Heavy metals uptake by sonicated activated sludge: Relation with floc surface properties

Julien Laurent*, Magali Casellas, Christophe Dagot

Groupement de Recherche Eau Sol Environnement, Université de Limoges, 16 rue Atlantis, Parc ESTER Technopôle, 87068 Limoges Cedex, France

A B S T R A C T

The effects of sonication of activated sludge on heavy metal uptake were in a first time investigated in respect with potential modifications of floc surface properties. The treatment led to the simultaneous increase of specific surface area and of the availability of negative and/or hydrophilic sites. In parallel, organic matter was released in the soluble fraction. Sorption isotherms of cadmium and copper showed that uptake characteristics and mechanisms were highly dependent on both heavy metal species and specific energy supplied. The increase of both specific surface area and fixation sites availability led to the increase of Cd(II) uptake. For Cu(II), organic matter released in soluble phase during the treatment seemed to act as a ligand and to limit adsorption on flocs surface. Three different heavy metals uptake mechanisms have been identified: proton exchange, ion exchange and (co)precipitation.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Wastewater treatment with activated sludge processes generates large quantities of excess sludge which must be eliminated. The disposal of this type of waste is subject to various environmental, social and economic problems. Disposal of heavy metals loaded sludge can lead to severe environmental hazards when used for agriculture or landfilling [1,2]. Thus, interest for the development of techniques allowing sludge volume and mass reduction is presently increasing [3]. Ultrasonic treatment is one of the most promising recent technologies for reducing sludge production in wastewater treatment plants [4]. Sludge is treated by ultrasounds in order to improve its biodegradability prior to anaerobic digestion [5,6] or recycling in aeration tank [3,7]. The effects of sonication on physico-chemical characteristics of sludge are well known: solubilization i.e. release of organic components: COD, proteins, nucleic acids, polysaccharides [8–10], reduction of the flocs size [8,11–13], biodegradability improvement [8,14].

Among mineral pollutants present in domestic wastewaters, heavy metals are of great concern due to their high toxicity [15]. Wastewater treatment plants (WWTPs) are expected to control the discharge of heavy metals in the environment [15]. However, the design criteria for biological WWTPs are mainly focused on the removal of organic matter by activated sludge microorganisms. The removal of heavy metals in those systems was (and still is) regarded as a side-benefit, if any [1,2]. During sonication and the subsequent biodegradation step, mainly organic matter is affected and the final quantity of waste-activated sludge decreases. Mineral and/or non-biodegradable components such as heavy metals are not affected and may accumulate in the sludge or be released in the aqueous phase i.e. the effluent of the WWTP. For example, Chipasa [16] showed an increase in the contents of heavy metals per dry weight unit during anaerobic digestion as a result of the microbial decomposition of sludge compounds.

Uptake of heavy metals by sludge of WWTPs was the subject of numerous studies. Experimentations have been carried out either to identify the mechanisms and the main parameters affecting heavy metal uptake in WWTPs or to use waste-activated sludge as a low cost adsorbent. The influence of a lot of physico-chemical and process parameters has been extensively studied and demonstrated: hydraulic residence time [17], sludge age [18], feed C/N ratio [19], pH [20], temperature [20], dissolved organic matter [21,22], the presence of multiple metallic elements [23,24], the composition of the extracellular polymer matrix [25] . . .

However, few data concerning any link between sonication of activated sludge and heavy metals uptake is available in the literature. The most significant results come from Kim et al. [26] who reported that after sonication of waste-activated sludge heavy metals were released in the aqueous phase (Cu, Pb, Zn

---

* Corresponding author. Tel.: +33 5 55 36 61; fax: +33 5 55 42 36 62.
  E-mail addresses: julien.laurent@cyberficus.com, cyberficus@gmail.com
(J. Laurent).

Nomenclature

A	area occupied by a single rhodamine B molecule (m^2/molecule)
C_e	equilibrium metal concentration (μmol/L)
COD	chemical oxygen demand (mg O_2/L)
DOC	dissolved organic carbon (mg C/L)
DOM	dissolved organic matter
EPS	extracellular polymeric substances
E_S	specific energy (kJ/kg TS)
K_L	Langmuir constant (L/μmol)
N	Avogadro number (6.023×10^{-18} molecules/mol)
p	statistic significance coefficient
Q_e	adsorbed metal (μmol/g TSS)
Q_{max}	Langmuir maximal adsorptive capacity (μmol/g TSS)
$Q_{RR}+$	ruthenium red maximal adsorptive capacity (mg/g TSS)
Q_s	adsorbed metal per unit of floc surface area (μmol/m^2)
r_p	Pearson's correlation coefficient
RH	relative hydrophobicity (%)
S	constant related to limitation of adsorption in sigmoidal Langmuir model (μmol/L)
SCOD	soluble chemical oxygen demand (mg O_2/L)
TCOD	total chemical oxygen demand (mg O_2/L)
TS	total solids (g/L)
TSS	total suspended solids (g/L)
VS	volatile solids (g/L)
VSS	volatile suspended solids (g/L)
WWTP	wastewater treatment plant

...). Nevertheless, it appears essential to evaluate the possible impact of this process on quality of both final effluent and waste-activated sludge generated. Thus, it is useful to determine to which extent sludge properties are modified by ultrasounds treatments and to assess the behaviour of heavy metals towards sonicated sludge.

The objectives of this study are a better knowledge of sludge characteristics modifications after an ultrasonic treatment and of their potential effects on heavy metals uptake by activated sludge. This uptake is estimated by batch biosorption tests. Solubilization of organic components, specific surface area, negative surface charge and relative hydrophobicity are measured and linked to heavy metals uptake capacities. Finally, evolutions of the uptake mechanisms are also investigated.

2. Methods

2.1. Sludge sonication

Activated sludge samples were obtained from the aeration tank in the municipal wastewater treatment plant of the city of Limoges (250 miles south of Paris, France) and stored at 4 °C before use. The main characteristics of this type of sludge were: total solids (TS): 3.5 ± 0.2 g/L, % of volatile solids (VS) in total solids (TS): $70 \pm 1\%$, total chemical oxygen demand (TCOD): 4400 ± 720 mg O_2/L.

The ultrasonic device used throughout this study was an ultrasonic homogenizer (Sonopuls, Bandelin). The operating frequency was 20 kHz, value belonging to the low frequencies range which leads to a better flocs disintegration [6]. The power supplied was 100 W. Batch experiments were carried out in a 1 L beaker and without temperature regulation. The volume of the treated sam-

ple was of 900 mL. The specific energy (E_S) supplied ranged from 0 to 214,400 kJ/kg TS and is defined as follows (1):

$$E_S = \frac{P \times t}{V \times TS_0} \tag{1}$$

where E_S is the specific energy supplied (kJ/kg TS); P is the ultrasonic power (W); t is the ultrasonic time (s); TS_0 is the total solids concentration before sonication and V is the sample volume (L).

2.2. Sludge characteristics

Sludge characteristics measurements were done in triplicate. Chemical oxygen demand (COD) was measured by the closed reflux colorimetric method (Dr. Lange). Polysaccharides were determined using the colorimetric method of Dubois et al. [27]. Protein and humic acids were determined using the method of Lowry et al. [28] modified by Frølund et al. [29]. In order to assess the degree of sludge solubilization, all these measurements were done on both total and soluble fractions of sludge. The soluble fraction is defined here as the fraction resulting from the filtration of the samples through a cellulose nitrate membrane (0.45 μm pore size). 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.

Measurements of total and organic solids were done on total sludge, which allowed the determination of total and volatile solids (TS and VS). They also were done on centrifugation pellets ($6000 \times g$; 20 min; 4 °C), which allowed the determination of total suspended and volatile suspended solids (TSS and VSS). All these parameters allowed the whole quantification of total and particulate matter concentrations. The matter concentration of the supernatant, i.e. the soluble phase, was then deduced and so was the composition of each part of the sludge. The measurements were obtained using the normalized method [30]. The standard deviation for triplicate samples was below 2%.

2.3. Flocs surface properties

2.3.1. Specific surface area

The specific surface area of biomass was determined by a dye adsorption method inspired of Smith and Coackley [31] and Sørensen and Wakeman [32]. 2 mL of a rhodamine B solution (seven concentrations varying in the range 0–500 mg/L) were added to an 18 mL sludge suspension (previously diluted to obtain a TSS concentration close to 0.25 g/L). The biomass–dye mixtures were shaken on a rotary shaker for 20 h at 180 rpm at ambient temperature according to Sørensen and Wakeman [32]. Once equilibrium was reached, each mixture was centrifuged ($6000 \times g$; 20 min; 4 °C) and the dye remaining in supernatant was measured using a spectrophotometer (Shimadzu UV-1700) at 554 nm wavelength. The specific surface area of the flocs (m^2/g) was determined by calculating the maximum dye adsorption capacity Q_{max}, using Langmuir adsorption model (4). The coefficients of the Langmuir isotherm model were obtained with the linearized form of the Langmuir equation. The maximum dye adsorption capacity was then used to determine the biomass specific surface area according to Eq. (2) [31]:

$$S = Q_{max} \times N \times A \tag{2}$$

where S is the specific surface area of the solids (m^2/g); Q_{max} is the maximum dye adsorption capacity (mol/g); N is Avogadro's number (6.023×10^{23} molecules/mol); A is the area occupied by a single dye molecule (1.95×10^{-18} m^2/molecule of the dye used in this study). Because of the weak values obtained for rhodamine B adsorption, standard deviation obtained from the linear regression for seven

Table 1
Solubilization of activated sludge at different specific energies (results obtained on triplicate samples)

Specific energy (kJ/kg TS)	TSS/TS (%))	VS/TS (%)	Soluble mineral matter/TS (%)	Soluble organic matter/TS (%)	SCOD/TCOD (%)	Ratio of soluble to total polysaccharides (%)	Ratio of soluble to total proteins (%)	Ratio of soluble to total humic acids (%)
0	94.7	70	3.8	1.4	0.6 ± 0.1	0.2 ± 0.01	0	4.1 ± 0.6
27,900	83.0	70	5.6	11.4	4.9 ± 0.7	3.9 ± 0.2	8.00 ± 1.2	16.4 ± 2.4
56,400	70.9	69	9.7	19.3	9.2 ± 0.7	5.6 ± 0.3	11.1 ± 1.7	23.1 ± 3.5
113,400	56.7	69	10.5	32.8	17.6 ± 1.8	13.8 ± 1	19.3 ± 2.6	34.1 ± 6.3
214,400	58.2	67	10.10	31.7	19.6 ± 0.8	13.2 ± 1.9	26.9 ± 5.7	37.2 ± 8.9

rhodamine B concentrations was 12–29%. This values were quite high but sufficient enough to assess significative evolutions of specific surface area after sonication.

2.3.2. Negative surface charge

Negative surface charge was estimated by adsorption of ruthenium red, a cationic dye. This molecule mostly binds to hydroxyls, carboxyls and sulfate groups [33]. Measurement was realized according to the protocol described by Löwen and Piirtola [34]. 2 mL of dye solution (seven concentrations varying in the range 0–1 g/L) were added to an 18 mL sludge suspension (previously diluted to obtain a TSS concentration close to 0.25 g/L). The biomass–dye mixtures were shaken on a rotary shaker for 3 h at 180 rpm at ambient temperature. Once equilibrium was reached, each mixture was centrifuged ($6000 \times g$; 20 min; 4 °C) and the dye remaining in supernatant was measured using a spectrophotometer (Shimadzu UV-1700) at 533 nm wavelength. The negative surface charge of the flocs was estimated by calculating the maximum dye adsorption capacity Q_{max}, using Langmuir adsorption model (4). The coefficients of the Langmuir isotherm model were obtained with the linearized form of the Langmuir equation. The standard deviation obtained from the linear regression for seven ruthenium red concentrations was 5–8%.

2.3.3. Relative hydrophobicity

The relative hydrophobicity (RH) was measured as adherence to hydrocarbons with a protocol deriving from Wilén et al. [35]. A 30 mL sample of sludge was agitated uniformly for 5 min with 15 mL hexadecane (Sigma) in a separatory funnel. After 30 min, when the two phases were completely separated, the aqueous phase was transferred into another glassware. The RH (%) was expressed as the ratio of TS concentration in the aqueous phase after emulsification (TS_e) to the concentration of TS in the aqueous phase before emulsification (TS_i) (3):

$$RH = \left(\frac{1 - TS_e}{TS_i} \right) \times 100 \tag{3}$$

The standard deviation for triplicate samples was 1–6%.

2.4. Biosorption of heavy metals, Ca^{2+}, Mg^{2+} and DOC measurement

In a first time, heavy metals uptake by activated sludge flocs was evaluated with cadmium (Cd) and copper (Cu) respectively. All metals were added as chloride salts ($CdCl_2 \cdot H_2O$, $CuCl_2$, $2H_2O$, purity >98%) using stock solutions of 10 g/L of Cd^{2+} and Cu^{2+}. 100 mL high density polyethylene bottles were used for batch metal sorption experiments to minimize metal sorption to the bottle surface. Sorption isotherms of each metal were obtained using 50 mL of mixed liquor with known TSS concentration spiked with eight different initial metal concentrations. The bottles were shaken for 3 h at 180 rpm on a rotary shaker at ambient temperature (20 ± 2 °C daily checked). Preliminary kinetic studies indicated that metal sorption by non-treated and sonicated sludge reached equilibrium after approximately 2 h. pH was not regulated and no buffers were added to keep the pH constant during the course of sorption. In

fact, these buffers can affect the sorption process by complexing metal ions in solution. Initial pH was equal to 6.8 ± 0.1 (Metrohm 744) and initial conductivity (WTW LF 538) was $570 \pm 80 \, \mu S/cm$. Once equilibrium was reached, pH of the sludge suspension was measured and the sludge suspension was filtered through a cellulose nitrate membrane (0.45 μm pore size). The filtrate was divided in two parts: one was acidified with a few drops of concentrated HNO_3 and stored at 4 °C until analysis; the other one was stored at −20 °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. The dissolved organic carbon was determined in the frozen filtrate with a TOCmeter (Dohrmann Phoenix 8000). Metal sorption data were fitted to Langmuir (4) and sigmoidal Langmuir (5) isotherm models:

$$Q_e = \frac{Q_{max} \times K_L \times C_e}{K_L + C_e} \tag{4}$$

$$Q_e = \frac{Q_{max} \times K_L \times C_e}{1 + K_L \times C_e + S/C_e} \tag{5}$$

where Q_e is the quantity of metal adsorbed per mass of TSS (μmol/g TSS); C_e is the equilibrium concentration of metal in solution (μmol/L); Q_{max} is the maximal adsorption capacity of metal on sludge floc (μmol/g TSS); K_L is the Langmuir constant related to affinity (L/μmol) and S is the constant related to the limitation of adsorption at low concentrations (μmol/L).

2.4.1. Statistical analysis and non-linear regression

Statistical analyses were carried out using the Gnumeric software[1]. The analysis was simplified by using univariate linear correlations. The Pearson's product momentum correlation coefficient was used to evaluate linear estimations. The Pearson's r_p coefficient is always between −1 and +1, where −1 means a perfect negative correlation and +1 a perfect positive correlation while 0 means absence of relationship. Correlations were considered statistically significant at a 95% confidence interval ($p < 0.05$). Non-linear regression was carried out using the GnuPlot software[2] to fit the sigmoidal isotherms obtained for copper with sigmoidal Langmuir model.

3. Results and discussion

For each studied specific energy, several sludge samples collected at different times were studied. The results presented here correspond to one set of measurements representative of the trends observed throughout the study.

3.1. Solubilization of sludge components

The use of ultrasounds did not change total matter quantity and TS concentration measured after sonication was constant so

---

[1] http://www.gnome.org/projects/gnumeric/.
[2] http://www.gnuplot.info/.

Table 2
Heavy metals released during sonication (results obtained on triplicate measurement)

Specific energy (kJ/kg TS)	Soluble Cd (µg/L)	Soluble Ni (µg/L)	Soluble Cu (µg/L)	Soluble Ca(II) (mg/L)	Soluble Mg(II) (mg/L)
0	<DL[a]	<DL	6 ± 1	29.2	7.1
27,900	<DL[a]	7 ± 1	26 ± 1	35.4	8.0
56,400	<DL[a]	10 ± 1	29 ± 1	45.1	12.7
113,400	<DL[a]	189 ± 7	162 ± 4	26.0	8.6
214,400	<DL[a]	60 ± 2	196 ± 4	25.3	6.0

[a] DL = detection limit of the apparatus used for analysis.

as for the total organic solids (VS) ratio (Table 1). Thus ultrasounds did not induce an evaporation or mineralization phenomenon. However, soluble matter concentration increased, whereas particulate matter concentration decreased (Table 1). These results are consistent with those obtained by Bougrier et al. [8] on waste-activated sludge: ultrasounds led to the solubilization of organic and mineral solids. Solubilization of mineral matter was low; the ratio of soluble mineral matter at 214,400 kJ/kg TS was only 10%, whereas the ratio of soluble organic matter was 32% (Table 1). The solubilization of organic matter was underlined by the increase of soluble COD, proteins, polysaccharides and humic acids with increasing specific energy supplied (Table 1). For all the parameters measured, ratio of soluble to total concentrations increased fast with increasing specific energy from 0 to 113,400 kJ/kg TS. For higher specific energies, the increase of soluble to total components concentrations was lower. Concerning organic components, the order of solubilization varied as follows: humic acids > proteins > polysaccharides. Humic acids reached the highest ratio of soluble to total concentrations for all specific energies studied. This suggests that sonication affected the floc extracellular matrix to a great extent knowing that humic acids are not intracellular components.

3.2. Heavy metals, Ca(II) and Mg(II) release

As activated sludge used in this study was obtained from a real WWTP, it contained heavy metals originating from wastewater. Thus, they had to be taken into account in a quantitative respect. In order to assess the potential release of these metals due to sonication, soluble concentrations of cadmium, nickel and copper were measured by FAAS immediately after sonication. It can be noticed that copper and nickel soluble concentrations were increasing in parallel with specific energy whereas cadmium concentrations could not be detected with the FAAS used in this study (Table 2). The question of cadmium behaviour remained to be elucidated by using a more sensitive analytical method. Thus, sonication led to the release of nickel and copper in soluble phase. These results are in accordance with those of Kim et al. [26] who observed a reduction of the amount of Cu, As, Se, Pb, Zn and Zn contained in the filtration cake of sonicated sludge. Heavy metals bound to sludge flocs components were solubilized together with these components. Moreover, it can be noticed that ultrasounds were used to accelerate the release of heavy metals during the realization of sequential extraction schemes applied on a sludge sample [36,37].

Soluble concentrations of Ca(II) and Mg(II) were also determined (Table 1). Their concentrations were increasing for specific energies ranging from 0 to 56,400 kJ/kg TS and decreasing for higher specific energies. This can be attributed to variations in the initial Ca(II) and Mg(II) content of the different samples. However, both, Ca(II) and Mg(II) follow the same trend with the increase of the specific energy. According to the divalent cations bridging theory, Ca(II) and Mg(II) cations are involved in bioflocculation [38]. Thus, as soluble organic matter released by sonication can act as a good flocculant [12], the decrease of Ca(II) and Mg(II) concentrations at higher spe-

cific energies can also be linked to a reflocculation phenomenon induced by the higher soluble organic matter content.

3.3. Evolution of floc surface properties

According to Dewil et al. [11], the reduction of the flocs size after sonication offers an extended surface area. Measurement of specific surface area by rhodamine B adsorption confirmed this assumption (Fig. 1). Determination coefficients (R^2) of the Langmuir model used for calculation varied in the range 0.73–0.92. For non treated sludge, the value was $28 \pm 4 \, m^2/g$ TSS, which was of the same order of magnitude as the ones obtained with the same method on both activated sludge ($5.5–9 \, m^2/g$ TSS), and anaerobically stored activated sludge ($13.5–24 \, m^2/g$ TSS) by Sørensen and Wakeman [32]. Zheng et al. [39] obtained a value of $55.3 \, m^2/g$ TSS on granular activated sludge. However, the values obtained in this study were lower than those obtained on activated sludge by Smith and Coackley [31] ($40–142 \, m^2/g$ TSS) and Kim et al. [40] ($91 \, m^2/g$ TSS) who used New Coccine instead of rhodamine B. Their adjusting of pH may cause the sludge to undergo chemically induced changes. Thus, due the different protocols and also different origins of sludge, direct and precise comparisons are difficult to set up.

Specific surface area increased in a two steps sequence with energy input. For specific energy between 0 and 113,400 kJ/kg TS, an important increase was noticed: from 28 ± 4 to $104 \pm 18 \, m^2/g$ TSS. For higher specific energies, the specific surface area increase was apparently lower (from $104 \pm 18 \, m^2/g$ TSS for $E_S = 113,400$ kJ/kg TSS to $114 \pm 26 \, m^2/g$ TSS for $E_S = 214,400$ kJ/kg TSS).

Evolutions of the negative surface charge (measured by ruthenium red adsorption method) and of the relative hydrophobicity appear at Fig. 2. Ruthenium red maximal adsorption capacity (Q_{RR+}) increased with energy input whereas relative hydrophobicity (RH) decreased. These evolutions occurred in a two steps sequence:
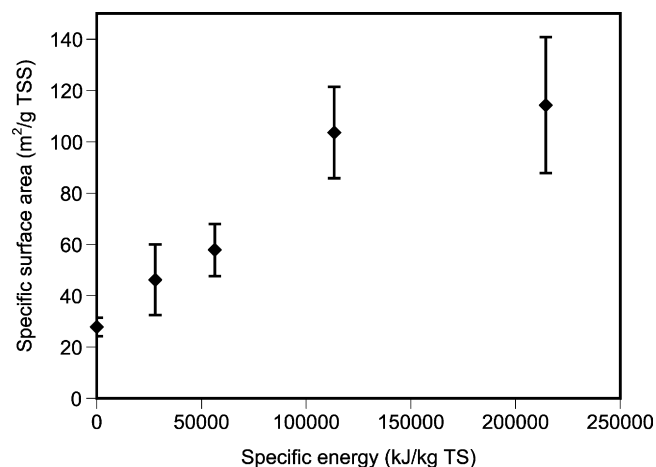

Fig. 1. Specific surface area increase with specific energy. The error bars represent the standard deviation calculated from the linear regression performed using the linearized Langmuir model. Seven points corresponding to seven rhodamine B concentrations were used for each value calculation.
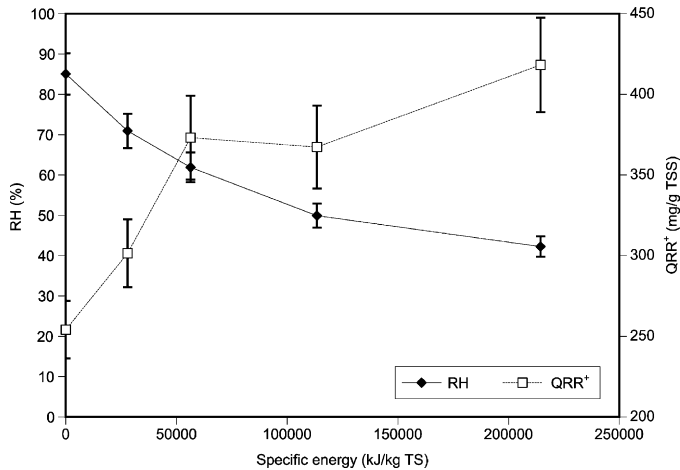
Fig. 2. Evolution of the relative hydrophobicity and adsorption capacity of ruthenium red with specific energy. For ruthenium red, the error bars represent the standard deviation calculated from the linear regression performed using the linearized Langmuir model. Seven points corresponding to seven ruthenium red concentrations were used for each value calculation. For RH, error bars represent the standard deviation obtained for triplicate samples.

for specific energy between 0 and 114,400 kJ/kg TS, both important increase of Q_{RR+} and important decrease of RH were noticed (Q_{RR+} increased from 254 ± 14 mg/g TSS to 373 ± 24 mg/g TSS and RH decreased from 85% to 49%). For higher specific energies, evolutions seemed to be lower ($Q_{RR+} = 418 \pm 24$ mg/g TSS and RH = 42% for $E_S = 214,400$ kJ/kg TS). The values obtained in this study for Q_{RR+} are of the same order of magnitude as the ones obtained by Figueroa and Silverstein [33].

The relative hydrophobicity of the sludge expressed in this study indicated the presence of both hydrophobic and hydrophilic groups at the surface of the flocs. On one hand, hydrophilic molecules are generally polar or charged while hydrophobic molecules are non-polar [41]. On the other hand, as mentioned above (see Section 2.3.2), ruthenium red binds essentially to hydroxyls, carboxyls and sulfate groups at the surface of the flocs. Thus, the decrease of RH and the increase of Q_{RR+} due to sonication can be linked to the increase of the availability of polar/charged groups such carboxyls, hydroxyls and sulfate at the surface of the flocs.

Evolutions of Q_{RR+} and RH appeared to be concomitant with specific surface area increase (Fig. 3). This is confirmed by the strong and significant correlations obtained between the three parameters
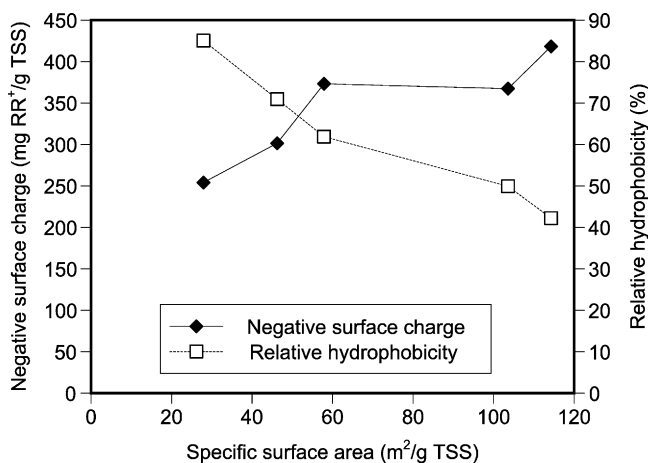

Fig. 3. Relative hydrophobicity and RR$^+$ maximal adsorption capacity vs. specific surface area.

Table 3
Pearson's correlation coefficient (r_p) an p-values (p) for linear correlations between specific surface area and relative hydrophobicity and adsorption capacity of ruthenium red

Parameter	r_p	p
RH	−0.9736	0.0051
Q_{RR+}	0.8800	0.0490

(Table 3). Thus, it can be assumed that sonication at the frequency used in this study (20 kHz) affected floc surface properties in an essentially physical manner. In fact, groups buried inside the EPS (extracellular polymeric substances) matrix were made free by the disintegration of the floc but no significant chemical modifications seemed to occur at the floc surface. This hypothesis is in accordance with the fact that hydromechanical shear forces produced by ultrasonic cavitation are predominantly responsible for the sludge disintegration [6]. These forces are namely predominant at frequencies inferior to 100 kHz [6,10,42]: high frequencies promote oxidation by radicals whereas low frequencies promote physical phenomena like pressure waves [43].

Surface binding and complexation with the negatively charged functional groups (carboxyls, hydroxyls, phosphoric ...) at the surface of the floc is one of the predominant mechanisms identified for heavy metal uptake by activated sludge [44]. Choi and Yun [45] also showed that carboxyl groups contributed to the metallic ions uptake by sewage sludge. Kim et al. [40] linked the increase of metal biosorption in a sludge spiked with the filamentous bacteria *Nocardia amarae*. They showed that this increase was due to the higher specific surface area of *Nocardia* cells. Thus, the results presented in this study showed that the number of available sites for biosorption of heavy metals was increasing with sonication.

3.4. Heavy metals adsorption capacity of the sonicated activated sludge

Cadmium and copper were studied because of their usual significant concentrations in wastewater [15,16]. To determine the amount of metal sorbed to activated sludge at various metal equilibrium concentrations, eight different initial metal concentrations ranging from 0 to 200 mg/L were used for batch sorption study as previously described in Section 2.4. Fig. 4 allows the comparison of metal biosorption isotherms for activated sludge treated by ultrasounds with specific energy ranging from 0 to 214,400 kJ/kg TS.

3.4.1. Cadmium

For each equilibrium concentration, the cadmium adsorptive capacity increased with specific energy. Cadmium isotherms were well described by Langmuir model as shown at Fig. 4a. The maximum adsorptive capacity (Q_{max}) obtained from the model increased in a two steps sequence with specific energy (Fig. 6). For specific energy between 0 and 113,400 kJ/kg TS, an important increase was noticed: from 298 to 888 μmol/g TSS. For higher specific energies, the Q_{max} increase was apparently lower (from 888 μmol/g TSS for $E_S = 113,400$ kJ/kg SS to 941 μmol/g TSS for $E_S = 214,400$ kJ/kg TS). Q_{max} evolutions seemed to be concomitant with the increase of specific surface area (see Section 2.3.1). To check this assumption, the cadmium sorption results were normalized with the specific surface area values obtained from the surface properties study (Section 2.3.1). The results obtained appear at Fig. 5a.
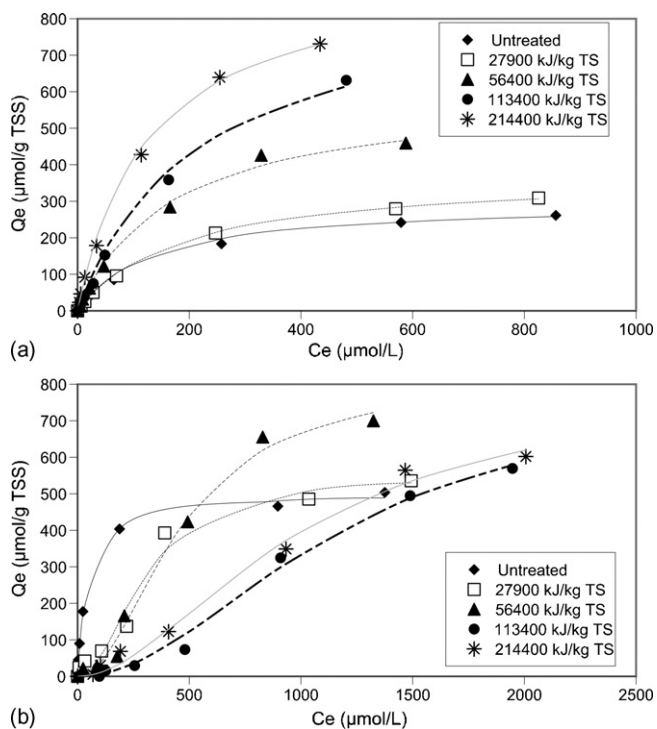
Fig. 4. Sorption isotherms for different specific energies obtained from one representative set of measurements. (a) Cd(II); (b) Cu(II). Data points represent the experimental results and curves represent the theoretical values obtained from the Langmuir model (cadmium) or sigmoidal Langmuir model (copper).

The convergence of isotherm curves supports the hypothesis that the increase of cadmium sorption capacity after sonication was due to the greater surface area thus available and consequently to the best availability of binding sites at the surface of sonicated
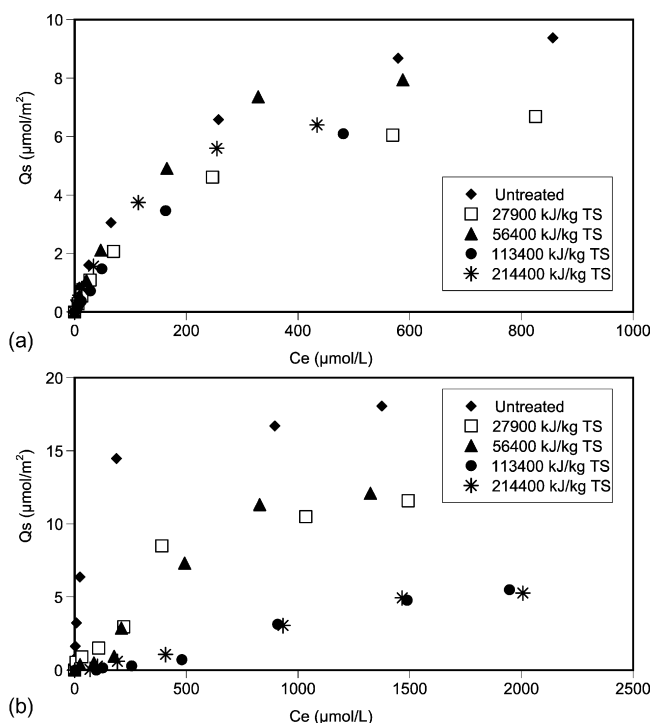
Fig. 5. Surface area normalized sorption isotherms for different specific energies. (a) Cd(II); (b) Cu(II).

flocs. Conversely, these results suggest that cadmium uptake mechanisms were not significantly altered by ultrasound treatment.

3.4.2. Copper

Copper sorption isotherms became more and more S shaped as the specific energy increased (Fig. 4b). The classical Langmuir model did not correctly describes the results obtained so the sigmoidal Langmuir model (see Section 2.4) was used. The copper adsorptive capacity decreased with increasing specific energy for low equilibrium concentrations. This decrease was not significant for highest equilibrium concentrations and moreover, maximum adsorptive capacity (Q_{max}) calculated from the model showed a slight increase in parallel with specific energy (Fig. 6). It ranged from 506 μmol/g TSS at 0 kJ/kg TS to 852 μmol/g TSS at 214,000 kJ/kg TS.

As showed in the solubilization study (see Section 3.1), sonication generates the release of organic compounds such carbohydrates, proteins and humic acids. Previous studies [21,22,46] have demonstrated that dissolved organic matter (DOM) has a strong influence on the uptake of heavy metals by activated sludge particulates. It could therefore be assumed that this newly present DOM could play the role of a ligand limiting adsorption of copper at low concentrations. This phenomenon could explain the S shape of the isotherms obtained with sonicated sludge. In fact, according to Limousin et al. [47] who reviewed the physical bases, the modeling and the measurement of adsorption isotherms, this type of S shaped isotherm can occur for metallic species when a soluble ligand is present. At low metal concentration, adsorption is limited by the presence of the ligand. Adsorption only occurs after ligand saturation. The point of inflection illustrates the concentration for which the adsorption overcomes the complexation.

The surface area normalized copper isotherms are shown at Fig. 5b. Isotherms obtained at different specific energies did not fall in concordance at all: the adsorptive capacity per square meter of surface area decreases with increasing sonication energies for each equilibrium concentration. This observation is concomitant with the increase of DOM with increasing sonication energies (Section 3.1). Therefore, copper uptake seemed to be altered by ultrasound treatment. This confirms the fact that although more binding sites were available after sonication, adsorption might be limited by complexation of the metal with DOM. However, Q_{max} calculated by the sigmoidal Langmuir model increased showing that the higher availability of metal binding sites due to sonication was still increasing copper binding capacity (Fig. 6).
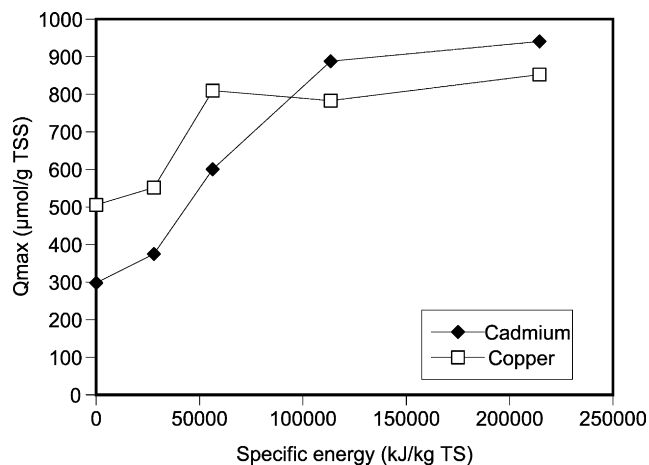

Fig. 6. Evolution of maximum adsorptive capacities for cadmium and copper with sonication. Q_{max} values were obtained for eight metal concentrations from Langmuir and sigmoidal Langmuir models for cadmium and copper respectively.

3.5. Investigation on the mechanisms of metal biosorption

Several mechanisms of heavy metals uptake by activated sludge have already been identified in the literature: surface binding and complexation with the negatively charged functional groups at the surface of the floc is one of the predominant mechanism identified for heavy metals uptake by activated sludge [44,48]. Ion exchange (Ca(II), Mg(II), H$^+$) is also involved [19,45,48,49] as well as precipitation [50]. In this study, the evolutions of some of these mechanisms were investigated with regards to the metal species and the specific energy previously applied to the sludge. Therefore, final pH and the final solution concentrations of DOC, Ca(II) and Mg(II) were determined.

3.5.1. Proton exchange

As previously mentioned (see Section 2.4), pH was not regulated during the course of the biosorption experiments. For each studied metal, the final pH value decreased with increasing equilibrium concentration suggesting that a proton exchange mechanism during sorption process was happening (Fig. 7). This phenomenon has been previously showed [19] and the proton exchange mechanism has been demonstrated on dried sludge [45,49]. However, in our study, fresh activated sludge was used and it was therefore impossible to quantify this mechanism because of the biological activity interference during biosorption test: since the bottles used for the test were closed, denitrification phenomenon caused the pH increase (up to 0.7 pH units) as demonstrated by the control tests realized without metal addition.

3.5.2. Cation exchange

The amount of Ca(II) and Mg(II) ions (counter ions) in solution increased with increasing quantity of adsorbed metal (Fig. 8). As suggested by Yuncu et al. [19], this release of divalent cations during biosorption of heavy metals by activated sludge can be linked to a cation exchange mechanism. In the context of this work, the term ion exchange refers to binding of a metal cation to a site which was initially occupied by another cation, this second ion being released upon the binding of the first ion. This phenomenon has also been observed by Hawari and Mulligan [48] during biosorption of Cd, Ni, Cu and Pb on calcium treated anaerobic biomass.

The quantity of Ca(II) and Mg(II) released in solution increased linearly with the quantity of adsorbed metal for each metal and each specific energy studied. Therefore, linear regression has been carried out for each metal and each specific energy. The slope value of the regression line obtained can be expressed in μeq of released
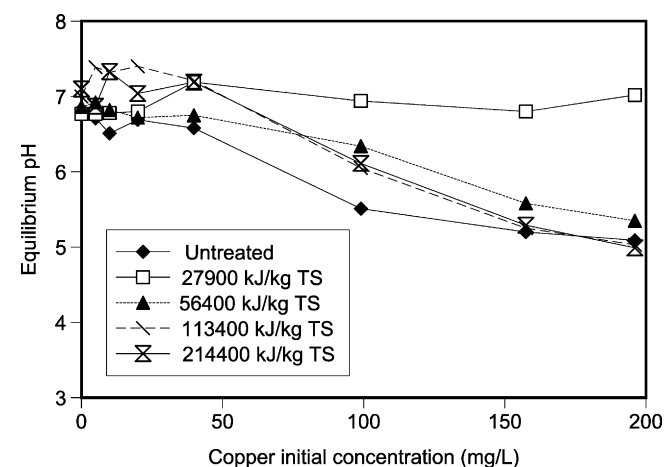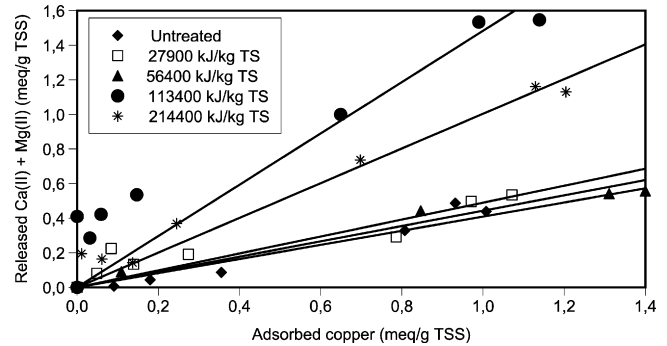

Fig. 8. Release of Ca(II) and Mg(II) during adsorption of copper on different sonicated sludges.

counter ions per μeq of adsorbed metal ion. Thus, it represents the cation release ratio: cations released (μeq)/metal ions adsorbed (μeq). Fig. 9 presents the ratio of desorbed cations as a function of specific energy for respectively Cd and Cu. This ratio was below 1 for most of the studied specific energies for both cadmium and copper. This indicates that the whole metal removal cannot be only explained by ion exchange alone as previously mentioned in the literature [19,48].

There were apparently no major differences in the divalent cations release ratios observed at different specific energies for cadmium (Fig. 9). The cations release ratios were comprised in a range varying from 0.55 ± 0.07 μeq/μeq at 0 kJ/kg TS to 0.88 ± 0.18 μeq/μeq at 113,400 kJ/kg TS. This underlines the fact that for this metal, there was no significant shift in the mechanism of metal uptake with increasing sonication energy. However, for copper, the cations release rate ranged from 0.44 ± 0.06 to 0.49 ± 0.10 μeq/μeq for specific energies between 0 and 56,400 kJ/kg TS and raised up to 1.00 ± 0.11 and 1.48 ± 0.32 μeq/μeq at 214,400 and 113,400 kJ/kg TS respectively. There is therefore a shift in the copper uptake mechanisms as ion exchange seemed to become more prevalent for higher specific energies. Several hypotheses can be formulated to explain this shift:

- the greatest availability, after high energy sonication, of binding sites with high affinity for copper that were firstly bound by Ca(II) and/or Mg(II) as counter ions,
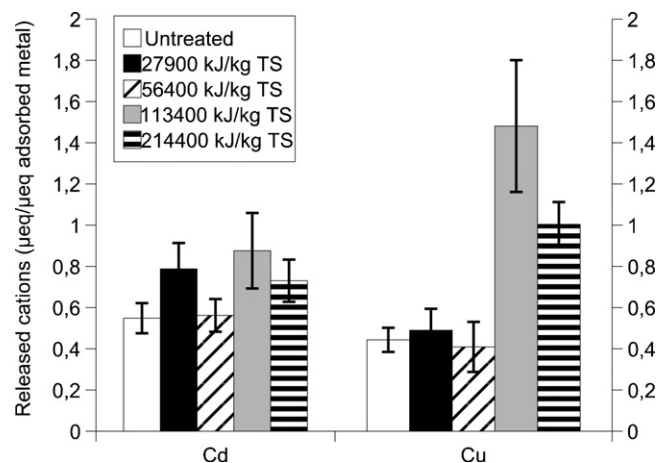

Fig. 7. pH decrease with biosorption for different specific energies (copper).

Fig. 9. Rate of divalent cations released during sorption process as a function of specific energy. The error bars represent the standard deviation calculated from the linear regression of cations released versus metal adsorbed curves for eight metal concentrations.
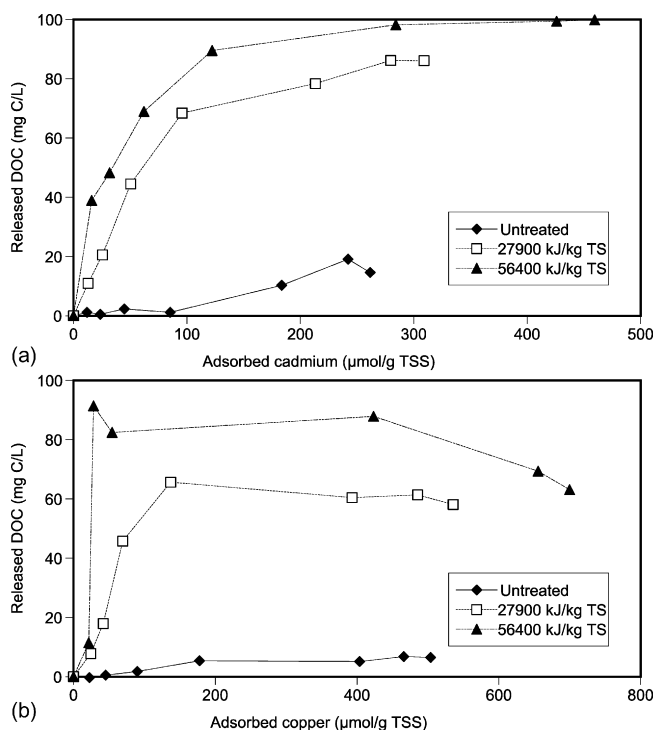
(a)

(b)

Fig. 10. DOC released as a function of adsorbed metal for specific energies ranging from 0 to 56,400 kJ/kg TS: (a) Cd; (b) Cu.

- a further destructuration of the floc leading to the release of Ca(II) and Mg(II) due to the action of Cu^{2+} ions because the floc structure after treatment at higher specific energies might be weaker.

3.5.3. DOC release or precipitation during metal adsorption

According to the divalent cation bridging theory which describes the best the role of cations in bioflocculation [38], divalent cations such Ca(II) and Mg(II) bridge negatively charged groups within the EPS. Thus, the release of Ca(II) and Mg(II) can also be related to the destructuration of the floc due to the presence of heavy metals leading to polymers release in the bulk solution. Yuncu et al. [19] measured the release of carbohydrates during the biosorption of heavy metals. It was attributed to the release of EPS during the ion exchange process. However, they noticed that the measurement of carbohydrates was not sufficient to assess the release of EPS during biosorption as there are other major constituents such as proteins in the EPS. This is the reason why the measurement of DOC was chosen in this study to estimate more completely the EPS release.

The increase of DOC equilibrium concentration with heavy metals adsorbed was effectively observed for specific energies ranging from 0 to 56,400 kJ/kg TS (Fig. 10). The release of DOC was more pronounced as the specific energy increased suggesting that flocs were more subject to heavy metal induced destructuration after sonication. Ideally, a higher polymer release is expected with a higher amount of adsorbed metal. However, DOC release was not proportional to adsorbed metal (Fig. 10): DOC release increased at a greater extent for lower amounts of adsorbed metal and reached a plateau for higher amounts of adsorbed metal. This can be attributed to other phenomena interfering during the biosorption test such as precipitation of the DOM–heavy metal complexes formed. This is confirmed by the decrease of the amount of DOC released by sludge treated at 56,400 kJ/kg TS for the highest copper equilibrium concentrations.

Indeed, the results obtained for higher specific energies confirmed this assumption because DOC concentrations were
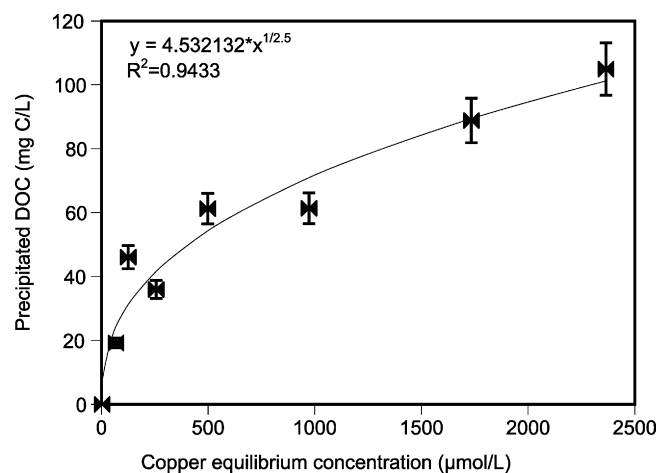

Fig. 11. Precipitation of DOC as a function of copper equilibrium concentration for sludge treated at 208,700 kJ/kg TS.

decreasing with increasing amount of adsorbed metal. This means that part of DOC was transferred to the solid phase. It could therefore be assumed that the solubility of the DOM–heavy metal complexes formed was overcome due to the higher DOM/heavy metal ratio of the sonicated sludge. Thus, these complexes precipitated, leading to the decrease of DOC concentration after heavy metals addition. Fig. 11 shows the results obtained after copper biosorption on sludge treated at 208,700 kJ/kg TS.

Therefore, it can be concluded that the final DOM content in the aqueous phase after biosorption results from two antagonistic phenomena: on one hand, DOC concentration increases because of the polymer release due to the ion exchange mechanism. On the other hand, the complexes formed between DOM released in the sonication process and heavy metals added precipitate, decreasing the final DOC concentration.

4. Conclusions

In this study, it is demonstrated that sonication is affecting heavy metals uptake by activated sludge to a great extent. Depending on the metal specie considered, the uptake can either be enhanced or limited. In fact, sonication induces important modifications of both soluble and particular phases composition and characteristics:

- Sonication of activated sludge led to solubilization of organic matter: COD, polysaccharides, proteins and humic acid concentrations increased in the aqueous phase.
- Sonication affected flocs surface properties by increasing the specific surface area and the negative surface charge whereas relative hydrophobicity decreased. As there were strong correlations between specific surface area increase, negative surface charge increase and relative hydrophobicity decrease, it can be assumed that sonication effects on floc surface properties are essentially physical: negative and/or polar sites are made free by floc disruption due to ultrasound waves.
- The increase in fixation sites availability at the surface of the flocs due to sonication led to an increase of the number of available active sites for fixation of metal ions at the surface of the floc. Thus, cadmium and copper maximal adsorptive capacities increased.
- Depending on the heavy metal species considered, sonication caused a shift in the metal affinity towards activated sludge: cadmium affinity was not significantly modified by sonication whereas copper uptake was affected by the increasing concentration of DOM acting as a ligand towards copper ions. The

DOM–copper complex formed first limited adsorption on sludge particulates. Once either the ligand was saturated or the solubility of the complex was reached, copper uptake increased by means of adsorption and/or precipitation. Moreover, it seemed that ion exchange mechanism was more pronounced during copper biosorption by sludge treated at highest specific energies.

- Three heavy metal uptake mechanisms by sonicated activated sludge have been identified: proton exchange, divalent cations exchange and precipitation with dissolved organic matter. However, the relative importance and evolution of these different mechanisms remains to be elucidated.

Acknowledgement

References

[1] L. Lazzari, L. Sperni, P. Bertin, B. Pavoni, Correlation between inorganic (heavy metals) and organic (PCBs and PAHs) micropollutant concentrations during sewage sludge composting processes, Chemosphere 41 (2000) 427–435.
[2] J. Scancar, R. Milacic, M. Strazar, O. Burica, Total metal concentrations and partitioning of Cd, Cr, Cu, Fe, Ni and Zn in sewage sludge, Science of the Total Environment 250 (2000) 9–19.
[3] S.H. Yoon, H.S. Kim, S. Lee, Incorporation of ultrasonic cell disintegration into a membrane bioreactor for zero sludge production, Process Biochemistry 39 (2004) 1923–1929.
[4] H. Ødegaard, Sludge minimization technologies—an overview, Water Science and Technology 49 (2004) 31–40.
[5] T. Mao, K.Y. Show, Performance of high-rate sludge digesters fed with sonicated sludge, Water Science and Technology 54 (2006) 27–33.
[6] A. Tiehm, K. Nickel, M. Zellhorn, U. Neis, Ultrasonic waste-activated sludge disintegration for improving anaerobic stabilization, Water Research 35 (2001) 2003–2009.
[7] X.Q. Cao, J. Chen, Y.L. Cao, J.Y. Zhu, X.D. Hao, Experimental study on sludge reduction by ultrasound, Water Science and Technology 54 (2006) 87–93.
[8] C. Bougrier, H. Carrère, J.P. Delgenès, Solubilisation of waste-activated sludge by ultrasonic treatment, Chemical Engineering Journal 106 (2005) 163–169.
[9] F. Wang, S. Lu, M. Ji, Components of released liquid from ultrasonic waste-activated sludge disintegration, Ultrasonics Sonochemistry 13 (2006) 334–338.
[10] P. Zhang, G. Zhang, W. Wang, Ultrasonic treatment of biological sludge: floc disintegration, cell lysis and inactivation, Bioresource Technology 98 (2007) 207–210.
[11] R. Dewil, J. Baeyens, R. Goutvrind, Ultrasonic treatment of waste-activated sludge, Environmental Progress 25 (2006) 121–128.
[12] E. Gonze, S. Pillot, E. Valette, Y. Gonthier, A. Bernis, Ultrasonic treatment of an aerobic activated sludge in a batch reactor, Chemical Engineering and Processing 42 (2003) 965–975.
[13] S. Na, Y.U. Kim, J. Khim, Physiochemical properties of digested sewage sludge with ultrasonic treatment, Ultrasonics Sonochemistry 14 (2007) 281–285.
[14] T.I. Onyeche, O. Schläfer, H. Bormann, C. Schröder, M. Sievers, Ultrasonic cell disruption of stabilised sludge with subsequent anaerobic digestion, Ultrasonics 40 (2002) 31–35.
[15] M. Karvelas, A. Katsoyiannis, C. Samara, Occurrence and fate of heavy metals in the wastewater treatment process, Chemosphere 53 (2003) 1201–1210.
[16] K.B. Chipasa, Accumulation and fate of selected heavy metals in a biological wastewater treatment system, Waste Management 23 (2003) 135–143.
[17] T.A. Ozbelge, H.A. Ozbelge, M. Tursun, Effects of hydraulic residence time on metal uptake by activated sludge, Chemical Engineering and Processing 44 (2005) 23–32.
[18] B. Arican, C.F. Gokcay, U. Yetis, Mechanistics of nickel sorption by activated sludge, Process Biochemistry 37 (2002) 1307–1315.
[19] B. Yuncu, F.D. Sanin, U. Yetis, An investigation of heavy metal biosorption in relation to C/N ratio of activated sludge, Journal of Hazardous Materials 137 (2006) 990–997.
[20] O. Gulnaz, S. Saygideger, E. Kusvuran, Study of Cu(II) biosorption by dried activated sludge: effect of physico-chemical environment and kinetics study, Journal of Hazardous Materials 120 (2005) 193–200.
[21] J. Wang, C.P. Huang, H.E. Allen, I. Poesponegoro, H. Poesponegoro, L.R. Takiyama, Effects of dissolved organic matter and ph on heavy metal uptake by sludge particulates exemplified by copper(II) and nickel(II): three-variable model, Water Environment Research 71 (1999) 139–147.
[22] J. Wang, C.P. Huang, H.E. Allen, Modeling heavy metal uptake by sludge particulates in the presence of dissolved organic matter, Water Research 37 (2003) 4835–4842.
[23] A. Hammaini, A. Ballester, M.L. Blazquez, F. Gonzalez, J. Munoz, Effect of the presence of lead on the biosorption of copper, cadmium and zinc by activated sludge, Hydrometallurgy 67 (2002) 109–116.
[24] A. Hammaini, F. Gonzalez, A. Ballester, M.L. Blazquez, J.A. Munoz, Simultaneous uptake of metals by activated sludge, Minerals Engineering 16 (2003) 723–729.
[25] G. Guibaud, N. Tixier, A. Bouju, M. Baudu, Relation between extracellular polymers' composition and its ability to complex Cd, Cu and Pb, Chemosphere 52 (2003) 1701–1710.
[26] Y.U. Kim, J. Ha, K.Y. Yoon, S.H. Lee, Effect of ultrasound on sludge from water treatment plant, Japanese Journal of Applied Physics, Part 1: Regular Papers and Short Notes and Review Papers 43 (2004) 7362–7363.
[27] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Analytical Chemistry 28 (1956) 350–356.
[28] E.H. Lowry, N.J. Rosebrough, R.A. Lewis Far, R.J. Randall, Protein measurement with the folin phenol reagent, Journal of Biological Chemistry 193 (1951) 265–275.
[29] B. Frølund, R. Palmgren, K. Keiding, P.H. Nielsen, Extraction of extracellular polymers from activated sludge using a cation exchange resin, Water Research 30 (1996) 1749–1758.
[30] AFNOR, NF T 90-105, Qualité de l'eau – dosage des matières en suspension – méthode par centrifugation, Association française de normalisation, Paris, 1997.
[31] P.G. Smith, P. Coackley, A method for determining specific surface area of activated sludge by dye adsorption, Water Research 17 (1983) 595–598.
[32] B.L. Sørensen, R.J. Wakeman, Filtration characterisation and specific surface area measurement of activated sludge by rhodamine B adsorption, Water Research 30 (1996) 115–121.
[33] L.A. Figueroa, J.A. Silverstein, Ruthenium red adsorption method for measurement of extracellular polysaccharides in sludge flocs, Biotechnology and Bioengineering 33 (1989) 941–947.
[34] M. Löwén, L. Piirtola, Characterization of activated sludge flocs, in: Bengt Hultman, Jerzy Kurbiel (Eds.), Proceedings of a polish-swedish seminar, Division of Water Ressources Engineering, Nowy-Targ, 1998, Available on line: http://www.lwr.kth.se/forskningsprojekt/Polishproject/JPS3s135.pdf.
[35] B.M. Wilén, B. Jin, P. Lant, The influence of key chemical constituents in activated sludge on surface and flocculating properties, Water Research 37 (2003) 2127–2139.
[36] B. Pérez-Cid, I. Lavilla, C. Bendicho, Comparison between conventional and ultrasound accelerated tessier sequential extraction schemes for metal fractionation in sewage sludge, Fresenius' Journal of Analytical Chemistry 363 (1999) 667–672.
[37] B. Pérez-Cid, I. Lavilla, C. Bendicho, Speeding up of a three-stage sequential extraction method for metal speciation using focused ultrasound, Analytica Chimica Acta 360 (1998) 35–41.
[38] D.C. Sobeck, M.J. Higgins, Examination of three theories for mechanisms of cation-induced bioflocculation, Water Research 36 (2002) 527–538.
[39] Y.M. Zheng, H.Q. Yu, G.P. Sheng, Physical and chemical characteristics of granular activated sludge from a sequencing batch airlift reactor, Process Biochemistry 40 (2005) 645–650.
[40] D.W. Kim, D.K. Cha, J. Wang, C.P. Huang, Heavy metal removal by activated sludge: influence of *Nocardia amarae*, Chemosphere 46 (2002) 137–142.
[41] B. Jin, B.M. Wilén, P. Lant, A comprehensive insight into floc characteristics and their impact on compressibility and settleability of activated sludge, Chemical Engineering Journal 95 (2003) 221–234.
[42] C. Bougrier, C. Albasi, J.P. Delgenès, H. Carrère, Effect of ultrasonic, thermal and ozone pre-treatments on waste-activated sludge solubilisation and anaerobic biodegradability, Chemical Engineering and Processing 45 (2006) 711–718.
[43] E. Gonze, L. Fourel, Y. Gonthier, P. Boldo, A. Bernis, Wastewater pretreatment with ultrasonic irradiation to reduce toxicity, Chemical Engineering Journal 73 (1999) 93–100.
[44] G. Guibaud, S. Comte, F. Bordas, S. Dupuy, M. Baudu, Comparison of the complexation potential of extracellular polymeric substances (EPS), extracted from activated sludges and produced by pure bacteria strains, for cadmium, lead and nickel, Chemosphere 59 (2005) 629–638.
[45] S.B. Choi, Y.S. Yun, Biosorption of cadmium by various types of dried sludge: an equilibrium study and investigation of mechanisms, Journal of Hazardous Materials 138 (2006) 378–383.
[46] A. Katsoyiannis, C. Samara, The fate of dissolved organic carbon (DOC) in the wastewater treatment process and its importance in the removal of wastewater contaminants, Environmental Science and Pollution Research 14 (2007) 284–292.
[47] G. Limousin, J.P. Gaudet, L. Charlet, S. Szenknect, V. Barthes, M. Krimissa, Sorption isotherms: a review on physical bases, modeling and measurement, Applied Geochemistry 22 (2007) 249–275.
[48] A.H. Hawari, C.N. Mulligan, Heavy metals uptake mechanisms in a fixed-bed column by calcium-treated anaerobic biomass, Process Biochemistry 41 (2006) 187–198.
[49] A. Hammaini, F. Gonzalez, A. Ballester, M.L. Blazquez, J.A. Munoz, Biosorption of heavy metals by activated sludge and their desorption characteristics, Journal of Environmental Management 84 (2007) 419–426.
[50] W.C. Chang, C.H. Hsu, S.M. Chiang, M.C. Su, Equilibrium and kinetics of metal biosorption by sludge from a biological nutrient removal system, Environmental Technology 28 (2007) 453–462.