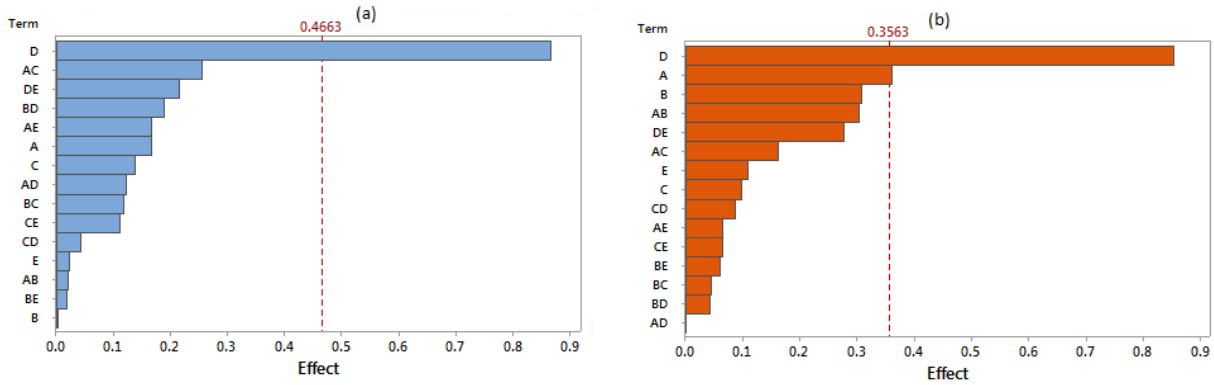

Figure S3. Pareto chart showing factors affecting the variability of the oxidative demand at 2 (a) and 10 (b) mg/L: the red dashed line represents the significance threshold at 0.05.

Table S1. Experimental plan (full factorial design) for ammonia nitrogen (A), nitrate (B) and nitrite (C) in coded units. The mean values of the oxidative demand are also reported as well as their confidence intervals at 95% of significance.

Experiment	Factor			Response	
	A	B	C	D_2	D_{10}
1	-	-	-	0.03 ± 0.01	0.01 ± 0.01
2	+	-	-	0.02 ± 0.01	0.03 ± 0.08
3	-	+	-	0.00 ± 0.01	0.04 ± 0.05
4	+	+	-	0.02 ± 0.03	0.06 ± 0.06
5	-	-	+	0.00 ± 0.02	0.04 ± 0.06
6	+	-	+	0.01± 0.01	0.00 ± 0.05
7	-	+	+	0.04 ± 0.02	0.70 ± 0.05
8	+	+	+	0.00 ± 0.01	0.12 ± 0.06

Effect of inorganics on decay rate: In Figure S4 the extension of the experimental sub-space of iron and orthophosphate is shown. As for Figure S5, it shows a graphical representation of the model reported in Table 4 in the manuscript.

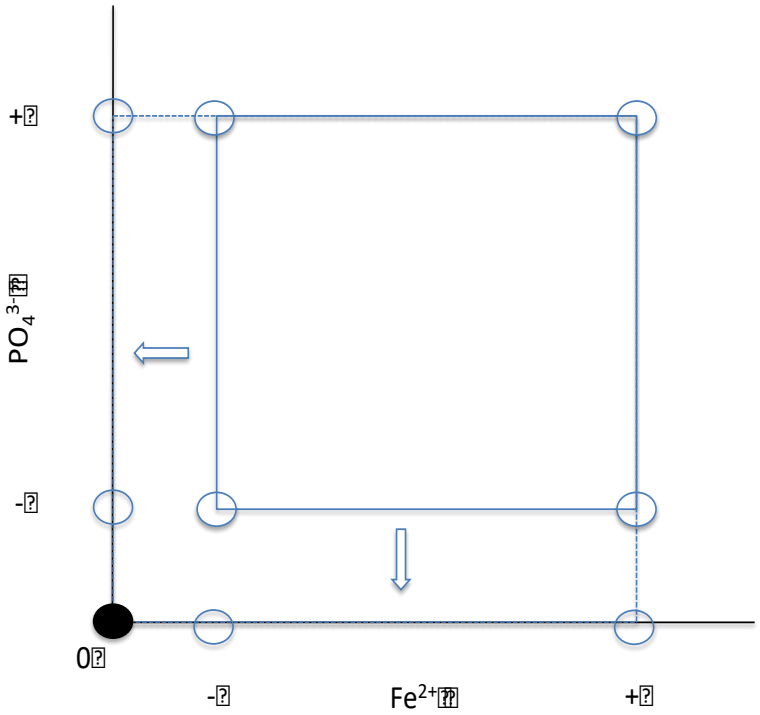

Figure S4. Extension of the experimental sub-space of iron and orthophosphate: the blue dots represent the experiments of the original DoE plan in the subspace of iron and orthophosphate, the white dots are the *ad hoc* experiments and the black dot is the blank test.

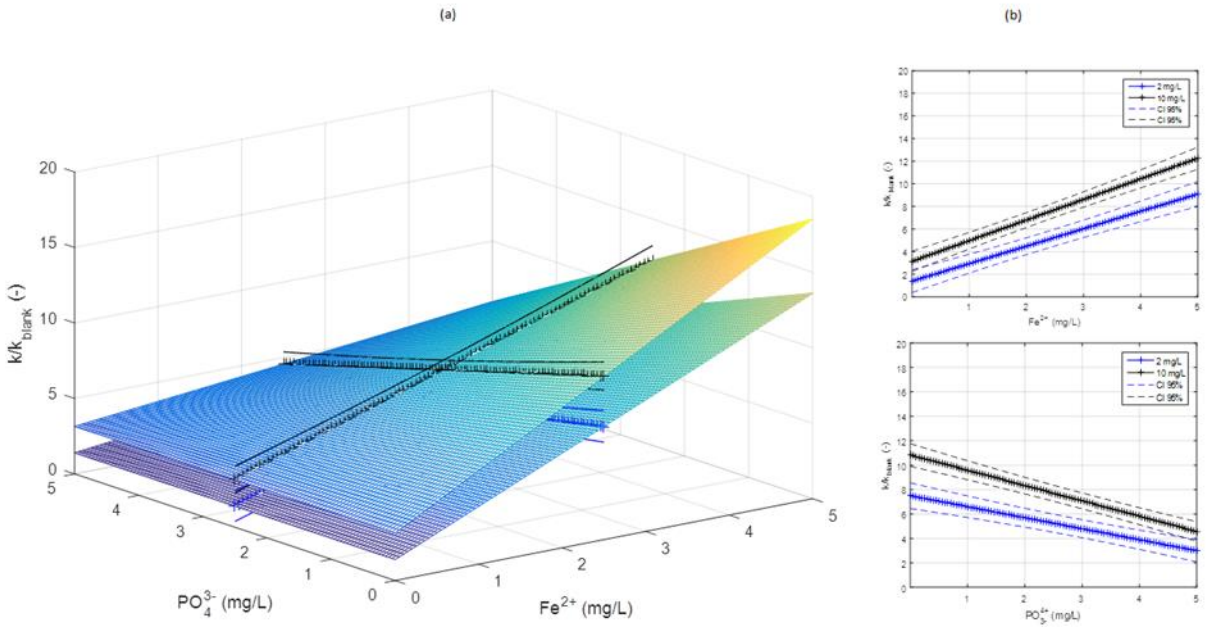

Figure S5. 3D (a) and 2D (b) plots of the model for the effect of inorganics on PAA decay rate. Confidence intervals at 95% of significance are reported.

Effect of organics on PAA decay: A representative example of experimental data and the interpolation functions for both models are shown in Figure S6, referred to the third replicate of experiment #11 of the experimental plan for organics (see DoE in Table 1) at 2 mg/L. As it can be observed in Figure S6, the R^2 value for the Haas and Finch model is far higher than the value for first-order kinetic model. Moreover, it can be noticed that after an initial oxidative demand, the PAA residual concentration decreases very slowly for the remaining time. Same results have been obtained for all the experiments of the experimental plan. Figure S8 reports the raw data for the experimental plans for organics. As it can be seen, the data at 10 mg/L exhibit an unexplained high variability within the replicates, leading to exclude these data from the statistical analysis. On the other hand, the raw data coming from the extra DoE at 5 mg/L have shown a variability within the replicates as low as the DoE at 2 mg/L.

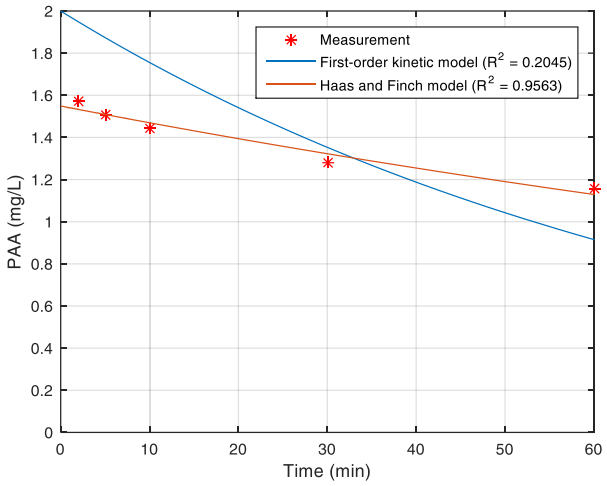

Figure S6. Residual PAA concentration vs. contact time: experimental data (dots) and interpolation functions for the first-order kinetic (blue line) and Haas and Finch (red line) models (2 mg/L, first replicate of experiment #11 of the DoE for organics).

Effect of organics on oxidative demand. The PAA decay at 2 and 5 mg/L was tested in deionized water spiked with urea. In particular, the concentration of urea was specifically selected to emulate the same load of organic nitrogen contained in the experiments with casein and peptone, corresponding to 2 and 8 mg/L as N. Results are shown in Figure S7 where blank test is also plotted.

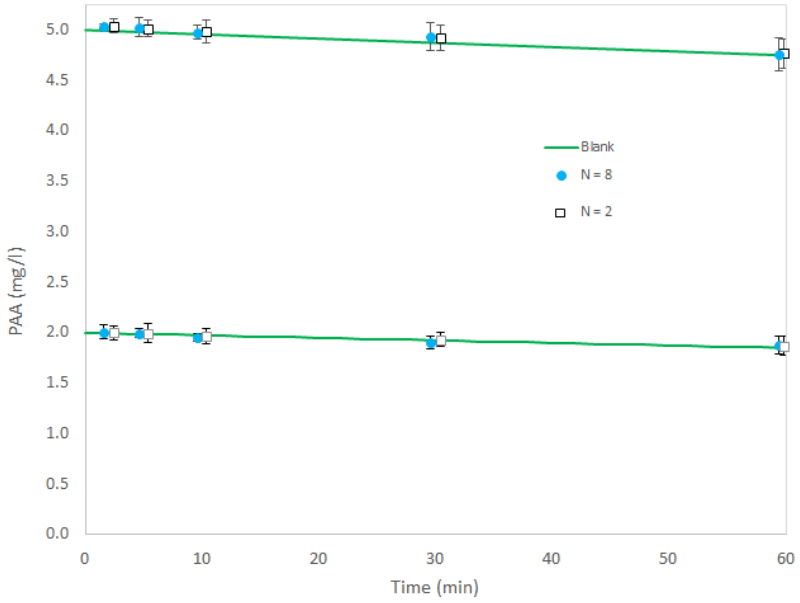

Figure S7. PAA decay over the time in experiments with organic nitrogen as urea.

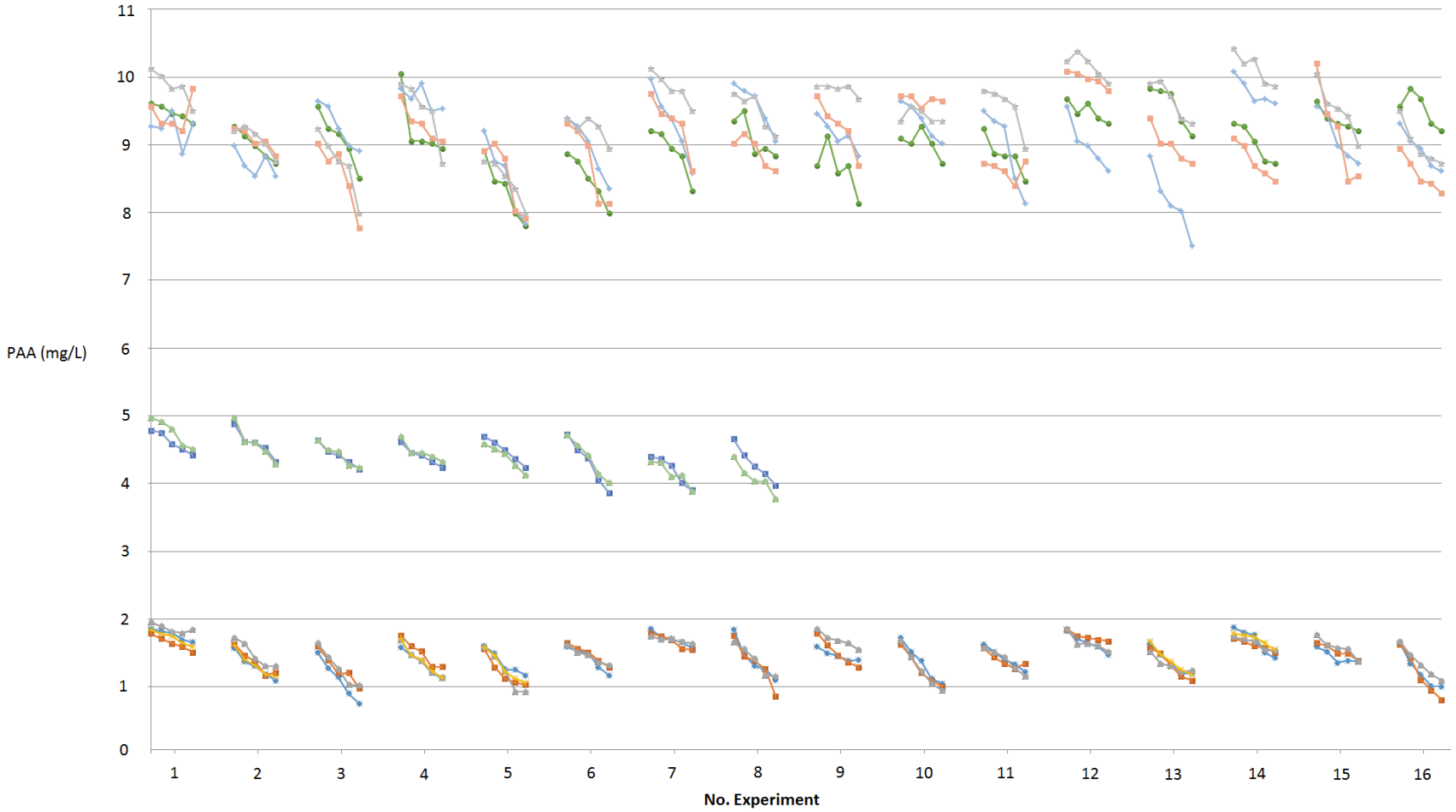

Figure S8. Raw data for the experimental plans for organics. The experimental plan at 10 mg/L has been replicated four time (instead of three) to better investigate the high variability within the replicates. The extra experimental plan at 5 mg/L have been replicated twice since the variability within the replicates was sufficiently low to estimate accurately the model coefficients of the Haas and Finch Kinetic model.

CHAPTER 6. EFFECT OF SUSPENDED SOLIDS ON PAA DECAY AND BACTERIAL INACTIVATION KINETICS

Abstract

The work addresses the effect of total suspended solids (TSS) on disinfection by peracetic acid (PAA) concerning two aspects: PAA decay and bacterial inactivation kinetics. As for the former aspect, the effect of TSS on PAA decay was evaluated at five solids concentrations (5, 40, 80, 120 and 160 mg/L), obtained from enriched effluent suspensions (EES) that were prepared from activated sludge samples. The influence of the soluble matter associated to the solids on PAA decay was evaluated separately, using the same EES after the removal of solids by filtration. The contributions of suspended and soluble fractions were found to be independent, and a predictive model formed by two additive sub-models was proposed to describe the overall PAA decay kinetics. Then, the disinfectant dose (mg/L min) was highlighted as the main parameter determining disinfection efficiency on a pure culture of *E. coli* and an inactivation kinetic model was developed based on the response of *E. coli* to various PAA doses. Finally, the effect of TSS (40 and 160 mg/L) on the inactivation of free-swimming *E. coli* was investigated at two PAA doses (5 and 20 mg/L min). TSS reduced inactivation extent from 0.33 to 0.48 logs at 5 mg/L min and from 1.37 to 1.67 logs at 20 mg/L min. It was hypothesized that this might be due to the formation of bacteria aggregates as defense mechanism against disinfection, enhanced by the presence of solids.

Keywords Disinfection, peracetic acid, *Escherichia coli*, suspended solids, decay rate kinetics, bacterial inactivation kinetics.

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The research activity was funded by the company PeroxyChem (Philadelphia, USA) and by Fondazione Cariplo (DrinkAble project, grant #2014-1285).

This study will be submitted for publication shortly to the Journal 'Science of the Total Environment'.

6.1. Introduction

Suspended solids in wastewater treatment plants (WWTPs) include a wide and heterogeneous group of particles whose characteristics and composition are determined by a combination of factors, including the characteristics of the influent wastewater, which mainly depend on the source (domestic, industrial, agricultural, storm) and on the type of the sewer (combined or separated), and the treatment processes occurring in the WWTP. A key element when dealing with suspended solids is the particle structure, that is not smooth and rigid but rather an irregular sponge-like matrix characterized by pores of different sizes in which bacteria can be shielded [1].

According to the related literature, suspended solids affect PAA disinfection as a consequence of two mechanisms: mainly (i) consumption of PAA entailing a reduction of the available concentration in the disinfection reactor and, thus, a lower PAA dose exposure for bacteria inactivation, and (ii) shielding bacteria against the oxidative action of the disinfectant.

As summarized in the State of the Art in Section 1, previous works have investigated the effect of soluble organic content on PAA in terms of macro-parameters such as COD and BOD, while the detrimental effect of suspended matter has been scarcely studied, although it has been observed in different works. Several authors have indicated that CSOs and primary effluents require higher PAA concentrations for disinfection than secondary and tertiary effluents [2–5]. Furthermore, Chhetri et al. [5] observed that a pre-treatment of CSO for the removal of suspended solids decreases the required disinfectant dose.

Regarding protective shielding afforded to bacteria, suspended solids play a major role. Microbial aggregates and microorganisms attached or embedded into particles, have demonstrated increased resistance to inactivation by different disinfectants compared to non-attached, free-swimming microorganisms [6–9]. However, it should be considered that approximately 99% of the overall bacterial population in wastewater is free-swimming (i.e., size < 10 μm) and only approximately 1% or less consists of particle associated bacteria (PAB) [10–12]. As for the particle size of the suspended solids, Falsanisi et al. [12] observed that it has a paramount importance during PAA disinfection, as the protection afforded by TSS was 0.6 and 1.3 logs for solids between 10 -120 μm and greater than 120 μm, respectively, whereas McFadden et al. [13] observed that solids size in the range between 10 and 100 μm had a minor effect on PAA disinfection.

It is important to highlight that, when dealing with PAA disinfection, another key aspect is related to the actual dose (mg/L min) as the main parameter determining disinfection efficiency, whose estimation must depend on the changing concentration of disinfectant at which bacteria are exposed over contact time [14]. Usually the design and operation of disinfection processes with chlorine-based compounds for simplicity, is based on the concept that the bacterial inactivation can be estimated by the product of the nominal concentration of the disinfectant and contact time. Consequently, the most widely used inactivation models are based on this concept and therefore on these two

parameters [15]. However, this approach neglects the disinfectant decay, thus it is not a reliable predictor for PAA disinfection performance. On the contrary, PAA decay should be considered in the calculation of the actual PAA dose, taking into account the disinfectant active concentration at any contact time [16].

The present study aims at elucidating the effect of suspended solids on PAA decay and *E. coli* inactivation performance. As for the first aspect, PAA decay was assessed in the presence of five different concentrations of TSS (5, 40, 80, 120 and 160 mg/L), representing secondary effluents of good (well settled) and medium (not well-settled) quality, and CSOs. In detail, enriched effluent suspensions (EES) were prepared with activated sludge samples to obtain test solutions at different TSS concentrations. In addition, the effect on PAA decay of the soluble matter in solution with suspended solids was also evaluated. A mathematical model to relate TSS concentration and residual concentration of PAA was developed, considering the contribution of suspended and soluble matter. Then, the effect of TSS on PAA disinfection efficiency has also been addressed. For this latter purpose, firstly PAA dose (mg/L min) was evaluated as the main parameter determining disinfection efficiency. The dose-response curve of a pure culture of *E. coli* in saline solution was determined and a log-inactivation model to describe *E. coli* inactivation as function of the actual disinfectant dose was studied. The independence of the extent of bacterial inactivation from the operating conditions was tested by exposing bacteria to same PAA doses obtained via different combinations of initial PAA concentration and contact time. Finally, the effect of TSS on PAA disinfection was assessed at two PAA doses in order to verify the existence of a potential defense mechanism for bacteria against disinfection afforded by TSS.

6.2. Materials and methods

6.2.1. Reagents

PAA technical grade solution (VigorOx® WWTII) was supplied by PeroxyChem, whose composition as weight percentage is 15% of peracetic acid, 23% of hydrogen peroxide and 16% of acetic acid. The PAA concentration in the commercial solution was checked monthly by iodometric titration [17]. The LB broth (10 g/L of tryptone, 5 g/L of yeast extract and 5 g/L of NaCl) and the saline solution (9 g/L of NaCl) were prepared in deionized water and sterilized in autoclave at 120°C for 20 min. All chemicals were reagent grade purchased from Sigma Aldrich, except for DPD (N,N-diethyl-p-phenylenediaminesulfate) salt and test kits that were provided by Hach Lange, whereas tryptone, yeast extract and C-EC Agar were supplied by Biolife.

6.2.2. Preparation of stock TSS and filtrate solutions

Concentrated stock TSS solutions were prepared from activated sludge samples collected from two conventional municipal wastewater treatment plants of Milan area (WWTP-A and -B, respectively). Both WWTPs are based on a treatment train composed of preliminary treatments, denitrification/nitrification biological reactors and clarification tanks. The WWTPs have different configurations of the biological process: WWTP-A is equipped with

a conventional activated sludge (CAS) biological reactor with suspended biomass, while WWTP-B combines CAS reactor with a Moving Bed Biofilm Reactor (MBBR) in which biomass additionally grows immobilized on plastic supports. The activated sludge samples were collected in the aeration basins. The stock TSS solutions were prepared by collecting the supernatant of the activated sludge samples after 2 hours of settling, which was assumed to be representative of the suspended solids in a secondary effluent before disinfection. The soluble fraction associated to the stock TSS solution (stock filtrate solution) was obtained by filtering a portion of the stock TSS solution using acetate-cellulose membranes (0.45 μm pore size, Sartorius). Both stock solutions were sterilized at 120°C for 30 min. The stock TSS solutions were analyzed for particle size distribution, absorbance at 254 nm (UV_{254}), TSS, COD, total nitrogen (N_{TOT}) and phosphate ion (PO_4^{3-}) concentrations, while the stock filtrate solutions were analyzed for UV_{254}, COD, total nitrogen and phosphate ion concentrations, as summarized in Table 1. Stock solutions were stored in the dark and at 4°C for up to two weeks. No significant changes were observed in particle size distribution after sterilization.

Table 1. Physical-chemical characteristics of the stock TSS and filtrate solutions.

Parameter		WWTP-A		WWTP-B	
		Stock TSS solution	**Stock filtrate solution**	**Stock TSS solution**	**Stock filtrate solution**
Particle size (mean±st.dev.)	μm	1.013±0.127	-	1.568±0.881	-
UV_{254}	cm^{-1}	0.292	0.057	0.168	0.047
TSS	mg/L	450	-	240	-
COD	mg_{O2}/L	408	195	204	137
N_{tot}	mg_N/L	22	12	17	9.9
PO_4^{3-}	mg_P/L	5.2	3	0.85	0.37

6.2.3. *E. coli* culture preparation and cell enumeration

The *E. coli* (DH5α) pure culture was provided by the Laboratory of Environmental Engineering of the Politecnico di Milano (LIA) and it was grown to exponential phase overnight in LB broth incubated at 37°C for 24 h in an orbital incubator SI600 (Stuart) with continuous shaking speed at 90 rpm. *E. coli* cells were centrifuged for 20 min at 4000 rpm, washed and re-suspended three times with saline solution to achieve a cell count of approximately 10^8 to 10^9 CFU/mL. The number of cells in the washed cell suspension was roughly estimated by measuring the optical density at 600 nm (Unicam UV/VIS 2 – optical path 10 mm) and the viable cells were enumerated as colony forming units by single plate - serial dilution spotting (SP-SDS) on chromogenic substrate C-EC Agar (Biolife) after incubation at 37°C for 18-24 h.

6.2.4. Experiments on PAA decay

1-hour decay tests were performed in completely mixed batch reactors (1 L) mixed by magnetic stirrer (380 rpm) in dark conditions at room temperature (20±1°C). As for the assessment of the influence of TSS on PAA decay, the stock TSS solution was diluted in deionized water to obtain five different TSS concentrations (5, 40, 80, 120 and 160 mg/L) and the effect on PAA decay was tested at three different initial concentrations of PAA (2, 5 and 8 mg/L). The stock filtrate solutions were prepared adopting the same dilution factors of the stock TSS solution in order to obtain the equivalent concentration of soluble matter associated to each TSS concentration. The pH of the solutions was adjusted to 7.5 at the beginning of the tests with sodium hydroxide (NaOH, 1 M) or sulfuric acid (H_2SO_4, 1 M), and it was monitored continuously (EcoScan pH6, Eutech Instruments). Samples were collected at five contact times (2, 5, 10, 30 and 60 min) to measure the residual PAA concentration. The blank decay determined in Section 4 and its respective equation was adopted in this study.

6.2.5. Experiments on E. coli inactivation

1-hour disinfection tests were performed in Erlenmeyer flasks of 500 mL where an aliquot of 25 µL of the washed cell suspension was inoculated into 250 mL of test solution, to reach an *E. coli* count of 10^6 CFU/100mL approximately. Table 2 shows the experimental plan for *E. coli* disinfection. In detail, the relation between the applied PAA dose and the resulting *E. coli* inactivation (dose-response curve) was determined in saline solution at five PAA doses (5, 10, 15, 20 and 25 mg/L min). The independence of the extent of bacterial inactivation from the operating conditions was tested in saline solution at three PAA doses (5, 10 and 20 mg/L min) varying the initial concentration of PAA (PAA_0, in mg/L) and contact time (t, in min), according to the combinations shown in Table 2. Regarding the evaluation of the effect of TSS on the disinfection performance of PAA, it was tested at two TSS concentrations (40 and 160 mg/L), solely with the stock TSS solution prepared with the activated sludge from WWTP-A.

Table 2. PAA doses (D_{PAA}) and combinations (PAA_0, t) of initial concentration of PAA (mg/L) and contact time (min) tested. The dose-response curve was built using the underlined combinations.

Test solution	Combination #	D_{PAA} (mg/L min)				
		5	10	15	20	25
Saline solution	1	<u>0.50, 10</u>	-	-	2.00, 10	-
	2	0.25, 20	<u>0.50, 20</u>	-	1.01, 20	-
	3	0.17, 30	-	<u>0.50, 30</u>	0.68, 30	-
	4	0.11, 45	-	-	<u>0.50, 41</u>	-
	5	0.09, 60	-	-	0.35, 60	<u>0.50, 52</u>
40 mg/L		0.50, 13	-	-	1.00, 11	-
160 mg/L		1.00, 22	-	-	2.00, 29	-

The extent of bacterial inactivation after disinfection tests was expressed as the log-inactivation of viable colony forming units, log (N/N_0), where N_0 and N are the microbial densities before and after disinfection, respectively. Each disinfection test was replicated four times.

6.2.6. Analytical procedures

The size distribution of stock TSS solutions was determined using Zetasizer Nano ZS90 (Malvern). The concentrations of TSS were measured according to Standard Methods [18] using 0.45 μm acetate-cellulose membranes (Sartorius). COD, total nitrogen and phosphate ion were measured using test kits (LCK 114, 138, 349 respectively) and a spectrophotometer Dr. Lange XION 500 (Hach Lange).

The residual PAA concentration was measured by means of the DPD colorimetric method describe in Chapter 4 - Evaluation of a colorimetric method for the measurement of low concentrations of PAA and H_2O_2 in water. The interference of the suspended solids with spectrophotometric measures due to light scattering was corrected by filtering the samples on syringe filters (0.45 μm acetate-cellulose membranes) prior to the absorbance measure.

Samples for microbiological analyses were collected in sterile flasks, after the addition of 0.5 mL sodium thiosulfate (0.1 N) and 0.5 mL of bovine catalase solution (1160 units/mL) to quench residual PAA and H_2O_2, respectively. Microbiological analyses were performed in two dilutions, each one repeated twice. *E. coli* were enumerated by plate-count standard technique based on a membrane filtration procedure (method 7030C [19]) using sterile mixed cellulose esters membrane (GN-6 Metricel® MCE Membrane Disc Filters, Pall). *E. coli* were cultured on chromogenic substrate C-EC agar (Biolife) and incubated at 37°C for 18-24 h. The colonies of *E. coli* were evidenced as green-blue colonies, fluorescent under the light of a Wood lamp (365 nm). Results were expressed as colony forming units (CFU) in a reference volume of 100 mL (CFU 100/mL).

6.2.7. Data processing

The standard curve for PAA measurement was obtained through a linear least-square regression ($R^2 = 0.9995$). The equation was: $ABS_{530} = \alpha + \beta \cdot PAA$, where ABS is the absorbance value at 530 nm and PAA is the concentration of PAA (mg/L). The estimated coefficients are: $\alpha = 0.0624$, $\beta = 0.5563$. The residual PAA concentrations measured during decay tests were plotted over time for each experiment (residual PAA concentrations at five contact times in triplicate, for a total of 15 data points) and interpolated with a non-linear least-square regression according to a modified first-order kinetic model proposed by Haas and Finch [20] (Equation 1).

$$PAA_t = (PAA_o - OD) \cdot e^{-kt} \quad \text{(e.1)}$$

where PAA_0 is the initial PAA concentration (mg/L), PAA_t is the concentration at time t, k is the decay rate constant (min^{-1}) and OD is the initial PAA demand (mg/L).

PAA dose (D_{PAA}) was quantified by estimating the area under the PAA decay curve until a defined contact time (t, in min) as in Equation 2 [21]:

$$D_{PAA} = \int_0^{CT} (PAA_o - OD) \cdot e^{-k \cdot t} dt = \frac{(PAA_0 - OD)}{k} \cdot (1 - e^{-k \cdot CT}) \quad \text{(e.2)}$$

The estimation of regression coefficients and the Monte Carlo simulations were performed in Mathworks Matlab R2017a, while the statistical analysis was performed using Minitab 17.

6.3. Results and discussion

6.3.1. Effect of TSS on PAA decay

The effect of TSS on PAA decay was assessed at five TSS concentrations, namely 5, 40, 80, 120 and 160 mg/L that were obtained by dilution of the stock TSS solutions in deionized water. The effect of soluble fraction associated to TSS was evaluated by diluting the stock filtrate solution in the same proportion of the stock TSS solution, correspondingly to each TSS concentration. Therefore, experimental tests on TSS solutions accounted for the contribution of suspended and soluble matter, whereas for filtrate solutions only the contribution of soluble matter was considered. Raw data from experimental tests and related fitting by the Haas and Finch kinetic model are shown in Figure S1 of Supporting Information, while the values of initial oxidative demand (OD) and decay rate constant (k) estimated by the non-linear least-square regression are reported in Tables S1 and S2 for WWTP-A and B, respectively in the Supporting Information. In the following sections, these estimated values are discussed.

6.3.1.1. Initial oxidative demand

The soluble matter associated to TSS exhibited scarce initial oxidative demand, ranging from -0.80% to 4.87% and from -0.45% to 7.86% of initial PAA concentration for solutions prepared with the activated sludge from WWTP-A and WWTP-B, respectively. In contrast, the estimated values for initial oxidative demand due to TSS can be grouped in two clusters. The first one corresponds to the experiments on TSS concentrations between 5 and 40 mg/L, for which the initial oxidative demand is not affected by the presence of solids. These values are similar to those observed in the experiments performed on the associated soluble fractions. The second cluster corresponds to the experiments on TSS concentrations of 80, 120 and 160 mg/L, for which the presence of TSS led to significantly higher initial oxidative demand with respect to the associated soluble fraction. Furthermore, for TSS concentration higher than 80 mg/L the initial oxidative demand was independent of TSS concentration, while it depended on the initial PAA concentration. In detail, initial PAA demand values of 0.4 and 0.8 mg/L were observed for initial PAA concentrations of 2 - 5 mg/L and for 8 mg/L, respectively. This finding suggests that the limiting factor in the rapid oxidation reactions occurring between PAA and organic matter is represented by PAA concentration.

6.3.1.2. Decay rate constant

Regarding the effect of TSS and associated soluble fraction on the decay rate constant, an increasing trend with TSS concentrations was observed. Figure 1 summarizes the estimated values, referred to as k_{TSS} and k_{sol} for the experiments performed on TSS and filtrate solutions, respectively. The effect of low concentrations of both suspended and soluble matter on PAA decay was negligible and comparable to the blank decay of PAA. In detail, k_{TSS} and k_{sol} values for 5 and 40 mg/L at the three initial PAA concentrations were comparable to k_{blank}. The lack of statistically significant differences was proven by an ANOVA test (p-value < 0.05).

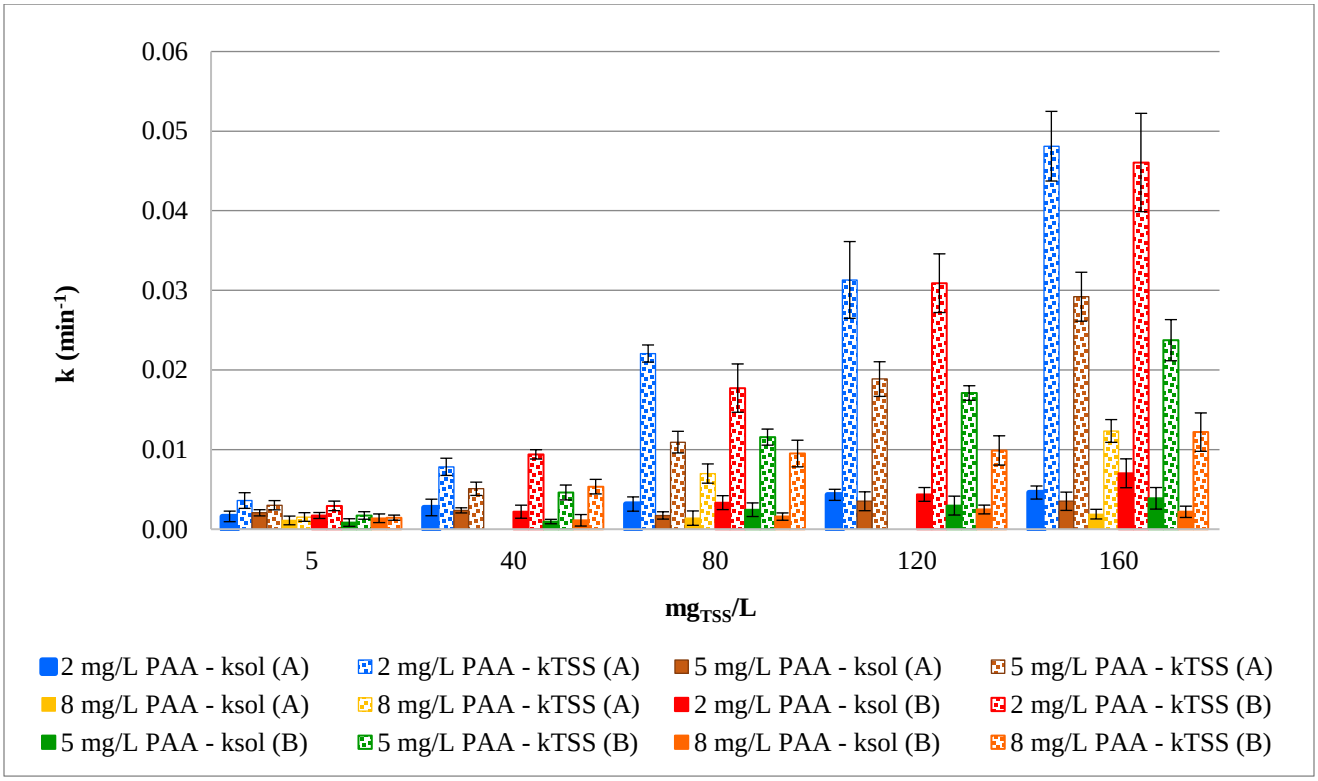

Figure 1. Decay rate constants of the Haas and Finch kinetic model (mean ± confidence interval at 95% of significance) as a function of TSS and PAA concentration for PAA decay tests on soluble ($k_{sol)}$ and TSS (k_{TSS}) solutions from WWTP- A (A) and WWTP-B (B).

At TSS concentrations higher than 40 mg/L the effect of the suspended matter on PAA decay became more relevant, showing an increasing trend with TSS concentrations. Indeed, k_{TSS} values are about five times higher than k_{sol} values at TSS concentrations higher than 40 mg/L, evidencing that TSS content is the main driver of observed PAA decay. Moreover, higher PAA concentrations led to lower decay rate constants. This was also observed in the blank decay tests and in the study of Pedersen et al. [22]. It was hypothesized that the higher the PAA concentration the more stable it is, therefore the dilution of the stock PAA solution contributes also to its decay.

As for the effect of the source of activated sludge, similar values for decay rate constants were obtained for both WWTPs. Indeed, no significant differences were observed between the experimental data from different WWTPs and the confidence intervals at 95% of significance of the parameters overlap for the experiments at the same TSS and PAA concentrations. Again, the lack of statistically significant differences was assessed by an ANOVA test (p-value < 0.05).

Subsequently, a model was developed to relate the decay rate constant with TSS concentration, composed of two sub-models separately describing the effect of suspended and soluble matter. The choice to describe the effect of suspended and soluble matter as two additive elements was based on the independence that was observed between the two components and on the likely assumption of negligible mutual interaction, according to Dignac et al. [23]. In the following, the structure of each sub-model is detailed and the results of the regressions performed on experimental data are reported.

As for the contribution of soluble matter on decay rate constant, it was effectively described based on COD concentration (COD_{sol}). A linear relationship was observed between the decay rate constants normalized by the blank decay rate (k_{sol}/k_{blank}) and COD_{sol}. According to the linear regression, the sub-model formulation is reported in Equation 3. As demonstrated in Section 4, the COD itself is not an effective predictor for PAA decay. However, since the effect of the soluble fraction associated to TSS was evaluated adopting different dilution factors from the same stock filtrate solution, it can be assumed that the different COD concentrations are proportional to the concentration of soluble matter affecting PAA decay. Moreover, a linear dependence on initial PAA concentration was highlighted, whose effect was incorporated in the term *a*, as evidenced in Equation 4. Finally, the blank decay rate constant was defined as reported in Equation 5, being the formulation derived from the work presented in Chapter 6.

$$\frac{k_{sol}}{k_{blank}} = 1 + x \cdot COD_{sol} \quad \text{(e.3)}$$

$$x = a_1 + a_2 \cdot PAA_0 \quad \text{(e.4)}$$

$$k_{blank} = 0.00128 - 6.24 \cdot 10^{-5} \cdot PAA_0 \quad \text{(e.5)}$$

where COD_{sol} is expressed in mg_{O2}/L, k is the decay rate of PAA, expressed in min^{-1}, and PAA_0 represents the initial PAA concentration expressed in mg/L.

The effect of suspended matter on the decay rate constant was described separately from the soluble part as an independent coefficient k*, as shown in Equation 6. In detail, under the assumption of independence between effects, the difference between k_{TSS} (overall decay rate ascribable to suspended and soluble matter) and k_{sol} (decay rate ascribable only to soluble matter) represents the decay rate due to suspended matter. A model to describe k*

was obtained by means of a non-linear regression on data obtained from the difference between k_{TSS} and k_{sol} normalized by the k_{blank}. The resulting model indicates that the effect of suspended matter depends on TSS concentration and initial PAA concentration. The effect is null in the absence of TSS in solution, as expected.

$$k^* = \frac{k_{TSS} - k_{SOL}}{\hat{k}_{blank}} = b_1 \cdot TSS^{\alpha} \cdot \ PAA_0{}^{\beta} \quad \text{(e.6)}$$

where TSS is the concentration of suspended matter (mg/L). The overall model accounting for the effect of soluble and suspended matter is obtained by merging Equations 3, 4 and 5, as presented in Equation 7, in which k_{TSS} normalized by the k_{blank} is defined:

$$\frac{k_{TSS}}{k_{blank}} = \frac{k_{sol}}{k_{blank}} + k^* = 1 + (a_1 + a_2 \cdot PAA_0) \cdot COD_{sol} + b_1 \cdot TSS^{\propto} \cdot PAA_0{}^{\beta} \quad \text{(e.7)}$$

Where a_1, a_2, b_1, α and β are the coefficients of the non-linear regression. The results of regressions on the dataset from each WWTP (parameter estimates and main statistics) are reported in Supporting Information for the sub-model for soluble matter (Table S3), for the sub-model for suspended matter (Table S4), and for the overall model (Table S5). In addition to the effectiveness of both sub-models in describing the related phenomena, satisfactory results were obtained by the overall model (R^2 values equal to 0.972 and 0.965, respectively for WWTP-A and WWTP-B). Since confidence intervals at 95% of significance for estimated parameters overlap between WWTP-A and WWTP-B, it was hypothesized that there are not significant differences between two WWTPs, therefore all the experimental data was analyzed as a single pooled dataset. Results of regression are reported in Table 3, while a graphical representation of the overall model is shown in Figure 2.

The overall model obtained from the single pooled dataset of WWTPs is effective at describing the effect of suspended and soluble matter on PAA decay rate constant, as highlighted by the high R^2 value. The negative values of a_2 and β coefficients indicates that the detrimental effect is more pronounced at low initial PAA concentrations, which is in agreement with the idea of a consumption mechanism determined by the reaction of PAA molecules with other compounds, suspended or dissolved.

It is interesting to notice that according to the interpolated model within 60 minutes the presence of 40 mg/L of TSS causes decreases of 16, 7 and 4% of PAA initial concentrations of 2, 5, 8 mg/L, respectively.

The decrease of the same initial PAA concentration turns into 61, 41 and 29% respectively in the presence of 160 mg/L of TSS. This evidences the effect of TSS content on PAA decay, which stresses the importance of an efficient settling process for suspended matter removal in order to optimize the disinfectant dosage. Furthermore, this outcome entails that when dealing with effluents with high content of suspended matter, the measurement of the

TSS concentration is a reliable and easy-to-measure parameter to determine the decay rate and therefore the half-life of a defined PAA initial concentration.

Table 3. Main statistics and regression estimates for the overall model of the effect of suspended and soluble matter on PAA decay rate constant obtained from the single pooled dataset of WWTPs.

	Soluble matter model		**Suspended matter model**		
Parameter	a_1	a_2	b_1	α	β
Estimates	0.062	-0.006	0.085	1.263	-0.543
Min 95% Confidence Interval	0.044	-0.009	0.044	1.165	-0.609
Max 95% Confidence Interval	0.079	-0.002	0.125	1.36	-0.476
Standard Error (SE)	0.009	0.002	0.021	0.049	0.034
t (df)	7.03	-3.505	4.074	25.59	-16.086
p-level	< e-11	< e-04	< e-05	< e-58	< e-35
R^2 (adj-R^2)	0.964 (0.963)				
n	164				

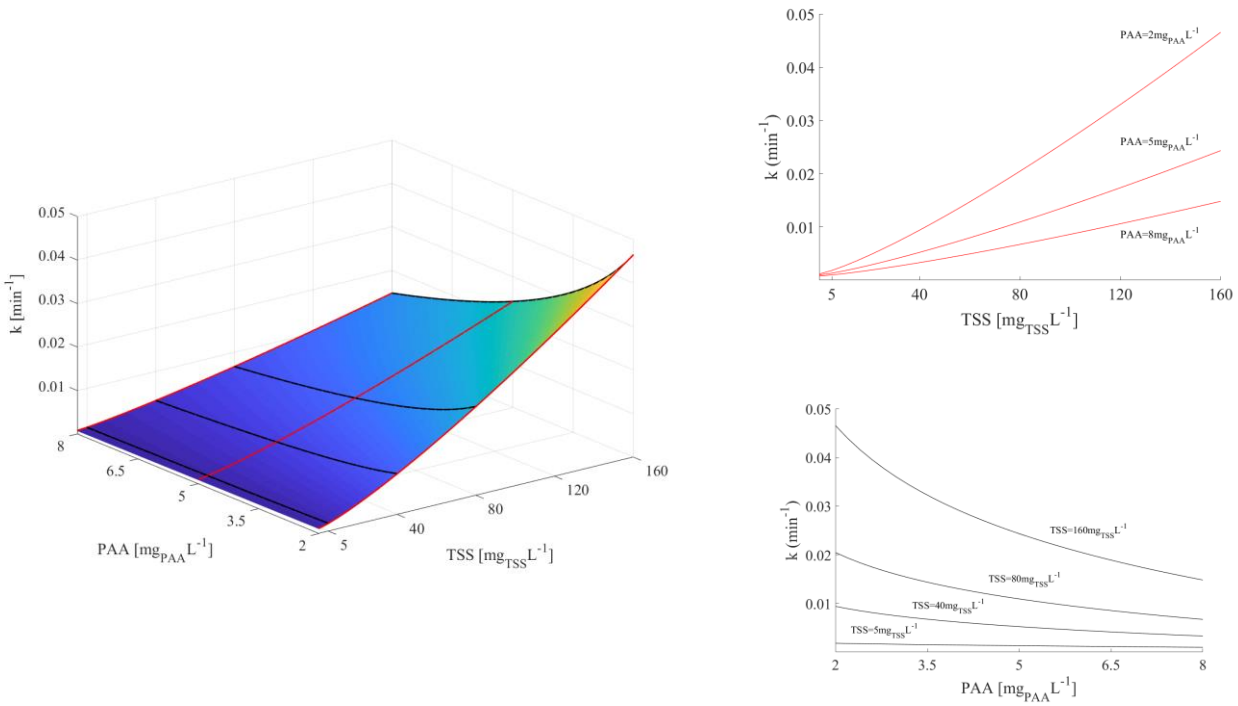

Figure 2. 3D (left) and 2D (right) plots of the overall model of the effect of suspended and soluble matter on PAA decay rate constant as a function of TSS concentration and initial PAA concentration.

6.3.2. *E. coli* inactivation

6.3.2.1. Dose-response curve

Disinfection tests for building the dose-response curve were performed in saline solution, in which PAA alone interacts with bacteria and no other compound interferes with the disinfection process. Furthermore, the saline solution is isotonic with the bacterial cells, which nullifies the osmotic stress to the cells. The response of *E. coli* when exposed to different PAA doses (5, 10, 15, 20 and 25 mg/L min) determined in saline solution is presented in Figure 3, where the log-inactivation (log N/N_0) values are presented as a function of PAA dose.

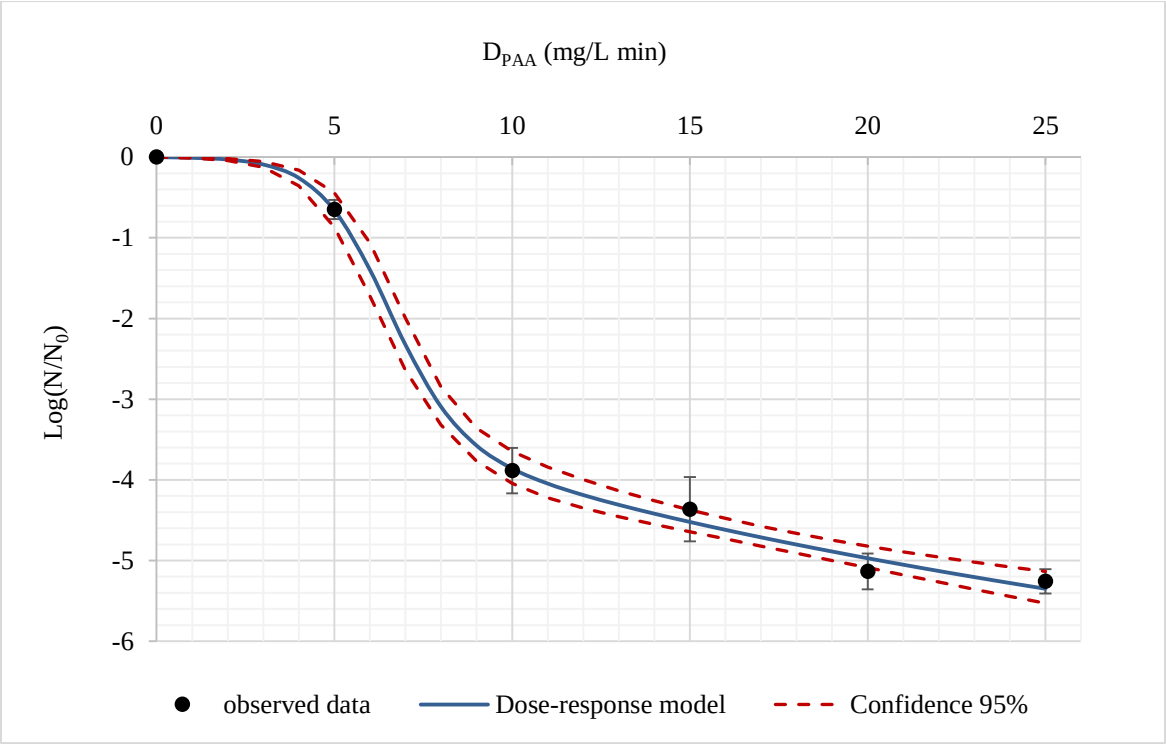

Figure 3. *E. coli* log-inactivation in saline solution at different PAA doses: experimental (mean ± standard deviation) and modelled data. Confidence intervals at 95% of significance are reported.

In detail, D_{PAA} was changed by varying the contact time (t) at the same initial PAA concentration (PAA_0), while taking into account the PAA decay as defined in Equation 5. Experimental results indicated that, after an initial lag of inactivity, *E. coli* was susceptible to rapid disinfection by PAA. Log-inactivation values ranged from -0.65 to -5.26, depending on the applied PAA dose. Comparable levels of *E. coli* inactivation for similar PAA doses were observed in the study of McFadden et al. [13], in which the same strain of *E. coli* was used, whereas a similar level of *E. coli* inactivation required much larger doses in other studies [15,21]. The differences in bacteria behavior can probably be attributed to the use of a pure lab culture of *E. coli*, characterized by lower resistance to inactivation than heterogeneous mixtures of bacteria naturally present in real effluents.

Nevertheless, a typical sigmoidal-shaped response of *E. coli* was observed, as shown in Figure 3, in which the initial lag phase is determined by an initial resistance to inactivation, followed by an exponential inactivation phase where the maximum inactivation rate is achieved, and finally by an asymptotic inactivation phase, during which the inactivation rate decreases [24]. The initial shoulder trend is usually attributed to the diffusion resistance of PAA through the cell membrane, which delays the inactivation effect [15]. Previous studies of PAA inactivation reported similar trends of initial lag phase in the presence of low doses of PAA [13,15,25]. On the other hand, the final tailing-off trend is commonly explained by the presence of suspended solids or a genetic heterogeneity of microorganisms, resulting in different capacities of resistance to disinfectant [15,26]. However, in this specific case, previous reasons could not explain the tailing-off trend because only NaCl is present in saline solution and only one type of DNA characterizes the *E. coli* culture. Therefore, it can be hypothesized that the trend is caused by bacterial aggregation. Indeed, bacteria tend naturally to aggregate in natural environments as protection mechanism against extreme conditions [9,27]. Consequently, bacteria located in the external part of the aggregate can be inactivated by the disinfectant, whilst bacteria in the core might survive PAA disinfection.

Some of the most commonly used inactivation models belong to a family of special cases of the generalized inactivation rate (GIR) model, according to Equation 8 [28–30].

$$\frac{dN}{dt} - k' C^n m N^x t^{m-1} \qquad \text{(e.8)}$$

where k' is the microbial decay constant; *n, m* and *x* are model parameters, *C* is the disinfectant concentration and t is the contact time. The GIR parameters lead to different inactivation models, namely Chick-Watson, Hom, Power Law (Rational) and Hom Power Law (HPL), which can be combined with the disinfectant decay kinetics, in order to consider the loss of disinfectant in time. The inactivation equations with the disinfectant demand and their integrated solutions have been described in previous works [29,31–33], providing a suitable option to consider the disinfectant loss during bacterial inactivation. However, analytical solutions may be not available for all disinfectant decay kinetics [28,31,32] and further assumptions are required to obtain a closed form of the inactivation models, such as (1) negligible instantaneous disinfectant demand and (2) negligible microbial inactivation during instantaneous disinfectant demand [28]. Under these assumptions, inactivation models accounting for zero-order disinfectant decay have been adopted in the past [28,34].

Most of these models with their different approaches have been developed and studied for chlorine or ozone; however, some assumptions are not suitable for PAA disinfection. For instance, PAA decay generally follows the first-order kinetic rate law as described previously and as found in previous works [15,35–38] and for effluents with high organic or TSS content the instantaneous disinfectant demand cannot be neglected.

Moreover, the approach of considering both the disinfectant decay and inactivation kinetic models such as the

Rational and the HPL, therefore non-first order inactivation in disinfection residuals ($x \neq 1$ in Equation 8), result in expressions that are complex with as many as four parameters that can lead to overparametrization and highly correlated parameter estimates [29,39].

The S-model for PAA disinfection kinetics proposed by Profaizer [40], on the other hand, takes into account the decay, however it considers the applied PAA concentration and the contact time as independent variables. Consequently, the natural PAA decay is not considered in the calculation of actual disinfectant concentration at any contact time.

Considering that an essential feature of kinetic modeling is simplification and idealization of complex phenomena, a log-inactivation model to describe experimental data on *E. coli* inactivation as a function of the PAA dose has been defined in the present work, whose formulation is reported in Equation 9.

$$log\left(\frac{N}{N_0}\right) = -k' \cdot {D_{PAA}}^{n}\left(\frac{1}{1+e^{h-D_{PAA}}}\right) \qquad \text{(e.9)}$$

where D_{PAA} (mg L^{-1} min) is the PAA dose at which bacteria are exposed determined by Equation 2, and k', *n* and *h* are the empirical model parameters. In detail, the model has been built assuming that the log-inactivation could be described in two parts. The first one, can be regarded as a special case of the GIR, where *m*, *x* = 1 and in which the product of the disinfectant concentration and contact time can be approximated to D_{PAA}, considering that: (1) the disinfectant concentration and contact time have the same weight at defining the log-inactivation and (2) D_{PAA} is the effective disinfectant concentration at which bacteria are exposed during *t*. This result is a similar expression to the Chick-Watson model but in terms of the dose, in which parameters k' and *n* quantify the inactivation level at high doses (tailing-off trend). However, this expression is not yet satisfactory to fit the inactivation data. Therefore, the second part corresponds to a sigmoid function, which improves the typical disinfection S-shaped dose-response curve, in a similar fashion as the inactivation model proposed by Profaizer [40], in which the parameter *h* determines the initial lag phase of the inactivation curve at low doses (shoulder trend). It is noteworthy that when $D_{PAA} < h$ in Equation 10, the denominator takes values higher than 1 resulting in the shoulder trend. This effect becomes negligible at high D_{PAA} values ($D_{PAA} > h$), as shown in Figure 4.

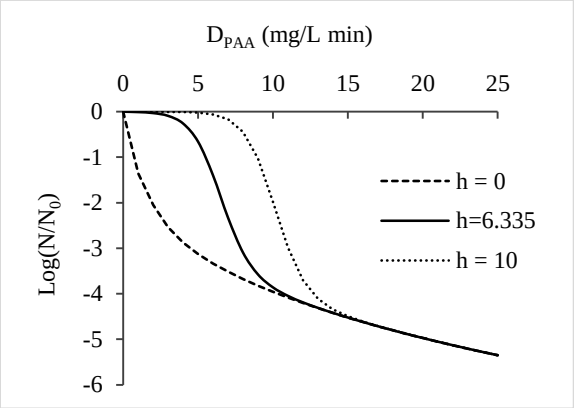

Figure 4. Graphical study of the behavior of the proposed model for log-inactivation of *E. coli* due to PAA disinfection as a function of parameters *h*.

The parameters of the proposed model for log-inactivation of *E. coli* due to PAA disinfection were estimated by a non-linear regression of the experimental data. Table 4 summarizes the main statistics and the regression estimates, while the modelled data are reported in Figure 3.

Table 4. Main statistics and regression estimates of the dose-response model for *E. coli*.

Parameter	k'	n	h
Estimates	1.851	0.328	6.335
Min 95% Confidence Interval	1.404	0.246	5.885
Max 95% Confidence Interval	2.297	0.412	6.785
Standard Error (SE)	0.215	0.040	0.218
t (df)	8.576	8.215	29.101
p level	< 0.05	< 0.05	< 0.05
R^2	0.990 (0.989)		
n	26		

6.3.2.2. Independence of *E. coli* inactivation from operating conditions

Similar inactivation levels were obtained for all the combinations tested for 20 mg/L min with log-inactivation values of -4.92±0.26 (mean ± standard deviation), as shown in Figure 5. An ANOVA for 20 mg/L min highlighted no significant differences among log-inactivation values observed for different combinations of initial PAA concentration and contact time for a defined dose (p-value of 0.334). Conversely, at 5 mg/L min an ANOVA showed significant differences among the inactivation values (p-values 0.002). Indeed, a graphical inspection of Figure 5 evidences a much higher inactivation for combination #5 ($[PAA]_0$ = 0.09 mg/L , t = 60 min). The statistical analysis

of the dataset for 5 mg/L min excluding combination #5 evidenced that there are no differences among log-inactivation values (p-value 0.067). Consequently, only combination #5 for the lowest PAA dose led to different levels of inactivation than its peers, displaying higher log-inactivation (-1.53±0.46) with respect to the average value obtained for other combinations (-0.59±0.12). Therefore, the level of *E. coli* inactivation obtained for a defined PAA dose was independent from operating conditions PAA_0 and t, up to 45 min for 5 mg/L min and 60 min and 20 mg/L min.

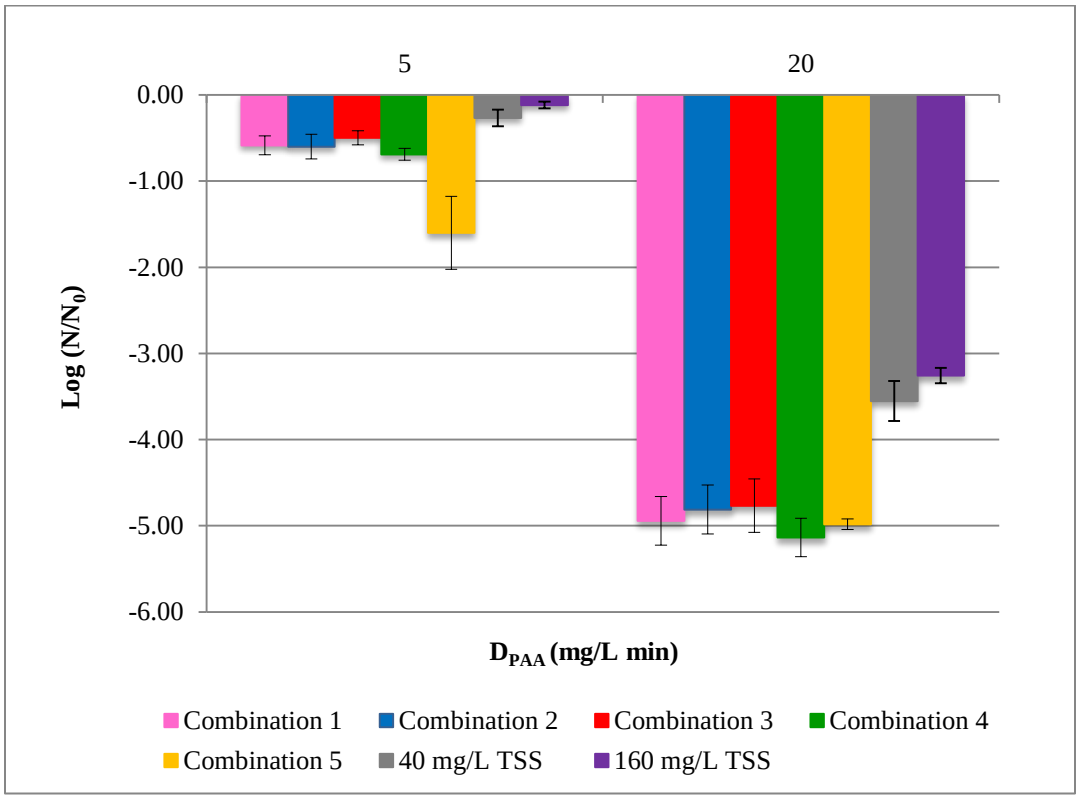

Figure 5. *E. coli* log-inactivation (mean ± standard deviation) in saline solution, 40 and 160 mg/L of TSS for different combinations of PAA_0 and t at defined PAA doses.

6.3.2.3. Effect of TSS on PAA disinfection

The effect of TSS on PAA disinfection was assessed on free-swimming E. coli and it was assumed that no PAB was present during the tests since the cell suspension of the E. coli culture was spiked in the tests solution at the beginning of the trial. Indeed, to developed within the porous of the particle, bacteria would require prior incubation time [7], necessary to produce extracellular polymers to guarantee the attachment of cells on the surface of the particle. Moreover, PAB growth is limited by the average size of the particles present in the solution that is comparable to the size of the bacteria. In addition, coliform bacteria are expected to be protected by TSS larger than 10 μm: this is the conventional size threshold dividing free-swimming bacteria from PAB [12,41].

Disinfection tests in saline solution quantified the log-inactivation at defined PAA doses without any other factor affecting the process, as described previously. Therefore, in case that PAA dose is the main factor determining log-inactivation of free-swimming bacteria, similar values are expected in both saline solution and in the presence of suspended matter.

As shown in Figure 5, lower log-inactivation values were observed in the presence of both concentrations of TSS tested (40 and 160 mg/L) compared to the values in saline solution at same PAA doses. The protection afforded by TSS to *E. coli* showed to be dependent on PAA dose. At 5 mg/L min the presence of 40 and 160 mg/L of TSS afforded a protection of approximately 0.33 and 0.48 logs respectively, whereas at 20 mg/L min the same concentrations of TSS increased the protection to 1.37 and 1.67 logs, respectively. A t-test between log-inactivation values at two TSS concentrations confirmed significant statistical differences at each PAA dose (p-values equal to 0.027 and 0.033 at 5 and 20 mg/L min, respectively).

Consequently, the presence of TSS in solution affects the disinfection efficiency of free-swimming bacteria, beyond its effect on PAA decay that was accounted for in the calculation of PAA dose according to Equation. 2. This can be attributed to a protection mechanism afforded to bacteria.

The mechanism of protection afforded by suspended solids to bacteria (free-swimming and PAB) is not well understood and many efforts have been addressed to elucidate it. It has been hypothesized that the solids effect on disinfection was due to PAA decay, leading bacteria to be exposed to lower doses of disinfectant [5,42]. Experiments performed at same levels of PAA dose allow to exclude this mechanism and proved the occurrence of interaction phenomena between bacteria and solids. According to a previous work [7], 20% of the dispersed *E. coli* can attach to a porous material in 20 min and find protection from disinfectant action. In this case, since the size of suspended solids is comparable to bacteria size, this hypothesis is ruled out. Therefore, it can be hypothesized that suspended particles act as a condensation nucleus and that they are able to favor aggregation of bacteria, as already observed for disinfection with PAA and other disinfectants [9,16,27].

6.4. Conclusions

The effect of suspended matter on PAA decay was studied. Increasing TSS concentrations lead to higher PAA decay rates, trend that becomes more pronounced at TSS concentrations above 40 mg/L and a non-linear regression was performed to interpolate a model to describe this behavior. As for the contribution the associated soluble matter to PAA decay, it displayed a linear relationship with the COD_{sol} and its effect was found to be additive to the one of the suspended matter, yet much less relevant particularly at high TSS concentrations. This outcome suggests that when implementing PAA for the disinfection of effluents with high solids load, such as primary effluents and sewer overflows, TSS concentration allows to determine the PAA consumption.

Regarding the adoption of the dose as the main parameter determining disinfection efficiency, it proved to be

invariant from the combination of initial concentration of PAA and contact time up to 45 min for the lowest dose tested (5 mg/L min) and up to 60 min for higher doses (20 mg/L min).

As for the role of the suspended matter during *E. coli* inactivation by PAA, a detrimental effect was observed given that the presence of the solids lead to lower bacterial abatements respect to ones observed in saline solution. This effect was dependent on both the TSS concentration and PAA dose. In detail, a more pronounced protective effect by the solids was observed at higher PAA doses, which suggests that the protection afforded by the solids might be proportional to the PAA dose applied.

Further studies are required to elucidate the protection mechanism afforded by the solids to bacteria against the disinfection and their role as potential condensation nucleus promoting bacterial aggregation.

6.5. References

[1] J.P. Dietrich, H. Başağaoğlu, F.J. Loge, T.R. Ginn, Preliminary assessment of transport processes influencing the penetration of chlorine into wastewater particles and the subsequent inactivation of particle-associated organisms, Water Res. 37 (2003) 139–149.

[2] J. Koivunen, H. Heinonen-Tanski, Peracetic acid (PAA) disinfection of primary, secondary and tertiary treated municipal wastewaters, Water Res. 39 (2005) 4445–4453.

[3] R. Gehr, D. Cochrane, Peracetic acid (PAA) as a disinfectant for municipal wastewaters: encouraging performance results from physicochemical as well as biological effluents, Proc. Water Environ. Fed. 2002 (2002) 182–198.

[4] T. Luukkonen, J. Teeriniemi, H. Prokkola, J. Rämö, U. Lassi, Chemical aspects of peracetic acid based wastewater disinfection, Water SA. 40 (2014) 73–80.

[5] R.K. Chhetri, A. Bonnerup, H.R. Andersen, Combined Sewer Overflow pretreatment with chemical coagulation and a particle settler for improved peracetic acid disinfection, J. Ind. Eng. Chem. 37 (2016) 372–379.

[6] G.P. Winward, L.M. Avery, T. Stephenson, B. Jefferson, Chlorine disinfection of grey water for reuse: Effect of organics and particles, Water Res. 42 (2008) 483–491.

[7] M.W. LeChevallier, T.S. Hassenauer, A.K. Camper, G.A. McFeters, Disinfection of bacteria attached to granular activated carbon, Appl. Environ. Microbiol. 48 (1984) 918–923.

[8] J.P. Dietrich, F.J. Loge, T.R. Ginn, H. Başağaoğlu, Inactivation of particle-associated microorganisms in wastewater disinfection: Modeling of ozone and chlorine reactive diffusive transport in polydispersed suspensions, Water Res. 41 (2007) 2189–2201.

[9] Z. Bohrerova, K.G. Linden, Ultraviolet and Chlorine Disinfection of Mycobacterium in Wastewater: Effect of Aggregation, Water Environ. Res. 78 (2006) 565–571.

[10] R.G. Qualls, S.F. Ossoff, J.C.H. Chang, M.H. Dorfman, C.M. Dumais, D.C. Lobe, J.D. Johnson, Factors controlling censitivity in ultraviolet disinfection of secondary effluents, Water Pollut. Control Fed. 57 (1985) 1006–1011.

[11] R.G. Qualls, M.P. Flynn, J.D. Johnson, The Role of suspended particles in ultraviolet disinfection, Water Pollut. Control Fed. 55 (1983) 1280–1285.

[12] D. Falsanisi, R. Gehr, L. Liberti, M. Notarnicola, Effect of suspended particles on disinfection of a physicochemical municipal wastewater with peracetic acid, Water Qual. Res. J. Canada. 43 (2008) 47–54.

[13] M. McFadden, J. Loconsole, A.J. Schockling, R. Nerenberg, J.P. Pavissich, Comparing peracetic acid and hypochlorite for disinfection of combined sewer overflows: Effects of suspended-solids and pH, Sci. Total Environ. 599–600 (2017) 533–539.

[14] D. Santoro, F. Crapulli, M. Raisee, G. Raspa, C.N. Haas, Nondeterministic Computational Fluid Dynamics Modeling of *Escherichia coli* Inactivation by Peracetic Acid in Municipal Wastewater Contact Tanks, Environ. Sci. Technol. 49 (2015) 7265–7275.

[15] S. Rossi, M. Antonelli, V. Mezzanotte, C. Nurizzo, Peracetic acid disinfection: a feasible alternative to wastewater chlorination., Water Environ. Res. 79 (2007) 341–350.

[16] A. Turolla, R. Sabatino, D. Fontaneto, E.M. Eckert, N. Colinas, G. Corno, B. Citterio, F. Biavasco, M. Antonelli, A. Mauro, G. Mangiaterra, A. Di Cesare, Defence strategies and antibiotic resistance gene abundance in enterococci under stress by exposure to low doses of peracetic acid, Chemosphere. 185 (2017) 480–488.

[17] B.D. Sully, P.L. Williams, The analysis of solutions of per-acids and hydrogen peroxide, Analyst. 87 (1962) 653.

[18] APHA/AWA/WEF, Standard Methods for the Examination of Water and Wastewater, 22nd ed., Washington, DC., 2012.

[19] APAT-IRSA/CNR, Metodi analitici per il controllo della qualità delle acque., Rome, Italy, 2003.

[20] C.N. Haas, G.R. Finch, Methodologies for the determination of disinfection effectiveness, AWWA Research Foundation and American Water Works Association, 2001.

[21] D. Santoro, F. Crapulli, M. Raisee, G. Raspa, C.N. Haas, Nondeterministic Computational Fluid Dynamics Modeling of Escherichia coli Inactivation by Peracetic Acid in Municipal Wastewater Contact Tanks,

Environ. Sci. Technol. 49 (2015) 7265–7275.

[22] L.F. Pedersen, P.B. Pedersen, J.L. Nielsen, P.H. Nielsen, Peracetic acid degradation and effects on nitrification in recirculating aquaculture systems, Aquaculture. 296 (2009) 246–254.

[23] M.F. Dignac, P. Ginestet, D. Rybacki, A. Bruchet, V. Urbain, P. Scribe, Fate of wastewater organic pollution during activated sludge treatment: nature of residual organic matter, Water Res. 34 (2000) 4185–4194.

[24] V. Mezzanotte, M. Antonelli, S. Citterio, C. Nurizzo, Wastewater Disinfection Alternatives : Chlorine, Ozone, Peracetic Acid, and UV Light, Water Environ. Res. 79 (2007) 2373–9.

[25] D. Santoro, T.A. Bartrand, D.J. Greene, B. Farouk, C.N. Haas, M. Notarnicola, L. Liberti, Use of CFD for Wastewater Disinfection Process Analysis: E.coli Inactivation with Peroxyacetic Acid (PAA), Int. J. Chem. React. Eng. 3 (2005).

[26] M. Profaizer, Peracetic acid as an alternative technology for drinking water disinfection: a new modelling approach (in Italian), PhD. Dissertation, Politecnico di Milano, 1998.

[27] J. Mir, J. Morató, F. Ribas, Resistance to chlorine of freshwater bacterial strains, J. Appl. Microbiol. 82 (1997) 7–18.

[28] D. Santoro, R. Gehr, T.A. Bartrand, L. Liberti, M. Notarnicola, A. Dell'Erba, D. Falsanisi, C.N. Haas, Wastewater disinfection by peracetic acid: assessment of models for tracking residual measurements and inactivation., Water Environ. Res. 79 (2007) 775–87.

[29] L.L. Gyiirek, G.R. Finch, Modelling water treatment chemical disnfection kinetics, J. Environ. Eng. 124 (1998) 783–793.

[30] L. Li, Effects of initial microbial density on disinfection efficiency in a continuous flow system and validation of disinfection batch kinetics in a continuous flow system, PhD. Dissertation, Drexel University, 2004.

[31] C.N. Haas, J. Joffe, Disinfection under Dynamic Conditions: Modification of Hom's Model for Decay, Environ. Sci. Technol. 28 (1994) 1367–1369.

[32] J.G. Jacangelo, N. Patania, R. Trussell, C. Gerba, C.N. Haas, Inactivation of Waterborne Emerging Pathogens by Selected Disinfectants, AWWA Research Foundation and American Water Works Association, Denver, CO, 2002.

[33] C.N. Haas, M.S. Heath, J. Jacangelo, J. Joffe, U. Anmangandla, J.. Hornberger, J. Glicker, Development and validation of rational design methods of disinfection, The Foundation and American Water Works Association, Denver, CO, 1995.

[1] J.P. Dietrich, H. Başağaoğlu, F.J. Loge, T.R. Ginn, Preliminary assessment of transport processes influencing the penetration of chlorine into wastewater particles and the subsequent inactivation of particle-associated organisms, Water Res. 37 (2003) 139–149. doi:10.1016/S0043-1354(02)00239-7.

[2] J. Koivunen, H. Heinonen-Tanski, Peracetic acid (PAA) disinfection of primary, secondary and tertiary treated municipal wastewaters, Water Res. 39 (2005) 4445–4453. doi:10.1016/j.watres.2005.08.016.

[3] R. Gehr, D. Cochrane, Peracetic acid (PAA) as a disinfectant for municipal wastewaters: encouraging performance results from physicochemical as well as biological effluents, Proc. Water Environ. Fed. 2002 (2002) 182–198. doi:10.2175/193864702785033527.

[4] T. Luukkonen, J. Teeriniemi, H. Prokkola, J. Rämö, U. Lassi, Chemical aspects of peracetic acid based wastewater disinfection, Water SA. 40 (2014) 73–80. doi:10.4314/wsa.v40i1.9.

[5] R.K. Chhetri, A. Bonnerup, H.R. Andersen, Combined Sewer Overflow pretreatment with chemical coagulation and a particle settler for improved peracetic acid disinfection, J. Ind. Eng. Chem. 37 (2016) 372–379. doi:10.1016/j.jiec.2016.03.049.

[6] G.P. Winward, L.M. Avery, T. Stephenson, B. Jefferson, Chlorine disinfection of grey water for reuse: Effect of organics and particles, Water Res. 42 (2008) 483–491. doi:10.1016/j.watres.2007.07.042.

[7] M.W. LeChevallier, T.S. Hassenauer, A.K. Camper, G.A. McFeters, Disinfection of bacteria attached to granular activated carbon, Appl. Environ. Microbiol. 48 (1984) 918–923.

[8] J.P. Dietrich, F.J. Loge, T.R. Ginn, H. Başağaoğlu, Inactivation of particle-associated microorganisms in wastewater disinfection: Modeling of ozone and chlorine reactive diffusive transport in polydispersed suspensions, Water Res. 41 (2007) 2189–2201. doi:10.1016/j.watres.2007.01.038.

[9] Z. Bohrerova, K.G. Linden, Ultraviolet and Chlorine Disinfection of Mycobacterium in Wastewater: Effect of Aggregation, Water Environ. Res. 78 (2006) 565–571.

[10] R.G. Qualls, S.F. Ossoff, J.C.H. Chang, M.H. Dorfman, C.M. Dumais, D.C. Lobe, J.D. Johnson, Factors controlling censitivity in ultraviolet disinfection of secondary effluents, Water Pollut. Control Fed. 57 (1985) 1006–1011. doi:10.2307/25042770.

[11] R.G. Qualls, M.P. Flynn, J.D. Johnson, The Role of suspended particles in ultraviolet disinfection, Water Pollut. Control Fed. 55 (1983) 1280–1285. doi:10.2307/25042084.

[12] D. Falsanisi, R. Gehr, L. Liberti, M. Notarnicola, Effect of suspended particles on disinfection of a physicochemical municipal wastewater with peracetic acid, Water Qual. Res. J. Canada. 43 (2008) 47–54.

[13] M. McFadden, J. Loconsole, A.J. Schockling, R. Nerenberg, J.P. Pavissich, Comparing peracetic acid and

hypochlorite for disinfection of combined sewer overflows: Effects of suspended-solids and pH, Sci. Total Environ. 599–600 (2017) 533–539. doi:10.1016/j.scitotenv.2017.04.179.

[14] D. Santoro, F. Crapulli, M. Raisee, G. Raspa, C.N. Haas, Nondeterministic Computational Fluid Dynamics Modeling of *Escherichia coli* Inactivation by Peracetic Acid in Municipal Wastewater Contact Tanks, Environ. Sci. Technol. 49 (2015) 7265–7275. doi:10.1021/es5059742.

[15] S. Rossi, M. Antonelli, V. Mezzanotte, C. Nurizzo, Peracetic acid disinfection: a feasible alternative to wastewater chlorination., Water Environ. Res. 79 (2007) 341–350. doi:10.2175/106143006X101953.

[16] A. Turolla, R. Sabatino, D. Fontaneto, E.M. Eckert, N. Colinas, G. Corno, B. Citterio, F. Biavasco, M. Antonelli, A. Mauro, G. Mangiaterra, A. Di Cesare, Defence strategies and antibiotic resistance gene abundance in enterococci under stress by exposure to low doses of peracetic acid, Chemosphere. 185 (2017) 480–488. doi:10.1016/j.chemosphere.2017.07.032.

[17] B.D. Sully, P.L. Williams, The analysis of solutions of per-acids and hydrogen peroxide, Analyst. 87 (1962) 653. doi:10.1039/an9628700653.

[18] APHA/AWA/WEF, Standard Methods for the Examination of Water and Wastewater, 22nd ed., Washington, DC., 2012. doi:ISBN 9780875532356.

[19] APAT-IRSA/CNR, Metodi analitici per il controllo della qualità delle acque., Rome, Italy, 2003.

[20] C.N. Haas, G.R. Finch, Methodologies for the determination of disinfection effectiveness, AWWA Research Foundation and American Water Works Association, 2001.

[21] D. Santoro, F. Crapulli, M. Raisee, G. Raspa, C.N. Haas, Nondeterministic Computational Fluid Dynamics Modeling of Escherichia coli Inactivation by Peracetic Acid in Municipal Wastewater Contact Tanks, Environ. Sci. Technol. 49 (2015) 7265–7275. doi:10.1021/es5059742.

[22] L.F. Pedersen, P.B. Pedersen, J.L. Nielsen, P.H. Nielsen, Peracetic acid degradation and effects on nitrification in recirculating aquaculture systems, Aquaculture. 296 (2009) 246–254. doi:10.1016/j.aquaculture.2009.08.021.

[23] M.F. Dignac, P. Ginestet, D. Rybacki, A. Bruchet, V. Urbain, P. Scribe, Fate of wastewater organic pollution during activated sludge treatment: nature of residual organic matter, Water Res. 34 (2000) 4185–4194. doi:10.1016/S0043-1354(00)00195-0.

[24] V. Mezzanotte, M. Antonelli, S. Citterio, C. Nurizzo, Wastewater Disinfection Alternatives : Chlorine, Ozone, Peracetic Acid, and UV Light, Water Environ. Res. 79 (2007) 2373–9. doi:10.2175/106143007X183763.

[25] D. Santoro, T.A. Bartrand, D.J. Greene, B. Farouk, C.N. Haas, M. Notarnicola, L. Liberti, Use of CFD for Wastewater Disinfection Process Analysis: E.coli Inactivation with Peroxyacetic Acid (PAA), Int. J. Chem. React. Eng. 3 (2005). doi:10.2202/1542-6580.1283.

[26] M. Profaizer, Aspetti modellistici e tecniche alternative nella disinfezione di acque potabili: l'acido peracetico, Ph. D. Thesis, Politecnico di Milano, 1998.

[27] J. Mir, J. Morató, F. Ribas, Resistance to chlorine of freshwater bacterial strains, J. Appl. Microbiol. 82 (1997) 7–18. doi:10.1111/j.1365-2672.1997.tb03292.x.

[28] D. Santoro, R. Gehr, T.A. Bartrand, L. Liberti, M. Notarnicola, A. Dell'Erba, D. Falsanisi, C.N. Haas, Wastewater disinfection by peracetic acid: assessment of models for tracking residual measurements and inactivation., Water Environ. Res. 79 (2007) 775–87. doi:10.2175/106143007X156817.

[29] L.L. Gyiirek, G.R. Finch, Modelling water treatment chemical disnfection kinetics, J. Environ. Eng. 124 (1998) 783–793.

[30] L. Li, Effects of initial microbial density on disinfection efficiency in a continuous flow system and validation of disinfection batch kinetics in a continuous flow system, Drexel University, 2004.

[31] C.N. Haas, J. Joffe, Disinfection under Dynamic Conditions: Modification of Hom's Model for Decay, Environ. Sci. Technol. 28 (1994) 1367–1369. doi:10.1021/es00056a028.

[32] J.G. Jacangelo, N. Patania, R. Trussell, C. Gerba, C.N. Haas, Inactivation of Waterborne Emerging Pathogens by Selected Disinfectants, AWWA Research Foundation and American Water Works Association, Denver, CO, 2002.

[33] C.N. Haas, M.S. Heath, J. Jacangelo, J. Joffe, U. Anmangandla, J.. Hornberger, J. Glicker, Development and validation of rational design methods of disinfection, The Foundation and American Water Works Association, Denver, CO, 1995.

[34] J. Anotai, Effect of calcium ion on chemistry and disinfection efficiency of free chlorine, Drexel University, 1996.

[35] L. Domínguez Henao, R. Delli Compagni, A. Turolla, M. Antonelli, Influence of inorganic and organic compounds on the decay of peracetic acid in wastewater disinfection, Chem. Eng. J. 337 (2018) 133–142.

[36] D. Falsanisi, R. Gehr, D. Santoro, A. Dell'Erba, M. Notarnicola, L. Liberti, Kinetics of PAA demand and its implications on disinfection of wastewaters, Water Qual. Res. J. Canada. 41 (2006) 398–409.

[37] M. Antonelli, S. Rossi, V. Mezzanotte, C. Nurizzo, Secondary effluent disinfection: PAA long term efficiency, Environ. Sci. Technol. 40 (2006) 4771–4775.

[38] T. Luukkonen, S.O. Pehkonen, Peracids in water treatment: A critical review, Crit. Rev. Environ. Sci. Technol. 0 (2016) 1–39.

[39] D.M. Bates, D.G. Watts, Nonlinear Regression Analysis and Its Applications, in: John Wiley & Sons, Inc., 2008: pp. i–xiv.

[40] M. Profaizer, Peracetic acid as an alternative technology for drinking water disinfection: a new modelling approach (in Italian). PhD. Dissertation, Politecnico di Milano, 1998.

[41] R.W. Emerick, F.J. Loge, T. Ginn, J.L. Darby, Modeling the Inactivation of Particle- Associated Coliform Bacteria, Water Environ. Res. 72 (2000) 432–438 (7).

[42] R.K. Chhetri, D. Thornberg, J. Berner, R. Gramstad, U. Öjstedt, A.K. Sharma, H.R. Andersen, Chemical disinfection of combined sewer overflow waters using performic acid or peracetic acids, Sci. Total Environ. 490 (2014) 1065–1072.

6.6. Supporting information

Effect of solids on PAA decay. An example of PAA decay data is shown in Figure S1, referred to experimental data obtained in decay tests with the lowest (5mg/L) and the highest (160mg/L) TSS concentrations at two PAA concentrations, namely 2 and 8 mg_{PAA}/L, compared to the corresponding data obtained in the stock filtrate solution. In addition, values of initial oxidative demand (OD) and decay rate constant (k) estimated by the non-linear least-square regression are reported in Tables S1 and S2.

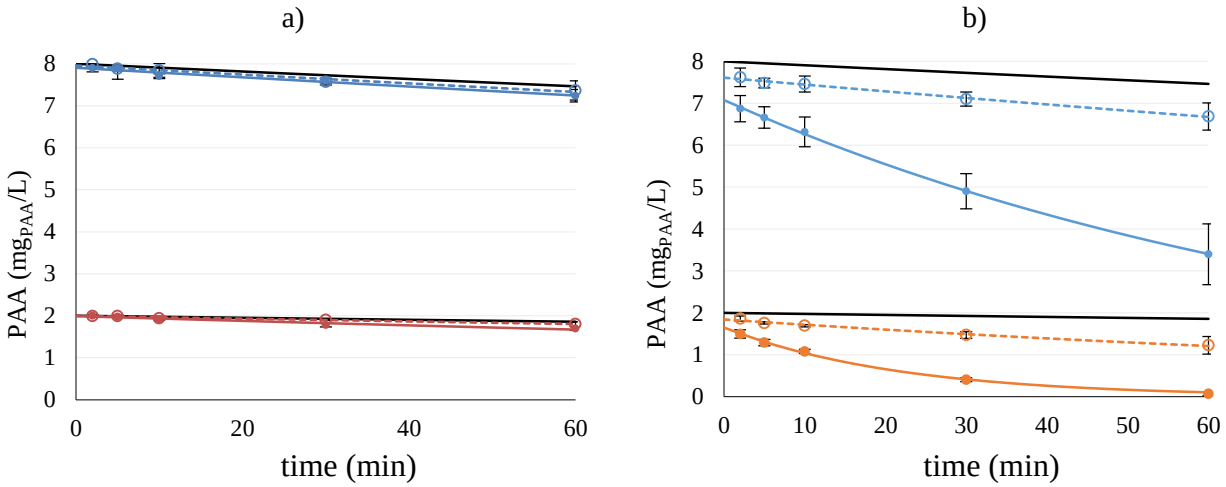

Figure S1. PAA concentration (mean±dev.st) vs. time as a function of PAA (2 and 8 mg_{PAA}/L) and TSS concentration: a) 5 mg_{TSS}/L and b) 160 mg_{TSS}/L (sludge from WWTP-B).

●, —: PAA decay with TSS: experimental data and interpolating curve;

○, - - -: PAA decay with filtrate and interpolating curve;

In black: PAA decay in deionized water.

Table S1. Estimated coefficients of Haas and Finch model (OD and k) for the experiments performed with the stock TSS solution prepared with activated sludge samples form WWTP-A. The mean values of the replicates are reported.

Stock Solution	TSS (mg_{TSS}/L)	PAA (mg_{PAA}/L)	OD (mg_{PAA}/L)	k (min^{-1})	R^2
TSS	5	2	0.01	0.00436	0.89
TSS	40	2	-0.02	0.00784	0.96
TSS	80	2	0.22	0.02206	0.99
TSS	120	2	0.37	0.03131	0.97
TSS	160	2	0.37	0.04811	0.99
TSS	5	5	0.00	0.00301	0.91
TSS	40	5	-0.08	0.00508	0.94
TSS	80	5	0.39	0.01094	0.97
TSS	120	5	0.46	0.01886	0.98
TSS	160	5	0.52	0.02920	0.99
TSS	5	8	0.05	0.00153	0.85
TSS	80	8	0.31	0.00699	0.97
TSS	160	8	0.87	0.01234	0.97
Filtrate	5	2	0.03	0.00160	0.68
Filtrate	40	2	0.04	0.00273	0.72
Filtrate	80	2	0.06	0.00316	0.83
Filtrate	120	2	0.10	0.00431	0.94
Filtrate	160	2	0.08	0.00460	0.93
Filtrate	5	5	-0.04	0.00206	0.91
Filtrate	40	5	0.00	0.00236	0.95
Filtrate	80	5	0.00	0.00173	0.84
Filtrate	120	5	0.02	0.00351	0.77
Filtrate	160	5	0.12	0.00351	0.78
Filtrate	5	8	0.12	0.00110	0.77
Filtrate	80	8	0.08	0.00139	0.62
Filtrate	160	8	0.26	0.00189	0.79

Table S2. Estimated coefficients of Haas and Finch (OD and k) model for the experiments performed with the stock TSS solution prepared with activated sludge samples form WWTP-B. The mean values of the replicates are reported.

Stock Solution	TSS (mg_{TSS}/L)	PAA (mg_{PAA}/L)	OD (mg_{PAA}/L)	k (min^{-1})	R^2
TSS	5	2	0.01	0.00293	0.90
TSS	40	2	-0.02	0.00941	0.99
TSS	80	2	0.19	0.01772	0.95
TSS	120	2	0.32	0.03090	0.98
TSS	160	2	0.35	0.04606	0.99
TSS	5	5	-0.04	0.00173	0.83
TSS	40	5	-0.09	0.00461	0.91
TSS	80	5	0.18	0.01157	0.98
TSS	120	5	0.31	0.01711	0.99
TSS	160	5	0.29	0.02374	0.98
TSS	5	8	0.09	0.00145	0.88
TSS	40	8	0.27	0.00535	0.93
TSS	80	8	0.71	0.00951	0.94
TSS	120	8	0.75	0.00989	0.93
TSS	160	8	0.98	0.01219	0.93
Filtrate	5	2	0.00	0.00172	0.89
Filtrate	40	2	0.03	0.00220	0.73
Filtrate	80	2	0.05	0.00334	0.85
Filtrate	120	2	0.15	0.00437	0.91
Filtrate	160	2	0.16	0.00702	0.86
Filtrate	5	5	-0.02	0.00084	0.53
Filtrate	40	5	0.06	0.00096	0.81
Filtrate	80	5	0.05	0.00245	0.76
Filtrate	120	5	0.07	0.00297	0.71
Filtrate	160	5	0.19	0.00388	0.76
Filtrate	5	8	0.04	0.00137	0.70
Filtrate	40	8	0.24	0.00112	0.47
Filtrate	80	8	0.20	0.00159	0.82
Filtrate	120	8	0.23	0.00248	0.88
Filtrate	160	8	0.39	0.00219	0.78

Decay rate constant model The results of regressions on the dataset from both WWTP-A and B (parameter

estimates and main statistics) are reported in the following tables for the sub-model for soluble matter (S3), for the sub-model for suspended matter (S4), and for the overall model (S5 and S6).

Table S3. Summary statistics and regression estimates for the model of decay rate due to soluble matter (k_{SOL})

	WWTP-A sludge		**WWTP-B sludge**	
Parameter	***a*$_1$**	***a*$_2$**	***a*$_1$**	***a*$_2$**
Estimates	0.059	-0.004	0.056	-0.004
Min 95% Confidence Interval	0.044	-0.007	0.045	-0.006
Max 95% Confidence Interval	0.074	-0.001	0.068	-0.002
Standard Error (SE)	0.007	0.001	0.005	0.001
t (df)	7.986	-2.987	10.116	-4.243
p_level	<0.05	<0.05	<0.05	<0.05
R^2 (adj-R^2)	0.399 (0.364)		0.661 (0.645)	
n	37		45	

Table S4. Summary statistics and regression estimates for the model of decay rate due to only suspended matter (k^*_{TSS})

	WWTP-A sludge			**WWTP-B sludge**		
Parameter	***b*$_1$**	***α***	***β***	***b*$_1$**	***α***	***β***
Estimates	0.069	1.313	- 0.550	0.107	1.209	- 0.568
Min 95% Confidence Interval	0.005	1.123	-0.65	0.014	1.033	-0.66
Max 95% Confidence Interval	0.134	1.502	-0.45	0.200	1.386	-0.47
St.Err (SE)	0.032	0.093	0.048	0.046	0.087	0.046
t (df)	2.188	14.109	-11.410	2.319	13.83	-12.274
p_level	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
R^2 (adj-R^2)	0.950 (0.945)			0.938 (0.933)		
n	37			45		

Table S5. Summary statistics and regression estimates for the overall model of decay rate applied to data from

WWTP-A.

WWTP-A	**Soluble matter model**		**Suspended matter model**		
Parameter	a_1	a_2	b_1	α	β
Estimates	0.079	-0.009	0.064	1.318	-0.505
Min 95% Confidence Interval	0.05	-0.015	0.020	1.180	-0.598
Max 95% Confidence Interval	0.108	-0.003	0.108	1.456	-0.412
Standard Error (SE)	0.014	0.003	0.022	0.069	0.047
t (df)	5.401	-3.116	2.898	19.023	-10.804
p-level	<0.05	<0.05	<0.05	<0.05	<0.05
R^2 (adj-R^2)	0.972 (0.97)				
n	74				

Table S6. Summary statistics and regression estimates for the overall model of decay rate applied to data from WWTP-B

WWTP-B	**Soluble matter model**		**Suspended matter model**		
Parameter	a_1	a_2	b_1	α	β
Estimates	0.056	-0.004	0.106	1.210	-0.568
Min 95% Confidence Interval	0.036	-0.008	0.037	1.078	-0.661
Max 95% Confidence Interval	0.077	-0.001	0.177	1.341	-0.475
Standard Error (SE)	0.010	0.002	0.035	0.066	0.046
t (df)	5.440	-2.315	3.034	18.277	-12.157
p-level	<0.05	<0.05	<0.05	<0.05	<0.05
R^2 (adj-R^2)	0.965 (0.963)				
n	90				