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IMPACT OF ACTIVATED SLUDGE FLOCS PROPERTIES AFTER SONICATION IN RELATION WITH HEAVY METAL UPTAKE

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Abstract: The effects of activated sludge sonication on flocs surface properties and heavy metal uptake was investigated. Negative surface charge and specific surface area were estimated by correlation with dye adsorption whereas relative hydrophobicity was measured by adhesion to hexadecane. Experimental results show that ultrasound treatment leads to a simultaneous increase of specific surface area and availability of negatives and/or hydrophilic sites. Thus, fixation sites for heavy metal uptake are made free by sonication. Both increase of specific surface area and fixation sites availability leads to an increase of uptake of Cd(II). For Cu(II), organic matter released in soluble phase during the treatment acts as a ligand and limits adsorption on flocs surface.

Keywords: ultrasounds, activated sludge, surface properties, adsorption, heavy metal

INTRODUCTION

Among mineral pollutants presents in domestic wastewaters, heavy metals are of great concern due to their high toxicity (Viessman and Hammer, 1993 ; Gardea-Torresday et al., 1996 cited by Karvelas *et al.* (2003). Wastewater treatment plants (WWTPs) are expected to control the discharge of heavy metals in the environment (Karvelas *et al.*, 2003). However, biological WWTPs are chiefly designed for removal of organic matter by activated sludge microorganisms and the removal of heavy metals in those systems may be regarded as a side-benefit (Lazzari *et al.*, 2000 ; Scancar *et al.*, 2000).

Wastewater treatment with activated sludge processes generates large quantities of excess sludge which must be eliminated. This disposal is nowadays subject to various environmental, social and economic problems, especially concerning heavy-metals loaded sludge which can represent an environmental hazard when used for agriculture or landfilling (Lazzari *et al.*, 2000, Scancar *et al.*, 2000). Thus, interest for solutions allowing sludge volume and mass reduction is increasing (Yoon *et al.*, 2004). Ultrasonic treatment is one of the most promising recent technologies to reduce sludge production in wastewater treatment plants (Ødegaard, 2004). Sludge is treated by ultrasound in order to improve its biodegradability prior to anaerobic digestion (Mao and Show, 2006; Tiehm *et al.*, 2001) or recycling in aeration tank (Cao *et al.*, 2006; Yoon *et al.*, 2004). 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 (Bougrier *et al.*, 2005; Wang *et al.*, 2006; Zhang *et al.*, 2007), reduction of floc size (Bougrier *et al.*, 2005; Dewil *et al.*, 2006; Gonze *et al.*, 2003 ; Na *et al.*, 2007), biodegradability improvement (Bougrier *et al.*, 2005; Onyeche *et al.*, 2002).

During sonication and the subsequent biodegradation step, only organic matter is affected and the final quantity of excess sludge decreases. Mineral and/or non-biodegradable components like 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 (2003) showed an increase in the contents of heavy metals on dry weight basis during anaerobic digestion as a result of the microbial decomposition of compounds of the sludge.

Uptake of heavy metals by sludge of WWTPs was the subject of many studies. Experimentations have been carried out either to identify the mechanisms and the main parameters affecting heavy metal uptake in WWTPs or to use excess sludge as a low cost adsorbent. The influence of a lot of physico-chemical and process parameters has been studied and demonstrated: hydraulic residence time (Ozbelge *et al.*, 2005), sludge age (Arican *et al.*, 2002), feed C/N ratio (Yuncu *et al.*, 2006), pH (Gulnaz *et al.*, 2005), temperature (Gulnaz *et al.*, 2005), dissolved organic matter (Wang *et al.*, 1999, Wang *et al.*, 2003), presence of multiple metallic elements (Hammaini *et al.*, 2002; Hammaini *et al.*, 2003), composition of the extracellular polymer matrix (Guibaud *et al.*, 2003)...

However, no data concerning the link between sonication of activated sludge and heavy metal uptake is available in the literature. It only could be noticed that Kim *et al.* (2004) observed after sonication of excess sludge the release in the aqueous phase of heavy metals. It is nevertheless essential to evaluate the possible impact of this process on the quality of both final effluent and excess sludge generated. Thus, it is useful to understand to which extent sludge properties are modified by ultrasound treatment and to assess the behaviour of heavy metals towards sonicated sludge.

The objectives of this study were to understand sludge characteristics modifications due to an ultrasound treatment and their effects on heavy metals uptake by activated sludge. Solubilization of organic components, specific surface area, negative surface charge and relative hydrophobicity were measured and linked to heavy metal uptake capacity.

MATERIALS AND METHODS

Sludge sonication

Activated sludges were obtained from the municipal wastewater treatment plant of the city of Limoges (France) and stocked at 4°C before use. The main characteristics of this sludge were: Total solids (TS): 3.5 ± 0.2 g/L, ratio of volatile solids (VS) on total solids (TS): 70 ± 1 %, total chemical oxygen demand (TCOD): 4,400 ± 720 mg O_2/L.

The ultrasonic apparatus used in this study was an ultrasonic homogenizer (Sonopuls, Bandelin). This apparatus worked with an operating frequency of 20 kHz as low frequencies leads to better floc disintegration (Tiehm *et al.*, 2001). The power supplied was 100W. Batch experiments were carried out in a 1L beaker and without temperature regulation. The treated sample had a volume of 900 mL. The supplied specific energy (E_S) is defined as follows (equation 1):

$$E_S = \frac{P * t}{V * TS_0} \qquad (1)$$

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).

The objective of the study was not the process optimization but the study of both sludge characteristics and heavy metal uptake evolutions after sonication. Therefore, a wide range of E_s values was used in this study : it ranged from 0 to 214,400 kJ/kg TS.

Moreover, such high Es values have already been used to disintegrate activated sludge : in their study, concerning excess sludge production reduction in a membrane bioreactor, Yoon *et al.* (2004) used a specific energy of 216,000 kJ/kg TSS.

Sludge characteristics

Chemical Oxygen Demand (COD) is measured by the closed reflux colorimetric method (Dr Lange). Polysaccharides are determined using the colorimetric method of Dubois *et al.* (1956). Protein and humic acids were determined using the method of Lowry *et al.* (1951) modified by Frølund *et al.* (1996). In order to assess the degree of sludge solubilization, all these measurements were done on both total fraction of sludge and soluble fraction, defined here as the fraction resulting from the filtration of the samples through a cellulose nitrate membrane with 0.45 µm pore size.
Measurements of total and organics solids were realised on total sludge, which allows the determination of total and volatile solids (TS and VS), and on solids of centrifugation (6,000 x g; 20 min; 4°C), which allows the determination of total suspended and volatile suspended solids (TSS and VSS). All this process permitted the whole concentration and particulate matter concentration to be obtained. The matter concentration of the supernatant, i.e. the soluble phase, was then deduced and so was the composition of the different parts of the sludge. The measurements were obtained using the normalised method (AFNOR, 1997).

Flocs surface properties

- Specific surface area

The specific surface area of biomass was estimated by a dye adsorption method (Smith and Coackley, 1983). According to Sørensen and Wakeman (1996), it is assumed that complete monolayer coverage had occurred when the dye adsorption isotherm reached a plateau. Thus, dye maximal adsorptive capacity can be linked to the specific surface area of activated sludge flocs with equation 2 (Smith and Coackley, 1983):

$$S = Q_{max} * N * A \qquad (2)$$

Where S is the specific surface area of the solids (m^2/g); Q_{max} is the maximum dye adsorption capacity (mole/g); N is Avogadro's number (6.023 x 10^{23} molecules/mole); A is the area occupied by a single dye molecule (1.95 x 10^{-18} m^2/molecule of the dye used in this study).
2 mL of a dye solution (Rhodamine B) with known concentration was added to a 18 mL sludge suspension primarily diluted to obtain nearly a SS concentration of 0.25 g/L. The biomass-dye mixtures were shaken on rotary shaker for 20h at 180 rpm according to Sørensen and Wakeman (1996). Once equilibrium had been reached, each mixture was centrifuged (6,000 x 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. The coefficients of the Langmuir isotherm model were obtained with the linearized Langmuir equation. The maximum dye adsorption capacity was then used to determine the specific surface area of biomass according to equation 2.

- Negative surface charge

Negative surface charge of activated sludge is usually determined using colloidal titration method (Wilén *et al.*, 2003).

However, probably due to non-stoichiometric polymer complex precipitation during the titration procedure, this method is not valid for charge determination of whole sludge (Mikkelsen, 2003). Thus, another protocol was used in this study. Negative surface charge was estimated by adsorption of a cationic dye: ruthenium red. This molecule binds essentially to hydroxyls, carboxyls and sulphate groups (Figueroa and Silverstein, 1989). Measurement was realised according to the protocol described by Löwén and Piirtola (1998). 2 mL of dye solution with known concentrations was added to a 18 mL sludge suspension primarily diluted to obtain nearly a SS concentration of 0.25 g/L. The biomass-dye mixtures were shaken on rotary shaker for 3h at 180 rpm. Once equilibrium had been reached, each mixture was centrifuged (6,000 x 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. The coefficients of the Langmuir isotherm model were obtained with the linearized Langmuir equation.

- Relative hydrophobicity

The relative hydrophobicity (RH) was measured as adherence to hydrocarbons with a protocol deriving from Wilén *et al.* (2003). 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 had separated completely, the aqueous phase was transferred into other 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) as showed in equation 3:

$$RH = 1 - \frac{TS_e}{TS_i} * 100 \qquad (3)$$

Biosorption of heavy metals

Heavy metal uptake by activated sludge flocs was evaluated with three metals: cadmium (Cd), copper (Cu). All metals were added as chloride salts. High density polyethylene bottles were used for batch metal sorption experiments to minimize metal sorption to the bottle surface. To obtain a sorption isotherm for each metal, 50 mL of mixed liquor with known TSS in 100 mL bottles were spiked with eight different initial metal concentrations. The bottles were then shaken for 3h at 180 rpm on a rotary shaker at ambient temperature. A preliminary kinetic study indicated that metal sorption by non-treated and sonicated sludge reached equilibrium after approximately 2h. pH is not regulated and no buffers were used to keep the pH constant during the course of sorption in order not to affect the sorption process. Initial pH was equal to 6.8±0.1 (Metrohm 744) and initial conductivity (WTW LF 538) was 570±80 µS/cm. Once equilibrium had been reached, pH of the sludge suspension was measured and the sludge suspension was filtered through a cellulose nitrate membrane with 0.45 µm pore size. The filtrate was divided in two parts: one was acidified with a few drops of concentrated HNO_3 and stocked at 4°C until analysis; the other was stocked at -20°C until analysis. The soluble metal concentrations were determined in the acidified filtrate by flame atomic spectrometry (Varian 220FS). The dissolved organic carbon was determined in the frozen filtrate with a TOCmeter (Dohrmann Phoenix 8000).

RESULTS AND DISCUSSION

Solubilisation

The use of ultrasounds did not change total matter quantity and total solids concentration was constant. The total mineral solids and total organic solids concentrations were constant. Thus ultrasound did not induce a mineralization phenomenon. However, soluble matter concentration increased, whereas particulate matter concentration decreased. Fig. 1 shows matter distribution. These results are consistent with those obtained by Bougrier *et al.* (2005) on waste-activated sludge : ultrasounds led to a solubilization phenomenon of organic solids and mineral solids. In our experimental conditions, 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%.

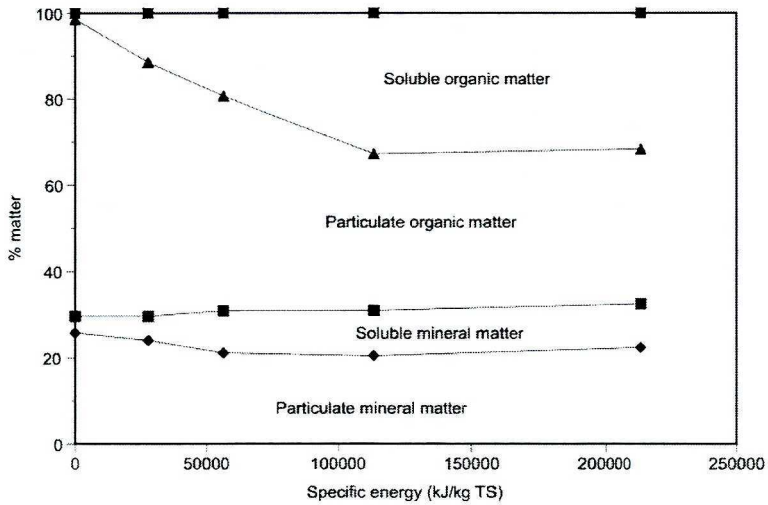

Fig. 1 Matter distribution as a function of specific energy

This solubilization of organic matter is underlined by the increase of soluble COD (Fig. 2), proteins, polysaccharides and humic acids (Fig. 3) with specific energy supplied.

The solubilization of organic may influence heavy metal uptake. In fact, the strong influence of dissolved organic matter on heavy metal uptake by activated sludge has been previously demonstrated (Wang *et al.*, 1999; Wang *et al.*, 2003). Dissolved organic carbon affects the repartition coefficient of heavy metals during the wastewater treatment because dissolved organic matter plays the role of ligand limiting uptake by sludge particulates (Katsoyiannis and Samara, 2007).

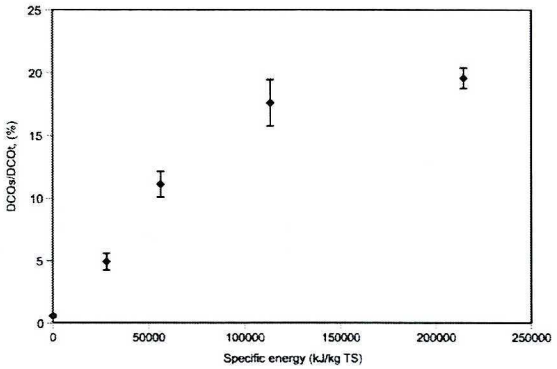

Fig. 2 Soluble DCO (DCOs) ratio vs. specific energy.

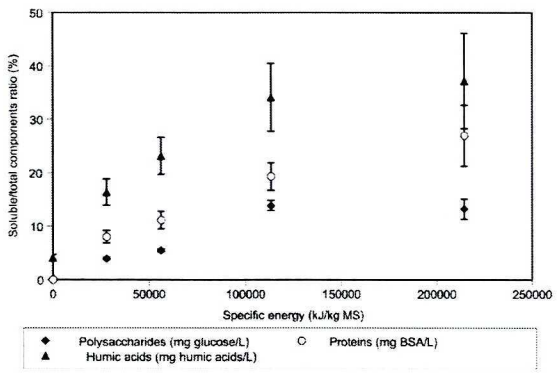

Fig. 3 Ratio of soluble organic components vs. specific energy

Floc surface properties

In this study, specific surface area was estimated considering that rhodamine B maximal adsorptive capacity is directly proportional to specific surface area.Sørensen and Wakeman, 1996.

Ultrasound treatment leads to floc size reduction (Bougrier *et al.*, 2005; Gonze *et al.*, 2003). Thus, flocs specific surface area must increase with specific energy. Experimental results confirmed this assumption (Fig.4) as specific surface area measured by rhodamine B adsorption increased. It ranged from de 28 ± 4 m^2/g SS for non treated sludge to114 ± 26 m^2/g SS for Es = 214000 kJ/kg MS.

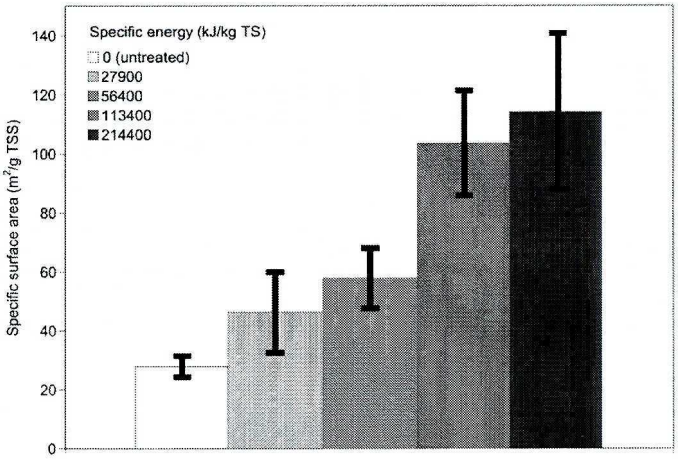

Fig.4 Specific surface area (estimated from rhodamine B) adsorption evolution with sonication

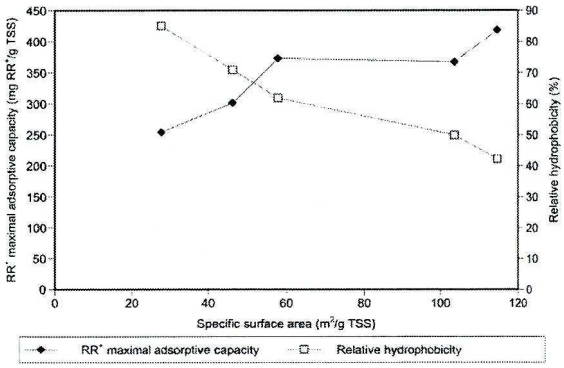

Fig. 5 Ruthenium red maximal adsorptive capacity and relative hydrophobicity of the flocs as a function of specific surface area obtained from rhodamine B adsorption

Fig. 5 shows relations between specific surface area with both maximal adsorptive capacity of ruthenium red (RR^+) and relative hydrophobicity measured by adhesion to hexadecane. RR^+ maximal adsorptive capacity increased with specific surface area whereas relative hydrophobicity decreased.

Although ruthenium red has mainly been used to assess extracellular polysaccharides of sludge flocs (Figueroa and Silverstein, 1989), it interacts with most of anionic charges on the sludge .The amount of adsorbed dye thus reflects dye interactions with negatively charged groups of

sludge (Boyette *et al.*, 2001).In fact, ruthenium red binds essentially to negatively charged groups of polysaccharides (carboxyl, hydroxyl) and sulphate groups (Figueroa and Silverstein, 1989).
The method used in this study to assess surface hydrophobicity derives from Wilén *et al.* (2003) with one main difference : sludge was not disintegrated before applying the protocol in order to assess only properties at the surface of the floc. The obtained RH value is an average value of both hydrophilic and hydrophobic groups at the surface the sludge. Hydrophilic molecules are generally polar or charged whereas hydrophobic ones are non polar (Jin *et al.*, 2003).

Thus, the increase of RR^+ maximal adsorptive capacity together with the decrease of relative hydrophobicity indicates an improved availability of negatively charged and/or polar groups at the surface of the flocs. Moreover, the relations of the two parameters with specific surface area are quasi linear (Fig. 5) demonstrating that ultrasounds action on flocs is essentially physical. Groups buried inside the EPS matrix are made free by floc desintegration but no significant chemical modification seemed to occur. This is confirmed by the fact that ultrasound at the frequency used in this study (20 kHz) have essentially hydromechanical effects due to ultrasonic cavitation (Tiehm *et al.*, 2001).

As adsorption and complexation of metal ions with negatively charged functional groups at the surface of EPS are one of the predominant mechanisms of heavy metal uptake by activated sludge (Guibaud *et al.*, 2005). Thus, evolutions of the surface properties shown in this study demonstrated that sonication may increase heavy metal uptake capacity of activated sludge.

Behaviour of heavy metals

In order to check to whether extent surface properties evolutions and organic matter release can affect heavy metal uptake by sonicated activated sludge, cadmium and copper biosorption tests have been carried out. Results obtained are showed in Table 1.

The mass maximal adsorption capacities (expressed in µmol/g TSS) increased with the specific energy supplied for the two metals studied (Table 1). This confirms the fact that the greater availability of fixation sites due to sonication is increasing heavy metal uptake capacity of activated sludge.

However, considering surface maximal adsorptive capacities (expressed in µmol/m2), it appears that the two metals studied had distinct behaviours (Table 1). In fact, cadmium surface maximal adsorptive capacity remained constant with specific energy: there is no major ultrasound induced shift in the cadmium uptake mechanisms. Concerning copper, the surface maximal adsorptive capacity decreased with specific energy: this indicates that a limitation of adsorption occurred as the specific energy increased. This is certainly due to metal complexation with the dissolved organic matter released (see above).

Table 1. Cadmium and copper maximal adsorptive capacities as a function of specific energy supplied.

Maximal capacities	adsorptive	E_s (kJ/kg TSS)				
		0	*27900*	*56400*	*113400*	*214400*
Mass (µmol/g MES)	*Cadmium*	298	375	600	888	941
	Copper	506	552	810	783	852
Surface ($\mu mol/m^2$)	*Cadmium*	11	8	10	9	8
	Copper	18	12	14	8	7

CONCLUSIONS

- Sonication of activated sludge leads to solubilization of organic matter: COD, polysaccharides, proteins and humic acid concentrations increased in the aqueous phase.
- Sonication affects 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 increase and relative hydrophobicity decrease, it can be assumed that sonication effects on floc surface properties are essentially physical: floc disruption due to ultrasound waves make negatively and polar sites more available.
- By increasing both dissolved matter content and fixation sites availability at the surface of the floc, sonication has two antagonistic effects on heavy metal uptake: on the one hand, dissolved organic matter limits the heavy metal uptake due to its complexation capacity with heavy metals. On the other hand, the greater availability of fixation sites increases heavy metal uptake capacity. The relative importance of these effects varies with the metal species considered. This is confirmed by the distinct behaviours of cadmium and copper towards sonicated activated sludges.

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