



Cadmium biosorption by ozonized activated sludge: The role of bacterial flocs surface properties and mixed liquor composition

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ABSTRACT

Cadmium uptake by activated sludge was studied following modifications of sludge composition and surface properties induced by ozone treatment. Ozone leads to the solubilization of sludge compounds as well as their mineralization, especially humic like substances. Small particles were formed following floc disintegration, leading to a decrease of average floc size. The study of surface properties underlined the mineralization as the number of surface binding sites decreased with the increase of ozone dose. Depending on ozone dose, cadmium uptake by activated sludge flocs was either increased or decreased. Different mechanisms were involved: below 10 mg O₃/g TS, the increase of floc specific surface area following floc size decrease as well as the release of phosphate ions yielded an increase by 75% of cadmium uptake, due to the better availability of biosorption sites and the increase of precipitation. Inversely, at higher ozone doses, the number of biosorption sites decreased due to oxidation by ozone. Moreover, dissolved organic matter concentration increased and provided ligands for metal complexation. Cadmium uptake was therefore limited for ozone doses ranging from 10 to 16.8 mg O₃/g TS.

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1. Introduction

The legislation concerning water treatment in Europe (91/271/EEC directive, 21/05/1991 and framework directive 2000/60/CE, 23/10/2000) requires more extensive and more efficient wastewater treatment. It implies an increasing production of sewage sludge in Europe (from 5.5 millions tonnes of dry solids per year in 1992 to 9 millions tonnes in 2005). Three main alternatives are currently used for sludge disposal: land application, land filling and incineration. However, these routes are prone to various socio-economical problems limiting or even hindering their use: sludge use in agriculture is strictly regulated concerning both potentially toxic elements concentrations (heavy metals, pathogens, persistent organic pollutants) and agronomic value, incineration is often expensive, sludge land filling is banned since 2002. Finally, sludge treatment and valorization costs account for up to 50% of wastewater treatment plants operating costs [1].

The interest for new technologies allowing sludge volume and mass reduction is therefore presently increasing. Ozonation of activated sludge has proven to be an effective process for reducing sludge production at source. It was indeed already set up at full scale

applications [2] and commercialized (Degrémont BioLysis O[®]). The process of sludge ozonation is generally described by the sequential reactions of floc disintegration, solubilization, followed by oxidation of the released organics into carbon dioxide (mineralization) [3–5].

Sludge reduction performances by ozone treatment are well documented in the literature [4]. However, the environmental aspects and the sustainability of such technologies have not been yet extensively investigated. Especially, few data concerning the fate of heavy metals following activated sludge treatment with an oxidative process such ozonation is available. Studying heavy metals behavior when using sludge reduction processes is nevertheless of great concern since their presence in wastewaters constitutes a major environmental issue. Indeed, they exhibit a potential high toxicity if rejected in the environment. The metal content of excess sludge should be carefully monitored due to strict regulations concerning sludge landspreading. Moreover, municipal wastewater treatment plants (WWTPs) play a major role for the control of the discharge of heavy metals in the environment [6].

Presently, only the results obtained by Zhang et al. [7] can be noticed when ozone is used as oxidant: they observed that several metallic species (Cr, Cu, Hg, Ni) were released in the sludge supernatant after ozonation at a dose of 50 mg O₃/g TS during 90 min. Cadmium behavior differed from the above cited metals as its concentration in sludge particulate fraction was increasing. However, the authors did not provide any interpretation of these phenomena. Also, Dewil et al. [8] studied the effect of Fenton's peroxidation

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on heavy metals content of dewatered sludge, they observed the release of Cd, Cu and Ni in the aqueous fraction (filtrate). In this last case however, oxidative treatment was not used to minimize sludge production during wastewater treatment.

The fate of heavy metals in WWTPs is the result of complex interactions between metal cations and the surrounding medium including surface complexation with negatively charged biopolymers, precipitation, ion exchange, physical adsorption, etc. [9,10]. These mechanisms are impacted by the physico-chemical and process parameters: hydraulic residence time [11], sludge age [12], feed C/N ratio [13], pH [14], temperature [14], dissolved organic matter [15], the presence of multiple metallic elements competing for sorption [16], the composition of the extracellular polymer matrix [17], etc.

These mechanisms are strongly correlated with activated sludge structure and composition. In the presence of a strong oxidant such as ozone, all sludge compounds may undergo important chemical modifications leading to important variations of sludge properties (functional groups present at the floc surface and in the soluble fraction, etc.). These modifications may then alter heavy metals interactions with mixed liquor. The decrease of the final quantity of waste activated sludge may also induce an over-accumulation of metals in the excess sludge produced.

The present study therefore focused on the relationship between activated sludge physico-chemical properties following ozonation and heavy metals interactions. Cadmium was chosen because of its possible significant concentrations in wastewaters and sewage sludge [6,9] and because of its classification as a priority substance by the Water Framework Directive (European Directive 2455/2001/CE modifying the 2000/60/CE directive). The mechanisms governing the fate of cadmium were established and discussed with respect to floc surface functionality and the composition of activated sludge soluble and particulate fractions.

2. Methods

2.1. Samples preparation

Activated sludge samples were obtained from the aeration tank in the municipal wastewater treatment plant of the city of Limoges (285,000 inhabitant-equivalent) and stored for a maximum duration of 24 h at 4 °C before use. Average characteristics of samples were as follows: total solids (TS): 4.1 ± 0.1 g/L, volatile solids/total solids (VS/TS): 70%.

Sludge ozonation was carried out with a Trailigaz 5LO ozone generator (Trailigaz Wedeco, Roissy, France). Gas flow rate, supplied power and gas pressure were fixed at 300 L/h, 150 W and 0.8 bar respectively. The ozone/sludge contactor consisted of a 1 L glass reactor provided with a fine bubble diffuser. The sludge volume to be treated was 800 mL. Ozone concentration at the outlet of the contactor was measured according to the iodometric method [18]: the gas leaving the sludge reactor was bubbled successively through two flasks each containing 400 mL potassium iodide (20 g/L KI, 7.3 g/L Na₂HPO₄, 2H₂O, 3.5 g/L KH₂PO₄). The ozone dose applied to the sludge was determined by subtracting the amount of ozone measured with an empty contactor (corresponding to the ozone production of the generator) and the amount of ozone measured after sludge ozonation when the same gas volume was injected in the system according to Eq. (1):

$$D = \frac{C_{O_3} \times V_{\text{trial}} - m(O_3)_{\text{residual}}}{V_{\text{sludge}} \times [\text{TS}]} \quad (1)$$

where D is the ozone dose consumed by the sludge (g O₃/g TS); C_{O_3} is the ozone concentration in the gas (g/L); $m(O_3)_{\text{residual}}$ is the total residual ozone mass measured during sludge treatment (g); V_{trial}

is the gas volume measured during sludge treatment (L); V_{sludge} is the sludge sample volume (800 mL); [TS] is the sludge sample total solids concentration (g/L).

For operational reasons, activated sludge samples were either filtrated (0.45 μm) or centrifuged (6000 × g, 20 min, 4 °C), according to the measurement carried out, to separate particulate and soluble fractions. Centrifugation was used for total suspended solids measurement and potentiometric titrations. Filtration was used for soluble biochemical compounds determination as well as during metal biosorption and precipitation tests. The supernatant after centrifugation might contain more particulate/colloidal material (small flocs) than the filtrate after filtration. However, this did not affect the trends discussed in this study. Also, the filtrate or centrifuged supernatant originating from these procedures may contain both soluble and colloidal compounds. For simplification purpose, the term “soluble fraction” refers to both soluble and colloidal fraction in the present study.

2.2. Chemical and biochemical composition

Sludge characteristics measurements were done in triplicate. Chemical Oxygen Demand (COD) was measured by the closed reflux colorimetric method (ISO 15705:2002). Polysaccharides were determined using the colorimetric method of Dubois et al. [19]. Proteins and humic like substances were determined using the method of Lowry et al. [20] modified by Frølund et al. [21]. The degree of solubilization was defined as the transfer of sludge components from the particulate fraction of the sludge to the soluble fraction (defined here as the 0.45 μm filtrate) and is calculated as the ratio of solubilized compounds at given ozone dose to initial particulate compound concentration [22]. For all colorimetric methods used in this study, the standard deviation for triplicate samples was 1–8% and 5–20% for soluble and total fractions respectively. Ionic species remaining in solution after treatment were determined on samples filtrated at 0.22 μm using ion chromatography (DIONEX 120) according to the standard method [23]. The used detector was conductimetric. A daily calibration allowed the determination of a given anion from the surface of the peak observed on the chromatogram. Analytical error was ±5%.

Measurements of total solids, volatile solids, total suspended solids and volatile suspended solids were performed according to the standard method [24]. The standard deviation for triplicate samples was below 2%.

2.3. FTIR analysis

IR spectra were performed on the dried washed biomass according to the procedure described by Laurent et al. [25]. The IR spectra were recorded using a Perkin-Elmer 1000 infrared spectrometer.

2.4. Determination of floc size by automated image analysis

Images captures were made using an optical microscope (Olympus CX 31) equipped with a CCD video camera connected to a PC via a grabbing card. Measurements were done using the software Visilog 6 (Noesis, Saint Aubin, France) according to the procedure described by Laurent et al. [26].

2.5. Determination of active sites number and acidity constants

In order to assess the potential chemical variations induced by ozone treatment, potentiometric titrations were carried out to determine pK_a values and proton binding site concentrations on both floc surface and soluble phase. The total number of ionizable functional groups was determined on both floc surface and soluble fraction by potentiometric titration coupled with surface proton

complexation modeling. The particulate fraction was studied after washing of the biomass by a sequence of three centrifugations ($6000 \times g$, 20 min, 4°C) followed by resuspension in 0.01 M NaNO_3 . The first centrifugation supernatant was used for titration of soluble fraction. Titrations were carried out using an automatic titrator (Metrohm 721 NET Titrino). The samples were divided in two parts: one was titrated with 0.01 M HNO_3 , the second was titrated using 0.01 M NaOH . Data interpretation using a non-electrostatic model of proton adsorption was performed using the software PROTOFIT [27]. This allowed both number and acidity constant of the different functional groups to be determined. The detailed protocol is given in a previous study [25].

2.6. Cd biosorption tests (total sludge)

Cadmium behavior towards activated sludge flocs was evaluated after ozone treatment by batch biosorption tests conducted at a constant pH level of 7. Cadmium was added as chloride salt ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$, purity >98%) using stock solutions of 10 g/L of Cd. 100 mL high density polyethylene bottles were used for batch metal sorption experiments to minimize metal sorption to the bottle surface. Sorption isotherms were obtained using 50 mL of mixed liquor with known TSS concentration spiked with seven different initial metal concentrations (0, 5, 10, 20, 40, 100 and 200 mg/L). pH of sludge-metal suspensions was fixed at a value of 7 using 1 M NaOH in order to reproduce conditions occurring in a real WWTP. In order to keep pH constant during the course of biosorption, HEPES buffer was also added in each bottle ($1.1915 \text{ g}/50 \text{ mL} = 10^{-1} \text{ M}$). HEPES buffer was used because it is recognized as non-complexing metals [17]. The bottles were then shaken for 3 h at 180 rpm on a rotary shaker at ambient temperature ($20 \pm 2^\circ\text{C}$ daily checked). Preliminary kinetic studies indicated that metal sorption by untreated and ozonized sludge reached equilibrium after approximately 2 h. Once equilibrium was reached, the sludge suspension was filtered through a cellulose nitrate membrane ($0.45 \mu\text{m}$ pore size). The filtrate was acidified with three drops of concentrated HNO_3 (69%, w/w) and stored at 4°C until analysis. The soluble metal, calcium and magnesium concentrations were determined in the acidified filtrate by a flame atomic absorption spectrometer (FAAS) Varian 220FS.

2.7. Cadmium behavior in the soluble fraction

In order to investigate the contribution of metal precipitation to metal removal occurring in samples, tests similar to those carried out on the total fraction were carried out on filtrates ($0.45 \mu\text{m}$) of

sludge after treatment and at metal additions of 10, 40, 100 and 200 mg/L, according to the procedure introduced by Brown and Lester [28] and previously used by Laurent et al. [29]. Mixed liquor was filtered through a $0.45 \mu\text{m}$ pore size membrane immediately after ozonation. Initial pH was fixed at 7 using 1 M NaOH and 1 M HCl . Then, cadmium was added. After 3 h of shaking, samples were again filtrated at $0.45 \mu\text{m}$. The metal concentration remaining in soluble fraction and phosphate content were analyzed by FAAS and ion chromatography respectively.

3. Results and discussion

3.1. Effects of ozonation on sludge composition and structure

One of the aim of a sludge reduction process such ozonation is to convert the particulate organic matter into soluble organic matter. This is achieved by cell lysis and/or by reducing the size of the organic matter particles. The released compounds may alter the equilibrium between the soluble (free + complexed) and the particulate (sorbed + precipitated) metal species.

Sludge structure and composition were studied for ozone doses ranging from 3.4 to 15.8 $\text{mg O}_3/\text{g TS}$.

Automated image analysis results (Table 1) underlined a progressive decrease of floc mean projected surface area from 130 to $22 \mu\text{m}^2$ for ozone dose ranging from 0 to 15.8 $\text{mg O}_3/\text{g TS}$. This was certainly the consequence of the release of very fine particles in the supernatant [7]. Floc size decrease may induce a better availability of flocs surface functional groups to bind metal cations.

Table 1 presents matter quantity and repartition. For the studied ozone dosage, a slight decrease of TS concentration was noticed as it ranged from $4.1 \pm 0.1 \text{ g/L}$ for untreated sludge to $3.7 \pm 0.1 \text{ g/L}$ for sludge treated at 15.8 $\text{mg O}_3/\text{g TS}$. This suggests that a complete oxidation of sludge particulate matter occurred: a part of sludge compounds was mineralized to CO_2 (10% at 15.8 $\text{mg O}_3/\text{g TS}$). This phenomenon was observed in previous studies for higher ozone doses: Ahn et al. [30] obtained 20% of mineralization for ozonation at 0.5 $\text{g O}_3/\text{g TS}$. Bougrier et al. [31] stated that mineralization was negligible below 0.2 $\text{g O}_3/\text{g TS}$. However, these authors studied a more concentrated sludge (16.1 g/L TSS) which could explain this contradictory result. At the same time, volatile solids solubilization was quite low: 8% at 15.8 $\text{mg O}_3/\text{g MS}$. However, as mineralization also occurred, solubilization was probably underestimated.

Biochemical measurements performed on sludge total fraction highlighted this mineralization: sludge total COD ranged from $5080 \pm 110 \text{ mg O}_2/\text{L}$ to $4520 \pm 170 \text{ mg O}_2/\text{L}$ for ozone dose between 0 and 15.8 $\text{mg O}_3/\text{g MS}$. Mineralization was almost focused on

Table 1
Properties of ozonized sludge (ND: non-detected).

	Dose ($\text{mg O}_3/\text{g TS}$)				
	0	3.4	7.9	11.8	15.8
Ions (mg/L)					
Cl^-	46.1	51.59	51.46	47.07	48.01
NO_2^-	0	0.35	0.63	0.55	0.21
NO_3^-	0.85	2.48	4.65	9.83	16
PO_4^{2-}	16.52	20.09	27.02	29.3	26.8
SO_4^{2-}	36.1	41.64	42.5	40.14	42.99
Na^+	28.16	32.84	34.68	30.77	30.19
NH_4^+	0.94	1.03	0.69	1.38	3.26
K^+	11.83	18.14	24.53	27.65	30.62
Mg^{2+}	5.55	7.24	8.72	8.66	9.73
Ca^{2+}	32.42	34.06	32.7	27.98	27.89
Floc mean projected surface area (μm^2)	130	65	54	40	22
Soluble organic matter (g/L)	ND	ND	ND	0.27	0.37
Particulate organic matter (g/L)	2.85	2.74	2.59	2.45	2.25
Soluble mineral matter (g/L)	0.17	0.10	0.19	0.19	0.17
Particulate mineral matter (g/L)	0.98	1.0	0.91	0.91	0.89

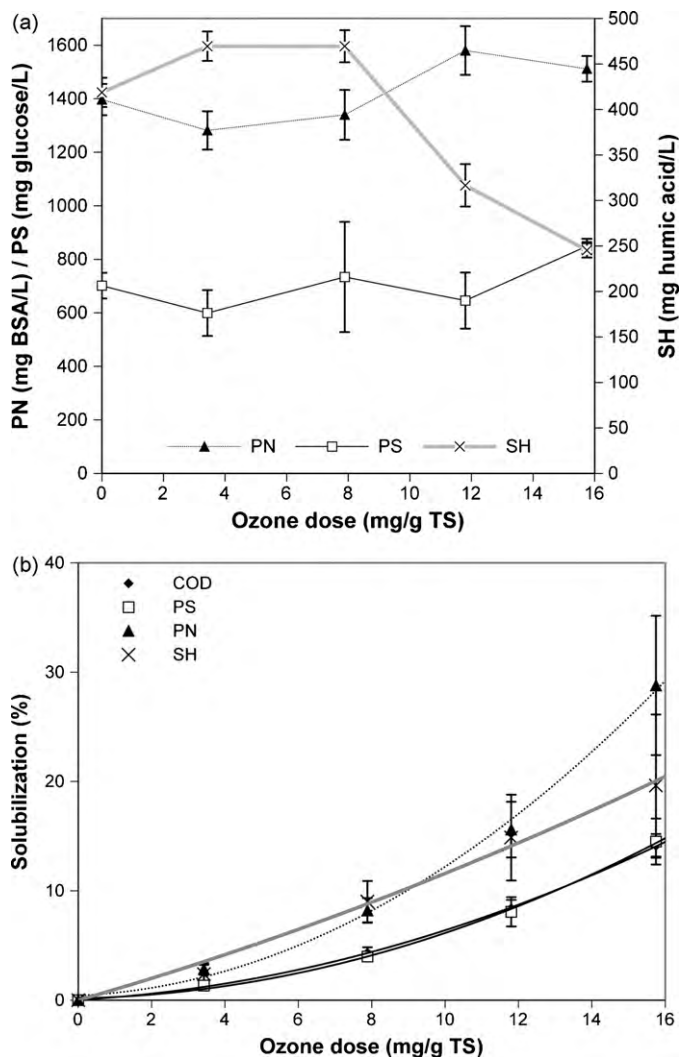


Fig. 1. Biochemical composition of ozonized sludge. (a) Total concentrations; (b) Solubilization as a function of ozone dose. PN: proteins, PS: polysaccharides, SH: humic like substances.

humic like substances as their concentration decreased suddenly from 450 ± 30 mg HA/L for doses below 7.9 mg O₃/g TS to 245 mg HA/L at 15.8 mg O₃/g TS (Fig. 1a). At the same time, proteins and polysaccharides total concentrations remained almost constant.

Solubilization of organic matter followed the order humic like substances = proteins > polysaccharides for doses below 11.8 mg O₃/g TS (Fig. 1b). For the highest dose tested (15.8 mg O₃/g MS), proteins were the most solubilized (28.8%) against humic like substances (19.6%) and polysaccharides (14.5%).

Concentrations of ionic substances in the aqueous fraction were also affected during ozonation (Table 1): nitrate, phosphate and potassium concentrations were notably increased in this fraction with increasing ozone dose.

3.2. Evolution of floc surface functional groups

The important modifications of activated sludge composition (solubilization of mineral and organic compounds) and structure (floc size decrease) are likely to induce a shift of both floc surface properties and soluble fraction reactivity. Metal behavior towards both particulate and soluble compounds of ozonized activated sludge is governed by specific interactions with active sites on biomass (cell wall and EPS) and in soluble fraction (solubilized

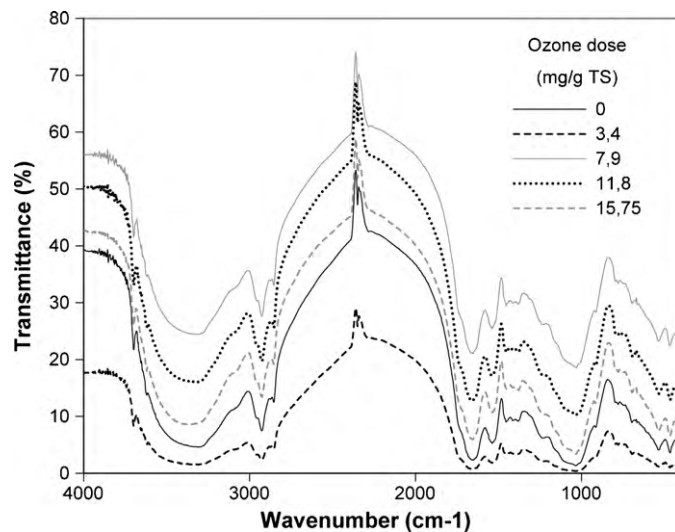


Fig. 2. FTIR spectra of ozonized activated sludge.

compounds). These binding sites are characterized by acid–base properties since most of them are amine, carboxyl, hydroxyl and phosphoric groups originating from proteins, polysaccharides and phospholipids. The extent of attraction of positively charged metallic species by sludge flocs and interactions with them result from the surface charge of these groups, which is ruled by their degree of dissociation with respect to the pH of the medium. Sludge biochemical composition shifted following ozonation: a large part of sludge compounds is transferred from particulate to soluble fraction (proteins, polysaccharides, humic like substances). Therefore, functionality of both floc surface and soluble fraction may have been altered qualitatively and quantitatively. A combination of spectroscopic and potentiometric methods was therefore used to investigate the shift of functional groups due to ozonation.

3.2.1. Flocs characterization by infrared (IR) spectra

Flocs IR spectra were not modified by ozone treatment (Fig. 2). The study of these spectra underlined the presence of functional groups able to react with protons or cations. The more intense characteristic bands observed can be attributed to proteins and polysaccharides functional groups (carboxyl, amine, hydroxyls). For instance, the peak at 1640 – 1660 cm⁻¹ was attributed to the stretching vibrations of C=O and C–N. For a complete description of the IR spectra of activated sludge, refer to Laurent et al. [25]. In order to assess the variations of surface functional groups in a quantitative way, potentiometric titration was carried out on control and ozonized samples.

3.2.2. Flocs and soluble fraction functional groups characterization by potentiometric titration

Potentiometric titration and Surface Complexation Modeling (SCM) allow the determination of the total number of ionizable functional groups in both particulate and soluble fractions, this number is equivalent to the Protonic Exchange Capacity (PEC). It also provides information concerning the various acidity constants associated with functional groups types. In order to assess the nature and the number of potentially active sites for metal biosorption, potentiometric titration was performed in both particulate fraction (floc) and centrifugation supernatant (colloidal + soluble fraction).

3.2.2.1. Repartition of ionizable functional groups (PEC). PEC repartition between soluble and particulate fractions was determined (Fig. 3). The concomitance of matter mineralization and solubi-

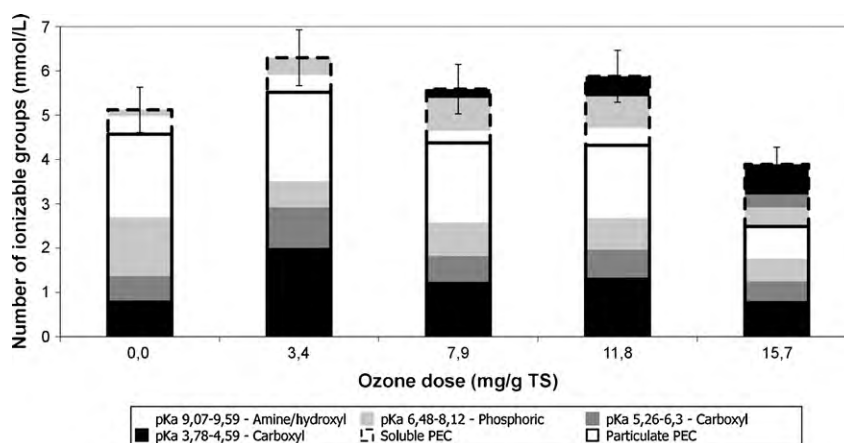


Fig. 3. Volumic concentrations of ionizable functional groups in soluble and particulate fractions and relative contributions of functional groups types associated with their acidity constant (pK_a) for both particulate fraction (flocs) and aqueous fraction of ozonized activated sludge.

lization yielded a two step sequence concerning the repartition of ionizable functional groups: in a first time, the oxidation of soluble compounds [32] led to a decrease of soluble active sites concentrations from 1.45 to 0.45 mmol/L for a dose of 3.4 mg O_3 /g TS. Solubilization became subsequently sufficient to observe an increase of soluble active sites concentration to 1.40 mmol/L when ozone dose ranged from 3.4 to 15.8 mg O_3 /g TS. At the same time, for the highest dose of 15.8 mg O_3 /g TS, the total number of active sites decreased from 5.90 to 3.89 mmol/L.

3.2.2.2. Nature of proton binding sites in particulate and soluble phases. Potentiometric titrations data modeling by a non-electrostatic model of proton adsorption allowed the determination of four mean acidity constants for the different samples as well as the corresponding site concentrations for both soluble and particulate fractions (Fig. 3). The acidity constants have been associated to functional groups types according to IR spectra and literature data [25,33,34]: 3.8–4.6 (pK_{a1}) and 5.3–6.3 (pK_{a2}) were considered as carboxyl groups, 6.5–8.1 (pK_{a3}) were considered mainly as phosphoric groups and 9.1–9.6 (pK_{a4}) were considered as amine or hydroxyl groups.

In the particulate fraction, the main observed mechanism was the important decrease of the number of sites with pK_a comprised in the range 9.3–9.6 for the highest ozone dose tested (15.8 mg O_3 /g TS). These groups partially correspond to hydroxyl function which is importantly carried by humic like substances. The quantity of these groups shifted from 1.87 mmol/L for untreated flocs to 0.72 mmol/L for 15.8 mg O_3 /g TS: their relative contribution at the floc surface therefore dropped from 36.6 to 18.5%. This decrease can therefore be associated with the oxidation/mineralization of these compounds due to ozone (see Section 3.1). At the same time, no significant evolutions could be evidenced concerning the other functional group types.

The relative contributions of functional group types determined in the soluble fraction also shifted during ozonation. As already evidenced during the study of activated sludge sonication by the same experimental protocols [25], the untreated soluble fraction functionality was not representative of sludge solubilized compounds but mainly resulted from initial wastewater composition. For a dose of 3.4 mg O_3 /g TS, the number of soluble functional groups decreased, especially those with pK_a comprised between 6.5 and 8.1 (phosphoric or phenolic groups) which concentration dropped from 1.17 mmol/g to 0.44 mmol/L (Fig. 3). This might be due to a rapid oxidation of these groups by ozone. Above 7.9 mg O_3 /g TS, solubilization of sludge compounds was highlighted by the increase of the number sites attributed to carboxyl function (pK_a ranging

from 4.2 to 4.6 and pK_a 5.6) which shifted from 0.20 to 0.96 mmol/L when ozone dose increased from 7.9 to 15.8 mg O_3 /g TS. Finally, carboxyl function accounted for 68.8% of soluble functional groups for the highest ozone dose. At the same time, no increase of the number of sites with pK_a 9.1–9.6 (amine or hydroxyl groups) could be observed and this fraction was even worn off at 15.8 mg O_3 /g TS: these groups were directly oxidized by the action of ozone in the particulate fraction or immediately after the solubilization of the corresponding compounds.

3.3. Cadmium uptake by ozonized sludge

Metal uptake by activated sludge involves several mechanisms such as sorption onto bacterial cells or EPS, bioaccumulation and precipitation [9]. The classical Langmuir model cannot therefore be used to provide a direct description of the mechanisms occurring during cadmium sorption: the required hypotheses of uniformity of binding sites, monolayer adsorption, etc. may not be respected [35]. Nevertheless, the use of a Langmuir type equation (2) provided good fitting of experimental data and allowed a quantitative comparison of datasets [29].

$$Q_e = \frac{Q_{max} \times K_L \times C_e}{1 + K_L C_e} \quad (2)$$

where Q_e is the quantity of metal adsorbed per mass of TSS ($\mu\text{mol/g TSS}$); C_e is the equilibrium concentration of metal in solution ($\mu\text{mol/L}$); Q_{max} is the maximal adsorption capacity of metal on sludge floc ($\mu\text{mol/g TSS}$); K_L is the Langmuir constant related to affinity ($L/\mu\text{mol}$).

Cadmium uptake isotherms are presented in Fig. 4. Cadmium uptake was affected by ozonation pretreatment (doses ranging from 4.2 and 16.9 mg O_3 /g TS) as ANOVA test performed on the five obtained datasets yielded a p value of 0.03. The determination of Langmuir equation parameters from experimental data by a non-linear regression method, using the spreadsheet developed by Bolster and Hornberger [36], yielded good correlation between experimental and calculated values (R^2 ranging from 0.9930 to 0.9997) for all samples. The maximum biosorption capacity Q_{max} as well as the affinity constant K_L showed variations according to the ozone dose tested (Table 2). Q_{max} increased first from $545 \pm 39 \mu\text{mol/g TS}$ for untreated sludge to reach a maximal value of $957 \pm 48 \mu\text{mol/g TS}$ between 4.2 and 8.1 mg O_3 /g TS. For higher ozone doses (until 16.9 mg O_3 /g TS), Q_{max} gradually decreased to a value close to its initial one. After an initial decrease for the weakest ozone dose (4.2 mg O_3 /g TS), the affinity constant K_L increased subsequently almost linearly with the ozone dose.

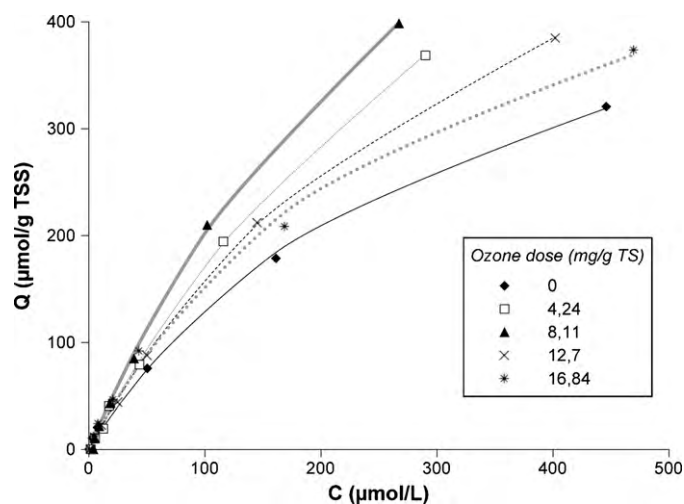


Fig. 4. Cadmium biosorption isotherms on ozonized sludge.

Q_{\max} evolutions could be explained by some of the activated sludge physic-chemical evolutions investigated in this study:

- For the weakest ozone doses (until 8.1 mg O_3/g TS), the observed Q_{\max} increase could be due to the generation of fine particles (see Section 3.1), leading to a larger specific surface area which provided more active sites for cadmium binding to sludge particles. Similarly, the increase of specific surface area after ultrasonic disintegration was previously shown to increase cadmium sorption capacity of activated sludge [37]. At the same time, the increase of soluble phosphate concentration could have favored cadmium precipitation (see Section 3.1).
- For doses above 8.1 mg O_3/g TS, phosphate ion concentration was not varying anymore (see Section 3.1 and Table 1) and the decrease of the total number of active sites for biosorption due to ozone oxidation (see Section 3.2) could explain the observed decrease of Q_{\max} .

3.4. Investigation on the mechanisms of cadmium biosorption

Among the mechanisms of metal biosorption by activated sludge, surface binding and complexation with the negatively charged functional groups at the surface of the floc has been identified as a predominant mechanism [38]. This mechanism can lead to both proton and divalent cation exchange between metal and $Ca^{2+} + Mg^{2+}$ [13,39]. $Ca^{2+} + Mg^{2+}$ are indeed involved in floc structure by bridging the negatively charged biopolymers according to the divalent cations bridging theory [40]. Cadmium precipitation cannot be neglected as the pH used in this study was neutral and due to the compounds present in the soluble fraction [28]. The evolutions of these mechanisms were investigated and discussed according to the ozone dose previously applied to the sludge. Therefore, soluble Ca^{2+} and Mg^{2+} concentrations were measured after biosorption tests performed on total sludge to assess ion exchange.

Table 2

Biosorption parameters (Langmuir and ion exchange ratio) for cadmium biosorption on ozonized activated sludge.

Dose (mg O_3/g TS)	Q_{\max} ($\mu\text{mol Cd/g TSS}$)	K_L ($10^{-3} \text{ L}/\mu\text{mol}$)	$(Ca^{2+} + Mg^{2+})$ release rate ($\mu\text{eq}/\mu\text{eq}$)
0	545 ± 39	3.17 ± 0.44	0.15 ± 0.01
4.24	946 ± 73	2.21 ± 0.25	0.15 ± 0.01
8.11	957 ± 48	2.68 ± 0.21	0.14 ± 0.01
12.7	730 ± 16	2.79 ± 0.11	0.17 ± 0.02
16.84	589 ± 50	3.58 ± 0.64	0.19 ± 0.02

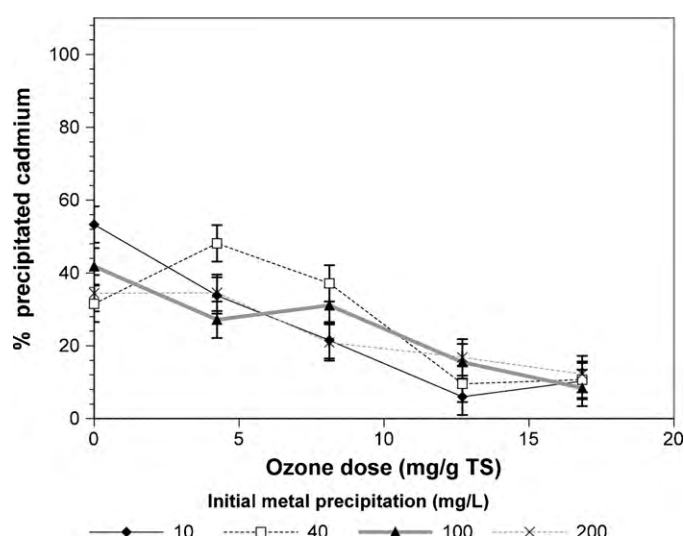


Fig. 5. Cadmium removal from the filtrate of ozonized sludge.

Precipitation was studied by adding known concentrations of cadmium in the filtrate of ozonized sludge and determining metal removal from soluble fraction by filtration at 0.45 μm .

3.4.1. Study of ion exchange mechanism

During biosorption tests performed on total sludge, the concentration of soluble Ca^{2+} and Mg^{2+} increased linearly with sorbed metal quantity. The slope of this curve was therefore used to calculate the $Ca^{2+} + Mg^{2+}$ release rate during cadmium biosorption, expressed as μeq released ($Ca^{2+} + Mg^{2+}$) per μeq sorbed Cd (Table 2). $Ca^{2+} + Mg^{2+}$ release rates were always $<1 \mu\text{eq}$ released cation/ μeq sorbed Cd as ion exchange mechanism was obviously not the sole mechanism involved in metal uptake.

For ozone doses below 8.1 mg O_3/g TS, ion exchange mechanism was not significantly affected as the ratio remained equal to $0.15 \pm 0.01 \mu\text{eq}/\mu\text{eq}$ (Table 2). For higher ozone doses, an increase of ion exchange is then observed as it rose up to $0.19 \pm 0.02 \mu\text{eq}/\mu\text{eq}$ at 16.8 mg O_3/g TS. It seems therefore that ion exchange during cadmium biosorption was favored beyond a threshold ozone dose. The highest floc destructurelation observed at higher ozone doses may have induced a better availability of surface sites previously bound to Ca^{2+} and/or Mg^{2+} ions.

3.4.2. Mechanisms of cadmium removal in soluble fraction ("precipitation" test)

The study of biosorption isotherms suggested the importance of both mineral and organic compounds of the soluble fraction. Metal cations can indeed undergo interactions with these compounds: precipitation (mineral phase), flocculation (organic matter), complexation (mineral and organic ligands). In order to investigate the cadmium behavior with soluble compounds as well as its degree of precipitation, experiments were carried out by adding metal to the soluble fraction of ozonized sludge.

It was first demonstrated that cadmium removal from soluble fraction occurred mainly by precipitation (Fig. 5): for all metal concentrations tested, cadmium precipitation was high: 25–60% of cadmium was removed from soluble fraction of untreated sludge after filtration at 0.45 μm . Precipitation decreased with increasing ozone dose and was only about 12% at all initial cadmium concentrations for filtrate of sludge pretreated at 16.8 mg O_3/g TS.

Several mechanisms were investigated to explain these observations:

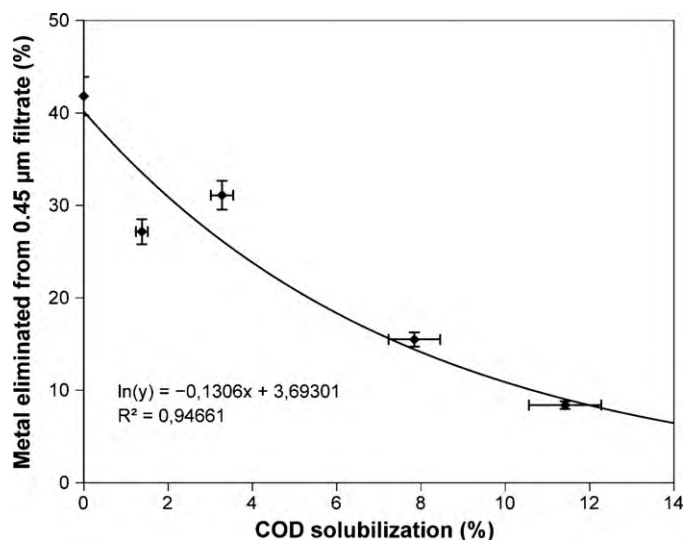


Fig. 6. Relationship between cadmium precipitation and DOC concentration (Cd initial concentration = 100 mg/L).

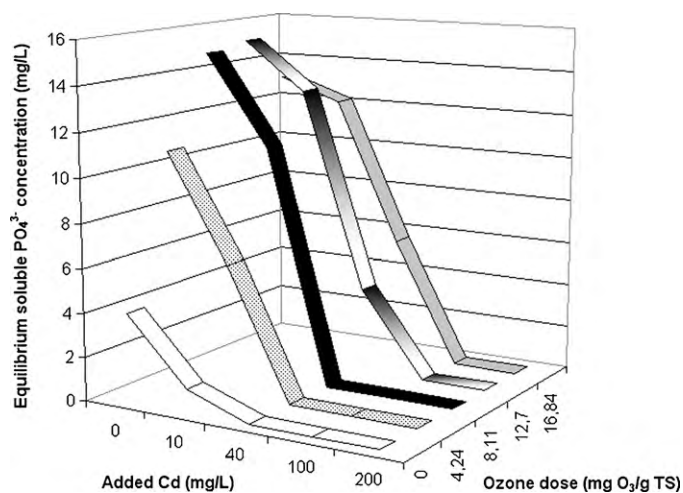


Fig. 7. Phosphate concentration in activated sludge filtrate as a function of added Cd and ozone dose.

- Complexation of the metal with the dissolved organic matter released during ozonation.

It was evidenced that the decrease of metal precipitation was due to an increase of its apparent solubility following complexation with compounds released in soluble fraction during the treatment. Indeed, metal precipitation and COD solubilization were significantly correlated (Fig. 6).

- Metal precipitation with phosphate ions released during ozonation.

The determination of soluble phosphate concentrations following ozonation showed the release of PO_4^{3-} ions (Table 1). PO_4^{3-} can form $\text{Cd}_3(\text{PO}_4)_2$ salt whose solubility is very low [41]. The precipitation of the released orthophosphate ions with Cd was indeed evidenced by the drastic decrease of PO_4^{3-} concentration in filtrate after the addition of growing Cd concentrations for each investigated ozone dose (Fig. 7). Phosphate ions were totally eliminated for the highest cadmium concentrations and could therefore constitute a limiting factor for metal precipitation. The decrease of Cd precipitation observed with the increase of ozone dose could therefore be also linked to the lower release of PO_4^{3-} in the soluble fraction.

4. Conclusions

This study evidenced that the evolutions of activated sludge physico-chemical properties following ozonation induced a shift in the extent and mechanisms of cadmium uptake: at ozone doses below $10 \text{ mg O}_3/\text{g TS}$, the formation of very fine particles in sludge supernatant first increased cadmium uptake capacity of activated sludge by offering an extended surface area for cadmium binding. The increase of soluble phosphate concentration also provided a higher degree of cadmium precipitation. When increasing ozone dose up to $17 \text{ mg O}_3/\text{g MS}$, the oxidation and the complete mineralization of some sludge compounds, especially humic like substances, induced a decrease of the number of active sites for cadmium sorption. The increase of dissolved organic matter concentration also limited cadmium precipitation by complexation with the metal cations. This soluble complexation seemed to favor ion exchange mechanism over precipitation during cadmium uptake. These phenomena were responsible for the reduction of cadmium uptake capacity for higher ozone doses.

The occurrence of the above cited modifications should be considered when introducing ozonation in biological wastewater treatment plants as the fate of cadmium may shift at the outlet of the process according to the characteristics of ozone treatment. The behavior of other metallic (Pb, Zn, Cu, Ni, etc.) and organic (PAHs, PCBs, pharmaceuticals, etc.) might also be affected. This could modify both excess sludge and treated effluent quality. The real environmental impact of excess sludge reduction processes such ozonation should therefore be carefully investigated before their implementation, especially concerning micropollutants.

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